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## Efficacy of Nanofertilizers on Spinach Growth Performance in Medially Polluted Soils

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### **Abstract:**

#### *Purpose*

Food security has been a global problem with the sporadic growing population especially in the developing world, Kenya included. Many crop growth enhancers have been used such as conventional fertilizers, biofertilizers to boost food productivity. However, use excessive commercial fertilizer with the notion that some will be eroded and leached eventually causes eutrophication and alters soil pH that increases heavy metal uptake in crops threatening food safety and it has also minimal plant nutrient utilization. Nanoparticles have been used to give slower and timely release of nutrients to plants due to their versatile desirable characteristics.

#### *Methods*

Pot study in green house for contaminated soils were conducted with eight treatments in triplicate; DAP added, NPK added, NPK-DAP added, DAP nanofertilizer, NPK nanofertilizer, NPK-DAP nanofertilizer and non-treated control (ck) treatments, for spinach in the greenhouse study done in Nyakoe, Kisii county. Nine pots were set for each treatment with one seedling each. Nanofertilizers were applied at normal application rates during planting. Spinach plants were left grown for 30 days to 90 days until they matured as the growth performance parameters were being recorded.

#### *Results*

The results shows that there was a significant difference in plant growth performance in pots where nanofertilizers were used, with spinach amended with synthetic ash (nHA) increasing the growth parameters as follows; height (64.29%), dry matter weight (17.55%), leaf diameter (34.54%) and number of leaves (32.39%) while bone ash treatment increased height by (65.34%), dry matter weight (17.52%), leaf diameter (44.34%) and number of leaves (37.24%) when compared to NPK-DAP fertilizer added control.

#### *Conclusion*

Nanofertilizers enhances the growth performance of cropseven when grown in medially polluted sandy-loamy soils of lower fertility, especially in peri-urban farming to boost food productivity to feed ever growing population.

**Keywords:** Nanohydroxyapatite, nanofertilizer, synthetic ash, bone ash, Nanoparticles, growth performance

### **1. Introduction**

Globally escalating population and rapid urbanization leaves agriculturalists and agronomists with an uphill task of feeding higher number of people from limited agricultural arable fertile land which is ever decreasing at an terrific rate due to much land being transformed to settlement areas by ballooning human settlement in rural and urban areas like in Kisii County where the study was done. Satellite images indicates that the earth is rapidly running out of fertile arable land due to overpopulation, eutrophication and leaching of nutrients as a result of excessive use of commercial fertilizers, therefore food production is incapable to feed the ballooning world population especially in developing world (Jaggard *et al.*, 2010; Baruah and Dutta, 2009; Khodakovskaya *et al.*, 2012). Hence there is a looming food crisis especially in the developing world; Kenya included (Cui *et al.*, 2010). According to FAO 2015, thousands of people die in developing world yearly especially south of Sahara due to hunger and multinutrition related diseases.

Food production for the 20<sup>th</sup> century has been boosted with use of commercial fertilizers by farmers to feed ever growing population which is expected to reach 8.5 billion by year 2030 and 9.8 billion in the year 2050 (Jaggard *et al.*, 2010; Nilwala *et al.*, 2011). Agronomists have tried several methods of improving food production including use of high yielding and fertilizer responsive crop varieties, top dressing and growing of fast maturing crops. However, due to scarce arable

land it leads to over tillage depleting soil nutrients which led to excessive application of commercial fertilizers with the notion that it will translate to higher crop yields (Rai *et al.*, 2012; Gopinath *et al.*, 2014). But, much of the applied commercial fertilizer is leached and eroded to water bodies making nutrients unavailable to the crops when they need it. Therefore, there is limited interaction between the plant and the soil nutrients leading to lower crop yields. Hence, conventional fertilizer is inefficiently used by crops leading to lower food productivity and famine in developing countries, heightening the food crisis as poorer crop yields are realized. The artificial fertilizer use efficiency is below 40% for nitrogenous fertilizers and below 30% for phosphatic fertilizers, which implies that food production will have to be much more inefficient than ever before, hence there is need to apply nanotechnological strategies in fertilizer manufacturing to produce responsive nanofertilizers to feed ever increasing population especially in developing world (Ma *et al.*, 2010;Shaviv, 2000; Gopinath *et al.*, 2014).

Nanotechnology can be used to produce nanoparticles to be used as fertilizer carriers to act as new facilities to improve crop nutrient use efficiency and reduce environmental pollution caused by eutrophication in water bodies (Melika *et al.*, 2015; Cui *et al.*, 2010; Chinnamuthu and Boopathi, 2009). Encapsulation of conventional fertilizers within the nanoparticle is one of these recent strategies of nanotechnology which can be implemented in three ways. Firstly, the nutrient can be encapsulated inside nano porous pocket like particles then it is released slowly to the crop slowly and sustainably. Secondly, the nanomaterial is coated with the polymeric thin film enriched with NPK and DAP commercial fertilizer then, its gradual released to crops when they need it (Zhang *et al.*, 2013; Riu *et al.*, 2015). Thirdly, the artificial fertilizer is delivered as nanoparticles or emulsions of nano scale dimensions to the growing crop (Rai *et al.*, 2012; Joshiet *al.*, 2018;Gopithaet *al.*, 2013). Nanofertilizers will also combine as nano scale devices in order to regulate the release of nitrates and phosphatic nutrients to the plants, thereby preventing undesirable nutrient losses to the erosion, soil, weeds, water and air (DeRosa *et al.*, 2010; Giraldo *et al.*, 2014; Klingenfussiet *al.*, 2014).

Nano scale materials such as; the nano clays and nanozeolites have honey comb-like layered crystal structures that increases nanofertilizer use efficiency (Chinnamuthu and Boopathi, 2009; Song *et al.*, 2012). This multistructured layers increases adsorption sites that can be filled with nitrates, phosphates, potassium ions and other essential nutrients required for plant growth. So, it acts as a sustainable nutrient supplier at a slower rate as required by the plant. However, Leggo, (2000) noted that the major use of zeolites in agriculture is in nitrogen fixation, storage and sustainable release to crops. The over application of nitrogenous fertilizers is major source of underground water pollution. Nitrate release mechanisms of nanofertilizers in the absorbed form in nanozeolites are much slower than that which is in ionic form in artificial fertilizers (Cui *et al.*, 2010; Chinnamuthu and Boopathi, 2009;Tarafdaret *al.*, 2014).

Millan *et al.*, (2009) reported that nanozeolite encapsulated with urea had a higher capacity to raise the solubility of phosphate nutrients and thus improving phosphorus uptake and eventual better crop yield. Similarly, Li (2003) showed the possibility of application of the surfactant- modified nanozeolite using hexadecyltrimethyl ammonium as nanofertilizer carrier to control nitrate nutrient release and deduced that surfactant-modified nanozeolite is a better sorbent for nitrate, slow release rate. This shows that surfactant-modified nanozeolite encapsulated with NPK nutrients can be used as nanofertilizers that can be nutrient carriers to release them at controllable rate.

Coating and encapsulation of nanoparticles with artificial fertilizer enable them to regulate the release of plant nutrients from the nanofertilizer capsule to the crop (Yuvakkumar *et al.*, 2011;Raliyaet *al.*, 2015). Ma *et al.*, (2013) demonstrated that application of nitrogenous, phosphatic and potassium nanofertilizers, micronutrients, bionutrients and amino acids increase the uptake and utility efficiency of nutrients by grain crops. Nanozeolites have been applied for the controlled release of chemical substances such as growth enhancers, pesticides, herbicides and medicine. This study indicate that fertilizer incorporated into nanoparticles have improved crop yield of some crops, for example it has been reported to improve maize grain yield by 31.2% (DeRosa *et al.*, 2010; Husen *et al.*, 2014; Maet *al.*, 2012). This present study aimed to compare the use of nanofertilizers made from bone and that chemically synthesized nanohydroxyapatite encapsulated with NPK and DAP commercial fertilizers to grow spinach. This study made use of waste bone materials from streets that were otherwise regarded as an environmental menace to synthesize nanofertilizers which will not only reduce heavy metal uptake in food crops but also minimize change of soil pH, reduce fertilizer wastage and eventually reduce pollution in rivers that causes eutrophication. These boosts crop productivity to guarantee food security for the rapid growing world population, as they have been found to boost crop yield such as maize by 31.2% (Prapatsornet *al.*, 2015; Jaggardet *al.*, 2010; Prasad *et al.*, 2012; Srinivasan *et al.*, 2010).

## 2. Methodology

### 2.1. Research Design

The study was carried using a randomized complete block design with each category type having eight treatments in three replicates. The study was done in a greenhouse pot experiment set at Nyakoe, Kisii County. Three sets of experiments were set with each having spinach as the study plant. For this spinach plant, two categories of experiments were set. In category I, pots used to grow Sukuma wiki were treated with synthetic nanoparticle, while category II experiments for spinach bone made nanoparticle amendments were used. The soil used in the study were obtained 10 m underground to ensure they are nutrient deficient to provide a better experimental control. The nutrient poor soil was artificially polluted with water polluted with Cd/Pb salts to concentration levels that are permissible to NEMA, by sprinkling the water while mixing thorough to give uniform homogeneous mixed soil. In each category of grown Sukuma wiki (kales), eight treatments were set as follows; the non-treated control (CK), DAP added treatment, NPK added treatment, DAP-NPK added treatment, synthetic nanohydroxyapatite added treatment (nHA), DAP added nanofertilizer

(nHA+DAP), NPK added nanofertilizer (nHA+NPK), DAP-NPK added nanofertilizer (nHA +DAP+NPK) respectively. The same eight treatments were set using bone made nanoparticle fertilizers. All the fertilizers and nanofertilizers were applied by basal dispersal one day before planting the seedlings and the soils were harrowed immediately into 5 cm deep soil layer.

Crops were left to grow to maturity to a period of between one and three months (30-90 days) and then harvesting was done on monthly basis that's 30 days, 60 days and 90 days and then left to grow to full maturity. Growth performance parameters of various plants studied was measured by parameters like crop height, number of leaves, leaf diameter, and plant weight biomass.

## 2.2. Preparation of Hydroxyapatite Nanofertilizers

Chemically precipitated HA nanoparticles was synthesized as described by Mateus *et al.* 2007 using aqueous solution of 2M calcium hydroxide  $\text{Ca}(\text{OH})_2$  and phosphoric acid ( $\text{H}_3\text{PO}_4$ ). Firstly, 0.6M phosphoric acid ( $\text{H}_3\text{PO}_4$ ) was added to a suspension of 2M calcium hydroxide  $\text{Ca}(\text{OH})_2$  drop wise at different rates to vary the size. Synthesized nanoparticles were stirred vigorously under mechanical agitation of 1000 rpm for a period of 24 hours. The resulting HA nanoparticles was thrice washed with distilled deionized water to remove excess phosphoric acid. The solid HA nanoparticles obtained were then oven dried at temperatures of 100 °C for period of 2 hours then they were further crushed with mortar and pestle and they were then filtered to get Nano sized nanoparticles.

For the bone nanoparticles, the bones were collected from streets and butcheries and any loose tissues were removed, washed, dried and then cut into small pieces by grinder then the ground bones were put into the muffle furnace set at 600 °C for a period of 24 hours to obtain bone ash. The bone ash was ground into very fine sized particles, then it was mixed with water at a weight ratio of 1:1 to form bone suspension which was then put into a vibratory miller for a period of 18 hours before filtering to get filter cakes. The filter cakes were oven dried at 80 °C for 2 hours then they were ground using mortar and pestle and they were then filtered to get Nano sized nanoparticles. The bone made nanoparticles was the used to make nanofertilizers using the same procedure describe below.

Synthesized HA nanoparticles was dispersed in dilute solution of commercial NPK or DAP under ultrasonic mixing of 30 kHz for 1 hour. The resulting hydroxyapatite nanoparticles were dispersed and stirred in a saturated DAP-NPK solution at 25 °C for 12 hours. The resultant mixture was allowed to settle and the excess DAP/NPK solution was decanted. The product was twice washed with distilled water to remove any loosely held fertilizer and then dried at 50 °C for a period of 7 hours. NPK nanofertilizers, DAP nanofertilizers. The procedure was repeated with synthetic nanoparticle fertilizers.

## 2.3. Pot Experiment

In the greenhouse used to conduct pot study set at Nyakoe, Kisii County, the experimental soil was collected, medially contaminated with Cd/Pb, air dried and then ground before being sieved to pass through 2 mm nylon mesh sieve. Pot experiments were conducted with eight levels of treatments; the non-treated control (CK), DAP added, NPK added treatment, DAP-NPK added treatment, synthetic nanohydroxyapatite added treatment (nHA), DAP added nanofertilizer (nHA+DAP), NPK added nanofertilizer (nHA+NPK), DAP-NPK added nanofertilizer (nHA+DAP+NPK) respectively. Three replicates were set in spinach plant treatment. The experimental soil was homogenously mixed to give uniform conditions. Three seedlings of spinach were planted per pot and left to grow until maturity was reached with constant watering after every 3 days. The spinach plant samples were harvested on monthly basis that's 30 days, 60 days and 90 days of growth.

## 2.4. Growth Parameters

### 2.4.1. Plant Height (Cm)

Height of the main stem from ground level to the highest tip of the emerging spinach plant leaf was measured at every time of harvest using tape measure and expressed in cm and recorded in Table 1.

### 2.4.2. Leaf Diameter (Cm)

Leaf diameter from one leaf end to other of the spinach plant leaves was randomly selected and measured at every time of harvest using Vernier caliper and expressed in cm and recorded in Table 3.

### 2.4.3. Number of Leaves Per Plant

Numbers of fully opened leaves from the main stem were physical counted and recorded in spinach at the time of maturity and recorded in Table 3. All spinach leaves were counted including those that were senesced as long as this could be identified.

### 2.4.4. Dry Biomass Weight

After maturity the spinach plants were uprooted and thoroughly dried before being weighed in analytical balance to determine dry weight in grams. The obtained results were recorded in Table 3.

## 2.5. Data Analysis

Data collected was analyzed using statistical analysis software (SPSS). Analysis of variance (ANOVA) was carried out to determine whether there were significant differences among treatments on spinach plant growth parameters over

the entire growth period. Fisher's LSD (t-test) was used to separate means at ( $P < 0.05$ ) significance level. The results were presented tabular form and graphically.

### 3. Results and Discussions

TREATMENT	Synthetic Spinach Heights			P-Value	Bone Spinach Heights			P-Value
	Harvest 1	Harvest 2	Harvest 3		Harvest 1	Harvest 2	Harvest 3	
Non-treated control	12.67±0.88 <sup>a</sup> (11.00-14.00)	16.00±0.58 <sup>a</sup> (15.0-17.0)	20.00±1.53 <sup>a</sup> (18.0-23.0)	0.008428	10.67±0.88 <sup>a</sup> (9.00-12.00)	16.67±0.88 <sup>a</sup> (15.00-18.00)	20.00±0.58 <sup>a</sup> (19.00-21.00)	0.000472
DAP Added	13.33±1.20 <sup>a</sup> (11.00-15.00)	17.00±0.58 <sup>a</sup> (16.0-18.0)	21.33±0.33 <sup>a</sup> (21.0-22.0)	0.00117	12.00±0.58 <sup>a</sup> (11.00-13.00)	19.00±0.58 <sup>b</sup> (18.00-20.00)	24.00±1.15 <sup>b</sup> (22.00-26.00)	0.000142
NPK Added	14.00±1.15 <sup>b</sup> (12.00-16.00)	16.33±0.88 <sup>a</sup> (15.0-18.0)	21.33±1.20 <sup>a</sup> (19.0-23.0)	0.008255	13.00±0.58 <sup>b</sup> (12.00-14.00)	21.33±0.88 <sup>b</sup> (20.00-23.00)	29.33±0.88 <sup>c</sup> (28.00-31.00)	2.09x10 <sup>-5</sup>
DAP+NPK Added	11.33±0.88 <sup>a</sup> (10.00-13.00)	18.00±0.58 <sup>a</sup> (17.0-19.0)	23.33±1.20 <sup>a</sup> (21.0-25.0)	0.000288	12.00±0.58 <sup>a</sup> (11.00-13.00)	21.33±1.20 <sup>b</sup> (19.00-23.00)	28.83±1.20 <sup>c</sup> (26.00-30.00)	9.6x10 <sup>-5</sup>
nHA Added	12.33±0.88 <sup>a</sup> (11.00-14.00)	29.33±1.20 <sup>c</sup> (27.0-31.0)	38.00±0.58 <sup>d</sup> (39.0-41.0)	2.17x10 <sup>-6</sup>	12.33±0.88 <sup>a</sup> (11.00-14.00)	32.33±1.45 <sup>c</sup> (30.00-35.00)	47.67±1.76 <sup>e</sup> (47.00-53.00)	4.83x10 <sup>-6</sup>
nHA +DAP Added	11.67±0.88 <sup>a</sup> (10.00-13.00)	25.00±0.58 <sup>b</sup> (24.0-26.0)	36.67±1.76 <sup>d</sup> (34.0-40.0)	1.81x10 <sup>-5</sup>	11.33±1.20 <sup>a</sup> (9.00-13.00)	29.00±1.15 <sup>c</sup> (27.00-31.00)	43.33±1.45 <sup>d</sup> (41.00-46.00)	6.51x10 <sup>-6</sup>
nHA+ NPK Added	10.33±0.88 <sup>a</sup> (9.00-12.00)	28.67±0.88 <sup>c</sup> (27.0-30.0)	33.00±1.15 <sup>c</sup> (31.0-35.0)	7.49x10 <sup>-6</sup>	11.67±1.20 <sup>a</sup> (10.00-14.00)	27.00±1.00 <sup>c</sup> (26.00-29.00)	44.33±1.20 <sup>d</sup> (42.00-46.00)	2.96x10 <sup>-6</sup>
nHA+DAP+ NPK Added	11.67±0.88 <sup>a</sup> (10.00-13.00)	21.67±1.45 <sup>b</sup> (19.0-24.0)	30.00±0.58 <sup>b</sup> (29.0-31.0)	5x10 <sup>-5</sup>	13.00±0.58 <sup>b</sup> (12.00-14.00)	28.33±1.20 <sup>c</sup> (26.00-30.00)	45.00±1.15 <sup>d</sup> (43.00-47.00)	1.73x10 <sup>-6</sup>
P-value	0.247631	8.33X10 <sup>-09</sup>	1.94X10 <sup>-09</sup>		0.543828	1.16X10 <sup>-07</sup>	1.73X10 <sup>-11</sup>	

Table 1: Spinach Height Growth Performance for the Three Harvests (N=72)

Mean values followed with same small letters within the same column are not significantly different at  $p = 0.05$  (SNK test)

#### 3.1 Growth Performance of Synthetic Grown Spinach

##### 3.1.1. Growth Performance of Synthetic Nanofertilizer Grown Spinach Heights

Harvest 1, synthetic spinach heights had no significant difference between the various as evidenced by p-values in Table 1. Harvest 1 had  $p = 0.24763$ , indicating that the amendments from commercial fertilizers and synthetic nanofertilizers had not interacted enough with the experimental soil to cause a significant difference in the spinach heights elongation within the first 30 days of planting spinach seedlings in pots. The NPK fertilizer added treatment produces the highest spinach height due to its sufficient nutrients in the earlier days of growth, synthetic NPK nano fertilizers on the other hand, produced the lowest spinach heights elongations, however this height was not significantly different from each other.

Harvest 2, synthetic spinach height elongation was very significantly different with each other after 60 days of growth showing that the soil additives had interacted enough time with experimental to cause such big height differences. This is clearly indicated by  $p = 8.33 \times 10^{-9}$ , hence the heights are significantly different. Synthetic ash (nHA) pot produced the highest elongation indicating the synthetic nanohydroxyapatite had nutrients which could be gradually released to the spinach to enhance sustainable growth performance. This height elongation was not significantly different with those produced by the pot's amendment with synthetic NPK nano fertilizers producing height of  $28.67 \pm 0.88$  cm. The other synthetic nano fertilizers also had better heights of spinach elongation as compared with the control and the fertilizer added treatments. The synthetic DAP nano fertilizers significantly differed from the synthetic DAP mixture NPK nanofertilizers that had height of  $21.67 \pm 1.45$  cm. However, for the commercial fertilizers added treatments, they had lowest heights elongation, and they were not significantly different from each other. In fertilizers added treatment although they were not significantly different from each other pots treated with DAP+NPK fertilizers had longest elongation of  $18.00 \pm 0.58$ . Harvest 2 had the lowest height elongation in the non-treated control pots of  $16.00 \pm 0.58$  cm. Harvest 3 height elongation for synthetic set-up differed very significantly from each other in various treatments as indicated by p-value of  $1.94 \times 10^{-9}$  in Table 1. The non-treated control produced the lowest spinach heights of  $20.00 \pm 1.53$  cm, because the non-treated control lacked sufficient nutrients to spur good growth performance of spinach seedlings since experimental soil was poorly equipped with essential plant nutrients. The non-treated control height elongation was closely followed by pots treated with commercial fertilizers DAP and NPK, which showed no significant height difference with the non-treated control. However, in this fertilizer added treatments DAP+NPK added pots produced slightly higher harvest 3 height of  $23.33 \pm 1.20$  cm which was very close with the others.

##### 3.1.2. Growth Performance Comparison between Synthetic Harvest Heights.

Comparison of first three harvests of synthetic greenhouse spinach showed very significant difference in all the eight treatments as indicated by the p-value shown by Table 1 above. For the non-treated control there was significant difference between the harvests done within the first 90 days of growth as shown by p-value of 0.008428, indicating that the minimal nutrients in the non-treated control pots produced highest height in harvest 3 of  $20.00 \pm 1.53$  cm while harvest 1 produced the lowest height of  $12.67 \pm 0.88$  cm in this treatment. The DAP fertilizer added treatment similarly had a significant difference between harvest 1 and harvest 3, with  $p = 0.00117$  as shown by Table 2 above.

Generally, in all fertilizer added pots, the third harvest produced the longest spinach seedling measured after 90 days while harvest 1 that was taken after 30 days recorded lowest spinach height elongation. There was significant difference between harvest 1 and harvest 3 in each case as shown by p-values shown in Table 2 as follows; DAP added treatment ( $p=0.00117$ ), NPK added treatment ( $p=0.008255$ ), DAP+NPK added treatment ( $p=0.000288$ ). The p-values obtained in commercial fertilizer added treatments are lower than those obtained in nanofertilizer added treatments.

The treatment of synthetic ash produced the longest spinach plant in the third harvest with the height of  $38.00\pm 0.58$  cm which was totally significantly different from the heights recorded in harvest 1. The p-value for the first three harvests is  $2.17 \times 10^{-6}$  indicating that the harvests are significantly different from each other.

The synthetic nanoparticles encapsulated with NPK and DAP fertilizers had also related results, whereby in all these treatments the third harvest produced the longest height elongation of spinach plant in comparison with harvest 1 in each case respectively, hence there was a significant difference as shown by the p-values given in Table 2 as follows; synthetic DAP nanofertilizer ( $p=1.81 \times 10^{-6}$ ), synthetic NPK fertilizer ( $p=7.49 \times 10^{-6}$ ) and mixture of NPK and DAP synthetic nanofertilizer had  $p=5.0 \times 10^{-5}$ .

### 3.2.1. Growth Performance of Bone Nanofertilizer Grown Spinach Heights.

For the bone grown spinach plants, harvest 1 height elongations did not differ significantly from each other in the various treatments as evidenced by the p-value which is greater than 0.05 ( $p=0.54382$ ) as shown by Table 1. This shows that the added fertilizers and bone nanofertilizers within the first 30 days had not interacted with the soil structure and soil nutrients to cause a difference in spinach height elongation of some treatments. However, in this first harvest non-treated control produced the poorest height elongation of  $10.67\pm 0.58$  cm due to lack of enough nutrients to support better spinach growth performance, which was significantly different from heights recorded in pots treated with DAP fertilizer ( $12.00\pm 0.58$  cm). Other fertilizer added treatment also produced lower spinach heights as follows; NPK added treatment ( $13.00\pm 0.58$  cm), and DAP + NPK added treatment ( $12.00\pm 0.58$  cm).

Harvest 2 spinach heights on pots treated with nanoparticles encapsulated with DAP and NPK fertilizers produced better spinach heights than those produced by pots treated with commercial fertilizers and non-treated control. Hence there was significance difference in the heights of spinach recorded in the various treatments as shown by the p-value of  $p=1.16 \times 10^{-7}$ . Due to lack of enough nutrients in nutrient poor experimental soil, that's why it recorded lowest spinach height after 60 days of growth. It was closely followed by commercial fertilizer added pots which had limited nutrients due to leakage of these nutrients to lower levels which can't be easily reached by spinach plants. However, bone nanohydroxyapatite had more sites to adsorb the plant nutrients and slowly release them to plants when needed. Other bone NPK nanofertilizers and bone DAP nanofertilizers also recorded impressive spinach heights elongation due to gradual release of these nutrients to spinach plants. The bone ash (nHA added) recorded the longest spinach height of  $47.67\pm 1.76$  cm followed by bone NPK nanofertilizers that had height of  $44.33\pm 1.20$  cm.

Harvest 3, heights which were the highest in each treatment differed very significantly in the various treatment of bone spinach as evidenced by p-value of  $1.73 \times 10^{-11}$  in Table 1. The non-treated control like other earlier harvests recorded the lowest third harvest of  $20.00\pm 0.58$  cm. The fertilizer added treatment had better growth performance than the non-treated control because they had enough nutrients that encouraged spinach height growth although most of these nutrients had been leached by the time third harvest was being done. However, they had better heights as shown in Table 4.16 above, DAP added treatment ( $24.00\pm 1.15$  cm), NPK added treatment ( $29.53\pm 0.83$  cm) and NPK+DAP added treatment ( $28.33\pm 1.20$  cm).

The bone nanoparticles had very impressive properties that guarantee gradual release of spinach plant nutrients that's why they produced the best results in the growth performance of the spinach plant as shown by Table 1. The bone ash added treatment (nHA added) posted the spinach plants with the highest heights of  $47.67\pm 1.76$  cm, indicating presence of the required properties to improve food productivity in the agricultural sector. Similarly, other bone made particles encapsulated with NPK and DAP nanofertilizers had better growth performance as shown by their impressive heights as follows, bone DAP nanofertilizers ( $43.33\pm 1.45$  cm), bone NPK nanofertilizers ( $44.30\pm 1.20$  cm) and bone NPK+DAP nanofertilizers had  $45.00\pm 1.15$  cm. this is due to gradual and slow sustainable release of nutrients to spinach plant as evidenced by studies done by; Morteza *et al.*, 2013.

### 3.2.2. Growth Performance Comparison between Bone Harvest Heights

The comparison of harvest 1 up to harvest 3 showed very significant differences in all the eight treatments indicating that maturity time of harvest greatly determines the growth performance of the spinach plant. The non-treated control had a significant difference between the first three harvests as shown by their p-value given in Table 1 ( $p=0.000472$ ), showed that the minimal nutrients available in the experimental soil was absorbed by spinach to sustain its dismal spinach height growth performance.

Generally, for all the commercial fertilizer added treatments there was a significant difference in heights recorded in between harvest 3 and harvest 1. The third harvest recorded the highest spinach height elongation because the plant nutrient although some are leached guarantees higher spinach height over a period of time. The first harvest recorded the lowest spinach elongation heights because the nutrients had not been up taken by enough amounts within 30 days to have higher spinach heights.

TREATMENT	Synthetic and Bone Spinach Heights H <sub>3</sub> comparison (cm)		
	Synthetic Harvest 3	Bone harvest 3	P-Value
Non-treated control	20.00±1.53 <sup>a</sup> (18-23)	20.00±0.58 <sup>a</sup> (19.00-21.00)	1.00
DAP Added	21.33±0.33 <sup>a</sup> (21-22)	24.00±1.15 <sup>b</sup> (22.00-26.00)	0.090733
NPK Added	21.33±1.20 <sup>a</sup> (19-23)	29.33±0.88 <sup>c</sup> (28.00-31.00)	0.005821
DAP+NPK Added	23.33±1.20 <sup>a</sup> (21-25)	28.83±1.20 <sup>c</sup> (26.00-30.00)	0.042316
nHA Added	38.00±0.58 <sup>d</sup> (39-41)	47.67±1.76 <sup>e</sup> (47.00-53.00)	0.006478
nHA +DAP Added	36.67±1.76 <sup>d</sup> (34-40)	43.33±1.45 <sup>d</sup> (41.00-46.00)	0.04336
nHA+ NPK Added	33.00±1.15 <sup>c</sup> (31-35)	44.33±1.20 <sup>d</sup> (42.00-46.00)	0.002443
nHA+DAP+NPK Added	30.00±0.58 <sup>b</sup> (29-31)	45.00±1.15 <sup>d</sup> (43.00-47.00)	0.000314
P-value	1.94X10 <sup>-09</sup>	1.73X10 <sup>-11</sup>	

Table 2: Comparison of Height Growth Performance between Synthetic and Bone Nano Fertilizers Treatments in Spinach (N=72)

Mean values followed with same small letters within the same column are not significantly different at  $p=0.05$  (SNK test)

The comparison shown in Table 2 indicate that the non-treated control and the DAP fertilizer added treatment had no significant difference with each other as shown by  $p>0.05$ . But, due to non-uniformity in amending other fertilizer added treatments, there was a significant difference in their harvest 3 heights. However, for all nanofertilizer treatments there was a significant difference in harvest 3 heights as  $p<0.05$  shown in Table 2 above. This shows that the bone and synthetic nanofertilizer treatment interacted with experimental soil to vary the number of adsorption sites that controls the gradual release of crop nutrients. The bone nanofertilizer had more stable multi-pocketed stable sites to have higher nutrient control rate as compared to respective synthetic nanofertilizers.

### 3.3. Other Growth Parameters

Treatment	Bone Spinach Othergrowth Parameters			Synthetic Spinach Othergrowth Parameters		
	L. diameter	Dry weight	No. leaves	L. diameter	Dry weight	No. leaves
Non-treated control	5.83±0.34	56.33±0.33	7.33±0.88	5.73±0.24	56±1.73	7.33±1.20
DAP Added	10.63±0.30	62.33±0.88	11±0.58	10.63±0.30	63.67±2.91	9.67±0.67
NPK Added	11.1±0.15	64±1.53	11.33±0.88	10.77±0.12	65.33±2.33	10.67±0.88
DAP+NPK Added	10.6±0.67	64.67±1.86	10.67±1.20	10.8±0.31	62.67±0.88	11.33±0.33
nHA Added	15.3±0.32	76±1.73	17±0.58	14.53±0.24	73.67±1.20	15±0.58
nHA +DAP Added	14.7±0.1	76.67±2.33	16±0.58	14.2±0.23	70±1.53	14.33±0.67
nHA+ NPK Added	14.37±0.24	77.67±1.20	17.67±0.88	14.33±0.24	71.33±1.76	16±0.58
nHA+DAP+NPK Added	14.67±0.45	76.67±2.33	16±1.53	14.27±0.23	69.67±0.88	16±0.58
P-value	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

Table 3: Other Growth Performance Parameters for Bone and Synthetic Spinach Harvest 3 (N=72)

TREATMENT	Synthetic and Bone Sukuma Heights H <sub>3</sub> comparison (cm)							
	%Syntheti c Harvest 3 height	% Bone Harvest 3 height	% synthetic No. leaves	% Bone No. leaves	% Synthetic L. diameter	% Bone L. diameter	% synthetic Dry weight	% Bone Dry weight
Non-treated control	-14.27 <sup>a</sup>	-29.40 <sup>a</sup>	-35.30 <sup>a</sup>	-45.57 <sup>a</sup>	-46.94 <sup>a</sup>	-45.00 <sup>a</sup>	-10.64 <sup>a</sup>	-12.90 <sup>a</sup>
DAP Added	-8.57 <sup>b</sup>	15.28 <sup>c</sup>	-14.65 <sup>c</sup>	3.09 <sup>bc</sup>	-1.57 <sup>b</sup>	0.28 <sup>b</sup>	1.60 <sup>b</sup>	-3.62 <sup>b</sup>
NPK Added	-8.57 <sup>b</sup>	3.53 <sup>b</sup>	-5.83 <sup>b</sup>	6.19 <sup>c</sup>	-0.28 <sup>b</sup>	4.72 <sup>b</sup>	4.24 <sup>b</sup>	-1.04 <sup>b</sup>
DAP+NPK Added	0 <sup>c</sup>	0 <sup>b</sup>	0 <sup>d</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
nHA Added	64.29 <sup>g</sup>	65.34 <sup>e</sup>	32.39 <sup>f</sup>	37.24 <sup>d</sup>	34.54 <sup>c</sup>	44.34 <sup>d</sup>	17.55 <sup>d</sup>	17.52 <sup>c</sup>
nHA +DAP Added	57.18 <sup>f</sup>	52.95 <sup>d</sup>	26.48 <sup>e</sup>	49.95 <sup>e</sup>	31.48 <sup>c</sup>	38.68 <sup>cd</sup>	11.70 <sup>c</sup>	18.56 <sup>c</sup>
nHA+ NPK Added	41.45 <sup>e</sup>	56.48 <sup>d</sup>	41.22 <sup>g</sup>	65.60 <sup>f</sup>	32.69 <sup>c</sup>	35.57 <sup>c</sup>	13.82 <sup>cd</sup>	20.10 <sup>c</sup>
nHA+DAP+NP K Added	28.59 <sup>d</sup>	16.67 <sup>c</sup>	41.22 <sup>g</sup>	49.95 <sup>e</sup>	32.13 <sup>c</sup>	38.39 <sup>cd</sup>	11.17 <sup>c</sup>	18.56 <sup>c</sup>
P-value	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

Table 4: Comparison of Percentage Height Growth Performance between Synthetic and Bone Nano Fertilizers Treatments with the Control in Spinach (N=72)

Table 3 shows the comparison of other spinach growth parameters like leaf diameter, dry weight biomass and number of leaves per spinach plant were very significantly between different treatments of the bone set-up and synthetic set-up as indicated by p-value p<0.01. Nanofertilizers recorded significantly better figures in the growth performance parameters. There is no significant difference in the fertilizers added pots of the bone experiment set-up and synthetic set-up.

The nanofertilizer treated pots had the highest increase in the growth performance indicators than the commercial fertilizer treated pots as shown by the above Table 4. The bone made nanofertilizers had significantly higher growth performance parameters than the nanofertilizers made from chemically precipitated nanohydroxyapatites. For instance, the bone ash (nHA) had better; 65.34% height and 44.34% leaf diameter and 37.24% number of leaves biomass while the synthetic ash (nHA) had better 17.55% dry weight.

#### 4. Discussions

For the synthetic nanofertilizer treatments, they had the longest spinach height elongations compared with other treatments. However, the synthetic ash had sufficient nutrients enabling it to produce the tallest spinach seedling in harvest 3 of 40.00±0.58cm which was not significantly different from the height recorded by synthetic DAP nanofertilizers with height of 36.67±1.76cm. This is proven that the synthesized nanohydroxyapatite have enough nutrients and have desirable characteristics of gradual release nutrients to spinach plants when they are demanded, thereby improving food productivity and reducing wastage of soil nutrients as concurred by Ma *et al.*, 2013. This signifies that the synthetic nanoparticle has large surface area, high density, higher number of adsorption sites that ensure that there is gradual release of plant nutrients to sustain higher spinach seedling height. Some scientists who have concurred with same results include Ma *et al.*, 2010, Prapatsornet *et al.*, 2015, and Riu *et al.*, 2015. Other nanofertilizers synthetic and encapsulated with fertilizer to gradually release to the spinach plant when they need it, NPK nanofertilizers had longer spinach height of 33.00±1.15cm and NPK +DAP nanofertilizers with 30.00±0.58cm.

This is because although commercial DAP and NPK fertilizers have enough plant nutrients to sustain growth, most of these nutrients are leached to lower zones that can't be easily reached by the short roots of the spinach plants, hence by the 90<sup>th</sup> day of growth very minimal nutrients are available to spinach plant to spur growth.

The third harvest was guaranteed the longest height than earlier harvests because the encapsulated NPK and DAP into the nanoparticles with larger surface area, higher adsorption capacity, high density and higher number of atoms that ensures slow and gradual release of nutrients to spinach plants when they need them. Several studies have posted related results such as Zhang *et al.*, 2013 and Gopithaet *et al.*, 2013.

For bone treatment set-ups, harvest 1 heights of nanoparticles and nanofertilizers produced highest spinach height elongations with the bone ash (nHA added) producing the longest spinach plant of (14.67±0.58 cm) in this harvest because of the unique properties of nanoparticles that ensures slow and sustainable release of plant nutrients. This is because bone nanoparticles had very impressive properties that guarantee gradual release of spinach plant nutrients that's why they produced the best results in the growth performance of the spinach plant as shown by Table 3. In harvest 3, the bone ash added treatment (nHA added) posted the spinach plants with the highest heights of 49.67±1.76 cm, indicating presence of the required properties to improve food productivity in the agricultural sector using little arable land (Jaggard *et al.*, 2010; Wang *et al.*, 2012). Similarly, other bone made particles encapsulated with NPK and DAP nanofertilizers had better growth performance as shown by their impressive heights as follows, bone DAP nanofertilizers (43.33±1.45 cm), bone NPK nanofertilizers (44.30±1.20 cm) and bone NPK+DAP nanofertilizers had 45.00±1.15 cm. this is

due to gradual and slow sustainable release of nutrients to spinach plant as evidenced by studies done by; Morteza *et al.*, 2013; Klingenfuss *et al.*, 2014; DeRosa *et al.*, 2010 and; Yaoyao *et al.*, 2019.

The comparison in spinach heights shows that the bone and synthetic nanofertilizer treatment differed significantly because they interacted with experimental soil to vary the number of adsorption sites that controls the gradual release of crop nutrients. The bone nanofertilizer had more stable multi-pocketed stable sites to have higher nutrient control rate as compared to respective synthetic nanofertilizers as noted by Baruah and Dutta, 2009.

Since, bone nanoparticle have better and superior and stable desirable nanoparticle properties such as a larger surface area for adsorption, more selective adsorption, higher density to particle weight and higher number of atoms it enables it to have a more controlled release of the plant nutrients to the spinach plants when they require the nutrients, therefore it emerges that bone nanoparticles are best for synthesis of nanofertilizers as noted that in all the four nanoparticle treatments, in each respective treatment the bone grown spinach had significantly higher height elongation at the third harvest. The nano hydroxyapatites from bone ash have more superior desirable qualities release the nutrients gradually to the plant when they are required, hence reduces nutrient wastage which guarantees higher and better plant growth performance. However, the nanoparticle encapsulated with NPK and DAP fertilizer had lower pH, scorching effect and lower soil organic matter that contributes to recording lower spinach height elongations than bone ash added treatment. Related posted agreeable results to that include researchers such as, Yuvakkumaret *et al.*, 2011, Melikaet *et al.*, 2015; Leeet *et al.*, 2012 and Joshi *et al.*, 2018.

However, the comparison of other spinach growth parameters like leaf diameter, dry weight biomass and number of leaves per kale plant were not very significantly different between the bone made nanofertilizers and synthetically made nanofertilizers. Although, the bone nanofertilizers recorded slightly better figures in the growth performance parameters. Similarly, there is no significant difference in the fertilizers added pots of the bone experiment set-up and synthetic set-up.

This is because nanoparticles have special adsorption sites with large surface area, hence when encapsulated with commercial NPK, DAP fertilizers they release nutrient gradually to plants that enhance growth of performance of spinach plants. On the other hand, the non-treated control had the lowest spinach heights because the plants lacked nutrients that could have spurred growth. Treatments give NPK and DAP fertilizers had higher spinach heights than control (non-treated). However, these treatments have lower heights than nano fertilizer treatments because of leaching of nutrients to lower zones that cannot be reached by spinach plants. Similarly, the commercial NPK and DAP fertilizers the lower soil that increases mobility of plant nutrients making them vulnerable to leaching. Commercial fertilizers they also have scorching effect that affects spinach growth performance. Hence the nanoparticles when encapsulated with commercial fertilizers has unique property of selective adsorption of nutrients that enables the slow and slow release of plant nutrients making them to have longest interaction with the bean seedlings up to 90 days to guarantee higher growth performance. Several studies have shared related results with different crops such as Liu *et al.* 2011 who used nanoparticles for slow release of nutrients and it improved the maize grain yield by 31.2% and other crops (Song *et al.*, 2012; Tarafdar *et al.*, Giraldo *et al.*, 2014; 2014 Melikaet *et al.*, 2015).

## 5. Conclusion

Spinach grown using nanofertilizer treatments recorded significant higher growth indicators in bone nanohydroxyapatite with following parameter performances; height ( $47.67 \pm 1.76$ cm), dry matter weight ( $76.67 \pm 2.33$ g), leave diameter ( $15.3 \pm 0.32$ cm) and number of leaves ( $17 \pm 0.58$ ). In synthetic set up the NPK nanofertilizer had height ( $38.00 \pm 0.58$ cm), dry matter weight ( $73.67 \pm 1.20$ g), leave diameter ( $14.53 \pm 0.24$ cm) and number of leaves ( $15 \pm 0.58$ ). Similarly, other nanofertilizer treatments posted significantly higher growth performance than commercial fertilizer added treatments and the non-treated control, especially in the third harvest. The spinach grown in pots treated with commercial fertilizers recorded significantly lower growth performance parameters after 90 days. For NPK added treatments; height ( $44.33 \pm 1.20$ cm), dry matter weight ( $77.67 \pm 1.20$ g), and number of leaves ( $17.67 \pm 0.88$ ). Generally, nanofertilizers had an increase in growth performance for instance for synthetic ash (nHA) growth parameters increased as follows; height (64.29%), dry matter weight (17.55%), leaf diameter (34.54%) and number of leaves (32.39%) and for bone ash (nHA) increasing as follows; height (65.34%), dry matter weight (17.52%), leaf diameter (44.34%) and number of leaves (37.24%).

The study has conclusively established that nanofertilizers when used in right proportions in spinach farming can increase crop yields of spinach that is used as vegetable to feed ever ballooning population to alleviate looming crisis in developing countries especially Kenya with invasion of desert locusts to many vegetable farms.

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