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Influence of Plant Density and NPK 15:15:15 Fertilizer on Some Agronomic Characters and Proximate Composition of Onion (*Allium cepa* L.) in Edo Rainforest of Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author EJF laid out the experiment, collected data and samples, analysed samples in the laboratory and wrote the first draft of the manuscript. Author JOE planned out the project and oversaw the whole experiment and laboratory analysis, reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The effect of plant population density and varying levels of NPK 15:15:15 fertilizer on the growth, yield and proximate composition of onion (*Allium cepa* L.) was investigated in a 2-year study, 2010/2011 and 2011/2012. The experiment was a factorial combination of three plant densities 333,333, 250,000 and 160,000 plants ha^{-1} and four levels of NPK 15:15:15 inorganic fertilizer at 0, 40, 80 and 120 kg /ha NPK laid out as a randomized complete block design with three replications. Results indicated that the three plant densities of 333,333, 250,000 and 160,000 plants ha^{-1} were statistically similar in both trials and enhanced the growth of onion in terms of stem diameter except at 6 weeks after transplanting (WAT). This trend was also observed for proximate composition of onion leaves and bulbs and number of leaves in 2010/2011. In 2011/2012, the lowest plant density of 160,000 plants/ha produced more leaves compared to the higher plant populations of 333,333 and 250,000 plants/ha. Chlorophyll content, CGR and bulb length were similar at the lower densities and significantly higher than those produced at the highest plant density of 333,333. In

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both trials bulb yield was significantly similar at densities of 333,333 and 250,000 plants/ha. Increase in fertilizer application significantly increased the moisture content. Crude protein and crude fiber but decreased the percentage dry matter content of leaves and bulbs. However, optimum bulb yield was obtained at density of 250,000 plant/ha with fertilizer application rate of 80 kg/ha NPK 15:15:15.

Keywords: Onion; fertilizers; chlorophyll content; crop growth rate; yield.

1. INTRODUCTION

Plant density is an important agronomic factor which can cause substantial increase or decrease in yield of most crops hence, appropriate plant density is vital for the interception of enough sunlight necessary for optimum photosynthesis .The information on the effect of density on the growth and yield of onion (*Allium cepa* L.) is scanty. Planting of onion at optimum density ensures proper plant growth through adequate utilization of moisture, light, and nutrients. Planting of onion at optimum density gives the best economic return. [1] obtained the highest yield with a density of 20 x 10 cm (500,000) plants/ha, [2], at 15 x 10 cm (666,666) plants/ha, [3] at 10 x 9 cm (1,110,000). The role of nutrients has also been documented by [4] to be one of paramount importance in boosting productivity and quality of onion bulb which is a heavy feeder of mineral elements. This fact corroborates its superficial root system that is rarely branched and lack root hairs which make the onion very inefficient in the uptake of water and nutrients. This has resulted in large amounts of chemical fertilizers being used in onion cultivation. The high demand of onion can only be met, by increasing its per hectare yield. This can be done by many ways of which the most important is the application of fertilizer especially that of nitrogen, phosphorus and potassium through the appropriate density management. Fertilizers are used to supplement the nutrients which the plant can obtain from the soil in order to increase crop yield without being detrimental to quality. The objective of this work is to determine the optimum fertilizer rate and appropriate plant density for onion growth, yield and quality in Edo rainforest zone of Nigeria.

2. MATERIALS AND METHODS

2.1Experimental Site

The Experiments were carried out in the 2010/2011 and 2011/2012 dry season at the Teaching and Research Farm of the University of Benin, Ugbowo Campus, Benin-City, in Edo State and Lies within the geographical coordinates of longitude 5º, 04" and 06º 43" E and latitude 05º 44" N and 07º 34" N. Meteorological data during the experimental period was obtained from Nigerian Institute for Oil Palm Research (NIFOR) and is presented in Fig. 1.

2.2 Analytical Methods of Experimental Soils

Prior to analysis, the soil samples were air dried and crushed to pass through a 2 mm sieve. Soil pH was determined using a pH meter. Organic carbon was determined by [5] wet oxidation method as modified by [6]. Total nitrogen was obtained by macro Kjeldahl methods as modified by [6]. Available P was extracted by [7] and the P was estimated by the blue colour method of [8] Exchangeable K and Na were determined using flame photometer, and Ca and Mg using the Atomic Absorption Spectrophotometer. The result of the analysis is as presented in Table 2. The experiment was laid out as a randomized complete block design (RCBD) with three replications. The treatments were composed of a factorial combinations of four rates (0, 40, 80 and 120 kg ha^{-1} NPK 15:15:15) fertilizer and three plant densities of 333,333, 250,000 and 160,000 plants ha^{-1}. Each replicate had 12 plots giving a total of 36 plots in this experiment.

2.3 Field Operations

The land was cleared with the debris worked into the soil with a hoe. Beds for planting were prepared and onion seeds (Kano red) were sown in the nursery and transplanted to the field when seedlings were seven weeks after sowing and at about 14 cm tall. Plots were mulched to conserve soil moisture and suppress weeds. The inorganic fertilizer application at (0, 40, 80 and 120 kg /ha of NPK 15:15:15 compound fertilizer were applied in two split applications. The first dose was applied two weeks after transplanting and the remaining half at six weeks after transplanting by side placement along the rows to the respective plots depending on the treatment. The field was weeded manually using hoe. A total of three hand weedings were done at 3, 6 and 8 weeks after transplanting. Insects were handpicked when necessary. Data collection started four weeks after transplanting. Four plants were randomly selected from each plot and tagged for the purpose of collecting data for number of leaves, stem diameter, chlorophyll content, crop growth rate, bulb length and bulb fresh yield per hectare.

2.4 Statistical Analysis

The data obtained were subjected to statistical analysis of variance (ANOVA) using SAS following the model for factorial experiment in a randomized complete block design and means separated by Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Plant density had a significant effect on number of leaves of onion at 6 WAT (Table 3). Highest number of leaves was observed with plant grown at 160,000 plants/ha. However, this was not significantly different from plant grown at 250,000 plants/ha but significantly different from the highest density of 333,333 which produced the least number of leaves per plant. Similarly, the effect of fertilizer application on number of leaves of onion in 2010/2011 (Table 3.) was highly significant at 4, 6, 8 and 10 WAT. At 4 WAT plants treated with 80 and 120 kg ha^{-1} NPK produced similar number of leaves which were significantly higher than leaves produced by plants which received 40 kg ha^{-1} NPK and no fertilizer (control) treatments.

Table 1. Chemical properties of the soil before and after planting

Soil properties	Pre	after plantation
pH (H_2O)	5.30	6.01
Organic matter g(100 g)$^{-1}$	0.83	2.18
Total Ng(100 g)$^{-1}$	0.06	0.07
Total P mg(kg)$^{-1}$	2.10	18.36
K (cmolkg^{-1})	0.26	0.29
Ca (cmolkg^{-1})	1.20	1.80
Mg(cmolkg^{-1})	0.60	0.80
Sand (%)	64.80	68.62
Clay (%)	27.20	26.66
Silt (%)	8.00	8.14
Textural class	Sandy loam	

In 2011/2012, mean number of leaves was significantly affected by density at all samples intervals (Table 3). At 4 WAT plants grown at higher density of 333,333 and 250,000 produced similar number of leaves, while the lowest plant density of 160,000 plants/ha produced highest number of leaves per plant. Significantly higher number of leaves observed from the lower density crops of 160,000 plants/ha could be due to the fact that the plants in that environment had adequate ground cover thereby preventing excessive loss of moisture from the soil,

Table 2. Effect of Plant density and NPK 15:15:15 fertilizer on number of leaves per plant 2010/2011 and 2011/2012 dry cropping season

Treatment	Number of leaves/plant								
	2010/2011 cropping season					2011/2012 cropping season			
	4	6	8	10	(WAT)	4	6	8	10
Plantdensity (ha^{-1})									
333,333	4.68^{a}	5.45^{b}	6.22^{a}	6.49^{a}		6.15^{b}	6.36^{b}	6.75^{b}	7.02^{b}
250,000	4.75^{a}	5.69^{ab}	6.46^{a}	6.70^{a}		6.38^{ab}	6.50^{b}	6.82^{b}	7.07^{b}
160,000	4.88^{a}	5.90^{a}	6.57^{a}	6.80^{a}		6.59^{a}	7.23^{a}	7.83^{a}	7.83^{a}
SEM	0.22	0.27	0.32	0.34		0.21	0.23	0.26	0.27
NPK 15:15:15fertilizer (kgha^{-1})									
0	4.05^{c}	5.21^{b}	5.69^{b}	5.92^{b}		6.05^{b}	6.59^{b}	6.68^{b}	6.72^{b}
40	4.79^{b}	5.45^{b}	5.83^{b}	6.06^{b}		6.27^{ab}	6.82^{ab}	7.26^{a}	7.47^{a}
80	5.62^{a}	6.45^{a}	6.86^{a}	7.13^{a}		6.41^{a}	6.97^{a}	7.27^{a}	7.49^{a}
120	5.77^{a}	6.72^{a}	7.16^{a}	7.42^{a}		6.50^{a}	7.03^{a}	7.43^{a}	7.80^{a}
SEM	0.24	0.29	0.30	0.32		0.22	0.25	0.27	0.28

WAT = Weeks after Transplanting; Means followed by the same letter in a column are not significantly different at 5% level of probability using DMRT; SEM = Standard error of a mea

Table 3. Effect of plant density and NPK 15:15:15 fertilizer on stem diameter per plant 2010/2011 and 2011/2012 dry cropping season

Treatment	Stem diameter/plant (cm)								
	2010/2011 cropping season					2011/2012 cropping season			
	4	6	8	10	(WAT)	4	6	8	10
Plant density (ha^{-1})									
333,333	0.68[a]	0.74[b]	0.88[a]	0.88[a]		0.65[a]	0.70[b]	0.80[a]	0.81[a]
250,000	0.70[a]	0.80[ab]	0.90[a]	0.91[a]		0.67[a]	0.80[a]	0.83[a]	0.84[a]
160,000	0.72[a]	0.85[a]	0.92[a]	0.93[a]		0.68[a]	0.82[a]	0.86[a]	0.87[a]
SEM	0.02	0.03	0.04	0.04		0.03	0.03	0.04	0.04
NPK 15:15:15 fertilizer ($kgha^{-1}$)									
0	0.52[b]	0.55[c]	0.60[c]	0.61[c]		0.44[b]	0.49[b]	0.54[b]	0.55[b]
40	0.60[b]	0.84[b]	0.86[b]	0.86[b]		0.74[a]	0.87[a]	0.94[a]	0.95[a]
80	0.73[a]	0.91[ab]	0.94[ab]	0.95[ab]		0.78[a]	0.90[a]	0.98[a]	0.98[a]
120	0.74[a]	0.98[a]	1.02[a]	1.02[a]		0.81[a]	0.91[a]	1.00[a]	1.02[a]
SEM	0.03	0.04	0.05	0.05		0.04	0.04	0.05	0.05

WAT = Weeks After Transplanting; Means followed by the same letter in a column are not significantly different at 5% DMRT; SEM= Standard error of a mean

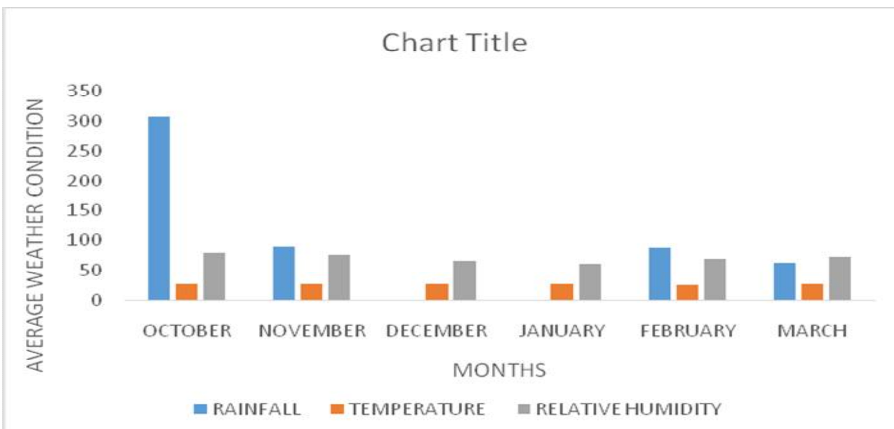

Fig. 1. Weather conditions at NIFOR station, Benin from October to March
Source: Nigerian Institute for Oil Palm Research (NIFOR

suppressing weed and also resulted in less competition for nutrient and other growth resources as opposed to the denser population of 333,333 which resulted in competition for growth resources. In all cases significantly lowest number of leaves was produced at the control.

Stem diameter of onion was significant at 6 WAT (Table 4). At 4, 8 and 10 WAT, the results were similar there was no significant differences observed in plants grown at all densities. The effect of fertilizer application on stem diameter of onion in 2010/2011 (Table 4.) was highly significant at all sampling intervals and similar to the results obtained for number of leaves. The significant increase in number of leaves, stem diameter and CGR observed from the density corroborates the findings of [9] that wider density enhances vegetative growth of plants through the development of more leaves. This might be attributed to the fact that plants widely spaced experience little or no competition for limited environmental resources compared to closely spaced plants. The higher values of stem diameter observed at higher density is not unexpected because the plants had adequate crop growth factors for their use. The significant differences observed with higher density at 6 – 10 WAT in some of the parameters measured could be attributed to the onset of bulb initiation since the plants need more nutrient at this stage, the higher plant density could not compensate for the nutrient demand compared with the lower density crop.

Table 4. Effect of plant density and NPK 15:15:15 fertilizer on chlorophyll content (mgg^{-1}), crop growth rate (CGR) $gm^{-2}wk^{-1}$, bulb length (cm) and bulb fresh yield ($t\ ha^{-1}$) of onion 2010/2011 and 2011/2012 dry cropping season

Treatment	2010/2011 cropping season				2011/2012 cropping season			
	Chlorophyll content (mgg^{-1})	CGR ($gm^{-2}wk^{-1}$)	Bulb length (cm)	Bulb yield ($t\ ha^{-1}$)	Chlorophyll content (mgg^{-1})	CGR ($gm^{-2}wk^{-1}$)	Bulb length (cm)	Bulb yield ($t\ ha^{-1}$)
Plant density (ha^{-1})								
333,333	15.84^{b}	0.50^{a}	3.72^{b}	13.92^{a}	13.36^{b}	0.40^{b}	3.10^{b}	13.84^{a}
250,000	23.32^{a}	0.52^{a}	3.98^{ab}	14.08^{a}	19.54^{a}	0.47^{a}	3.79^{a}	14.68^{a}
160,000	24.87^{a}	0.52^{a}	4.24^{a}	9.03^{b}	20.08^{a}	0.48^{a}	3.83^{a}	8.19^{b}
SEM	2.18	0.04	0.26	1.34	2.15	0.02	0.22	1.27
NPK 15:15:15 fertilizer ($kgha^{-1}$)								
0	8.33^{b}	0.26^{b}	2.69^{c}	4.45^{c}	8.12^{c}	0.27^{b}	2.82^{b}	5.15^{c}
40	20.11^{a}	0.55^{a}	3.83^{b}	10.11^{b}	15.96^{b}	0.45^{a}	3.79^{a}	10.49^{b}
80	21.62^{a}	0.59^{a}	4.51^{a}	14.83^{a}	20.93^{a}	0.47^{a}	4.05^{a}	14.82^{a}
120	22.24^{a}	0.60^{a}	4.71^{a}	15.81^{a}	21.76^{a}	0.48^{a}	4.06^{a}	15.15^{a}
SEM	2.31	0.05	0.28	1.41	2.22	0.03	0.26	1.35

WAT = Weeks After Transplanting; Means followed by the same letter in a column are not significantly different at 5% level of probability using DMRT; SEM= Standard error of a mean

Chlorophyll content index was significantly affected by density, plants grown at lower densities 250,000 and 160,000 plants/ha produced similar chlorophyll content index that were significantly higher than that of 333,333 plants/ha. Similarly, the effect of fertilizer application on chlorophyll content index of onion in 2010/2011 (Table 5) was highly significant. Plants treated with NPK fertilizers produced similar chlorophyll content index and were significantly higher in chlorophyll content index than plants which received no fertilizer (control). The increase in chlorophyll content of leaves as observed in the lower dense crops could be attributed to the fully expanded leaves found with wider density which perhaps results in less competition for space and less shading effect by other plants and so the leaves may have been positioned for better interception of light energy for photosynthesis. The superiority expressed by the lower dense crops over the higher dense conformed with the previous findings by [10] and [11] on onion. In addition [12] confirmed that higher density results in the growth of less vigorous plant when compared with lower density. The results of onion density experiment reported by [13] showed that not only were the leaves of onion more from the low density crops but they had thicker and larger leaves. This was probably accounted for by a relatively reduced competition among plants per unit area for available soil nutrients in the lower density plots than the higher density ones. In 2011/2012, 80 and 120 kg ha^{-1} produced similar chlorophyll content index and were significantly higher in chlorophyll content index than plants which received 40 kg ha^{-1} and the control. Crop growth rate was not significantly affected by density. However, fertilizer application significantly affected (CGR). Plants treated with 40, 80 or 120 kg ha^{-1} NPK produced similar (CGR) and were significantly higher than plants which received no fertilizer (control). In 2010/2011 (Table 5), density significantly affected bulb length of onion, plants grown at either 250,000 or 160,000 plants/ha produced similar bulb length. This trend was also similar for plants grown at either 333,333 or 250,000 plant densities. However, plants grown at 160,000 plant density significantly increased bulb length above that produced at the highest density of 333,333 plants/ha. The effect of fertilizer application on bulb length of onion in 2010/2011 (Table 5) was highly significant, bulb length increased with increase in fertilizer application up to 80 kg ha^{-1} NPK and a further increase did not result in a significant increase in bulb length. In 2011/2012 (Table 5).

Plants grown at 250,000 and 160,000 plants/ha produced similar bulb length which were significantly increased above the 333,333 plant density. Effect of fertilizer application on bulb length of onion was highly significant. Plants treated with 40, 80 and 120 kg ha^{-1} NPK produced similar bulb length and were significantly above that produced at the control. The increase in bulb length as a result of increase in plant density could probably be due to the growth factors which were not limiting in the micro environment and the plants had enough space for bulb elongation. The marked increase in bulb length as a result of lower density could be explained by the work of [14] that onion plant is a poor competitor for either space or nutrients. The lack of available space for increase in bulb length could account for the decrease in mean bulb length from denser population. Density significantly affected fresh bulb yield of onion. Bulb yield increased with increase in plant density. Plants grown at higher densities of 333,333 and 250,000 density were at par in fresh bulb yield and significantly produced higher bulb yield above the lowest plant density of 160,000 plants/ha. Similarly, the effect of fertilizer application on fresh bulb yield of onions in both years (Table 5) was highly significant. Increase in the rate of fertilizer application from 0 to 80 kg ha^{-1} increased bulb yield but a further increase did not result in a significant increase. In contrast to growth parameters, significantly highest bulb yield was recorded with 250,000 and 333,333 plants/ha and the least yield recorded for the lower density of 160,000 plants/ha, this result could be attributed to greater crop biomass found with the highly dense as supported by [15]. The results of this experiment has shown clearly that bulb yield can be increased at a density of 333,333 and 250,000 plants/ha. Lower density reduced yield due to total reduction in plants per hectare and consequently space is not fully utilized. These results are evidently in accordance with those of [16,9,10]. The increase in the vegetative and yield characters observed as a result of increase in fertilizer application rate could be due to the nutrients received by such plants as was evident in the control plots were values for bulb length and diameter were the lowest perhaps due to the inherent soil nutrient which was limiting in their micro environment. Similar results have been recorded by [10] on onion. This is a clear indication that onion plant responds positively to increase in fertilizer application while the reverse was the case in the control. [18] found that nutrient content of leaves increased with increase in fertilizer and so he lend support to the suggestion as he observes that the nitrogen content of onion leaf was directly proportional to soil N level. Moisture, ash, crude protein, crude fibre and dry matter content of onion leaves were not significantly increased by density (Table 6). When the effect of fertilizer application was considered, there was a significant increase in moisture content of onion leaves. Application rate of 120 kg ha^{-1} NPK increased moisture content compared to either 40 or 80 kg ha^{-1} NPK. Similarly (Table 7) moisture, ash, crude protein, crude fibre and dry matter content of bulbs were not significantly increase by density. The effect of fertilizer application on moisture content of onion bulbs was significant 120 kg ha^{-1} NPK significantly records higher moisture content compare to the no fertilizer treatment and plants treated with either 40 or 80 kg ha^{-1} which recorded significantly similar values. Fertilizer application significantly did not affect the ash content of leaves. The effect of fertilizer application was significant on crude protein of onion leaves. In this case, crude protein increased with increase in fertilizer application. Significantly higher values for crude protein was recorded for plants treated with 80 and 120 kg ha^{-1} NPK compared to 40 and 0 kg ha^{-1}. Crude protein of bulb increased significantly with increase in fertilizer application up to 40 kg ha^{-1}. The control received the least value for crude protein content. Significantly higher values for crude fibre was recorded for plants treated with 80 and 120 kg ha^{-1} NPK compared to 40 and 0 kg ha^{-1}. In (Tables 6 and 7), there was no significant increase in crude fat as affected by fertilizer application. Plants treated with 0, 40 and 80 kg ha^{-1} NPK produced similar dry matter content of leaves and bulbs which were significantly higher than plants which received 120 kg ha^{-1} NPK fertilizer. The crude protein, total ash, moisture content and crude fibre were not significantly affected density. The higher proximate values were obtained for crude protein in the leaves compared to bulbs could be attributed to the fact that N is, a constituent of all amino acids in proteins and lipids that act as a structural compounds of the chloroplast; [17].

The proximate composition of the leaves and bulbs showed that the leaves had higher moisture and crude fibre content than the bulbs and this suggest that consumption of the leaves will aid in easy digestion and removal of waste product from the body thus reducing the risk of colon cancer. [19] reported that diets high in fibre have been shown to decrease risks of heart diseases, obesity and help lower cholesterol. Generally it was observed that lowest moisture content was produced by the control as compared with NPK treated plants. This value is closely similar to the result reported by [20].

This shows that the higher dose of NPK fertilizer increases the moisture content of onion bulbs and leaves. Moisture is very important in fresh onion as well as in the period of storage of any food material. Availability of high moisture is suitable condition for the growth of microorganism and thus decreases shelf life of onion. Concentration of crude protein increased with increase in fertilizer application. This result shows strong agreement with the result recorded by [21].

Table 5. Effect of plant density and NPK 15:15:15 fertilizer on the proximate composition of onion leaves

Treatments	Moisture content (%)	Ash (%)	Crude protein %)	Crude fibre (%)	Crude fat (%)	Dry matter (%)
Plant density (ha^{-1})						
333,333	89.86^a	1.18^a	3.50^a	1.14^a	0.82^a	10.44^a
250,000	89.59^a	1.10^a	3.50^a	1.15^a	0.83^a	10.41^a
160,000	89.44^a	1.18^a	3.51^a	1.16^a	0.87^a	10.56^a
SEM	0.46	0.12	0.06	0.02	0.09	0.15
NPK15:15:15 fertilizer (kgha^{-1})						
0	89.50^b	1.25^a	3.47^b	1.11^c	1.02^a	10.50^a
40	89.57^b	1.16^a	3.52^b	1.16^b	0.90^a	10.43^a
80	89.60^b	1.12^a	3.60^a	1.21^a	0.90^a	10.40^a
120	91.69^a	1.10^a	3.69^a	1.23^a	0.84^a	8.31^b
SEM	0.49	0.13	0.09	0.02	0.18	0.16

WAT = Weeks after transplanting means followed by the same letter in a column are not significantly different at 5% level of probability using DMRT; SEM= Standard error of a mean

Table 6. Effect of plant density and NPK 15:15:15 fertilizer on the proximate composition of onion bulbs

Treatments	Moisture content (%)	Ash (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Dry matter (%)
Plant density (ha^{-1})						
333,333	88.56^{a}	1.51^{a}	3.47^{a}	1.10^{a}	0.90^{a}	11.44^{a}
250,000	88.62^{a}	1.57^{a}	3.52^{a}	1.11^{a}	0.90^{a}	11.38^{a}
160,000	88.65^{a}	1.66^{a}	3.47^{a}	1.11^{a}	0.93^{a}	11.35^{a}
SEM	0.28	0.14	0.08	0.01	0.09	0.10
NPK 15:15:15 fertilizer (kg ha^{-1})						
0	88.67^{b}	1.78^{a}	3.39^{b}	0.98^{b}	1.02^{a}	11.43^{a}
40	88.49^{b}	1.69^{a}	3.53^{a}	1.13^{a}	0.91^{a}	11.51^{a}
80	88.60^{b}	1.52^{a}	3.64^{a}	1.14^{a}	0.90^{a}	11.40^{a}
120	88.97^{a}	1.54^{a}	3.67^{a}	1.16^{a}	0.76^{a}	11.03^{b}
SEM	0.32	0.17	0.11	0.03	0.22	0.12

WAT = Weeks after transplanting; Means followed by the same letter in a column are not significantly different at 5% level of probability using DMRT; SEM= Standard error of a mean

4. CONCLUSION

Application of inorganic NPK (15:15:15) fertilizers at 80 and 120 kg ha^{-1} NPK produced the highest bulb yield. Decrease in plant density from 333,333 to 160,000 plants/ha resulted in increase in average bulb weight but decrease in bulb yield per hectare. Higher plant density of 333,333 and 250,000 plants/ha were associated with higher bulb yield but a decrease in average bulb weight. It can therefore be concluded that for optimum onion production in this locality 80 kg/ha NPK fertilizer should be applied at plant density of 250,000 plants/ha.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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