Effect of Spacing and Harvest Frequency on the Growth and Leaf Yield of Moringa (*Moringa oleifera* Lam), a Leafy Vegetable Crop

Amaglo, N. K.[1], Timpo, G. M.[1] , Ellis W.O[2] and Bennett, R.N.[3]

1. Department of Horticulture, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

2. Department of Biochemistry, Faculty of Biosciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3. CECEA-Departamento de Fitotecnia e Engenharia Rural, Universidade de Trás-os-Montes e Alto Douro (UTAD), 5001-801 Vila Real, Portugal

ABSTRACT

A series of experiments were performed to investigate spacing and harvest frequency effects on the growth, leaf yield and quality of Moringa oleifera Lam at the Department of Horticulture of the Kwame Nkrumah University of Science and Technology, Kumasi. The experiments were performed between 19th May 2004 and 21st March 2005. The spacing treatments (5 x 5 cm, 5 x 10 cm and 5 x 15 cm) and the harvest frequency studies (30, 35 and 40 days) were arranged in a 3 x 2 factorial Randomised Complete Block Design (RCBD). Five (5) individual plants, randomly selected from each harvest plot, were separated into their different tissues as stems, twigs and leaves and their fresh and air-dried weights were recorded. Samples of 100 grams from each tissue were analyzed for crude protein, carbohydrates, fiber, fat and ash contents. The results showed that, during a period of 60 days after sowing, plant height increased steadily reaching 87.76, 80.76 and 73.57 cm for the 5 x 5, 5 x 10 and 5 x 15 cm spacings respectively ($P<0.05$). Similarly average stem diameter during the same period reached 5.28 mm, 6.84 mm and 7.64 mm for the close, medium and wide spacings respectively ($P<0.05$). Average number of leaves produced per plant increased with time and reached 10.09 and 10.76 for medium and wide spacings respectively. In the close spacing the number increased steadily but declined 8.49 to 7.84 in the 8th week. The wider spacing produced a greater number of leaves and higher shoot yield per plant than the medium and close spacings ($P<0.05$). But, the total shoot yield per hectare was higher in the close than the medium and wide spacings ($P<0.05$).Total shoot losses were higher in the close spacing than the medium and wide spacing. The studies showed that optimum spacing in a well-drained sandy loam soil was 5 x 15 cm (1.333 million plants per hectare). After the initial harvest 60 Days after sowing, successive harvests should be made at 35 day intervals when it was found to be richest in nutrients, particularly crude protein content.

INTRODUCTION

Vegetables are the succulent plant parts that may be eaten as major food plants, as supplementary foods, or side dishes in the raw or cooked form alone, or in combination with meat or fish, in stews, soups and various preparations (Okigbo, 1983). They provide a source of food often low in calories and dry matter content and are often consumed in addition to starchy basic foods in order to make them more palatable (Grubben, 1977). They are universally recognized to have a great nutritional value and form an essential part of a balanced human diet.

Diets deficient in vegetables and fruits lead to various ailments including cancers, neurodegenerative diseases, immune system dysfunction, and heart diseases (McBride, 1992; Wood, 1992). Vegetables, especially leafy vegetables, therefore feature regularly in gardens, markets and homes. In Senegal, leafy vegetables account for as much as 50 to 85 % of the household budget of some consumers, while in Cameroon total vegetable production was estimated to be 93.600 tonnes in 1998 (Spore, 2005). These traditional leafy vegetables are now recognized as an ally in the fight against deficiencies of macro and micro nutrients although they have long been overshadowed by other green leafy vegetables of European origin such as cabbage and lettuce which can have a lower nutritional content, and especially from the conventional intensive production methods. One such important traditional leafy vegetable is *Moringa oleifera,* Lam. This multi-purpose food plant, originating from India, is produced and used in many African countries (e.g. Ghana, Senegal and Malawi), in South America (Nicaragua and Bolivia), and surprisingly in New Zealand. It also continues to be an important food plant in parts of India

The World Declaration and the Plan of Action on Nutrition, adopted by 159 countries, at the International Conference on Nutrition organized by the United Nation's Food and Agriculture Organization (FAO) and World Health Organization (WHO) in 1992, states that strategies to combat micronutrient malnutrition should: *"Ensure that sustainable food-based strategies are given first priority particularly for populations deficient in vitamin A and iron, favoring locally available foods and taking into account local food habits".* Studies have shown Moringa can be a cheap, all year round, high quality food for both humans and animals. It is also rich in health-promoting phytochemicals such as carotenoids, phenolics (chlorogenic acids), flavonoids (quercitin and kaempherol glycosides), various vitamins and minerals (Foidl, *et. al.*, 2001; Becker and Siddhuraju, 2003; Bennett *et al.*, 2003).

In certain regions of the world where large scale cultivation is practiced, the tree receives little or no horticultural attention because it is often regarded as an agro-forestry plant or grown as boundary trees. In Ghana, Moringa is often grown as a live fence or a backyard tree. To put more land under cultivation as a means of increasing production to meet the growing demands of the crop will be expensive, difficult and damaging to the environment (Okigbo, 1984). Growers therefore need to increase their production by adopting appropriate strategies and techniques which will lead to sufficient and reliable yields without depleting the natural resource base. It is therefore essential to establish the best agronomic practices for cultivation and utilization.

This study was initiated to establish the productivity levels of *Moringa oleifera* as a leafy vegetable in the semi-deciduous forest zone of Ghana. The specific objectives were to determine the optimum spacing required for growing *Moringa oleifera* as a leafy vegetable and the effects of spacing on the yield. It was also done to determine the most appropriate harvest frequency required for maintaining *Moringa oleifera* fields meant for leaf production as a continuous crop.

MATERIALS AND METHODS

Experimental Design. A 3x2 factorial in a Randomized Complete Block Design (RCBD) was used. There were three levels in the spacing factor (5 x 5, 5 x 10 and 5 x 15 cm) and three levels in the harvest frequency factor (30, 35 and 40 days) giving 9 treatment combinations in each block (Table 1).

Table 1 Treatment Combinations

1. 5 x 5 cm at 30 days harvest	**2. 5 x 15 cm at 35 days harvest**
3. 5 x 10 cm at 30 days harvest	**4. 5 x 5 cm at 40 days harvest**
5. 5 x 15 cm at 30 days harvest	**6. 5 x 10 cm at 40 days harvest**
7. 5 x 5 cm at 35 days harvest	**8. 5 x 15 cm at 40 days harvest**
9. 5 x 10 cm at 35 days harvest	

Location and Climate of Experimental Site. The studies were conducted at the Horticulture Department of the Kwame Nkrumah University of Science and Technology, Kumasi (Latitude 5 degrees, 36 minutes North; Longitude 0 degrees, 10 minutes East) between 19th May 2004 and 21st March 2005. The area lies in the semi-deciduous forest zone of Ghana. The rainfall pattern is bimodal (two wet and two dry seasons). The mean annual rainfall is 1563 mm of which about 55% occurs from March and July and 30% occurs between September and November. There is usually a short dry season in August and a long one between December and March. Monthly temperature averages range from 27^{o}C to 29^{o}C in the year with February, March and April usually being the hottest months. The relative humidity during the period of the experiment varied from 66% in early mornings to 27% at noon.

Land Preparation and Seed Sowing. The experimental plot was located on Akroso series of the Forest Ochrosols. This soil consists of yellowish brown, moderate drained sandy loam developed from colluvial material on the middle to lower slopes. The land had previously been cropped with *Amaranthus hybridus.* A total land area of 5.3 x 13.4 meters was used for the study. This was sub-divided into three blocks (Blocks I, II & III) from which soil samples were taken at two depths (0-15cm and 15-30cm) for analysis of nutrient levels. The field was ploughed, harrowed and leveled to a cloddy till. The nine (9) treatments were randomly distributed in each of the three blocks. The size of each plot was 1.1 x 1.1 meters square and plots were separated from each other by 0.25 meter walkways with a boarder of 0.50 x 0.50 cm created around the treatment plots. During the course of the study, the plots were well maintained by the picking of weeds, watering when required and application of compost after the first six months of growth at a rate of 1.5 tons per hectare to arrest declining yields. Seeds were obtained from a few identified volunteer plants located at the Faculty of pharmacy medicinal plants garden of KNUST (Santasi and Patasi suburbs of Kumasi). Clean de-hulled seeds were sown directly at a depth of two (2) cm on each plot on the 19th May 2004 based on the treatment allocation to each plot. Seeds germinated within ten (10) days of sowing.

Growth and Development Measurements. The cultivated Moringa seedlings were allowed to grow for 60 days while monitoring growth and development. The parameters studied included plant height, number of leaves and stem girth at 10 cm above ground level at weekly intervals for 60 days prior to harvesting. The 60 days duration was to allow the rooting system to develop enough to be able to survive the shocks at the initial cut/ harvest at 20 cm height above ground level. After the initial 60 days after sowing, subsequent harvests were made at 30, 35 and 40 days intervals respectively based on treatments assigned to each plot. The shoots were harvested manually by cutting with a knife at 20 cm above ground level. At harvest, only the inner rows of each plot were cut and bulked for yield determinations.

Measurement of Agronomic and Other Parameters. The fresh weight of shoots harvested per plot was determined using a weighing scale in the field. Five (5) randomly selected plants were taken from each plot after harvesting and their fresh weights taken using an electronic beam balance in the laboratory. They were then separated into different tissues of stems, petioles (twigs) and leaves and weighed. These tissues were put into brown paper envelopes, labeled and dried at 60°C for 72 hours using an electric oven. The dry weight of each sample was then recorded using an electric beam balance. The crude protein, carbohydrates, fiber, fat and ash contents of the petioles, stems and leaves were determined at the Biochemistry Department of the Kwame Nkrumah University of Science and Technology. Nutritionally relevant phytochemicals (glucosinolates and phenolic antioxidants) present in the different fractions of were also analyzed at UTAD, Portugal using previously described methods (Bennett *et al.*, 2003).

Statistical Analyses. Statistical analysis (Analysis of Variance (ANOVA)) of the data generated was done using the Genstat software. The differences between treatment means were determined using the Duncan's Multiple Range Test.

RESULTS

Effect of Spacing on Vegetative Growth during the First 60 days

The results showed that seeds germinated uniformly after 10-14 days of sowing. The mean plant height increased with time, showing significant differences ($P<0.05$) from the 6th week. The closer spacing (5 x 5cm) gave the highest increase in plant height followed by the medium spacing (10 x 5cm) then the wider spacing (15 x 5 cm) (Figure 1). The average number of leaves produced per plant with time is shown in Figure 2. There was a general increase in the number of leaves produced per plant with time in all treatments except with the closer spacing which showed a sharp decline from the 7th to the 8th week. The wider spacing produced more leaves per plant and this was significantly different ($P<0.05$) relative to plants of the medium and closer spacing throughout the study period. For stem diameter, it increased with time in all treatments and were significantly different ($P<0.05$) from the 5th week onwards. Individual plants with wider spacing gave had a larger girth followed by the medium spacing, then that of the closer spacing (Figure 3).

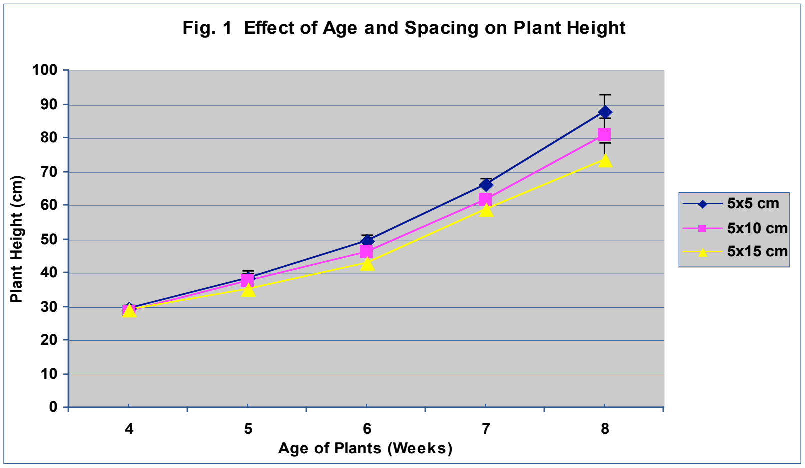


Fig. 1 Effect of Age and Spacing on Plant Height
Plant Height (cm)
100
90
80
70
60
50
40
30
20
10
0
4
5
6
7
8
Age of Plants (Weeks)
5x5 cm
5x10 cm
5x15 cm

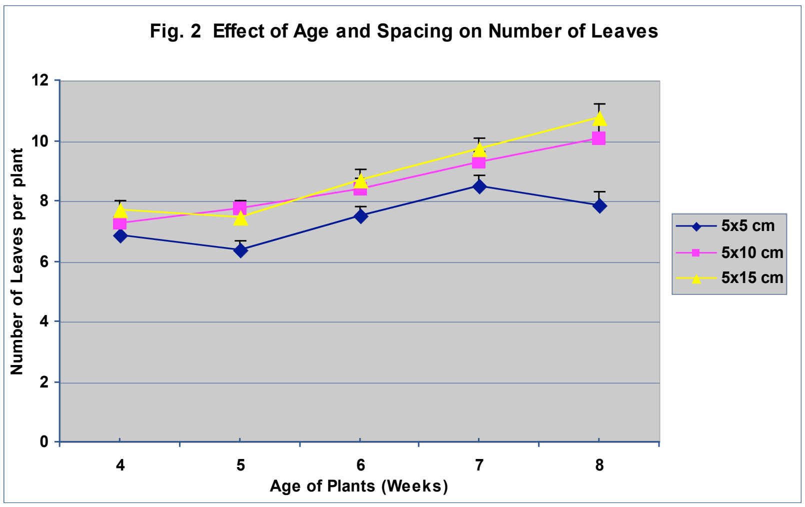


Fig. 2 Effect of Age and Spacing on Number of Leaves
Number of Leaves per plant
12
10
8
6
4
2
0
4
5
6
7
8
Age of Plants (Weeks)
5x5 cm
5x10 cm
5x15 cm

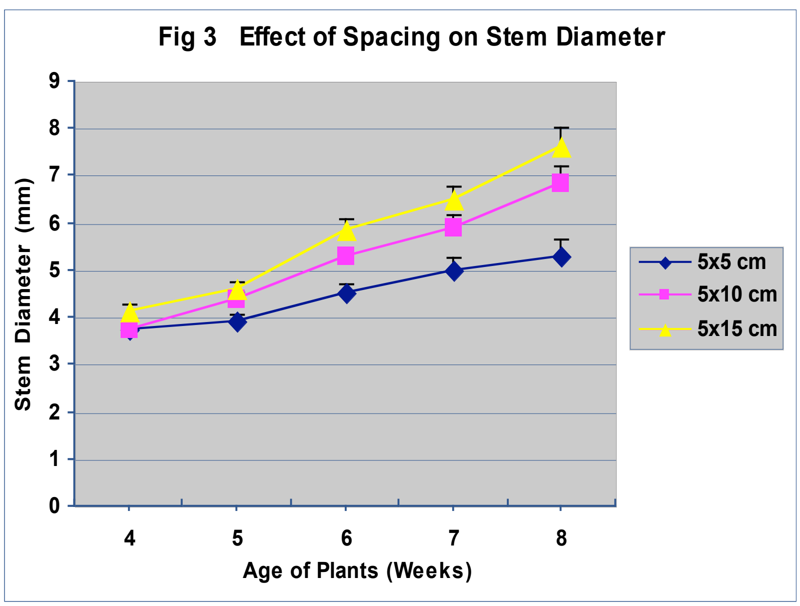
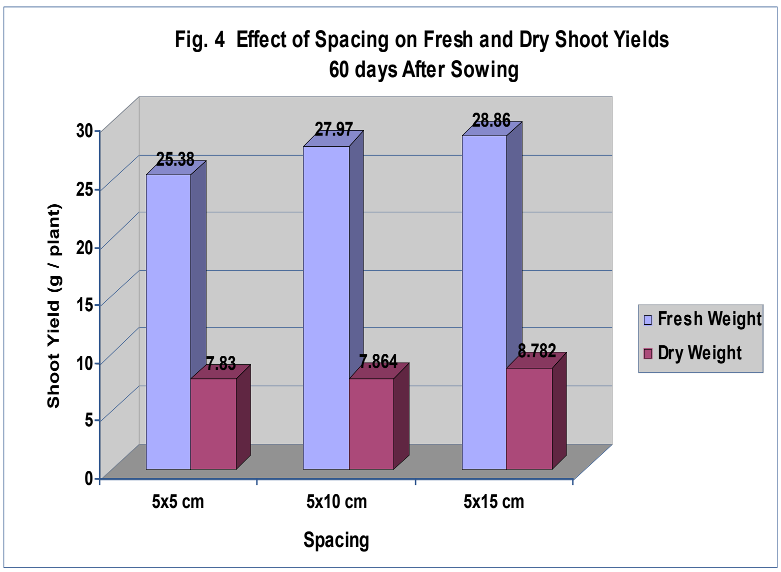


Fig 3 Effect of Spacing on Stem Diameter
Stem Diameter (mm)
9
8
7
6
5
4
3
2
1
0
4
5
6
7
8
Age of Plants (Weeks)
5x5 cm
5x10 cm
5x15 cm

Effect of Spacing on Shoot Yield (Aerial Tissue) 60 Days after Sowing

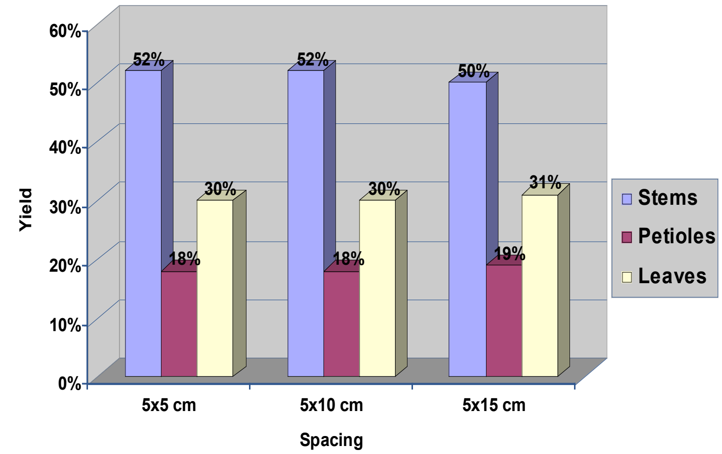
There was no statistical difference in fresh and dry shoot yield per plant at the first cutting at 60 days after sowing. The observed data showed that the widest spacing gave the highest fresh and dry shoot yield per plant followed by the medium and closer spacing respectively (Figure 4). This was however not significantly different. The results showed that the fresh and dry shoot yield per hectare were significantly different ($P<0.05$) with the closest spacing giving the highest yields of 101.52 and 31.32 tons of fresh and dry shoots respectively. The medium spacing gave 55.84 tons fresh shoots and 15.73 tons of dry shoots yield per hectare. The least shoot yield per hectare was from the widest spacing and that gave a 38.47 and 11.71 tons fresh and dry shoots yield respectively.



Effect of Spacing on Stem, Petioles and Leaf Yields

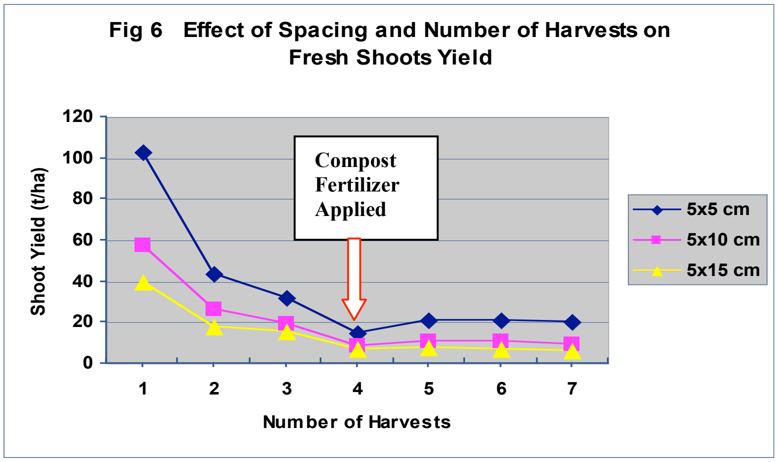
The stems formed 52% of the entire shoot yield in the close and medium spacing treatments but 50% in the case of the wide spacing. The petioles were 18% of the entire shoots in both the close and medium spacing but 19% in the case of the wide spacing treatments. The leaves formed 30% of the entire shoots in both the close and medium spacing while the wide spacing treatments gave 31% of shoot as leaf yield (Figure 5).

Fig. 5 Effect of Spacing on Yield Components per Plant 60 Days After Sowing

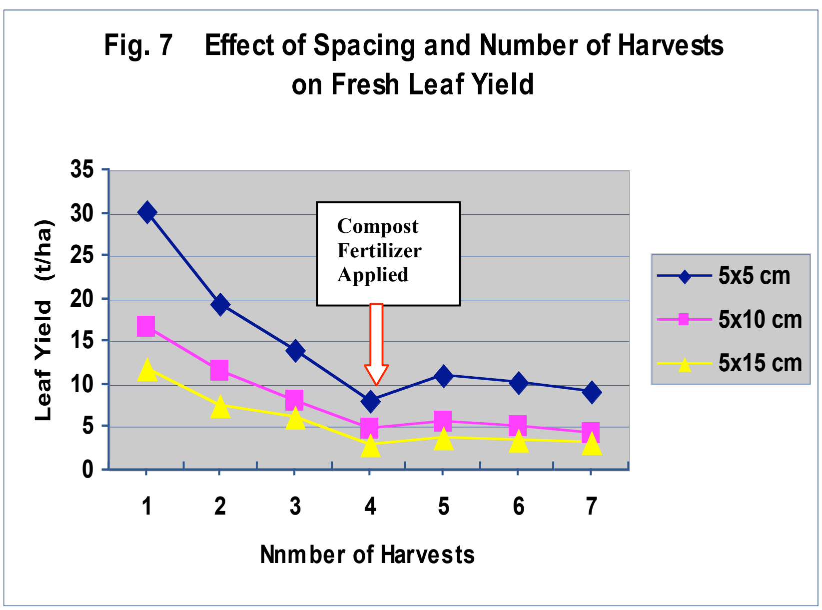


Effect of Spacing and Number of Harvests on Shoot Yield

After the first cutting/harvest 60 days after sowing, there were six harvests in all. Shoot yield per plant generally declined at each successive harvest. This decline was very sharp during the first three harvests. After applying 66.11 tons of compost the subsequent yields initially increased followed by a slight decline (Figure 5). Although spacing and frequency of harvest treatments did not significantly ($P<0.05$) affect shoot yield per plant, the wide spacing of 5x15cm gave highest shoot yields per hectare with the closest spacing gave the lowest. The 40 days frequency treatments equally gave the highest shoot yield per plant followed by the 35 days harvest with the 30 days giving the lowest. The interactions between the two factors (spacing and frequency) did not show any significant difference ($P<0.05$) (Figure 6).



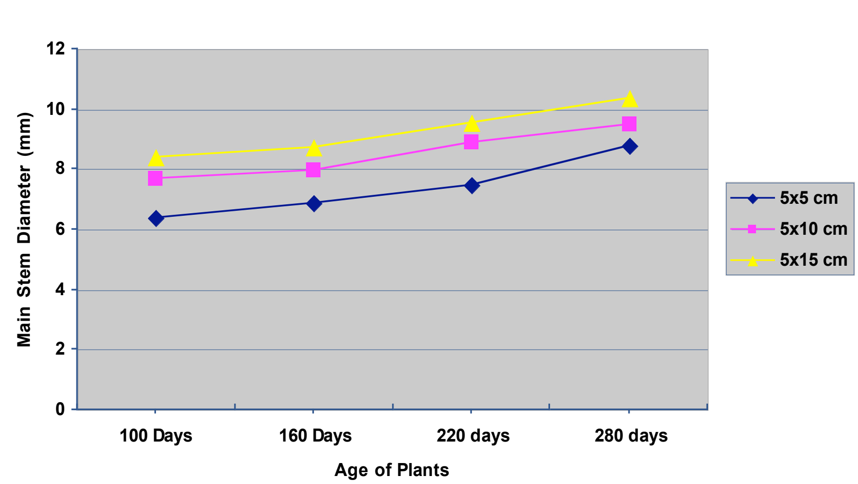
There was a generally sharp decline in shoot yield during the first three harvests followed by a rise from the 5th harvest. There was a significant difference ($P<0.05$) in shoot yield per hectare due to spacing but not with harvest frequency. The closest spacing (5x5cm) gave the highest shoot and leaf yield per hectare followed by the medium spacing (5x10cm) with the wide spacing giving the lowest yield per hectare. This trend was observed for all six harvest times. The interactions were not significantly different. Similarly, fresh leaf yields per hectare followed the same trend as shoot yield per hectare (Figure 7).



Effect of Spacing and Harvest Frequency on Plant Survival

The study showed that stem girth increased with time with plants of wide spacing having the thickest stems followed by those of medium spacing. The closely spaced plants gave the smallest stem thickness with time (Figure 8). It was also observed that a large number of plants died during the period between 100-280 days after sowing. This period also coincided with the dry season and the plants were monitored closely from the 100^{th} day after sowing.

Fig. 8 Effect of Spacing and Age on Main Stem Diameter



After discarding the boarder plants the remaining plants within each treatment plot were counted and the differences were significantly different ($P<0.05$). In the case of the closely spaced plants, out of an average of 250.4 plants within the treatment plot, 15.7 were lost within the 100-160 days period, with another 70.9 being lost within the 160-220 days period and another 37 plants lost within the next 60 days period. From an average 127.6 plants on the medium spacing treatment plots, 2.2 plants were lost within the period between 100-160 days with another 24.3 and 19.7 of them being lost during the period between 160-220 and 220-280 days respectively. Similarly 3.4 plants were lost on the wide spacing treatment plots within the period between 100-160 days after sowing, with another 17.5 and 10.4 lost within the 160-220 and 220-280 days periods respectively.

The effect of harvest frequency and age on the number of surviving plants followed a similar trend as that of the spacing effect. Thus, the average total number of plants which were lost from each treatment per hectare during the period 100-280 days after sowing for the close, medium and wide spacing treatments was 1, 972, 000; 724, 200 and 416, 000 respectively. This constituted 49.36%, 36.24% and 31.58% respectively of the close, medium and wide spacing treatment plots.

The data generated from the effect of harvest frequency on moisture, crude protein, carbohydrates, fiber, lipids and ash content of Moringa stems, petioles and leaves indicate that the leaves of Moringa contained more crude protein, carbohydrates, lipid and ash than the stems and petioles. The 35 days harvested leaf tissues gave the highest crude protein content of 8.4 % followed by the 30 and 40 days harvested leaf tissues, which had 6.9 % and 5.7 % respectively. In addition at 35 days the leaves also contained the highest levels of the health-promoting phytochemicals: glucosinolates, phenolics and flavonoid glycosides (data not shown).

DISCUSSION

Growth and Development

The growth and developmental trends observed showed significant increases in plant height and leaf numbers in all treatments. For plant height, the closer spacing showed the highest increases with the wider spacing showing relatively lower increases. The inverse trend was observed for the number of leaves per plant. According to Lyons, (1968), increasing plant density accelerates the rate of plant growth hence the increased heights in closer spacing. Growth depends on the interplay between external and internal factors, in a highly ordered and organized system. As plant population increases per unit area, a point is reached where plants begin to compete for essential growth factors like nutrients, sunlight, and water. The effect of increasing competition is similar to decreasing the concentration of growth factors (Janick, 1972; Norman, 1992). An increase in plant density does not affect the performance of individual plants while the plant density stays below the level at which competition occurs between plants. At 5x5 cm spacing the competition for essential growth factors like nutrients, sunlight, and water was so intense that, the lower leaves of the plants in this treatment died off. The lower leaves within the 5x10 cm and 5x15 cm spacing treatments received enough growth factors i.e. sunlight, etc and were healthy because at their level of competition the essential growth factors were still above the threshold level.

Effect of Spacing on Shoot Yield 60 days after Sowing

The spacing treatments 5 x 5 cm, 5 x 10 cm and 5 x 15 cm were equivalent to 4, 2 and 1.333 million plants per hectare respectively. The fresh shoot yields per plant 60 days after sowing on these spacing treatments were not significantly different even though the observed results showed the highest yield for the smaller spacing followed by the medium then the wide spacing. However the trends in the total fresh shoot yield per hectare even though similar were significantly different ($P < 0.05$). Reports by Norman, (1992) and Foidl, (2001) indicate that increasing plant density does not affect individual plants if the plant density is below the level at which competition occurs between plants. However, when the plant density is too high and there is competition between plants, yield decreases. For each crop there is an acceptable marketable size and quality. Even though competition may exist at high plant densities, such spacings may be used provided the crop harvested falls within the marketable size range. Yield per plant decreases as total biomass production per unit area increases with increased planting density. The lower production per plant is compensated for by the higher number of plants per unit area. The fresh shoot yield of 101.52 metric tones per hectare obtained for the 5 x 5 cm spacing is just slightly higher than 97.40 metric tons per hectare as recorded in Nicaragua by Foidl (2001). Unlike lettuce and cabbage which have nearly 100 percent of its fresh shoots as vegetable yield, the Moringa shoots in this study have only 30 to 31 % of fresh shoots as vegetable yield.

Spacing and Continuous Harvests

The results of the study showed that fresh shoot or leaf yields reduced drastically with continuous harvesting. After the third harvest a general fertilization with compost resulted in a slight increase which then reached a plateau. This may be an indication that there is high competition for nutrients and other growth factors and thus the continuous intake of nutrients in the soil by plants results in depletion with a corresponding decline in shoot and leaf yields. Thus it shows that high shoot productivity may only be maintained by continuously replenishing the nutrient intake by plants from the soil by a good fertilizer application program; preferably an organic fertilizer input since this will be of lower cost and will not have the negative environmental and health impacts (nitrate accumulation in water and the plant) of mineral fertilizers. As Moringa continues to grow between cuttings the number of plants per hectare is dramatically reduced owing to the different growth rates among the plants. Akinbamijo, *et. al.,*

(2003) reported that as Moringa plants compete for sunlight the larger plants shade out the slower growing or smaller plants. At 35 days, the average height of the plants is still between 1.6 and 2.0 meters and so the competition for light is not yet very great. Differences in height between plants at this stage as noted by Akinbamijo, *et. al.,* (2003) ranged between 10 and 40 cm.

Survival of Plants with Time

The trend showing continuous stem diameter increases with time, with plants in wide spacing followed by those of medium spacing then the closely spaced is expected since plant growth is an irreversible increase in size and may occur through increases both in cell size and in the number of cells (Laurie and Ries, (1950); Black and Edelman, (1970)). It also was reported by Janick, (1972) that increasing competition is similar to decreasing the concentration of growth factors. Thus the closely spaced plants have decreased growth factors being nutrients, space, etc leading to a higher competition for growth factors among individual plants. This explains why the widely spaced plants showed the thickest stems and the closely spaced plants the smallest stems. It was also observed that a large number of plants died during the period between 100 to 280 days after sowing. This period also coincided with the dry season and the plants were monitored closely at 60 days intervals. Thus the average total number of plants which survived the dry period (100-280 days after sowing) constituted 50.64%, 63.79% and 69.42% for the close, medium and wide spacing treatments respectively. This observation may be attributed to the decrease in growth factors and the increased competition between individual plants leading to the death of many more plants from the closest spacing relative to the other treatments. It also indicates that very good field management (watering and fertilization) is necessary in order to provide the optimum level of nutrients needed to reduce competition among the individual plants. This will then greatly reduce the death of plants and ensure sustainability in the long run.

Nutrient Content and Trends Observed

The observation of having more nutrients in the leaves than in the stems and petioles is favorable in the sense that the leaves form the vegetable yield components of the shoots. The 35 days harvested fresh leaf tissues gave the highest crude protein content of 8.4 %. This level of crude protein in Moringa leaves makes it the highest source of crude protein when compared to other traditional leafy vegetables like ***Amaranthus spp*** (3.6%), *Solanum macrocarpon* (4.6%), etc. Moringa can also be valued for its high antioxidants content. It is also a significant observation that the contents of health-promoting phytochemicals and antioxidants in the leaves are the highest and the most varied since the leaves serve as vegetable instead of the stems and petioles. It is also important to harvest the leaves at the right time in order to get the best amount of phytochemicals and this was found according to the study, to be 35 days frequency.

CONCLUSION

The results of the study showed that spacing had a significant effect on the growth and yield of Moringa. A pronounced effect was observed for leaf production, stem size and overall shoot yield. Thus in the production of Moringa as a source of leafy vegetable would require that among other factors, the spacing for cultivation is given attention. After taking into account a number of factors that affected the overall efficiency including the loss of plants after successive cuttings and the ease of working on fields, the optimum density in sandy, well drained and fertile soils was found to be 1.33 million plants per hectare with spacing of 5 x 15cm. After the initial harvest at 60 Days after sowing, the subsequent harvests should be done at every 35 Days and additional fertilization is essential for continuous good yields and plant survival.

REFERENCES

Akinbamijo, O. O., *et al*., (2003) *Moringa* fodder in ruminant nutrition in The Gambia. International Trypanotolerance Centre, P. M. B. 14, Banjul, The Gambia.

Becker, K., and Siddhuraju P. (2003) Antioxidant Properties Of Various Solvent Extracts of Total Phenolic Constituents From Three Different Agro-Climatic Origins Of Drumstick Tree (*Moringa oleifera*) J. Agric Food Chem.51:2144-2155.

Bennett, R.N., Mellon, F.A., Foidl, N. et al., (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. J Agric Food Chem. 51: 3546-3553.

Black, M. and Edelman, J. C., (1970). Plant growth. Heinemann Educational Book Limited, London. P. 193

Foidl, N., Harinder P. S., Markar and Klaus Becker (2001). The potential of *Moringa oleifera* for agricultural and Industrial uses. In: The Miracle Tree Edited by Lowell J. Fuglie, Darkar, Senegal pp 45-76

Grubben, G. J.H. (1977): Tropical vegetables and their genetic resources. International Board for Plant Genetic Resources. Rome, 1979

Janick, J. (1972). Horticultural Science. 2nd Edition. W.H. W. H. Freeman and Company, San Francisco. 586pp.

McBride, J. (1992). Woes of a fruitless diet. Agricultural Research, 40(5): 13.

Norman, J.C. (1992): Tropical vegetable crops. Arthur H Stockwell Limited, London, 110-252p.

Okigbo, B. N. (1984). Editorial International Institute of Tropical Agriculture (IITA) Research Briefs, 5 (4). Pg. 1

Spore, (2005) *Leafy Vegetables, A treasure to be plucked*, Information for Agricultural development in ACP countries, Issue 116 April, The Netherlands, pp 3, 10

Wood, M. (1992). Vitamin C Shortage Undermines Antioxidant Defense System. Agricultural Research, 40(6): 9