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Stressful Effect of Different Rates of Nitrogen Starter Dose on Nodulation, Growth, Proximate Composition and Yield of Soybean (*Glycine max*)

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Abstract

Despite the fact that high nitrogen content in the soil can impede nodule formation in soybean, the need for addition of nitrogen fertilizer as a starter dose to supplement the actions of N-fixing bacteria is a necessity because it restores the drained nutrient and results in high yield. To evaluate the stressful effects of different rates of N fertilizer starter dose, a pot experiment was conducted to evaluate the effect of N fertilizer starter dose on nodulation, growth, proximate composition and yield of soybean. Urea was applied at the rates 0, 50, 75 and 100 kgN/ha at planting using side placement method. The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. Growth assessment was through taking data on plant height, number of branches, number of leaves and internode length. Also, data on nodulation, yield and yield attributes were taken. Proximate compositions (crude protein, crude fat, crude fiber and ash content) were also determined. It was found that 100 kgN/ha of N-fertilizer improved the total nodulation by 33.95% compared with the control. The yield was greatest with application of 100 kgN/ha compared with other application rates because there was 20% yield increase compared with the control. For the proximate composition, crude protein content was 7% above the control with application of 50 kgN/ha. Furthermore, crude fat content was 37% better than the control with the application of 100 kgN/ha. It was concluded that for profitable soybean production in the study area, the use N fertilizer like urea at 50 kgN/ha as a starter dose is recommended because of its cheapness and high yield that resulted from its application to the crop. Finally, nitrogen starter dose up to 100kgN/ha did not constitute stress to production of soybean variety under test.

1. Introduction

Like other crops, soybean requires nitrogen for its growth and development. Apart from the fixed nitrogen which will be absorbed from the soil or the root nodules, nitrogen can still be added to the soil for soybean production at different growth stages starting from time of planting as a starter dose. Except when the nitrogen supply is above the optimum required, application of nitrogen fertilizer will not be toxic or stressful to the lives of plants. When the supply is at toxic or stressful level, there could be inhibition of symbiotic nitrogen fixation [1].

Despite the ability of soybean to fix nitrogen through its nodules, occasions could warrant addition of a starter dose to cater for the optimum need by the crop. On this, Hardy et al. [2] had a discovery that nitrogen fixation begins 14 days after planting only when soybean is cultivated under optimum moisture and temperature and as such a small amount of starter nitrogen will be useful at early vegetative stage. Similarly, starter nitrogen is capable of supplying nitrogen before nitrogen fixation begins in the root nodules [3]. In the same vein, the use of nitrogen starter dose aims at provision of soil nitrogen which is readily available to soybeans at seedling stage and could be a source of yield increment. Moreover, early application of starter dose nitrogen has been found to have significant increase in grain yield soybean and growth attributes as well as plant biomass [4].

Application of lower rate (45kgN/ha) of starter nitrogen dose combined with density of 45 plants per metre square increased the yield of soybean yield [5]. Furthermore, application of low rate (less than 15kgN/ha) of starter nitrogen at planting led to yield increase over years compared to no starter dose application [6].

Contrary to that, application of starter dose nitrogen could lead to nitrogen fixation reduction in soybean plant as well as drop in yield [7]. It should be noted that starter dose nitrogen may have no effects whatsoever on grain yield, shoot fresh mass, leaf area and plant height [8]. However, in irrigated and non-irrigated soybean fields, higher application rate of starter dose nitrogen significantly increased grain yield of the crop in the mid-southern part of USA [9].

Though different researchers have determined the effects of starter dose on soybean plant, the results are not conclusive. Therefore, this experiment was conducted to determine the stressful effects of different rates of nitrogen starter dose on nodulation, growth, proximate composition and yield of soybean (*Glycine max*).

2.0. Methodology

2.1. Experimental Site

The research was carried out at the green house, Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria.

2.2. Experimental Design and Treatment Application

The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. The treatments were four (4) levels of nitrogen- 0, 50, 75 and 100kgN/ha. The fertilizer source was urea. Application of fertilizer was done at planting.

2.3. Plant Materials, Planting and Cultural Practices

The soybean variety used was Sam Soya 2. The capacity of the experimental pots was 7 litres. The soil used was collected from a nearby field and sieved to remove large particles. A 5kg of the soil was weighed and put in each pot. Six seeds were planted at 2 cm depth and the resulting seedlings were thinned to four in each pot.

The plants were fully irrigated (at field capacity) till the termination of the experiment. Each experimental pot contained four plants. The pots were manually and regularly weeded till the end of the experiment to keep the plants weed free. Insect pests were controlled using Lambda Cyhalothrin at 15ml/10litres of water. This operation started 5 weeks after planting and was repeated fortnightly until harvest.

2.4. Data Collection

2.4.1. Chlorophyll determination

Leaf chlorophyll content was determined homogenizing 1g of a leaf sample in 15ml of ethanol. The mixture was then filtered and the filtrate was covered aluminum foil to prevent it from being broken down by sunlight. The concentration of chlorophyll was then measured as a function of intensity of absorbed light in a spectrophotometer. Absorbance was measured at 647 and 664 nm wavelengths with UV spectrophotometer. Total and actual chlorophyll were calculated using the following formulae:

Chlorophyll a = (13.19 x A_{664})-(2.57 x A_{647}) (1)

Chlorophyll b = (22.1 x A_{647})-(5.26 x A_{664}) (2)

Total chlorophyll = Chlorophyll a + chlorophyll b (3)

A_{664} and A_{647} are absorbances at wavelengths 647 and 664nm respectively.

2.5. Determination of Proximate Composition of Soybean

2.5.1 Preparation of sample for proximate analysis

Dried samples of leaves were ground into fine powder. From the ground samples, crude fat, crude protein, crude fibre and ash contents were determined using the methods described by Kirk and Sawyer [10] and James [11].

a. Nitrogen determination

This was done using Kjeldahl method described by Chang [12]. 0.5g of each plant sample was mixed with 10ml of H_2SO_4 in a digestion flask. A tablet of selenium was then added to the mixture and the resulting mixture was heated under a fume cupboard until the mixture turned to a clear solution (sample digest). The digest was made up to 100ml using distilled water and kept in a volumetric flask. 10ml of the digest was mixed with equal volume (10ml) of 45% sodium hydroxide solution in Kjeldahl distillation apparatus. The mixture was distilled into 10ml of 40% boric acid containing three drops of mixed indicators (bromocresol green and methyl red). A total of 50ml distillate was collected and titrated against 0.02N EDTA until the colour turned from green to deep red (the end point). Reagent without plant sample (blank) was also distilled and titrated. Percentage of nitrogen was then calculated as follows:

$$\%\text{Nitrogen}= \frac{100\text{x}N\text{x}14\text{x}Vt}{W\text{x}1000\text{ X }Va} xTxB \quad (4)$$

W= Mass of sample (0.5g)

N= Normality of titrant (0.02N H_2SO_4)

Vt= Total digest volume (100ml)

Va= Volume of analyzed digest (10ml)

T= Sample titre value

B=Blank titre value

Note:1ml of 1N H_2SO_4 =14mg

b. Crude Protein Determination

Nitrogen content was first determined using Kjeldahl method described by Chang [12] as stated above under nitrogen determination. Crude protein was then determined from the amount of nitrogen got using Equation 5.

%Crude Protein= %N x6.25 (5)

c. Determination of crude fat

The determination was through gravimetric method described by Krick and Sawyer [10]. 5g of plant sample was wrapped in a porous paper (whatman filter paper) and put in a thimble. The thimble was put in a soxlet reflux flask and mounted on weighed extraction flask (W_1) containing 200ml of petroleum ether. The upper part of the reflux flask was connected to a water condenser. The solvent (petroleum ether) was heated to boil, vapourize and condense into soxlet reflux flask. Through this process the sample in the thimble was shortly covered with the solvent after it was put there until soxlet reflux flask was filled and then siphoned. The oil extract was carried down to the boiling flask. This process was allowed to go on repeatedly for four hours before the defatted sample was removed. The solvent was recovered and the oil extract was left in the flask. The flask containing the oil extract was dried in an oven at 60^oC for 30minutes to remove any residual solvent. The flask was then cooled in a desiccator and weighed (W_2). The percentage of oil (fat) extract was then determined using Equation 6.

$$\%Fat = \frac{W2-W1}{\text{Mass of plant sample}} x100 \quad (6)$$

Where:
W_1=Mass of empty extraction flask
W_2=Mass of flask + Oil (fat) extract

d. Determination of Total Ash Content

This was determined through furnace incineration gravimetric method as described by James[11]. 5.0g of prepared plant sample was weighed into a porcelain crucible of mass W_1. The sample was burnt to ashes at 550^oC in a muffle furnace. After it has completely burnt into ashes, it was cooled in a desiccator and the mass of the crucible and ash was determined and recorded as W_2. Percentage of ash in the sample was determined as follows:

$$\%Ash= \frac{W2-W1}{\text{Mass of plant sample}} x100 \quad (7)$$

Where:
W_1=Mass of empty extraction flask
W_2=Mass of crucible + Ash

e. Determination of crude fibre

This was determined by the procedure described by James [11]. 5.0g of the prepared plant sample was weighed and boiled in 150ml of 1.25% H_2SO_4 solution for 30minutes under reflux. The boiled sample was washed in several portions of hot water using a two-fold cloth to trap plant particles. The sample was returned to the flask and boiled again in 150ml of 1.25% sodium hydroxide for 30 minutes under the same condition. After the sample was washed in several portions of hot water, the sample was allowed to drain and dry before being transferred into a weighed crucible where it was dried to a constant mass at 105^oC using an oven. The mass of crucible + the dry sample was recorded as W_2. The dried sample was then transferred into a muffle furnace and burned into ashes. Percentage of crude fibre was determined as follows:

$$\%Crude\ Fibre = \frac{W2-W3}{\text{Mass of plant sample}} x100 \quad (8)$$

Where:
W_2=Mass of crucible + sample after washing, boiling and drying
W_3=Mass of crucible + Sample of ash

Days to 50% flowering

This was recorded when about half of the total plants in each treatment flowered.

Days to maturity

This was taken when about 70% of the pods in each pot changed from green to brown colour.

Plant height

The plant height was measured the soil level to the tip of the last leaf using a measuring. The average height was calculated and recorded for each treatment after maturity.

Number of leaves

Two plant stands were randomly selected per pot and their leaves were counted. The average number of leaves was recorded for each treatment.

Number of branches

The number of branches was counted at maturity. The average value was computed for each treatment.

Number and mass of nodules

Nodulation was assessed when there was 50% flowering in each pot by carefully uprooting three (3) plants randomly from each treatment. The adhering soil was removed by washing gently over a metal sieve. The nodules from each plant were removed separately and spread on the sieve to drain water from their surfaces. The nodules were then counted and the average number of nodules per plant was recorded and the mass of nodules per plant was determined using a sensitive scale.

Pod yield per plant

The number of pods per plant was counted and recorded at maturity by counting the number of pods in all the plants in each pot and finding the average.

Seed yield per pod (g)

Number of seeds per pod was counted and the mass of seeds per pod was determined using a sensitive scale.

Fifty seed mass (g)

Fifty seeds were counted and weighed from each pot using a sensitive scale. The average value for each treatment was then recorded.

2.6. Data Analysis

Collected data were subjected to Analysis of Variance (ANOVA) using IBM SPSS version 20 while mean separation was done using Duncan's multiple range tests.

3.0. Results and Discussion

3.1. Effect of nitrogen starter dose on total nodulation and nodule mass

Plants from 100 kg/ha urea produced the highest number of nodules followed by those from 75kgN/ha while the lowest number of nodules were from the control plants. Plants from 100kg/ha urea were 33.33% higher in nodule production than the control plants (Table 1). As for nodule mass, the control plants produced the lightest nodules while pots with 100 kgN/ha produced the heaviest nodules followed by plants treated with 50 kgN/ha. Plants treated with 100 kgN/ha produced nodules that were 60.97% heavier than the control plants (Table 1). This result implies that nitrogen fertilization has a direct relationship with nodulation in the test crop. Furthermore, application of 100kgN/ha did not constitute stress to the plants to result in nodulation reduction. Increase in number of nodules and nodule mass with increase in nitrogen application could be attributed to increase in root cell division aided by the presence of nitrogen. In the same vein, application of nitrogen might have enhanced absorption of more water and minerals which led to better cell enlargement and elongation with resultant increase in the number of nodules and their masses. It has been reported that nitrogen application in soybean would be of help in the formation of nodules with eventual increase in symbiotic nitrogen fixation rate [13]. So, there is direct relationship between nitrogen application and number of nodules along with the nodule mass. Furthermore, increase in number of nodules and their dry masses has been reported with application of 40kgN/ha [14].

However, high rate of nitrogen fertilization can suppress nodulation and nitrogen fixation in soybean plants resulting in low grain yield [15]. This is an induced toxicity through nutrition. Furthermore, high level of nitrogen application can result in very luxuriant shoot growth, shoot lodging, poor formation of pods and the plants become highly prone to high insect infestation and disease infection [16]. In a similar study, average number of number of nodules per plant was significantly suppressed by application of 140 and 280kgN/ha resulting in a general trend of an inverse relationship between average nodule size and application rate of nitrogen [17]. In the same vein, Ntambo et al. [18] observed that increasing level of nitrogen decreased nodule number and nodule dry mass per plant. Kaschuk et al. [19] also found negative effects on number of nodules produced as well as their dry masses which was accompanied by low yield when they applied 30kgN/ha as starter dose and 50kgN/ha for top dressing[19]. Decrease in number of root nodules as well as their masses has been attributed to a decrease in translocation of photosynthates from the shoot to the nodules [20].

Table 1: Effect of nitrogen starter dose on nodulation and nodule weight of soybean

Application Rate (kgN/ha)	Number of nodules	Nodule Mass (g)
0	$6.53^{a} \pm 0.05$	$9.91^{b} \pm 0.03$
50	$13.00^{a} \pm 4.16$	$0.63^{a} \pm 0.17$
75	$17.00^{a} \pm 6.03$	$0.61^{a} \pm 0.20$
100	$18.00^{a} \pm 2.31$	$1.23^{a} \pm 0.68$

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.2. Effect of nitrogen starter dose on plant height, internode length, number of branches and number of leaves of soybean

The tallest plants (58cm) were from application of 100 kgN/ha. This was followed by the control (55cm) while the shortest plants (46cm) were from pots with 50 kgN/ha. The tallest plants were 20.68% above the control while the shortest plants were 16.36% below the control plants (Table 2). Application of 100 kgN/ha resulted into longest internode (4.31cm) followed by plants from 75 and 50 kgN/ha application rates (4.15 and 3.54cm) respectively. The shortest internodes were from the control plants. The tallest internodes were 24% above the shortest ones (control plants) (Table 2). The highest number of branches (57) was recorded from pots treated with 100 kg/ha followed by plants from pots with 75 kg/ha urea (46.67) while the lowest number of branches was recorded from plants with application rate of 50kgN/ha (Table 2). Leaf production was highest in pots with 100 kgN/ha followed by those with 75 kgN/ha while the lowest number of leaf production was from pots with 50 kg/ha. Plants from pots with 100 kgN/ha were 37.06% better than those with the lowest number of leaves (Table 2). Plant height, internode length, number of branches and leaves had direct relationship with nitrogen application rate. These results could be attributed to enhancement of chlorophyll of the leaves (because nitrogen is a component of chlorophyll) which then led to higher photosynthetic rate and eventual better assimilate production. The photosynthate produced was channeled to the cells of the leaves and stems and enlargement of the cells was achieved leading to leaf expansion, long internode, tallness of plants and increased number of branches. Furthermore, better growth attributes might be the result of proper secretion and functioning of certain growth hormones (auxins and cytokinins) which have nitrogen as one of their components. So, the hormones then caused increased leaf production, enhanced plant height, well-spaced nodes and branch multiplication. Moreover, the results might be the outcome of succulence effect created by ample supply of nitrogen to the plants though such might be eventually detrimental to the plant lives because they will be made prone to insect infestation and disease infection. Similar to the result from this experiment was the discovery of Raghuveer and Hosmath [21] who found out that height of plants, accumulation of dry matter, population of branches per plant were increased with increase in nitrogen application rate in soybeans. Furthermore, Mehmet [22] recorded maximum plant height with application of 90kgN/ha. In the same vein, Mrkovacki et al. [23] found the tallest plant height and the highest dry matter with application of nitrogen at 60 kg/ha at flowering stage. Summarily, application of 50 kgN/ha led to decline in number of leaves, height and number of branches with the exception of internode length.

Table 2: Effect of nitrogen starter dose on growth traits of soybean

Application Rate (kgN/ha)	Plant height (cm)	Internode length (cm)	Number of branches	Number of leaves
0	55.00[a] ± 7.00	3.45[a] ± 0.38	45.00[a] ± 3.00	123.00[a]±11.06
50	46.00[a] ± 2.88	3.54[a] ± 0.21	41.67[a] ± 4.04	122.00[a] ±8.02
75	48.00[a] ± 8.88	4.15[a] ± 0.47	46.67[a] ± 5.03	140.00[a]±14.57
100	58.00[a] ± 12.66	4.31[a] ± 1.13	57.00[a] ±14.42	168.00[a]±41.05

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.3. Effect of nitrogen starter dose on number of pods per pot and pod yield per pot of soybean

Highest number of pods per pot (54) was recorded from the pots with 100 kgN/ha followed by the control plants while the lowest number of pods (43) was found in with 50 kgN/ha. The best treatment (100kgN/ha) was 26.55% better than the pots with lowest number of pods (Table 3). The highest yield per pot was from pots treated with 100 kgN/ha followed by plants from pots treated with 50 kgN/ha while the lowest yield was realized from control plants. Yield from pots with 100 kg/ha urea was 19.57% higher than that of the control (Table 3). The increase in pod and grain yield might be attributed to nutrient synergy between nitrogen and potassium (which is responsible grain production and filling). As a result, luxuriant vegetative growth from nitrogen application was successfully transformed to appreciable reproductive features (pods and grains). The number of pods was directly proportional to the mass of pod per pot. This could be used to predict the yield of the plant based on this. In line with the results of this work, Singh et al. [24] also found high yield of soybean with split application of nitrogen.

Table 3: Effect of nitrogen starter dose on number of pods per pot and pod yield per pot of soybean

Application Rate (kgN/ha)	Number of pods	Pod yield (g/pot)
0	46.00[a] ± 13.90	87.67[a] ± 20.81
50	43.00[a] ± 14.36	89.33[a] ± 29.87
75	43.00[a] ± 3.05	88.67[a]± 12.74
100	54.00[a] ± 9.16	109.00[a] ± 23.64

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.4. Effect of nitrogen starter dose on seed yield and fifty seed mass of soybean

The highest seed yield was from plants treated with 100kgN/ha followed by those treated with 50kgN/ha while the lowest yield was from the control plants. After application of 50kgN/ha, there was a decline in the yield after which there was a surge again which led to production of highest yield. The yield from 100kgN/ha was 22.89% better that of the control (Table 4). The heaviest seeds were produced with application of 50kgN/ha urea followed by 100kgN/ha while the lightest seeds were produced by the control plants. Seeds from application of 50kg/ha nitrogen were 13.43% heavier than those from the control plants. After the increment recorded with application of 50kgN/ha, there was a decline with application of 75kgN/ha after which a surge was observed again (Table 4). Application of high nitrogen rate which resulted in higher yield might be attributed to enhancement of plant partitioning of assimilate to the economic yield. Moreover, nitrogen is a component of chlorophyll and so better trapping of radiant energy which was used for production of photosynthates. Higher seed mass that resulted from application of 50kgN/ha could be based on the fact that the seeds in plants with 100kgN/ha were more than those from plants with 50kgN/ha application. This then resulted in size reduction which was compensated for by increase in number of seeds. This resembles what happens when a particular crop is produced at high density and low density. The yield per plant of the plants at low density will be more than those from high density plantation. Increase in yield with increase in nitrogen starter dose was also found by Jeffery et al.[9] when they applied starter nitrogen dose to soybean and the yield increase was attributed to increase in grain number. Similarly, Mathew et al.[25] reported highest grain yield with the highest dose of starter fertilizer dose (75kgN/ha). Contrary to our finding, several researchers like Wang et al. [26] have reported positive effect of starter nitrogen dose on grain mass.

Table 4: Effect of nitrogen starter dose on seed yield and fifty seed mass of soybean

Application Rate (kgN/ha)	Seed yield (g/pot)	Mass of Fifty Seed (g)
0	$10.91^{a} \pm 1.38$	$6.06^{a} \pm 0.54$
50	$11.42^{a} \pm 8.89$	$7.00^{a} \pm 0.28$
75	11.37a ± 1.45	$6.60^{a} \pm 0.70$
100	$14.15^{a} \pm 1.88$	$6.72^{a} \pm 0.91$

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.5. Effect of nitrogen starter dose on days to flowering and days to maturity of soybean

Days to flowering was decreased by application of nitrogen starter dose. The lowest number of days to flowering was recorded for plants with application rate of 50kgN/ha followed by those with application rate of 75kgN/ha while the highest number of days to flowering was recorded from plants with application rate of 100kgN/ha and the control plants. Plants from pots application of 50kg/ha urea were two days ahead of the slowest plants (Table 5). The number of days to maturity was also affected by application of starter nitrogen. The lowest number of days to maturity was recorded for plants with application rate of 50kgN/ha followed by those with application rate of 75kgN/ha while the highest number of days to maturity was recorded for control plants. Plants from application of 50kgN/ha were five days ahead of the slowest plants (Table 5). Lateness in flowering and maturing of plants with 100kgN/ha could be attributed to enhancement of luxuriant vegetative growth as evident in number of leaves, branches, internode length and plant height (Table 2). This development was at the expense of transition to reproductive stage. This development characterizes abundance of nitrogen supply in plants. This then led to delay in flowering and maturing. Plants from 50 and 75kgN/ha which have less luxuriant vegetative growth produced flowers and reached maturity earlier than the ones with abundant supply of nitrogen (those with 100kgN/ha). This implies that delay in flowering and maturity, if the need be, can be nutritionally achieved by supplying our crops with abundance of nitrogen and vice-versa.

Table 5: Effect of starter dose of N on days to flowering and days to maturity

Application Rate (kgN/ha)	Days to Flowering	Days to Maturity
0	$45.00^{a} \pm 2.08$	$114.00^{b} \pm 1.15$
50	$43.00^{a} \pm 0.57$	$109.00^{a} \pm 0.56$
75	$44.00^{a} \pm 1.73$	$110.00^{ab} \pm 1.15$
100	$45.00^{a} \pm 2.08$	$113.00^{b} \pm 3.21$

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.6. Effect of nitrogen starter dose on chlorophyll content, ash content and total nitrogen of soybean

The highest chlorophyll content was found in plants treated with 75kgN/ha followed by the control plants while the lowest chlorophyll content was found in plants treated with 100kgN/ha (Table 6). The leaf chlorophyll content did not have direct relationship with soil nitrogen supply. It might equally be that the optimum nitrogen supply to produce the optimum leaf chlorophyll was 75kgN/ha. Therefore, further increase to hundred could only lead to diminishing return. So, having higher yield with application of 100kgN/ha may not have 100% direct relationship with the leaf chlorophyll content which should have had a direct relationship with it because nitrogen is a component of chlorophyll. This could be that absorption of nitrogen from the soil was not influenced by its supply to the soil. This is also evident in the leaf nitrogen content (Table 6). Moreover, leaf nitrogen content did not correlate with the leaf chlorophyll content. This could be explained on the basis of having other nutrient elements which are components of chlorophyll like phosphorus and magnesium while others like iron, copper and manganese are for chlorophyll synthesis. In summary, it could be said that nitrogen application beyond 75kgN/ha would negatively influence leaf chlorophyll content. In a similar discovery, Gai et al.[27] found decrease in chlorophyll content with higher application of nitrogen. It was said that excessive application of nitrogen had inhibitory effect which was not only limited to chlorophyll decrease but also root activities and root mass.

The highest level of ash content was found in the leaves of plants treated with 50kgN/ha followed by those from plants treated with 100kgN/ha while the lowest ash content was from the control (Table 6). Ash content is an indication of the mineral content of the leaf. Since the highest leaf ash content was through application of 50kgN/ha, it means 50kgN/ha let to better synergy of different mineral nutrient and was evident in the higher ash content found in the leaves. Beyond 50kgN/ha, less mineral nutrient synergy was achieved with consequent low ash content. This could have been because of toxicity resulting from high application rate of nitrogen.

Total nitrogen content was highest in plants treated with 50kgN/ha followed by the control plants while the lowest nitrogen content was found in plants treated with 75kgN/ha urea (Table 6). This was similar to the finding of Gai et al. [27]. In the same vein, it was discovered that application of 46kgN/ha led to highest nitrogen uptake [28]. Nitrogen uptake was enhanced at low nitrogen application for soybean. This was evident in the leaf nitrogen content which was highest in plants with lowest nitrogen application rate (50kgN/ha). This might be because of the already fixed nitrogen through symbiotic bacteria in the root nodules which made the supply through fertilizer application to become optimum for optimum absorption by the plant. Beyond 50kgN/ha, optimum level was exceeded and absorption was brought down probably as a result of toxicity resulting from oversupply of mineral nutrient.

Table 6: Effect of nitrogen starter dose on chlorophyll content, ash content and total nitrogen

Application Rate (kgN/ha)	Chlorophyll content (µmolcm^{-2})	Ash content (%)	Total Nitrogen (%)
0	$0.0603^{a} \pm 0.001$	$1.35^{a} \pm 0.115$	$4.54^{b} \pm 0.017$
50	$0.059^{a} \pm 0.002$	$3.81^{d} \pm 0.015$	$5.13^{d} \pm 0.0+20$
75	$0.061^{a} \pm 0.002$	$2.70^{b} \pm 0.105$	$4.46^{a} \pm 0.010$
100	$0.058^{a} \pm 0.004$	$3.39^{c} \pm 0.190$	$4.48^{c} \pm 0.015$

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

3.7. Effect of nitrogen starter dose on crude fat, crude protein and crude fibre of soybean

The highest crude fat content was found in plants treated with 100kgN/ha followed those treated with 75kgN/ha while the lowest crude fat content was found in the control plants (Table 7). Increase in crude fat content was enhanced by the highest level of nitrogen application. This might be attributed to low nitrogen in the leaves as opposed to nitrogen content of plants with 50kgN/ha which was high and led to production of highest crude protein content. Moreover, it might be because of high soybean yield which is normally accompanied by high fat production. The photosynthate produced by the leaves was converted to fat and part of it was equally translocated to the seeds for storage. In a similar finding, Singh et al. [24] had crude fat content increased with

increase in nitrogen application even beyond 60 kgN/ha. Contrary to this finding and ours, Kumawat et al.[29] found decrease in crude fat content with increase in nitrogen application.
Crude protein was highest in plants treated with 50kgN/ha followed by those from the control plants while the least crude protein contents was from plants treated with 75kgN/ha (Table 7). There was a surge after application of 50kgN/ha after which there was a decline with application of 75kgN/ha and finally there was another surge with application of 100kgN/ha. So, the trend was not linear as that of crude fat. This could be attributed to the highest nitrogen content of the leaf (Table 7). This is because amino acid is derived from nitrogen and protein is derived from amino acid. So, protein is the protein reservoir. Despite low nitrogen application rate (50kgN/ha), absorption and transformation to crude protein were high. This might be because the soil nitrogen reached the optimum requirement for the crop under test when 50kgN/ha nitrogen fertilizer was added to the Contrary to our result, Kumawat et al. [29] discovered that protein content increased with increase in nitrogen levels from 20-80 kgN/ ha.

The highest crude fibre content was found in plants treated with 75kgN/ha followed by those from plants treated with 100kgN/ha urea while the lowest crude fibre content was from plants treated with 50kgN/ha. There was a decline with application of 50kgN/ha which was followed by a surge after which there was a final decline in crude fibre content (Table 7).The level of leaf fibre content is an indicator of tenderness of leaves and its suitability for feeding animals. The higher the fibre content, the lower the quality of the leaves. In this experiment, the plants treated with 50kgN/ha had high quality leaves because of low fibre content. This might have resulted from having higher protein content which impeded accumulation of huge crude fibre.

Table 7: Effect of nitrogen starter dose on crude fat, crude protein and crude fibre of soybean

Application Rate (t/ha)	Crude Fat (%)	Crude Protein (%)	Crude Fibre(%)
0	$6.53^{a} \pm 0.05$	$9.91^{b} \pm 0.03$	$2.55^{a} \pm 0.16$
50	$8.05^{b} \pm 0.39$	$10.67^{c} \pm 0.14$	$2.52^{a} \pm 0.12$
75	$8.53^{b} \pm 0.71$	$8.18^{a} \pm 0.26$	$2.83^{b} \pm 0.02$
100	$10.42^{c} \pm 0.49$	$9.80^{b} \pm 0.06$	$2.64^{ab} \pm 0.06$

The values (means of three replicates ± SE) in the same column followed by the same letters are not significantly different from each other at $P < 0.05$.

4.0. Conclusion

From this work, it was found that 100 kgN/ha of N-fertilizer improved the total nodulation by 33.95% compared with the control. The yield was greatest with application of 100 kgN/ha compared with other application rates because there was 20% yield increase compared with the control. For the proximate composition, crude protein content was 7% above the control with application of 50 kgN/ha. Furthermore, crude fat content was 37% better than the control with the application of 100 kgN/ha. Therefore, it was concluded that for profitable soybean production in the study area, the use N fertilizer like urea at 50 kgN/ha as a starter dose is recommended because of its cheapness and high yield that resulted from its application to the crop. Finally, nitrogen starter dose up to 100kgN/ha did not constitute stress to production of soybean variety under test.

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