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***Euphorbia heterophylla* L. and Nitrogen Fertilizer Effects on the Chemical Composition and Photosynthetic Apparatus of *Macrotyloma geocarpa* (Harms) Marechal and Baudet**

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Abstract: Kersting's groundbean (*Macrotyloma geocarpa* (Harms) Marechal and Baudet), a grain legume was subjected to different nitrogen levels and spurge weed (*Euphorbia heterophylla* Linn.) competition to study the combined effects of these factors on its growth. Weed competition was achieved by growing Kersting's groundbean with spurge weed, while fertilizer stress was imposed by planting Kersting's groundbean without fertilizer treatments. There was another treatment, which was a combination of the two stresses. Fertilizer application of the crops was achieved by broadcasting NPK fertilizers at 0, 15 and 30 kg N ha⁻¹ in the Kersting's groundbean seedlings plots. The biochemical composition of the plants such as photosynthetic pigment accumulation, nitrogen, ash, crude fibre, fats, carbohydrates and lignin contents did not show a regular pattern at successive harvest times.

Key words: Fertilizer, weed, *Macrotyloma geocarpa*, biochemical composition, photosynthetic pigments

INTRODUCTION

Yields of grain legumes are smaller and generally more variable than those of many other crop species. In developed countries, grain yields of legumes have not increased rapidly as those of cereal crops. Between 1940 and 1981, for instance, winter wheat (*Triticum aestivum*) yield in UK increased at approximately twice the rate of those for pea (*Pisium sativum*) and faba bean (*Vicia faba*) 82, 44 and 31 kg ha⁻¹, respectively (Heath and Hebblethwaite, 1985). In France, between 1981 and 1996, yield increases were 75 kg ha⁻¹ for pea (*Pisum sativum*) and 120 kg ha⁻¹ for wheat (*Triticum aestivum*) (Carrouee, 1996 Personal Communication).

There is a need to increase the performance of pulse crops, particularly in developing countries, where most of the grain produced is for human consumption and demand for them is increasing as a result of population increase.

Macrotyloma geocarpa is an indigenous grain legume cultivated in parts of Tropical Africa for food. It produces its seeds underground (Hepper, 1963). Its cultivation is not as widespread as cowpea and other legumes. The leaves are sometimes eaten in soup. Dried seeds of approximately 100 g can yield 348 calories and contains 9.7% moisture, 19.4 g proteins, 1.1 g fat, 66.6 g total carbohydrate, 5.5 g fibre, 3.2 g ash, 100 mg calcium etc.

The agronomic problems associated with pulse differ between geographical areas. In Asia, Africa and oceanic regions (Johansen *et al.*, 1992), North America (Muehlbauer and Kaiser, 1992) and Europe (Monti *et al.*,

1992), drought and biotic stresses appear to be the major limiting factor while other stresses such as extreme temperature and nutrient deficiencies have less frequent impact. Crop management and plant breeding are among the various ways by which stresses could be alleviated.

The pods mature underground and are indehiscent usually divided by 1 or 2 constrictions into 2 or 3 joints seeds which are oblong to oblong-ovoid, about 0.6–1.3 cm long, kidney-shaped with a white hilum, white, red, black, or mottled in colour. The seeds resemble the seeds of *Phaseolus vulgaris* but smaller and very hard when dried. Since the seeds are buried in the soil they are safe from attacks by flying insects that severely limit or destroy pulses like soybeans whose pods remain in the air. It takes between 4-5 months to mature. Seeds ripen, as leaves turn yellow. Plants are later dug up and left on the ground to dry and later beaten with sticks or in a mortar to remove seeds. These are later dusted with insecticide to prevent attack by weevils. The protein is rich in essential amino acids, such as lysine 6.2% and methionine 1.4%.

The roots grow well in fine sand or silt where phosphorus and nitrogen are available but roots do not move into regions of moist gravel or coarse sand easily even when fertilizer is applied. The extent of the root system is related to texture and structure of soils as well as available nutrients. Some grain legumes such as varieties of cowpea will do well with about 20 kg N ha⁻¹ and 8 kg ha⁻¹ (Ezedinma, 1961; Fennel 1962). Optimum response to fertilizer as well as overall yield levels depends on timely sowing (Bandyopadhyay and De, 1986). Nitrogen deficiency is known to cause a reduction in the photosynthetic capacity of plants (Simpson *et al.*,

1983). The quantum yield for CO₂ uptake decreased heavily with leaf nitrogen content. The critical nitrogen concentration of a plant can be defined as the minimum nitrogen concentration required for maximum growth rate at any time (Sheehy *et al.*, 1996), leaching and erosion have been the cause of low levels of nutrients particularly nitrogen in cultivated soils.

Weeds are known to constitute a major limiting factor to grain legumes production and probably the most important yield depressing factor to grain legumes in Nigeria (Fadayomi, 1979). Weeds compete for nutrients, moisture, light and space. Weeds with good competitive ability show a faster rate of root elongation and development than the crop (Ayeni *et al.*, 1984). Such weeds cause yield losses ranging from 50 to 86% (Moudy, 1973; Remison, 1978; Akobundu, 1979). Weed competition is most serious when crop is young and the critical time for weeding has been reported to be between 4 and 6 weeks after planting (Fadayomi, 1979; Ayeni *et al.*, 1984). Weeds may also serve as a host for insects, pests and pathogens (Akinyemiju, 1987; Akinyemiju and Echendu, 1987).

In the present study nitrogen as a fertilizer and *Euphorbia heterophylla* that has been identified as a common weed in grain legume fields were applied to *Macrotyloma geocarpa* and the photosynthetic pigments as well as other chemical constituents accumulation followed over a period of time.

MATERIALS AND METHODS

The site of the experiment is situated in the Biological Gardens unit of Obafemi Awolowo University Ile-Ife, Nigeria. Soil samples were tested for pH and found to be 6.8, which is still ideal for the growth of legumes, which ranges between 6.5-7.0. Seeds of *Macrotyloma geocarpa* were collected from Plant Science Department, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The site was cleared and ridges were made. This was divided into three replicates R₁, R₂ and R₃ and space between each replicates being 1 m. Each replicate was divided into six representing,

Weed +Nitrogen control (WN₀)

Weed +Nitrogen dose 1 (WN₁)

Weed +Nitrogen dose 2 (WN₂)

Nitrogen Control (N₀)

Nitrogen dose 1 (N₁)

Nitrogen dose 2 (N₂)

with a space of 60 cm between each one which was 3x2.4 m.

Analysis started on the of fertilizer application that is zero day and at 7 day intervals thereafter.

Seeds of *Macrotyloma geocarpa* were planted in a space of 60 cm x 60 cm. NPK fertilizer was applied 18 days after germination. Application was 15 kg and 30 kg N h⁻¹.

Pigment analysis

Chlorophyll accumulation: Five gram of *Macrotyloma geocarpa* shoot was ground in 20 mL 80%(v/v) acetone using a mortar and pestle. The brei was filtered through a Whatman's filter paper. The pigment quantities in the acetone extract were determined on a camspec visible linear readout spectrophotometer at wavelengths of 664 and 647 nm. Chlorophylls a, b and total chlorophyll contents were determined using the formula by Coombs *et al.* (1985). Chlorophyll a =13.19 A₆₆₄ -2.57 A₆₄₇ (mg g⁻¹ dry wt.)

Chlorophyll b =22.1 A₆₄₇ -5.26 A₆₆₄ (mg g⁻¹ dry wt.)

Total chlorophyll =7.93 A₆₆₄+19.53 A₆₄₇ (mg g⁻¹ dry wt.)

A₆₆₄ was the absorbance at 664 nm

A₆₄₇ was the absorbance at 647 nm

Chemical Composition

Determination of percentage Nitrogen: This was determined using the modified Kjeldahl method. Two g dried and ground plant material was weighed into a Kjeldahl flask. A scoop of a digestion mixture which consists of Mercuric oxide as a catalyst plus Potassium Sulphate to raise the boiling point was added and 30 mL concentrated H₂SO₄.

The mixture was digested for two h and allowed to cool. 150 mL of Distilled water was added. The mixture was then transferred into a 500 mL Erlenmeyer flask and 5 mL of boric acid (plus methyl orange indicator) added to it.

The flask was then placed under the receiving tube of the distillation unit so that the end of the tube is below the H₂BO₃. Hundred milliliter of 4% NaOH was carefully added to the flask, 2 pieces of moss zinc was then added. This was then treated on a low heat and the distillation continued until the contents in the Erlenmeyer flask was about 250 mL.

The distillate was titrated with standard HCl until the blue colour disappeared. Blank determination was then carried out. The 'net volume of acid' is the volume of acid required for sample titration less that of the blank.

$$\text{Percentage total nitrogen} = \frac{\text{Net volume of} \times \text{conc. of acid} \times 14 \times 100}{\text{Weight of sample digested (mg)}}$$

Percentage crude protein = 6.25×% total nitrogen. 6.25 being a correction factor for converting total nitrogen into crude protein.

Percentage ether extract (crude fat): To 2 g of dried sample at 100°C, petroleum ether was added until the 300 mL Soxhlet extraction was half full. The ether was

put to boil gently on a hot plate for 2 h. When the content was nearly dried, it was transferred into ether stick bottle. The ether was distilled until the flask was practically dry. This was dried in a Gallenkamp drying oven at 80°C to a constant weight. The difference between the initial weight and the extracted residue was equivalent to the crude fat, which was converted as percentage of the initial weight.

Percentage crude fibre: One g of the residue from ether extract was hydrolysed first with dilute H₂SO₄ and then with dilute Na OH in a crude fibre beaker and later filtered through Whatman's No 4 filter paper. The residue was washed with boiling water, and later with 1% HCl and again with boiling water until the mixture was free of acid. It was then washed with 95% ethanol once and after three tries with petroleum ether. The drained residue was dried overnight in a Gallenkamp drying oven at 80°C, cooled, weighed and later ashed at 500°C for 3 h. Percentage crude fibre was calculated as follows (Anonymous, 1980):

$$\text{Crude fibre} = \frac{\text{Loss in weight on ashing}}{\text{Weight of sample}} \times 100$$

Determination of percentage ash content: Two gram of dry weight sample was transferred into a crucible and at 650°C for 2 to 3 h in a Gallenkamp furnace. After cooling the weight of the ash was taken and used to determine the percentage of inorganic matter present at each harvest as follows:

$$\% \text{Ash content} = \frac{\text{Ash weight}}{\text{WS}} \times 100$$

Where, WS is the total plant biomass.

Estimation of total soluble carbohydrates: To 0.5 g of dried ground material, 100 mL of hot ethanol was added in a conical flask. A pinch of CaCO₃ (about 1mg) was used to acidify it. The mixture was heated to boiling point for 30 min in a water bath. The coloured supernatant containing alcohol soluble carbohydrates was separated from the non-soluble sediment. The total soluble carbohydrate content of the sample was estimated through a modified phenol H₂SO₄ assay. One milliliter of 5% phenol solution and 5 mL of conc. H₂SO₄ to 1 mL of decanted alcohol-soluble sample were mixed together and the mixture was thoroughly shaken and later cooled to room temperature. The assay of the sample was carried out at 490 nm, wavelength on a Cecil 343 single sample spectrophotometer.

Total insoluble carbohydrate: To the alcohol insoluble sediment obtained above, 10 mL of conc. H₂SO₄ was added in order to digest the sediment and convert it in to a thick dark solution which was later diluted 20 times with 5 mL conc. H₂SO₄. The mixture was left for determination

through the process carried out for the soluble carbohydrates determined above.

Lignin determination: To the remaining alcohol insoluble sediment in soluble carbohydrate estimation performed earlier, 10 mL of conc. H₂SO₄ was added. The mixture was diluted 20 times with 5 mL of conc. H₂SO₄. Five milliliter of the solution was mixed with 5 mL of 5%(v/v) phloroglucinol in water. The mixture was used as the blank. At 450 nm wavelength, the spectrophotometric determination was on a CECIL 343 single sample spectrophotometer.

RESULTS

Chlorophyll a content increased gradually in all the treatments throughout the experiment. The accumulation of chlorophyll a was highest in the N₁ plants followed by N₀, WN₀, WN₂ and WN₁, while the accumulation of chlorophyll a was lowest in N₂ plants at the end of the experiment. There was variability in the chlorophyll a content based on weed competition. The chlorophyll a content did not however vary on the basis of nitrogen levels only (Fig.1).

There were fluctuations that involved decreases and increases in the levels of chlorophyll b throughout the experiment. The accumulation of chlorophyll b was highest in N₁ followed by WN₂, N₀, WN₁, WN₀ and N₂, respectively (Fig. 2). The chlorophyll b content had a high degree of variability based on weed competition especially in WN₀ plants but this did not depend on nitrogen levels.

Initially, the highest total chlorophyll was recorded in N₀ plants followed by N₁, WN₂, WN₀ and WN₁, while the lowest was recorded in N₂ plants. There was a gradual increase in all the treatment throughout the experiment. It was observed at the end of the experiment that WN₁ plants had the highest value followed by N₀, WN₂, N₂, N₁ and WN₀, respectively (Fig. 3).

The total chlorophyll had a high mean variability depending on the weed interference and nitrogen levels.

The levels of nitrogen fluctuated a great deal and these decreased towards the end of the experiment. The percentage nitrogen was highest in N₀ plants followed by WN₁, WN₂, N₁ and WN₀, while N₂ plants had the lowest percentage at the end of the experiment. The variation observed in the percentage nitrogen was dependent on both weed competition and nitrogen levels (Fig. 4).

The crude protein followed the same pattern as in as percentage nitrogen. There was a lot of fluctuation that involved increases and decreases in the levels of crude protein. At the end of the experiment, N₀ plants had the highest total percentage crude protein followed by WN₁, WN₂, N₁ and WN₀, while N₂ had the lowest percentage.

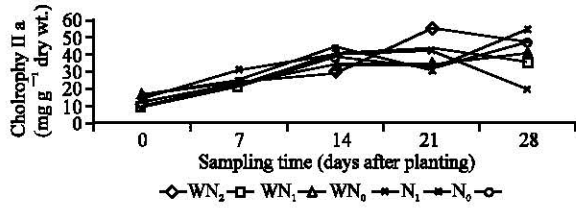


Fig. 1: Effect of weed and fertilizer on the chlorophyll a accumulation of *Macrotyloma geocarpa*

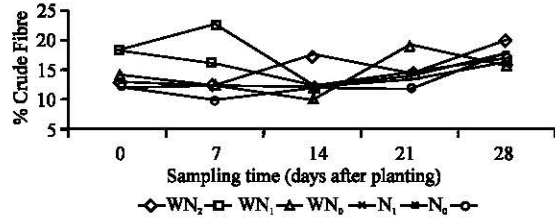


Fig. 6: Effect of weed and fertilizer on the percentage crude Fibre of *Macrotyloma geocarpa*

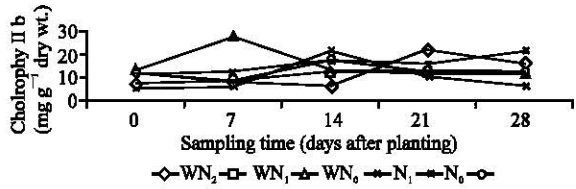


Fig. 2: Effect of weed and fertilizer on the chlorophyll b accumulation of *Macrotyloma geocarpa*

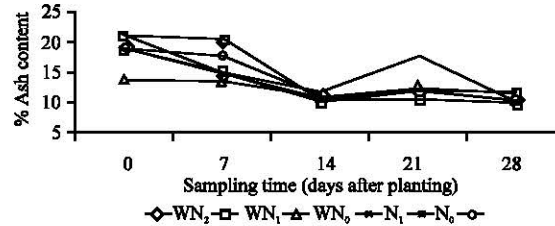


Fig. 7: Effect of weed and fertilizer on the percentage ash content of *Macrotyloma geocarpa*

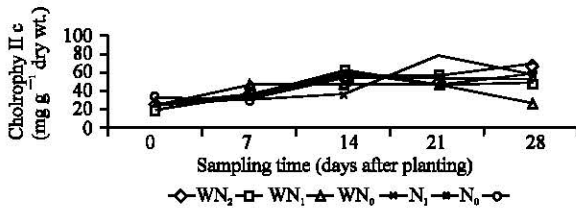


Fig. 3: Effect of weed and fertilizer on the total chlorophyll of *Macrotyloma geocarpa*

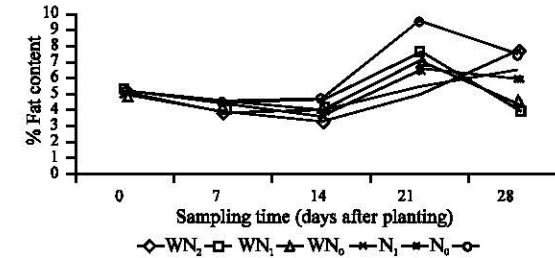


Fig. 8: Effect of weed and fertilizer on the percentage fat content of *Macrotyloma geocarpa*

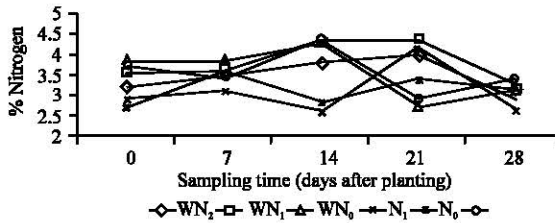


Fig. 4: Effect of weed and fertilizer on the percentage nitrogen of *Macrotyloma geocarpa*

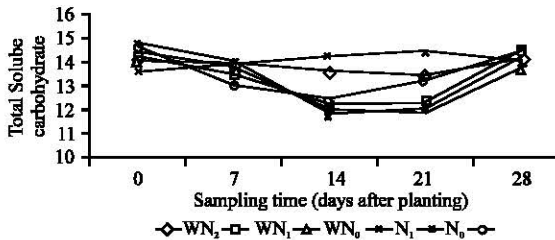


Fig. 9: Effect of weed and fertilizer on the total soluble carbohydrate of *Macrotyloma geocarpa*

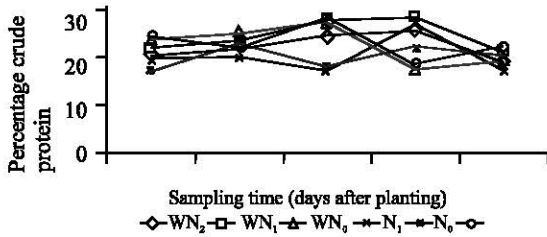


Fig. 5: Effect of weed and fertilizer on the percentage crude Protein of *Macrotyloma geocarpa*

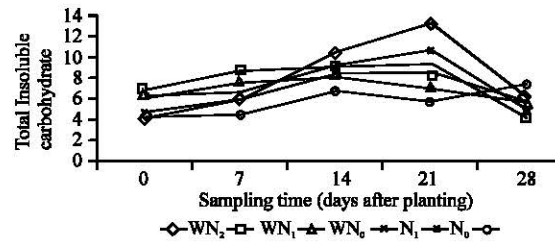


Fig. 10: Effect of weed and fertilizer on the Total insoluble carbohydrate *Macrotyloma geocarpa*.

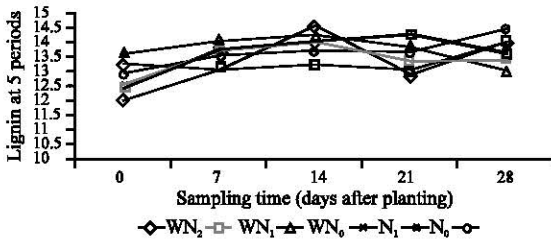


Fig. 11: Effect of weed and fertilizer on the Lignin content of *Macrotyloma geocarpa*

Variation was dependent on both weed competition and nitrogen levels (Fig. 5).

Apart from the increases in the levels of percentage crude fibre in WN₁ and WN₂ plants in the early part of the experiment, there was a gradual increase in all the treatments throughout the experiment. At the end of the experiment, percentage crude fibre was highest in WN₂ plants followed by N₀, WN₁, N₂ and N₁ while WN₀ plants had the lowest percentage crude fibre. The coefficient of variation in the crude fibre was only due to weed competition only (Fig. 6).

Ash content was initially high in all the treatments. There was a general decrease in percentage ash content till the 14th day of the experiment. Thereafter, the % ash content increased till the 21th day followed by a decrease till the end of the experiment. There was variation in all the treatments. Percentage ash content was highest in N₀ followed by WN₀, WN₂, N₁ and WN₁, while N₂ plants had the lowest percentage ash content at the end of the experiment (Fig. 7).

There was a decrease in percentage fat content in all the treatments till the 7th day followed by an increase for a greater part of the experiment. At the end of the experiment fat content was highest in N₁ followed by N₀, N₂, WN₁ and WN₀, while N₂ was lowest. There was no variation in all the treatments (Fig. 8).

Soluble carbohydrate was initially high in all the treatments. This was followed by a decrease in N₀, WN₁, WN₀ and N₁ plants and later an increase that lasted till the end of the experiment. The increase in WN₂ plants lasted till the 21st day before an increase that lasted till the end of the experiment. In N₂ plants there was a gradual increase throughout the experiment. At the end of the experiment, the amount of soluble carbohydrate was highest in WN₁ plants, followed by N₁, N₀ and N₂, while WN₀ plants had the lowest total soluble carbohydrate. There was variation in all the treatments (Fig. 9).

Total insoluble carbohydrate increased gradually in all the treatments up till the 21st day before a decrease till the end of the experiment except in N₀ plants where there was a gradual increase throughout the experiment. At the

end of the experiment, the total insoluble carbohydrates was highest in N₀ plants followed by WN₂, N₂, WN₀ and WN₁ while N₁ had the lowest total insoluble carbohydrates (Fig 10). Variability of the insoluble carbohydrates was dependent on the weed competition only. The plants treated with different nitrogen levels only had a high degree of closeness.

The total amount of lignin increased gradually in N₁, WN₁, N₀, WN₀ and N₂ plants for a greater part of the experiment. In WN₂ plants, the increment lasted till the 14th day; this decreased on the 21st day and was constant till the end of the experiment. Total lignin was highest in N₀ plants followed by N₁, N₂, WN₁, WN₀ and WN₂, respectively. There was no variation in the treatment means (Fig. 11). The plants that were treated with nitrogen levels only had means that were closer than the plants that had weed interference.

DISCUSSION

This study sets out to highlight, the effects of interspecific competition, nitrogen fertilizer application and effects of the two (weed + fertilizer) treatments combined on the overall growth, performance and reproductive yield of *Macrotyloma geocarpa*. All the investigations were carried out under the same experimental and environmental conditions, except for the variation in the doses of nitrogen fertilizers and weed interference. Any observed differences therefore in the accumulation of photosynthetic pigments as well as the chemical composition of the crop, in the control experiment (N₀ - no weed and no fertilizer) and other treatments were due to the effects of the applied treatments. The high significant differences obtained in the overall growth and yield of the crop between nitrogen fertilizer treatments at different levels imply that crop grain yield performance is predominantly determined by a constant ratio of growth rate to relative nitrogen uptake as the productivity in the control was lower than that in which there was application at the end of the experiment. This agrees with Ezedinma (1964) that there is a linear relationship between growth rate of a crop and the relative nitrogen uptake. Nitrogen fertilizer will affect the rate of this process only when the nitrogen fertilizer increases the external (soil) concentration of nitrogen. This declines with crop age. Growth response to nitrogen application was observed throughout. Ezedinma (1964) (cited by Osiname (1978) noted that nitrogen application to cowpea at planting eliminated any retardation in growth and development which might follow the loss of cotyledons shortly after emergence and before the nodules become functional.

The generally high chlorophyll a and total chlorophyll contents observed in all the treatments were due to availability of nitrogen supply either by nodulation where nitrogen fertilizer was not applied as in the N_0 and WN_0 , or application of inorganic nitrogen fertilizer. Singh and Rachie (1985) emphasized that application of nitrogen reduced nodulation of cowpea plants, because of adequate nitrogen supply from the nutrient nitrogen at the expense of that fixed by symbionts. However nodules were found not to be essential for legumes growth since appropriate nitrogen fertilizers can replace them. In this study, *Macrotyloma geocarpa* plants under the experiment such as WN_1 , WN_2 , N_1 and N_2 plants had a delay in nodule formation, but the plant overall development, crops maturation date and total bean yield was not directly affected due to application of nitrogen fertilizers.

The decrease noted in Chlorophyll b content of the crop seedling under interspecific competition may be connected with their low nitrogen contents. The weed which is non-leguminous competitor could not fix N biologically and therefore shared from little available nitrogen in the soil. The findings of Vos (1995), that the demand for N of every well-grown crop is the product of the dry weight of the crop and the fraction of N in it. Chlorosis has always been the primary symptom of nitrogen deficiency in plants. This can be said to be limiting the rate of photosynthesis and therefore, support early report that weed competition causes a reduction in crop-yield and a decrease in chlorophyll b content. Also the observation agrees with a report of Sagan *et al.* (1993) that N-deficiency affected an early end of pea flowering. Cessation of flowering is only affected if the deficiency starts before the onset of flowering on the stem and, if it has a long duration (Jeuffroy and Sebillate, 1997). Although grain legumes can fix nitrogen in symbiosis with rhizobia, nitrogen deficiencies have been observed in pea field (Dore, 1992). In such situations there are always few branches per plants.

Protein and ash contents of the plants decreased slightly with age. For example there was a clear difference on the 2nd, 3rd and 5th week in ash, on 5th week in protein probably due to the increased physiological activities. Percentage ash refers to the inorganic materials in the plants and it is a useful index of measuring accumulation of organic matter. The low protein content at the end of the experiment can be connected with maximum photosynthetic rate. Afolabi (1987) made a similar observation in sugarcane (*Saccharum officinarum*). This is because the greatest limitation to the rate of photosynthesis in C-3 plants is the amount of ribulose Biphosphate Carboxylase (RUBPC), which makes up about half the total leaf protein. When metabolic rate is

raised the amount of RUBPC will equally increase and consequently, a lowering of the plant's protein content as more protein is used up in metabolism. It may equally be due to the available soil nitrogen towards the end of the experiment.

Percentage ash content refers to the inorganic materials in the plant and is a useful index for measuring accumulation of organic matter. The overall low rate of photosynthesis is the non-availability of nitrogen content that caused a low organic carbon content and consequently, a high ash content in the plants treated with nitrogen fertilizer. This agrees with Boardman (1977) that fertilized plants promote a better accumulation of percentage ash and fosters active transport of solutes across membranes.

Chemical contents like crude fibre, ash, soluble and insoluble carbohydrates and lignin constitutes cell membranes and supporting structures and some of the materials that make up cell wall. Their high concentration in plants thus confers mechanical support against environmental stresses. The above assertion corresponds with that of George *et al.* (1995) that increase in cell wall components like calcium tend to confer some degree of mechanical support to rice (*Oryza sativa*) plant tissues. Nobel (1983) observed that greater cell components lead to increase in mechanical stretching. The high ash content of the plants on the day zero to day 7 was in line with the observation of Nobel (1983).

The low crude fibre under interspecific competition was due probably to the fact that, some of it was converted to soluble carbohydrates to increase the required high solute potential. The high metabolic rate of these plants was no doubt, responsible for their observed low fat content. The observable higher amounts of soluble carbohydrates in the fertilized plants probably increased the solute potentials of cells of the nitrogen fertilized plants and consequently increased the water capacity of the cells and the water retentive ability of the shoot consequently enhanced. Hofler *et al.* (1941) observed that drought resistant species in general have a high soluble potential, which helps them to absorb as well as conserve water. This can be found in N_1 , N_2 , WN_1 and WN_2 plants in the experiment and it is in agreement with that of Hofler *et al.* (1941).

The amount of insoluble carbohydrates in the *Macrotyloma geocarpa* were generally higher in the N_1 plants than in the N_0 plants. Beakbane and Thompson (1939) showed that an increase in the number of wood parenchyma cells of the bark increased the capacity for production for starch and cellulose i.e. insoluble carbohydrates. It is conceivable that the insoluble carbohydrates become hydrolysed into the soluble carbohydrates.

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