

A STUDY ON INITIAL ESTABLISHMENT OF MULTI-PURPOSE MORINGA (*Moringa oleifera.Lam*) WITH FOCUS ON STAND DENSITIES, NITROGEN, PHOSPHORUS, pH, MEDIA TYPE AND SEED PRIMING.

By

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ABSTRACT

Moringa is a multi-purpose tree which has gained a lot of publicity because of its many varied uses, which range from the medicinal applications, industrial, sanitary to the nutritional. Moringa has very high nutritional properties that would be useful as a food supplement, especially in those marginalized communities. As regards Moringa nutrition, much research has been documented, as opposed to research on agronomic needs of the plant. Hence, studies were carried out to determine whether initial establishment of Moringa is affected by stand densities, nitrogen, phosphorus and pH, and whether media type and seed priming influences the germination, emergence and initial seedling establishment. These studies were conducted as four separate experiments. The first experiment tested five population densities. The second experiment was a 5 x 3 x 3 factorial experiment with five pH levels (3.2, 6.2, 7.6, 8.2, 8.7) three phosphorus levels (0 g/plant, 8 g/plant, 16 g/plant) and three nitrogen levels (0 g/plant, 4 g/plant, 8 g/plant), and was carried out in the greenhouse. The third experiment was a 4 x 3 x 2 factorial with four pre-treatment methods, three organic sources and two soil texture sources, and also carried out in the greenhouse. The fourth experiment was a 4 x 3 x 2 factorial with four nitrogen levels (0 g/pot, 2 g/pot, 4 g/pot, 8 g/pot), three organic sources (pine bark, sawdust, none) and two textures (clay, sand). All these experiments were carried out at the University of Zimbabwe, Crop Science Department site. The results obtained indicated that high plant density produced high biomass yields and longer stems, but with smaller stem diameters, while low density produced higher individual growth of the plants as evidenced by the thicker stem diameters produced. The results further indicated that use of 1% potassium nitrate and water as seed primers best improves the germination, emergence and initial seedling establishment of Moringa compared to dehulling or heating. Clay proved more suitable than sand for Moringa seedling establishment. pH and phosphorus interaction had an effect on biomass and growth of Moringa. The most ideal pH for initial seedling establishment and growth was indicated as 7.6 (CaCl₂ scale), while P and N with the highest growth results was 4 g/plant P and 4 g/plant N.

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CONTENTS

PAGE

ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iii
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
LIST OF APPENDICES.....	ix
ABBREVIATIONS.....	xi

CHAPTER ONE: INTRODUCTION.....1

1.1 Background.....	1
1.2 Specific Objectives.....	3
1.3 Hypotheses.....	3

CHAPTER TWO: LITERATURE REVIEW.....4

2.1 Botany of the Moringa Plant.....	4
2.2 Some Uses of Moringa.....	4
2.2.1 Medicinal Applications.....	4
2.2.2 Cosmetic Uses and Industrial Uses.....	5
2.2.3 Sanitation and Agricultural Use	5
2.2.4 Fuel Source and Other Uses	5
2.2.5 Food and Nutritional Uses	5
2.3 Seedling Establishment Using Seedlings or Cuttings.....	6
2.4 Brief Status of Zimbabwean Soils Inhabited By Small-holder Farmers.....	6
2.5 Moringa Agronomy and pH Tolerance	7
2.6 Effect of Phosphorus Nutrition in Crop Productivity	8
2.7 Effect of Nitrogen Nutrition on Crop Productivity and Growth.....	9
2.8 Plant Density and Biomass Accumulation.....	9
2.8.1 Other Conclusive Studies on Related MPTS Which Might Apply to Moringa.....	9
2.8 Effect of N and P Interaction on Crop Growth.....	10
2.9 Effect of Seed Priming on Seed Germination.....	10
2.10.1 Hydro Priming as a Seed Pre-treatment Method.....	11

2.10.2	Osmotic Priming as a Seed Pre-treatment Method.....	11
2.10.2.1	Potassium Nitrate (KNO ₃) Used as Osmotic Primer.....	12

CHAPTER THREE: MATERIALS AND METHODS.....13

3.1	Experiment 1: A Study of the Initial Establishment of Multi - Purpose Moringa (<i>Moringa oleifera</i> . Lam) At Various Plant Densities, their effect on Biomass Accumulation and Leaf Yield.....	13
3.1.1	Study Site.....	13
3.1.2	Experimental Design	13
3.1.3	Experiment Management.....	13
3.2	Experiment 2: A Study of Moringa Seedling Establishment, Initial Plant Growth and Nutrient Uptake in Potted Media as Influenced by Interactions of pH, P and N.....	15
3.2.1	Study Site.....	15
3.2.2	Experimental Design.....	15
3.2.3	Experiment Management.....	15
3.3	Experiment 3: A Study of the Effect of Pre-Treatment Method and Media Type on Germination, Emergence, Initial Establishment and Growth of <i>Moringa oleifera</i>	17
3.3.1	Study Site	17
3.3.2	Experimental Design.....	17
3.3.3	Experiment Management	17
3.4	Experiment 4: A Study into the Nitrogen Uptake and Use of <i>Moringa oleifera</i> (Lam) Grown in Different Soil Textures and Organic Sources.....	18
3.4.1	Study Site.....	18
3.4.2	Experimental Design.....	18
3.4.3	Experiment Management.....	18
3.5	Measurements	18
3.6	Data Collection	19
3.7	Data Analysis.....	19

CHAPTER FOUR: RESULTS20

4.1	Experiment 1: A study of the Initial Establishment of Multi-Purpose Moringa (<i>Moringa oleifera</i> .lam) at Various Plant Densities, their Effect on Biomass Accumulation and Leaf Yield	20
4.1.1	Effect of Population Density on Above Ground Whole Plant Biomass	20
4.1.2	Effect of Population Density on Below Ground Whole Plant Biomass.....	20
4.1.3	Effect of Population Density on Leaf Biomass Yields.....	21

4.1.4	Effect of Population Density on Stem- Length and Stem Diameter measurements.....	22
4.2	Experiment 2: A Study of Moringa Seedling Establishment, Initial Plant Growth and Nutrient Uptake in Potted Media as Influenced by Interactions of pH, P and N	24
4.2.1	Effects of pH, Nitrogen and Phosphorus on Seedling Emergence Counts	24
4.2.2	Effects of pH, Nitrogen and Phosphorus on Stem-lengths Measurements	25
4.2.3	Responses of Root Biomass Yields to pH and Nitrogen.....	27
4.2.4	Total Above Ground Plant Biomass Yield Responses to N and pH.....	29
4.3	Experiment 3: A Study into the Effect of Pre-Treatment Method and Media Type on Seed Germination, Emergence and Initial Seedling Establishment of <i>Moringa oleifera</i>	31
4.4	Experiment 4: A Study into the Nitrogen Uptake and Use of <i>Moringa oleifera</i> (Lam) Grown in Different Soil Textures (clay and sandy soils) and Organic sources (Pine bark and sawdust).....	33
4.4.1	Seedling Emergence Responses to Nitrogen, Soil Texture and Organic source.....	33
4.4.2	Response of Stem-length to Nitrogen, Organic Matter and Texture.....	35
4.4.3	Effect of Nitrogen, Texture and Organic Source on Plant Biomass Yields.....	36
4.4.4	Effect of Nitrogen, Texture and Organic Source on Root Biomass Yield.....	37
CHAPTER FIVE: GENERAL, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS.....		38
5.1	Effect of Population Density on Moringa Growth.....	38
5.2	Effects of pH and its Interactions with N, P and Time on Moringa Growth.....	38
5.3	Effect of media type, texture, organic source and seed priming on Moringa growth.....	40
5.4	Conclusions.....	41
5.5	Recommendations.....	42
REFERENCES		43

APPENDICES.....	49
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LIST OF TABLES

Table 4.1 Effect of Population Density on above ground plant dry matter (g/pot) obtained at Crop Science Department site, July-August 2007.....	20
Table 4.2 Effect of Population Density on Root Fresh-weight (g/pot) from Crop Science Department site, July-August 2007.....	21
Table 4.3 Effect of Population Density on Leaf Dry Matter (g/pot) and Leaf Fresh-weight (g/pot) obtained from the Crop Science Department site, July-August 2007.....	22
Table 4.4 Effect of Population Density on stem diameter (mm) obtained from the Crop Science Department site, July-August 2007.....	23
Table 4.5 Effect of Population Density on stem length (mm) obtained from Crop Science Department site, July-August 2007.....	23
Table 4.6 Effect pH (CaCl ₂) on seedling emergence counts obtained from Crop Science Department site, July-August 2007.....	24
Table 4.7 Effect of organic source and media texture on root DM (g/pot) obtained from Crop Science Department site, July-August 2007.....	28
Table 4.8 Effect of pre-treatment on root FW (g/pot) obtained from Crop Science Department site, July-August 2007.....	32
Table 4.9 Effect of Time (weeks) on seedling emergence counts from Crop Science Department site, July-August 2007.....	34
Table 4.10 Effect of Time (weeks) on stem length (mm) from Crop Science Department site, July-August 2007.....	35
Table 4.11 Effect of nitrogen (g/pot) and organic source as single factors on plant biomass (g/pot) from Crop Science Department site, July-August 2007.....	37
Table 4.12 Effect of media texture on root biomass (g/pot) from Crop Science Department site, July-August 2007.....	37

LIST OF FIGURES

Figure 4.1 pH (CaCl ₂) and phosphorus (g/pot) effect on seedling emergence counts from Crop Science Department site, July-August 2007.....	25
Figure 4.2 pH (CaCl ₂) and nitrogen (g/pot) effect on Moringa stem length (mm) from Crop Science Department site, July-August 2007.....	26
Figure 4.3 Nitrogen (g/pot) and time (weeks) effect on Moringa stem length (mm) from Crop Science Department site, July-August 2007.....	27
Figure 4.4 Nitrogen (g/pot) and pH (CaCl ₂) effect on Moringa root DM (g/pot) at Crop Science Department site, July-August 2007.....	29
Figure 4.5 Nitrogen (g/pot) and pH (CaCl ₂) effect on Moringa above ground plant dry matter (g/pot) observed from Crop Science Department site, July-August 2007.....	30
Figure 4.6 Nitrogen (g/pot) and pH (CaCl ₂) effect on Plant FW (g/pot) obtained from Crop Science Department site, July-August 2007.....	31
Figure 4.7 Effect of organic source and media texture on root DM (g/pot) obtained from Crop Science Department site, July-August 2007.....	33
Figure 4.8 Nitrogen (g/pot) and Organic source effect on seedling emergence counts from Crop Science Department site, July-August 2007.....	35
Figure 4.9 Effects of nitrogen (g/pot)*organic sources*texture on stem length (mm) from Crop Science Department site, July-August 2007.....	36

LIST OF APPENDICES

APPENDIX A: ANOVA FOR POPULATION DENSITY EXPERIMENT ONE

Appendix 4A.1 Analysis of variance for above ground plant dry matter (g/pot) obtained at Crop Science Department field (Experiment 1).....	49
Appendix 4A.2 Analysis of variance for above ground plant fresh-weight (g/pot) from Crop Science Department field (Experiment1).....	49
Appendix 4A.3 Analysis of variance for below ground (root) plant fresh-weight (g/pot) obtained at Crop Science Department field (Experiment 1).....	49
Appendix 4A.4 Analysis of variance for below ground (root) plant dry matter (g/pot) obtained from the Crop Science Department field (Experiment 1).....	49
Appendix 4A.5 Analysis of variance for leaf dry matter (g/pot) at Crop Science Department field (Experiment 1).....	49
Appendix 4A.6 Analysis of variance for leaf fresh weight (g/pot) at Crop Science Department field (Experiment 1).....	50
Appendix 4A.7 Analysis of variance for stem diameter (mm) at Crop Science Department field (Experiment 1).....	50
Appendix 4A.8 Analysis of variance for stem length (mm) at Crop Science Department field (Experiment 1).....	50

APPENDIX B: ANOVA FOR pH, PHOSPHORUS AND NITROGEN INTERACTION EXPERIMENT TWO

Appendix 4B.1 Analysis of variance for seedling counts from Crop Science Department field (Experiment 2).....	50
Appendix 4B.2 Analysis of variance for stem length (mm) from Crop Science Department field (Experiment 2).....	51
Appendix 4B.3 Analysis of variance for below ground (root) plant fresh weight (g/pot) at Crop Science Department field (Experiment 2).....	51
Appendix 4B.4 Analysis of variance for below ground (root) dry matter (g/pot) at Crop Science field (Experiment 2).....	51

Appendix 4B.5 Analysis of variance for above ground plant fresh weight (g/pot) at Crop Science Department field (Experiment 2).....	52
--	----

Appendix 4B.6 Analysis of variance for above ground plant dry matter (g/pot) at Crop Science Department field (Experiment 2).....	52
--	----

APPENDIX C: ANOVA FOR GERMINATION EXPERIMENT THREE

Appendix 4C.1 Analysis of variance for below ground (root) plant dry matter (g/pot) from Crop Science Department site (Experiment 3).....	52
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Appendix 4C.2 Analysis of variance for below ground (root) plant fresh weight (g/pot) from Crop Science Department site (Experiment 3).....	53
--	----

Appendix 4C.3 Analysis of variance for above ground plant dry matter (g/pot) from Crop Science Department site (Experiment 3).....	53
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Appendix 4C.4 Analysis of variance for above ground plant fresh weight (g/pot) from Crop Science Department site (Experiment 3).....	53
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APPENDIX D: ANOVA FOR NITROGEN USE EXPERIMENT FOUR

Appendix 4D.1 Analysis of variance for seedling emergence counts from Crop Science Department site (Experiment 4).....	54
---	----

Appendix 4D.2 Analysis of variance for stem length (mm) from Crop Science Department site (Experiment 4).....	54
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Appendix 4D.3 Analysis of variance for above ground plant fresh weight (g/pot) from Crop Science Department site (Experiment 4).....	55
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Appendix 4D.4 Analysis of variance for above ground plant dry matter weight (g/pot) from Crop Science Department site (Experiment 4).....	55
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Appendix 4D.5 Analysis of variance for below ground (root) plant fresh weight (g/pot) from Crop Science Department site (Experiment 4).....	55
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Appendix 4D.6 Analysis of variance for below ground (root) plant dry weight (g/pot) from Crop Science Department site (Experiment 4).....	56
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ABBREVIATIONS

%	Percent
AN	Ammonium nitrate
g/pot	grams per pot
mls	Millilitres
MPTs	Multi-Purpose Trees
N	Nitrogen
N0	Refers to 0 grams/pot nitrogen
N2	Refers to 4 grams/pot nitrogen
N4	Refers to 4 grams/pot nitrogen
N8	Refers to 8 grams/pot nitrogen
OM	Organic matter
OS	Organic source
P	Phosphorus
P₂O₅	Phosphate
pH	The concentration of hydrogen ions present in the media.
S	Sulphur
SSP	Single Super Phosphate
WP	Wettable powder

CHAPTER ONE: INTRODUCTION

1.1 Background

Moringa (*Moringa oleifera*. Lam) is a fast-growing multi-purpose tree that originated from the Indian subcontinent and is considered to be suitable only for lowland cultivation at altitudes less than 600m. However, the adaptability of the tree has been demonstrated by discovery of natural stands at altitudes of 1200m in Mexico and in excess of 2000m in Zimbabwe (Foild, Makkar and Becker, 2001). Moringa tolerates a wide range of pH levels (5-9) and responds well to mulch, water and fertilizer (Price, 1985). Currently Moringa has since established itself in the Southern parts of Zimbabwe (where it is known as the ‘Tonga’ tree), along the Zambezi valley, with greater numbers being found to exist in Binga and Victoria Falls (Trends, 2005). Recently, given its widely publicized benefits, many people are planting it in most parts of the country.

Moringa is extensively promoted worldwide for nutrition supplementation as it is rich in protein (5-10%), in minerals (iron and calcium) and in vitamins such as vitamin C and carotene (Church World Service, 2000). In view of its high nutritional value, it becomes very important as a human food as it can supplement a number of food crops. In comparison, gram for gram, Moringa has more beta-carotene than carrots (*Daucus carota*), more protein than peas (*Pisum sativa*), more vitamin than oranges (*Citrus citrii*), more calcium than milk, more potassium than bananas (*Musa spp*) and more iron than spinach (*Spinacea oleracea*) (Palada and Chang, 2003).

Currently, studies on Moringa seem to be focusing more on its nutritional and medicinal values, than on its agronomy as a cultivated crop. Literature on its agronomic aspects is based mainly on Indian studies. For instance, in Zimbabwe, local studies are on evaluation of Moringa as a protein source in fish farming (Jacob Japssen (Tree Africa)-personal communication), while currently AREX (Agricultural Research and Extension Services) has suspended studies on Moringa following problems encountered with initial establishment of the crop. AREX has changed focus to Jatropha studies (Sibusiso Mavankeni (AREX Research Officer)-personal communication). Thus, under local Zimbabwean conditions, knowledge on production requirements for Moringa is not well established or documented,

and the production practices of isolated trees, widely promoted in Zimbabwe, is based on conjecture.

Given the high nutritional value of Moringa and its implication as an important human food supplement, it becomes imperative that studies into the most ideal conditions for its initial establishment in the nursery are carried out. Such studies will ensure high establishment rates of the seeds sown out, as the seed has also proven to be very difficult to acquire. High initial seedling establishment results in increased production levels, thus availing more Moringa produce to those in need of it as a food source.

Whilst semi-popular literature claims that pH range is 5-9 (Palada and Chang, 2003), the range appears too wide, hence the need to carry out studies which are more conclusive on the ideal pH ranges for optimum initial seedling establishment. Maintaining an ideal pH range is of utmost importance to optimum crop production as either pH extremes inhibits uptake of nutrients such as phosphorus (which is an immobile nutrient once applied in the media) (Mengel and Kirkby, 1982).

Also contrary to claims that Moringa grows well in soils without additions of fertilizer, preliminary observations at the University of Zimbabwe show that locally produced Moringa grows vigorously under high organic matter conditions (Dr Chris Nyakanda (University of Zimbabwe)-personal communication). There is need to carry out studies to establish exactly how Moringa responds to additions of inorganic fertilizers whether for the initial establishment or for subsequent growth of the tree. Moringa has lateral rooting which is weak and which responds negatively to high concentrations of applied inorganic fertilizers (Fuglie, 1999). This fact serves to re-emphasize the need to establish, firstly, the correct levels of applied inorganic nutrients, such as nitrogen, such that it enhances Moringa productivity without bringing about a negative response by the roots. Secondly, correct crop densities have to be established so that too dense populations which bring about competition among the plants are avoided; and very low populations that cause luxurious uptake and wastage of nutrients such as nitrogen (leaching losses) are avoided. Establishing and maintaining the ideal tree density will lead to increased nitrogen use efficiency since all the nitrogen applied per given area will be taken up by the Moringa plants, which would increase their productivity.

Promotion of Moringa in Zimbabwe is in the low input systems where human nutrition is

compromised, whilst these same areas are extensively characterized by soils with high levels of acidity, often below pH 5, as well as low levels of phosphorus and nitrogen (Nyamangara, Mugwira and Mpofu, 2000). This implies that Moringa expansion in smallholder farming will most likely involve the use of any locally available organic matter source in combination with local soils inherently low in fertility and of high acidity as nursery media. Hence the need to study the effect of various media on germination and initial Moringa establishment.

1.2 Specific objectives

The study aims to establish the:

1. The optimum population density for maximum Moringa growth and biomass accumulation.
2. Optimum nitrogen and phosphorus levels for initial establishment and optimum growth of Moringa.
3. Most ideal pH level for optimum initial Moringa establishment and growth and its effect on plant nutrient uptake and biomass accumulation, and whether any interactions exist among phosphorus, nitrogen and pH.
4. Most ideal seed priming method and media type for optimum germination and initial seedling establishment and growth.

1.3 Hypotheses

1. There is an optimum population density that promotes highest Moringa growth and biomass accumulation.
2. There is an optimum nitrogen and phosphorus level for initial establishment and optimum growth of Moringa.
3. pH has an effect on initial Moringa establishment, plant nutrient uptake and biomass accumulation and interactions do exist among phosphorus, nitrogen and pH.
4. Whilst seed priming increases germination percentage and rate, media type has no effect on Moringa germination and initial seedling establishment.

CHAPTER TWO: LITERATURE REVIEW

2.1 Botany of the Moringa Plant

The *Moringaceae* family has 12 known species, with *Moringa oleifera* Lam being the most widely known and utilized species. It has many pseudonyms; ‘horse-radish’ tree (due to taste of the root preparation - Florida), ‘drum-stick’ tree (shape of the pods - India), ‘mothers best-friend’ (leaves are cooked and fed to babies - Philippines), benzolive tree (Haiti), Malungay and nebeday (Senegal). These serve as an indication of its significance around the world (Price, 1985). The tree ranges in height from 5 - 12m with an open, umbrella shaped crown, straight trunk (10 - 30cm thick) with corky, whitish bark. It produces a tuberous taproot which helps explain its observed tolerance to drought conditions (Ramachandran, Peter and Gopalakrishnan, 1980). The evergreen, deciduous foliage (climate dependent) has leaflets 1 - 2cm in diameter, flowers that are pleasantly fragranced (white or cream coloured) and are borne profusely in auxiliary drooping panicles 10 - 25cm long. The fruits (pods) are initially light green, slim and tender, eventually becoming dark green and firm up to 120cm long, depending on variety. Fully mature, dried seeds are round or triangular shaped, the kernel surrounded by a lightly wooded shell with three papery wings. Moringa is a multi-purpose tree (MPT) with a variety of uses ranging from medicinal, sanitary, agricultural to even nutritional.

2.2 Some Uses of Moringa

2.2.1 Medicinal Applications

Moringa flowers, leaves and roots are used in folk remedies for tumours, whilst the seed is used specifically for abdominal tumours. Moringa root is used in Nicaragua for dropsy and the root juice can be applied externally to counter irritations of the skin, while the leaves are applied as poultices to sores, rubbed on the temples for headaches and are said to have purgative properties. Moringa bark, leaves and roots can be taken to promote digestion, while the moringa oil can be applied externally for skin diseases (Ramachandran *et al*, 1980). The bark can also be used to treat diarrhoea cases. Moringa flowers and roots contain a compound, pterygospermin, which has powerful antibiotic and fungicidal effects which have been put to various usages in the health sector (Hartwell, 1967-1971).

2.2.2 Cosmetic and Industrial Uses

Moringa seed contains ben oil used in making perfumes and soap, and can also be used as a preservative and as a machinery and watch lubricant because it won't spoil (Fuglie, 1999).

2.2.3 Sanitation and Agricultural Uses

One crushed kelor (Moringa) seed can clear 90 percent of the total coliform bacteria in a litre of river water within 20 minutes (Wilson, 1992). The seedcake left after oil extraction can be used as a soil fertilizer or in the treatment of turbid water where it is applied at a dose not exceeding 250 mg/litre of water (Price, 1985). It coagulates the solid matter and suspended bacteria and these impurities then sink to the bottom.

The juice from fresh Moringa leaves can be used to produce an effective plant growth regulator which increases yields by 25 – 30 percent for many crops: onions, bell pepper, soya, maize, sorghum, coffee, tea, chili, melon, since it contains Zeatin, a plant hormone belonging to the cytokinines group, which can be foliarly applied (Price, 1985).

2.2.4 Fuel Source and Other Uses

Moringa wood makes acceptable firewood but poor charcoal because it is very soft. It also makes excellent pulp for paper manufacture. The bark is sometimes used for tanning and to make mats and rope. A blue dye is being made from the wood in Senegal and Jamaica which is being put to use in the textile and other relevant industries (Foidl *et al*, 2001).

2.2.5 Food and Nutritional Uses

Moringa leaves are used for food, with almost every part of the plant having a food value (Palada and Chang, 2003; Ramachandran, Peter and Gopalakrishnan, 1980). For instance, seed is said to be eaten like a peanut in Malaya while the leaves are widely used as a highly nutritious vegetable, a supplement as greens in salads, in vegetable curries, as pickles and also for seasoning. Moringa leaf has significant quantities of vitamins A, B and C, calcium, iron and protein (Verma, Banerji, Misra and Nigam, 1976), qualities which assist in combating malnutrition in humans. Moringa flowers are cooked and consumed either mixed with other foods or fried in a flour batter and have shown to be rich in potassium and calcium which have proven to be vital sources of these minerals especially for developing countries. Moringa seeds are utilized either as a green 'pea' in their immature state or fried in their mature state

and they are added to curries, canned and sold in stores in India, a vital side dish or vegetable. The dried Moringa leaves are used to make herbal tea or Moringa powder which is used to increase energy or as a spice. The mature Moringa seed contains about 40 percent oil which is of excellent quality (73 percent oleic acid similar to olive oil) for cooking and has been sold for many years as “ben oil” used in cooking. It takes approximately 11kg of Moringa seed to produce 2.6 litres of oil (Price, 1985).

2.3 Seedling Establishment Using Seed or Cuttings

According to Fuglie (1999), seeds require little or no pre-treatment prior to germination with viability rates for fresh seeds reported to be up to 80 percent, which reduces to about 50 percent after 12 months storage. Seeds can be sown directly or in seedbeds, and they have no dormancy periods, thus they can be sown as soon as they mature. Seeds are usually planted in the nursery using a light soil mixture of 3 parts soil to 1 part sand (Church World Service, 2000). Germination occurs within 5 - 12 days or even up to 30 days, depending on the age of the seed and the pre-treatment method used, which might include: cracking the shells, de-hulling, soaking seeds with shells overnight, de-hulling seeds and soaking seeds for 24 hours then putting in a plastic bag and store in a warm place (drawer or cabinet). The seedlings can then be planted out when they are 50 - 90 cm tall (Fuglie, 1999).

Moringa seedlings which are initially raised from seeds are said to develop plants which have a giant taproot that enables them to withstand extreme droughts and highly acidic, alkaline or salty soils (Fuglie, 1999).

Cuttings can also be used to establish Moringa trees and these result in fastest growth. However, it has been observed that trees grown from cuttings produce inferior fruits which have a shallower root development, making them more drought-susceptible (Church World Service, 2000). This aspect therefore does not make seedling production from cuttings a very sound option for the intended farmers as they are located naturally in low rainfall areas, with low fertility, poorly structured soils.

2.4 Brief Status of Zimbabwean Soils Inhabited By Smallholder Farmers

Almost 70 percent of Zimbabwe is made up of sandy soil originating from coarse granite, with those in the south derived from gneiss and Triassic sands and the northwest of the country comprising Kalahari sands. These sands in relation to other soil types in Zimbabwe

are low in nitrogen (N), phosphorus (P) and sulfur (S). Many crops grown on these sandy soils exhibit multiple nutrient deficiencies of N, P and S (Grant, 1981). Historically on the other hand, the red soils in Zimbabwe have been regarded as the most fertile and form the most productive soils due to their inherent fertility and good hydrodynamic and physical properties (Nyamangara *et al*, 2000). The red soils belt is where most commercial farms are located, whereas, the majority of the small holder farmers occupy these inherently low fertile and acidic soils as a result of the imbalances created during the colonial era land distribution and post-independence land reforms programs.

Moringa production in these soils will be a food and income source thus farmers will gain more from their nutrient deficient soils and low rainfall areas if they are equipped with the information and skills to produce this crop.

2.5 Moringa Agronomy and pH Tolerance

Not much literature exists regarding agronomic practices for Moringa production; the little literature available is based on Indian conditions, with only very little research focusing on Moringa agronomy. Some agronomic trials with Moringa showed that the plant can grow well in hilly areas and in weathered soils of low fertility (Tinh Bien districts, India) (Manh, Dung and Xuan, 2003). However, information about growth of Moringa in the acid regions of India is almost absent (Manh *et al*, 2003). The information available on cultivation procedures for Moringa is limited, except in certain regions of India where it's cultivated on a large scale (Fuglie, 1999).

Since conclusive information on responses of Moringa to pH is absent, responses of other Multi-Purpose Tree Species (MPTS) to pH may be instructive. Adaptation to acidity varies markedly among legume MPTS, with *Flemingia* and *Gliricidia* species being reported to be extremely well adapted to acidic soils (pH 4.5), while *L. leucocephala*, *G. sepium* and *Desmanthus virgatus* species can tolerate highly alkaline soils. Response may also vary among leucaena varieties. Leucaena varieties such as Cunningham, K29, K132 and K420 are more tolerant to acidity than others (Brewbaker, Hedge, Hutton, Jones, Lowry, Moog and Van den Beldt, 1985). Conversely, studies by Ghai, Rao and Batra (1985), have shown that *Sesbania* species are tolerant of alkalinity during germination with *S. bispinosa* showing successful growth in acid soils (Mirchandani and Khan, 1983; Mune and Krishnamurthy, 1984), while on the other hand, *S. sesban* and *S. grandiflora*, have both shown an outstanding

feature of tolerance to both saline and alkaline conditions, though their tolerance to high acidity is yet unknown (Hansen and Munns, 1985). Nutrition however, might also have an influence on the growth aspects of Moringa.

2.6 Effect of Phosphorus Nutrition in Crop Productivity

In the wake of the almost non-existent studies on Moringa as regards its nutrition, agronomy and many other aspects such as ideal seed germination conditions, reference yet again is made to studies which have been carried out in other crops with regards to phosphorus nutrition and its role in crop growth and productivity, in the hope that it will give some insight on responses of Moringa to this nutrition.

Phosphorus (P) is important in root development and formation and increases root tolerance to soil-borne diseases such as avocado (*Persea americana*) root rots (Cooke, 1975). Phosphorus is absorbed in the ionic form, which is dependent on the soil pH. P is easily immobilized in the soil by being fixed with poorly soluble compounds, though there is a marked increase in P uptake in the presence of ammonium nitrate (AN), in comparison to nitrate nitrogen (Francis and Liogier, 1991; Marcu, 2005). Since Moringa seedling roots are said to be very vulnerable until they are established, P can be added to encourage root development (Ramachandran *et al*, 1980).

According to studies conducted on leucaena by Ruaysoongern, Shelton and Edwards (1989), *L. leucocephala* indicated relatively high requirements for Phosphates (225kg/ha) in relation to other tree legume species such as *Acacia villosa*. Leucaena is particularly sensitive to P deficiency; therefore in soils low in P, quite high rates need be applied. This might be a point to take note as regards Moringa production; since it has weak, vulnerable roots during initial crop establishment, there seems to be a need for fairly high P availability for Moringa at this establishment stage.

Further studies have indicated that P uptake resulted in yield increases, increased dry matter production, greater plant height, reduced days to flowering and increased fruit numbers per tree in acacias (Singh 1971 and 1972; Singh and Rai, 1974; Wanknade and Morey 1982; Gill, Thakur and Thakur, 1974). Conversely, other studies revealed that P did not increase the yield or the root and shoot dry matter (Mahajan and Khanna, 1968; Weston and Zandstra, 1989).

2.7 Effect of Nitrogen Nutrition on Crop Productivity and Growth

Studies on *Desmanthus virgatus* (Parbery, 1967a) and on *S. cannabina* (Lu, Yuan and Zhong, 1984) indicated that N increased the growth of and the total dry matter (DM). Further increases in N application resulted in higher total DM/plant than plants with lower N applications and consequently, biomass of other MPT's and this effect can not be ruled out on Moringa growth.

2.8 Plant Density and Biomass Accumulation

Studies by Mahn *et al* (2005) on different spacing of Moringa (40*20, 40*30cm, and 40*40cm) indicated that the different spacing did not affect plant height and biomass yield of Moringa. The estimated biomass yield was 8.6, 11.1 and 7.6 tonnes/hectare (first cutting); 7.6, 7.9 and 6.3 tonnes/hectare (second cutting) and 6.3, 6.3 and 4.9 tonnes/hectare (third cutting) for spacing of 40*20; 40*30cm and 40*40cm respectively. These studies were carried out on sulfate acidic soils and results suggest that Moringa can develop on the sulfate acid soil but the young plant did not adapt well to water logging condition, in contrast to mature plants. These studies further suggest that the plants can be harvested 7 times/year, yielding an annual biomass of between 43 to 52 tonnes/hectare. These studies apply elsewhere under sulfate acidic environments as opposed to Zimbabwean conditions which are less wet, warmer and comprise of sandy soils that are inherently of low nitrogen and phosphorus.

2.8.1 Other Conclusive Studies on Related MPTS Which Might Apply to Moringa

Numerous studies have been conducted on other MPTS. For example *Gliricidia* leaf biomass (DM) production under a range of climatic and edaphic conditions, various management regimes and different variables such as establishment methods, plant spacing, plant density, harvesting frequency and intensity, and the values (DM production) attained varied (Wong and Sharudin, 1986; Srisikandarajah, 1987; Atta-Krah and Sumberg, 1987; Ivory, 1990; Gutteridge and MacArthur, 1998). While spacing and population studies by Ella, Jacobsen, Stur and Blair (1989) revealed that as plant spacing was reduced, biomass yield per plant decreased owing to competition. However, total forage yield/unit area increased, giving the highest leaf yields at the highest density tested. In addition, studies on *Sesbania* spacing and population density indicated lower stem diameters and increased height at high densities but increased biomass as plant population increased (Dutt and Pathania, 1986; Zsuffa, 1984).

Further studies with leucaena plant densities and eucalyptus, indicated the mean total biomass

differed significantly between species; *M. azedarach* accumulated the maximum biomass (14.9 t/ha), which was 24% higher than *L. leucocephala* and 43% higher than that of *E. tereticornis*. Eucalyptus varied the most in biomass over the population densities tested. Total biomass in all species increased as population density increased, although gains from 15000 to 20000 plants/ha were non-significant (Mishra, Sharma and Verma, 1992).

2.9 Effect of N and P Interaction on Crop Growth

Numerous studies into nitrogen and phosphorus in various crops have indicated that P application increased N uptake, which is indicative of interactions between P and N (Gupta and Saxena, 1981). All growth parameters are often at their maximum with the highest P application (Chattopadhyay, Das and Sarkar, 1976; Dutt and Pathania, 1984). For instance, N and P fertilization significantly ($P < 0.05$) increased canopy height, growth rate, and number of primary branches per plant, stem diameter, leaf and fuel-wood DM yield in *Sesbania* (Kanyama-Phiri, Dzowela and Kategile, 1993).

There are other factors which might have an effect on the overall growth responses of plants which are not entirely nutrient related, some of which might be the pre-treatment conditioning done to the seed before being sown. This pre-treatment conditioning is known as seed priming and it has shown to influence rate of seed germination, emergence and initial seedling establishment of treated seed.

2.10 Effect of Seed Priming on Seed Germination and Emergence

Seed priming is a presowing treatment involving controlled hydration of seeds sufficiently to allow pregerminative metabolic events to occur, without triggering radicle protrusion through the seed coat. This results in enhanced germination and emergence. The enhanced performance might be attributed to the hydrolytic processes initiated by presoaking, releasing simple sugars which are then immediately utilized and possibly membrane repair through enzymes activated during the hydration process (Copeland and McDonald, 1995).

Priming may also lead to the weakening of radicle restraining tissues, embryo development, increased macromolecule synthesis and increased activity of several enzymes participating in the mobilization of seed reserves. These processes then result in improved capacity for rapid cell differentiation and growth (Basra, 2002). Emergence of presoaked seeds induces physiological changes such as sugar and organic compound accumulation in the leaves and

ion accumulation in the roots, which quickens the growth processes of the emerged seedlings (Cayuela, Perez-Alfolcea, Caro and Bolarin, 1996). All these physiological processes imply improved and faster seedling emergence and better initial seedling establishment.

Hydro priming, matric priming and osmotic priming techniques are used to increase the germination rate, total germination percentage and seedling uniformity (Bradford, 1984; Alvarado and Bradford, 1988; Basra, 2002; Cheng and Bradford, 2001; Geisenberg and Stewart, 1986; Mauromicale and Cavallaro, 1995). The most commonly used methods are hydro and osmotic priming, as they have shown to perform more favourably in comparison to matric priming.

2.10.1 Hydro Priming as a Seed Pre-treatment Method

Hydro priming involves soaking seeds in plain water before drying for storage or before sowing. The seed needs to be wet to soften the seed coat and a presoak provides this necessary moisture. A seed in the soil may not be 100 percent in contact with the soil or water and those areas not in contact will pull moisture from the areas of the seed in contact with soil or moisture, and this interferes with the imbibition process of the seed. When the seed coat is softened, the seedling has less energy to use on emerging out of the seed coat and can thus concentrate on growing (Nelson, 2003).

2.10.2 Osmotic Priming as a Seed Pre-treatment Method

Osmotic priming (osmoconditioning) exposes seeds to low water potentials and involves the hydration of seeds in solutions containing organic or inorganic solutes, which might include polyethylene glycol (PEG), mannitol, and sodium chloride, potassium nitrate (KNO_3) and potassium phosphate (Bradford, 1984; Bradford, 1986; Alvarado and Bradford, 1988; Basra, 2002; Cheng and Bradford, 2001; Geisenberg and Stewart, 1986; Mauromicale and Cavallaro, 1995). When dealing with seed pre-treatment, the duration of soaking and the concentration of the osmoticum used must be considered in order to determine optimum germinating conditions (Coolbear, Grierson and Heydecker, 1980). Comparisons of osmoticum indicate that KNO_3 performed better in terms of emergence time, dry weight and leaf area, followed by PEG, when compared to non treated seeds (Green, 1980, Bradford, 1984).

2.10.2.1 Potassium Nitrate (KNO_3) Used As Osmotic Primer

Seeds subjected to potassium nitrate soaking have shown to germinate faster, better and to

have an improved rate of initial establishment as seedlings thus improving the overall crop stand, and consequently the yield. The nitrate ion has been found to be very beneficial in helping revive old seed (Basra, 2002) because presence of nitrate ions during imbibition may provide additional substrate for amino acid and protein synthesis which enhances germination during priming (Khan, Tao, Knypl, Borkowsk and Powell, 1978).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experiment 1: A Study of the Initial Establishment of Multi - Purpose Moringa (Moringa oleifera. Lam) at Various Plant Densities, their effect on Biomass Accumulation and Leaf Yield.

3.1.1 Study Site

The experiment was carried out at the University of Zimbabwe Crop Science Department fields, in Plot 10 located in Mount Pleasant area, which is found in Natural Region II with an annual rainfall of 600 – 1000mm, an altitude of 1500mm above sea level and average temperatures of 20 - 30°C. The soils comprise mainly of red clayey soils.

3.1.2 Experimental Design

A Randomised Complete Block Design (RCBD) in three blocks was used. The experiment was a one factor experiment with five plant population densities: which were:

1. 12 346 plants/ha spaced at 0.9 x 0.9m
2. 24 692 plants/ha spaced at 0.65 x 0.65m
3. 49 384 plants/ha spaced at 0.45 x 0.45m
4. 98 764 plants/ha spaced at 0.35 x 0.35m
5. 197 528 plants/ha spaced at 0.25 x 0.25m

The gross plot sizes were 4m x 4m and the net plot size was 3m x 3m.

3.1.3 Experiment Management

Initially, the seed was de-hulled, soaked overnight and planted out in seedling trays in the greenhouse and nursed till they were 50-150mm tall, then planted out into the field.

Planting holes were prepared and watered a night before planting out of seedlings. The planting out process was carried out during the late afternoon to reduce the damaging effect of direct sunlight on the seedlings. However, transplanting of seedlings into the field proved challenging as most of the seedlings failed to establish as well as expected, due to their weak rooting system which easily got damaged, injured or broken during the transplanting process. As a result, more seedlings were raised in the nursery to cater for the gap filling needs and to enable planting out of treatments block by block as a way to manage the seedling shortages caused by the uneven Moringa seed germination and the establishment problems encountered.

At planting out, 10 grams of Single Super Phosphate (20% P₂O₅, min 12 %S) (SSP) was applied at each planting hole and ammonium nitrate (34, 5% nitrogen) (AN) was applied 3 weeks after planting out, at 5 grams per plant. Two other applications were carried at monthly intervals, 10 grams of AN being applied at each plant station.

Hardening of the seedlings was carried out 2 weeks before planting out, by moving the seedlings out of the greenhouse and into the shed house where they slowly acclimatized to the outside environment

Pests of major concern were the cutworms (*Agrotis segetum*), which were very destructive of the planted out seedlings; gap filling was done as and when needed. The *Agrotis segetum* infestation was controlled by two initial applications of Karate (Fenvalerate 20%, inert

ingredients 80%) at a rate of 15mls/15litres of water, using a knapsack sprayer, an even and full cover spray was achieved. The persistence of the cutworm problem was further contained by application of Carbaryl 85%WP (Carbaryl 85%, inert ingredients 15%) as bait. The prevalent disease encountered in the field was damping off (*Rhizoctonia species*) during the first three weeks of establishment after planting out. This problem was controlled in two ways; firstly, the seedlings were drenched in Dithane M45 (Mancozeb 80%, inert ingredients 20%) before being planted out, and secondly, based on the scouting and observations, the plants were sprayed with the same fungicide.

There was need for two initial weeding in the first month due to the rainfall which provided ideal conditions for rapid weed emergence. The major weeds were *Bidens pilosa*, *Commelina bengalensis* and *Amaranthus hybridus*. Hoe weeding was the method employed and the plots were weeded once every month to reduce competition as the Moringa plants were growing very slowly due to the prevailing low temperatures of the May to early August months. It was also very critical to have close control of cutworm, in order to sustain a good stand. The plants were established in February – March, and finally harvested at the end of July. Towards the end of the trial, there was need for water supplementation.

3.2 Experiment 2: A Study of Moringa Seedling Establishment, Initial Plant Growth and

Nutrient Uptake in Potted Media as Influenced by Interactions of pH, P and N.

3.2.1 Study Site

This experiment was conducted at the University of Zimbabwe green house at the Crop Science Department, in Mount Pleasant area, which is found in Natural Region II. The site experiences an annual rainfall of 600 – 1000mm and is at an altitude of 1500mm above sea level. Average temperatures range between 20 - 30°C.

3.2.2 Experimental Design

A 5 x 3 x 3 factorial experiment was laid out in a Split plot Design, with nitrogen being the main-plot factor, whilst the sub-plot factors were pH and phosphorus. The experiment was replicated three times with five pH levels (CaCl₂ scale): 3.2, 6.2 (initial pH), 7.6, 8.2 and 8.7; three levels of P: 0 g/pot, 8 g/pot and 16 g/pot and three levels of N: 0 g/pot, 4 g/pot and 8 g/pot.

3.2.3 Experiment Management

The potting media was made of 2 parts sand: 1 part pine bark: 1 part loamy clay soil, with

each pot being of 20cm diameter and carrying 4 kg of media. The media was sent for analyses before establishment of the trial to determine the initial pH and nutrient status of the media before amendments were done. The initial P application was done based on the treatment needs, once only, just before planting out.

Initially the intention was to work with pH levels 3.5, 5.0, 6.5, 8.0 and 9.0. However, after the inoculation and settling down process of the media, the pH levels attained had to be used for the experiment, as inoculating and settling of the media took a long time making it impossible to repeat the process, given the time frame for completion of the experimentation. The 3.2 pH level was attained by inoculating the media with 70g Iron (Ferrous) Sulphate (FeSO_4)/pot, whilst pH 6.2 was the initial media pH and it required no amends. To attain pH 7.6, the pot media was mixed with 10g lime (Calcium carbonate or calcitic lime)/pot and for pH 8.2; it was mixed with 20g lime/pot. pH 8.7 was achieved by mixing with 180g lime/pot. These mixes were then left to incubate for 3 weeks, with weekly pH measures being taken and light watering done twice weekly. At the end of the fourth week, the pH levels had stabilized adequately to enable establishment of the experiment.

Initially, the seedlings were transplanted into the various treatments but most of the seedlings failed to take, and as a result seven seeds were sown out per pot to improve the chances of emergence. Three weeks after emergence and seedling establishment, the seedlings were thinned down to two plants per pot and a week later, the first application of N was done as per treatment allocation.

The seedlings were drenched with Dithane M45 (Mancozeb 80%, inert ingredients 20%) to control damping off (*Rhizoctonia species*) and seed and root rots of the *Aspergillus species* which were very prevalent. Seeds were dressed with Thiram 80%WP (Thiram 80%, inert ingredients 20%) as a precautionary measure against fungal infections, prior to sowing them out.

The pests which were of prevalence in the greenhouse during seedling emergence and establishment were the ants, which were burrowing into the pots and feeding on the ungerminated seed and red spider mites (*Tetranychus species*) which attacked the seedlings some four weeks after emergence, causing a lot of mottling and leaf curling and substantial leaf drops. The diamond back moth (*Plutella xylostela*) was also troublesome during the two to three week period after crop emergence, causing a lot of defoliation of the young plants. Minute concentrations of Malathion (Malathion 50%, solvents and emulsifiers 50%) were

applied to control the pests as the young moringa seedlings were very susceptible to phytotoxicity. Antkill (Chlordane 30%, inert ingredients 70%) was sprayed onto the greenhouse floors to keep out the ants.

Watering was kept at light applications twice daily during the first two weeks, and then gradual reductions were implemented after that stage. Afterwards, water was applied as required and draining water was avoided to prevent leaching out nutrients applied as treatment factors.

3.3 *Experiment 3: A Study of the Effect of Pre-Treatment Method and Media Type on Germination, Emergence, Initial Establishment and Growth of Moringa oleifera.*

3.3.1 Study Site

This experiment was conducted at the University of Zimbabwe green house at the Crop Science Department, Mount Pleasant.

Site characteristics are as given under section 3.2.1

3.3.2 Experimental Design

A 4 x 3 x 2 factorial experiment in a RCBD was carried out, with three replications. The first factor was four seed priming methods (water soaked for 24 hours, 1% potassium nitrate soaked for 24 hours, oven-dried at 50°C for 24 hours and no priming). The second factor was organic source with three levels (pine bark, well decomposed organic manure and no organic matter). The third factor was soil texture at two levels (clay and sand). Both texture sources, sand and clay, were each mixed separately, with each organic source at 3 parts texture source (either clay or sand, separately) to 1 part organic source using either well decomposed organic matter or pine bark. That is, for instance, 3 parts clay was mixed with 1 part pine bark, then 3 parts clay was again mixed with 1 part of the well decomposed organic manure. The same

was done for the sand texture at 3 parts sand: 1 part of each organic source. The pots used were 12.5cm in diameter.

3.3.3 Experiment Management

Single Super Phosphate (20% P₂O₅, min 12.0% S) was applied at 5 grams per pot uniformly when sowing the seed and a light irrigation (river, dam or municipal water can be used) was applied avoiding draining water as this would have leached out the phosphate. The media was kept moist at all times but not drenched so as to enable proper aeration and aid in emergence of the germinating seedling. The seedlings were drenched with Dithane M45 (Mancozeb 80%, inert ingredients 20%) two weeks after emergence to reduce fungal infections, whilst the greenhouse floors were also sprayed with Antkill (Chlordane 30%, inert ingredients 20%) to discourage ants getting onto the experimental area, as they fed on the ungerminated seeds.

3.4 Experiment 4: A Study into the Nitrogen Uptake and Use of *Moringa oleifera* (Lam)

Grown in Different Soil Textures (clay and sandy soils) and Organic Sources.

3.4.1 Study Site

This experiment was conducted at the University of Zimbabwe green house at the Crop Science Department, Mount Pleasant.

Site characteristics are as given under section 3.2.1.

3.4.2 Experimental Design

The experiment was a 4 x 3 x 2 factorial experiment which was laid out in a RCBD and replicated three times, with the first factor being organic source which had three levels (pine bark, saw dust and none - no organic source). The second factor was nitrogen with 4 levels (0 g/plant, 2 g/pot, 4 g/plant and 8 g/plant) and lastly the third factor was texture, at two levels, being clay and sand.

3.4.3 Experiment Management

The media was made up of each texture type, for instance clay, being mixed with each organic source at 2 parts texture source: 1 part organic source, with the other media types being comprised of either clay or sand only, with no organic matter additions. At establishment, seven *Moringa* seeds were planted out in each pot (20cm diameter) and 5g SSP

was applied in each pot at sowing. Three weeks after crop emergence and seedling establishment, the seedlings were thinned down to two seedlings per pot, and five weeks after crop emergence, the different N levels were applied according to each treatment factor. There was need to repeat the applications twice more at nine weeks after crop emergence, then lastly at thirteen weeks after crop emergence, as indicated by the deficiency exhibited by the plants during the trial duration.

The pests and disease problems were similar to those encountered in experiment 2 since the trials were being carried out in the same greenhouse for ease of management and monitoring.

3.5 Measurements

Moringa growth and biomass accumulation, soil nutrient levels and plant nutrient uptake levels (nitrogen, phosphorus, potassium, calcium, magnesium, sulphur), and the following parameters were measured fortnightly (potted experiments) and at three weekly intervals:

- Plant height
- Leaf harvesting
- Stem diameter at 15cm DBH at base height
- Destructive sampling at the end of the trial (root biomass, whole plant biomass)

The Soil Science Department at the University of Zimbabwe provided services for analysis of plant nutrients assimilated, initial media nutrient and pH analysis and media pH inoculation.

3.6 Data Collection

Two week after seeds were sown, seedling emergence counts were conducted on a daily basis for the first week, and thereafter, counts were conducted at five day intervals for the pH interaction and nitrogen use experiments. Five weeks after emergence, the seedling height measurements for the above mentioned experiments commenced and were taken at weekly intervals. At the end of these experiments, the whole plant biomass was taken separately for the roots and the above ground parts. Plant nutrient analysis was also done for the potted experiments at the Soil Science Laboratories. Plant height measurements were also taken for the germination experiments at weekly intervals and after five weeks, the above ground and below ground biomass were taken for the whole plant, at the end of the trial.

For the density experiment in the field, plant height measurements and stem diameters at 15cm DBH at base height were taken at three weekly intervals and leaf harvests (three leaves were harvested per plant) at three weekly intervals. At the end of the trial, the whole plant biomass was taken separately as above and below ground parts.

3.7 Data Analysis

The data was subjected to analysis of variance (ANOVA) and mean separation was done using Fisher's Protected Least Significant Difference method (LSD). The analyses were done using Genstat 6 Release 3.22 for Windows Statistical Computer Package.

CHAPTER FOUR: RESULTS

4.1 Experiment 1: A Study of the Initial Establishment of Multi – Purpose Moringa (Moringa oleifera. Lam) at Various Plant Densities, their Effect on Biomass Accumulation and Leaf Yield.

4.1.1 Effect of Population Density on Above Ground Whole Plant Biomass

Increasing population density had the effect of increasing the plant dry matter weight of Moringa ($p < 0.05$) (Table 4.1). The highest treatment mean was for the largest plant density per given area (197 528 plants/hectare) which produced a plant dry matter of 174.42grams. This was however, not significantly different ($p < 0.05$) from the mean achieved for the second highest population density (98 764 plants/hectare) which yielded 100.98grams.

Table 4.1 Effect of Population Density on above - ground plant dry - matter (g/pot) Obtained at Crop Science Department, July - August 2007

Population (plants/ha)	Mean (DM g/plot)
197 528	174.42 ^{a#}
98 764	100.98 ^{ab}
49 384	68.07 ^b
24 692	41.33 ^b
12 346	31.82 ^b
P value	0.037
SED	46.5
LSD_{0.05}	96.7

Significance

*

Means followed by the same letter are not significantly different at $P \leq 0.05$
 * Significant at $p < 0.05$
 SED Standard errors of differences between means
 LSD_{0.05} Least significant differences of means (5% level)

4.1.2 Effect of Population Density on Below Ground Plant Biomass

The results showed significant differences ($p < 0.05$) for both plant fresh-weight and dry matter, with the highest mean for both parameters being for the highest population density (197 528 plants/hectare) (Table 4.2). The second highest population density mean was however, not significantly different from the other population densities at $p < 0.05$. The highest mean (793.1 grams) plant dry matter, for the highest plant density (197 528 plants/hectare), was significantly different ($p < 0.05$) from all the other treatment means.

Table 4.2 Effect of Population Density on Root Fresh Weight (g/plot) and Root Dry matter (g/plot) Obtained from Crop Science Department , July - August 20007

Population (plants/ha)	Mean fresh weight (g/pot)	Mean dry weight (g/pot)
197 528	3300 ^{a#}	793.1 ^a
98 764	2012 ^{ab}	415.5 ^b
49 384	1167 ^b	255.9 ^b
24 692	696 ^b	160.5 ^b
12 346	579 ^b	133.3 ^b
P value	0.019	0.002
SED	832.9	157.2
LSD_{0.05}	1722.9	325.1
Significance	*	*

Means followed by the same letter are not significantly different at $P \leq 0.05$
 * Significant at $p < 0.05$
 SED Standard errors of differences between means
 LSD_{0.05} Least significant differences of means (5% level)

4.1.3 Effect of Population Density on Leaf Biomass Yields

The highest population density (197 528 plants/hectare) and the second highest population density (98 764 plants/hectare) produced the highest leaf dry matter and leaf fresh-weight figures (Table 4.3). The lowest plant density (12 346 plants/hectare) produced the lowest leaf fresh-weight and leaf dry matter (Table 4.3). The highest means were obtained at the highest plant density, 82.82 grams for dry-matter and 404 grams for the fresh-weight. In both cases, these means were significantly different ($p < 0.05$) from the other treatment means.

Table 4.3 Effect of Population Density on Leafy Dry Matter (g/plot) and Leaf Fresh Weight (g/plot) Obtained from the Crop Science Department, July - August 2007

Population (plants/ha)	Leaf dry matter (g/pot)	Leaf fresh weight (g/plot)
197 528	82.82 ^a	404.0 ^{a#}
98 764	42.72 ^b	205.5 ^b
49 384	22.49 ^{bc}	106.8 ^{bc}
24 692	11.22 ^c	52.9 ^c
12 346	6.37 ^c	28.7 ^c
P value	<.001	<.001
SED	11.59	59.3
LSD_{0.05}	23.10	118.2
Significance	***	***

Means followed by the same letter are not significant at $P \leq 0.05$

*** Significant at $p < 0.001$

SED Significant errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

4.1.4 Effect of Population Density on Stem-Length and Stem Diameter

Population density reduced the stem diameters at high populations while increasing them at low densities (Tables 4.4 and 4.5). Conversely the stem-length increased at high density treatments. The results obtained indicated significant differences ($p < 0.05$) among the plant population densities for both stem-length and stem diameter. The treatment with the highest mean for stem diameter (5.234 mm/plant) was the lowest plant population density (12 346 plants/hectare), and this treatment mean was significantly different ($p < 0.05$) from the rest of the other treatment means. The third largest population (49 384 plants/hectare) produced the highest ($p < 0.05$) stem length of 118mm.

Table 4.4 Effect of Population Density on stem diameter (mm) obtained from the Crop Science Department site, July - August 2007

Population (plants/ha)	Stem diameters (mm/plant)
197 528	4.947 ^{b#}
98 764	5.020 ^b
49 384	5.053 ^b
24 692	4.502 ^c
12 346	5.234 ^a
P value	<.001
SED	0.1138
LSD_{0.05}	0.2230
Significance	***
# Means followed by the same letter are not significant at $P \leq 0.05$	
*** Significant at $p < 0.001$	
SED Standard errors of differences between means	
LSD _{0.05} Least significant differences of means (5% level)	

Table 4.5 Effect of Population Density on Stem-Length (mm) Obtained from Crop Science Department, July - August 2007

Population (plants/ha)	Stem length (mm/plant)
197 528	122.36 ^{a#}
98 764	121.32 ^a
49 384	118.28 ^b
24 692	102.06 ^c
12 346	122.44 ^a
P value	<.001
SED	2.828
LSD_{0.05}	5.544
Significance	***
# Means followed by the same letter are not significant at $P \leq 0.05$	
*** Significant at $p < 0.001$	
SED Standard errors of differences between means	
LSD _{0.05} Least significant differences of means (5% level)	

4.2 Experiment 2: A Study of Moringa Seedling Establishment, Initial Plant Growth and Nutrient Uptake in Potted Media as Influenced by Interactions of pH, P and N.

4.2.1 Effects of pH, Nitrogen and Phosphorus on Seedling Emergence Counts

The pH, N and P all had both negative and positive effects on Moringa growth parameters as indicated in the results which follow. There was significance at $p < 0.05$ for pH the pH*phosphorus interactions (Table 4.6, Figure 4.1). The pH with highest treatment means were the pH 7.6, 8.2 and 8.7 treatments and they were not significantly ($p < 0.05$) different from each other (Table 4.6). pH 3.2 and 6.2 had the lowest seedling counts.

Table 4.6 Effect of pH (CaCl₂) on seedling emergence counts obtained from Crop Science Department, July - August 2007

pH (CaCl ₂)	Emergence counts
3.2	1.407 ^{c#}
6.2	3.269 ^b
7.6	3.444 ^a
8.2	3.426 ^{ab}
8.7	4.083 ^a
P value	<.001
SED	0.3444
LSD_{0.05}	0.7055
Significance	***

Means followed by the same letter are not significantly different at $P \leq 0.05$

*** Significant at $p < 0.001$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

Highest seedling emergence counts were obtained at combinations of pH 7.6 and 0 g/pot phosphorus (Figure 4.1). Increasing phosphorus levels to 4 g/pot or 16 g/pot reduced emergence counts at this (7.6) pH level. A similar trend of reduced emergence with increasing phosphorus levels was observed at pH 6.2. Responses to phosphorus did not exist at pH 8.2 and 8.7.

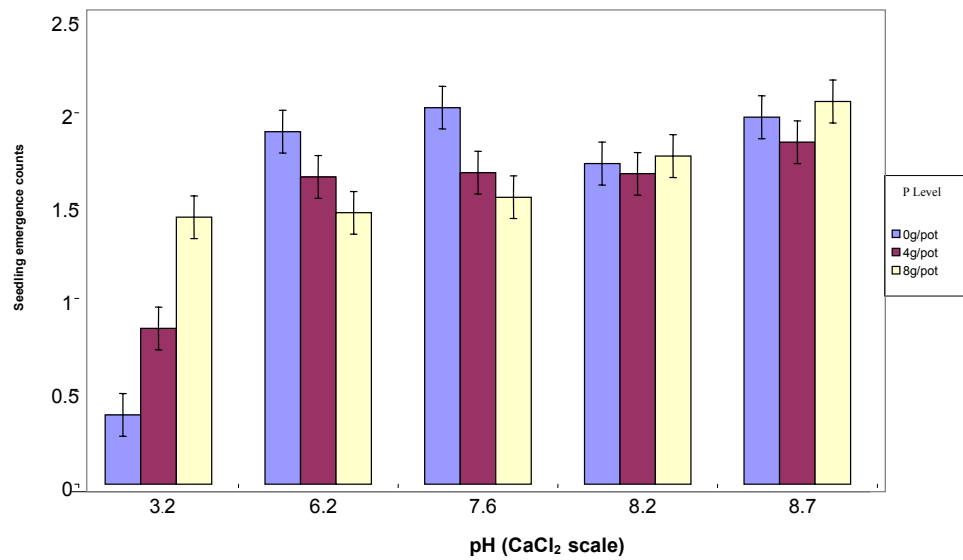


Figure 4.1 pH (CaCl₂) and phosphorus (g/pot) effect on seedling emergence counts from Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

4.2.2 Effects of pH, Nitrogen and Phosphorus on Stem-length

Stem-length was highest with the 4 grams N/pot treatment, which was significantly different from the other treatment means. The results were highly significant ($p < 0.05$) for nitrogen, pH, Time, nitrogen*pH interaction and nitrogen*time interaction (Figure 4.2, Figure 4.3).

For the pH*nitrogen interaction the highest stem length was produced at pH level 7.6 and nitrogen level 4grams/pot (Figure 4.2). However, this height was not significantly different ($p > 0.05$) from that at pH 6.2 for the same (4 g/pot) nitrogen rate.

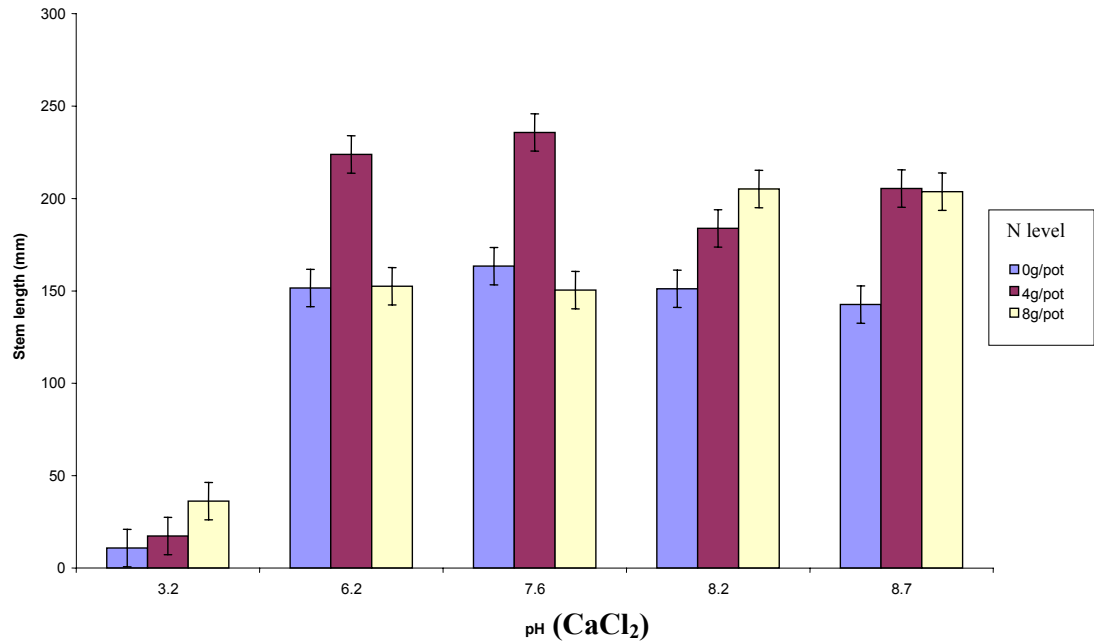


Figure 4.2 pH (CaCl₂) and nitrogen (g/pot) effect on Moringa stem-length (mm) from Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

There was also significance ($p < 0.05$) for nitrogen*time interaction (Figure 4.3) for stem length of Moringa. Whilst stem height gradually increased with time for 4 g/pot nitrogen, height remained generally constant at 0 g/pot nitrogen and height declined after 4 WACE for the 8 g/pot nitrogen.

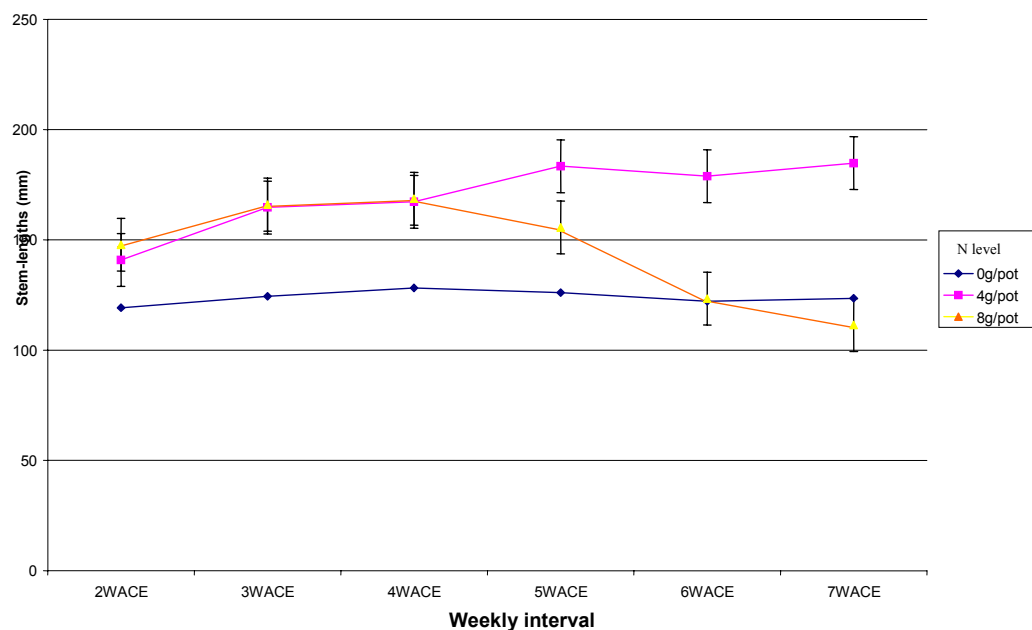


Figure 4.3 Nitrogen (g/pot) and time (weeks) effect on Moringa stem-length (mm) from Crop Science Department site, July – August 2007. Bars represent significant difference at $\alpha = 0.05$.

4.2.3 Responses of Root Biomass Yields to pH and Nitrogen

The results indicated effects of nitrogen, pH and the nitrogen*pH interaction on the root fresh biomass (Table 4.7). The nitrogen* pH interaction was significant only for the root dry weight (Figure 4.4).

The highest treatment mean was produced at pH 8.7 for both root fresh weight and dry matter, whilst pH 7.6 level had the second highest mean figure, which was however, not significantly different from that of pH 8.2 (Table 4.7)

Table 4.7 Effect of nitrogen (g/pot) and pH (CaCl₂) on root fresh - weight (g/pot) as single factors obtained from Crop Science Department site July - August 2007

Nitrogen (g/pot)	Mean fresh weight (g/pot)	pH	Mean fresh weight (g/pot)
		3.2	0.09 ^d
		6.2	7.07 ^c
0g	11.11 ^a	7.6	13.26 ^b
4g	12.30 ^a	8.2	12.63 ^b
8g	6.69 ^b	8.7	17.13 ^{a#}
P value	<.001		<.001
SED	1.440		1.860
LSD_{0.05}	2.853		3.683
Significance	***		***

Means followed with the same letter are not significantly different at $P \leq 0.05$

*** Significant at $p < 0.001$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

Root dry matter results obtained indicated that root dry matter was highest at pH 8.7 and 4 grams/pot nitrogen level (Figure 4.4). Applications of 4 g N/pot caused root biomass to increase with increasing pH up to pH 8.7. Increases in dry root biomass also occurred at 0 g N/pot, but increases were lower than those obtained at 4 g N/pot. Root dry matter was generally stagnant across all pH levels for the 0 g N/pot.

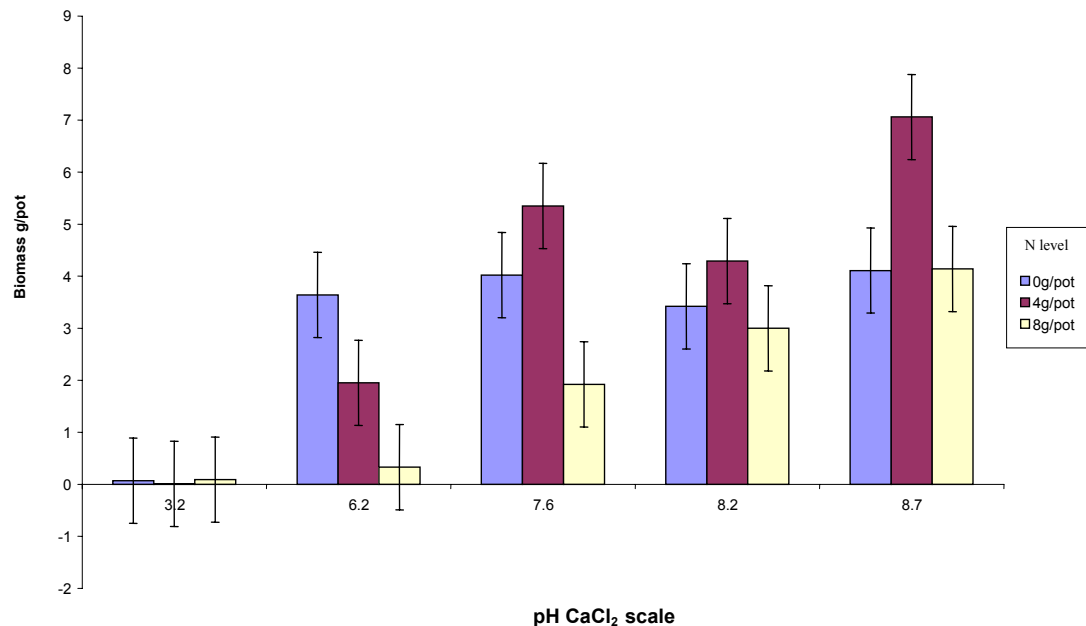


Figure 4.4 Nitrogen (g/pot) and pH (CaCl₂) effect on Moringa root DM (g/pot) at Crop Science

Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$

4.2.4 Total Above Ground Plant Biomass Yield Responses to N and pH

The levels of nitrogen and pH influenced plant biomass production of Moringa as indicated by the significance ($p < 0.05$) for nitrogen, pH and nitrogen*pH interaction for both Plant fresh-weight and Plant Dry-matter (Figure 4.5). The application of 4 g N/pot gave the highest yield responses with increasing pH levels to pH 7.6 (23 g/pot) and declined thereafter. Lowest biomasses were obtained at 0 g N/pot across all pH levels. Even though biomass was higher at 8 g N/pot compared to 0 g N/pot, the levels were also stagnant across all pH levels.

The results indicated highest plant dry matter at pH level 7.6 and nitrogen level of 4grams/pot. The application of 4 grams/pot nitrogen at all levels of pH (except at pH 3.2) resulted in increase in plant dry matter produced up to pH 7.6 after which it declined. With increasing pH at 8 g N/pot, an increase in dry matter occurred, but remained constant across all the pH levels. At 0 g N/pot increasing pH levels indicated increasing plant dry matter, with the highest being at pH 6.2, with further pH increases beyond 6.2 resulting in decreased plant dry matter.

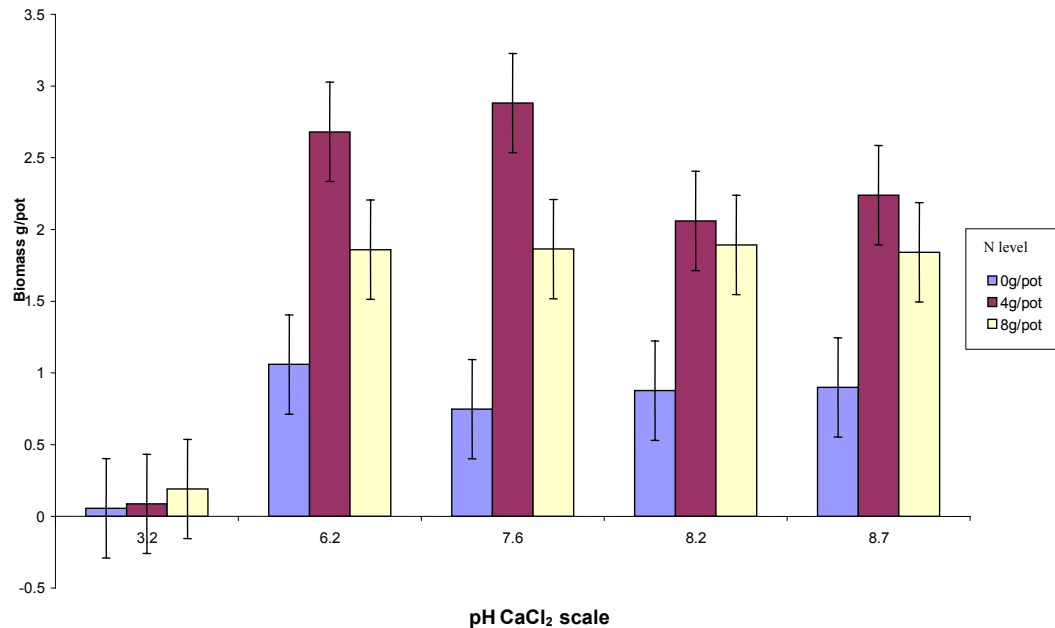


Figure 4.5 Nitrogen (g/pot) and pH (CaCl₂) effect on Moringa above ground plant dry-matter (g/pot) observed at Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

The amount of nitrogen available for plant use effected the plant fresh biomass produced as indicated by the significant differences ($p < 0.05$) in all the nitrogen treatment means (Figure 4.6), with the highest plant fresh-weight (6.772grams) being for 4 grams nitrogen/pot, followed by 8 grams nitrogen/pot (4.466grams) and the least was for 0 gram nitrogen/pot (2.350 grams) and these were all significantly different ($p < 0.05$) from each other.

The results indicated significant ($p < 0.05$) influence of the nitrogen*pH interaction (Figure 4.6), on plant fresh-weight production for Moringa during initial establishment. The highest plant fresh-weight mean was for pH 7.6 and nitrogen level 4 grams/pot (10.1grams), followed by nitrogen level 4 grams/pot at two pH levels, 6.2 and 8.2. Nitrogen applications at 4 g N/pot generally resulted in greatest increases in fresh weight with increasing pH, peaking at pH 7.6. Applications at 8 g N/pot also gave increased fresh weight with increasing pH, up to pH 8.7 but responses were lower than those for 4 g N/pot. Fresh weights gave a low and decreasing trend with increasing pH values for no nitrogen application.

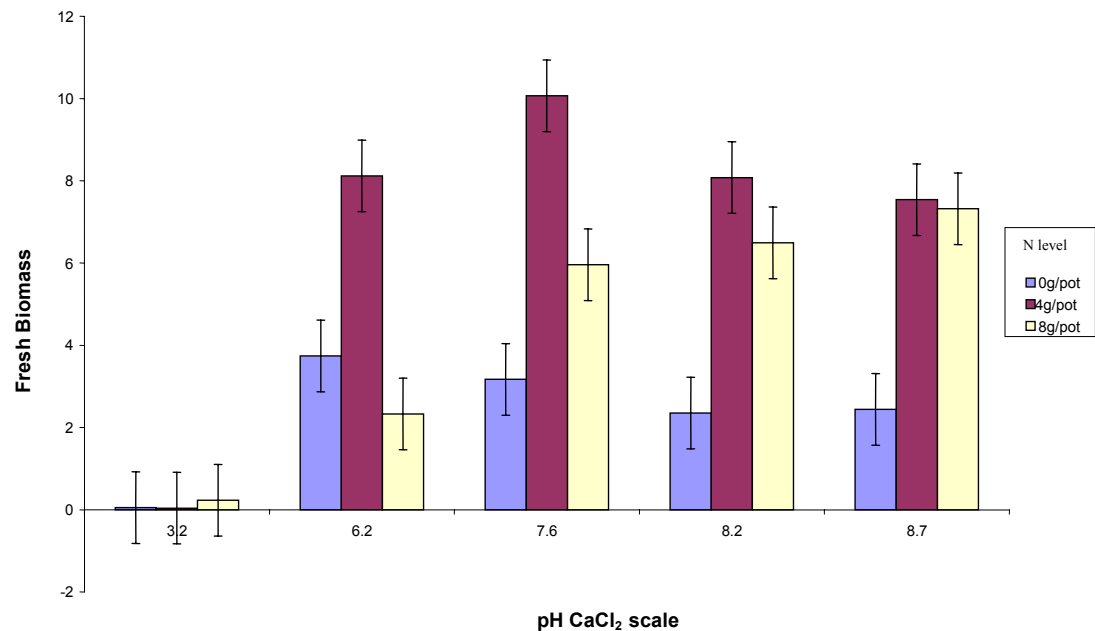


Figure 4.6 Nitrogen (g/pot) and pH (CaCl₂) effect on Plant FW (g/pot) obtained from Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

4.3 Experiment 3: A Study into the Effects of Pre-Treatment Method and Media

Type on Seed Germination, Emergence and Initial Seedling Establishment of Moringa oleifera.

The results indicated that clay soil produced the greatest root fresh weight in contrast to sandy soil and these treatment means were significantly different ($p < 0.05$) from each other. In addition, pretreatment improves the germination of Moringa as evidenced by the significance ($p < 0.05$) shown by potassium nitrate primed seed yielding the highest treatment mean on root fresh weight (Table 4.8). However, the 1% potassium nitrate treatment mean was not significantly different ($p < 0.05$) from that of water primed seed, whilst the water primed seed was not significantly different from oven dried and dehulled seed. Texture was highly significant at $p < 0.05$ for the plant fresh weight. There was also significance ($p < 0.05$) for organic source, texture, pretreatment and on organic source*texture interaction in the root dry matter weight (Figure 4.7).

Table 4.8 Effect of pre-treatment on root FW (g/pot) obtained from Crop Science Department site, July - August 2007

Pretreatment	
KNO ₃ soaking	2.267 ^{a#}
Water soaking	1.804 ^{ab}
Oven drying	1.319 ^b
Dehulling only	1.025 ^b
P value	0.048
SED	0.458
LSD_{0.05}	0.922
Significance	*

Means followed by the same letter are not significantly different at $P \leq 0.05$

* Significant at $p < 0.05$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

The observations from these studies (Figure 4.7) indicated that organic source and texture improve seedling emergence and initial establishment of Moringa as evidenced by the high emergence means produced in the clay soil for root dry matter. These means were significantly different from those obtained for sand at $p < 0.05$.

In the interaction observations (Figure 4.7), clay in combination with well decomposed organic manure, produced the highest biomass treatment means, which were significantly different ($p < 0.05$) from all the other treatment means, across all treatment factors. Furthermore, clay on its own with no organic source addition produced more biomass in comparison to the sand treatments or the pine bark treatments.

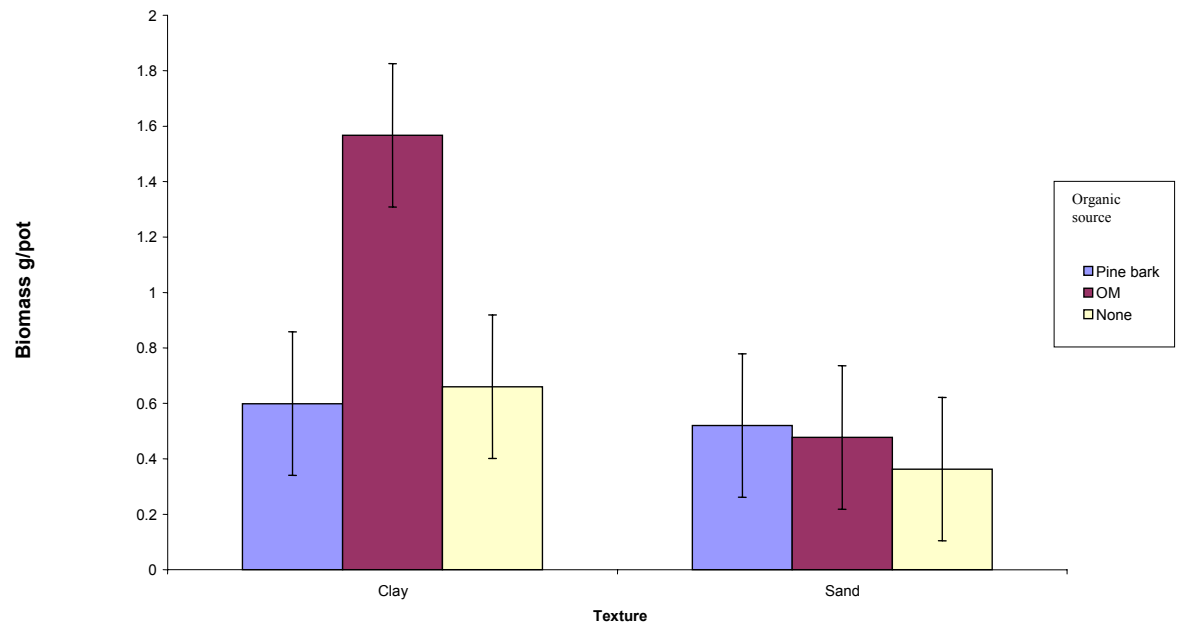


Figure 4.7 Effect of organic source and media texture on root DM (g/pot) obtained from Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

The results in Figure 4.7 indicated that well decomposed organic matter increases the root DM significantly ($p < 0.05$), whilst clay also produced significantly higher root DM (g/pot) in comparison to sand as a texture for seedling production at $p < 0.05$. However, there was no interaction between the two sources of organic matter and texture of soil media.

4.4 Experiment 4: A Study into the Nitrogen Uptake and Use of Moringa oleifera

(Lam) Grown in Different Soil Textures (clay and sandy soils) and Organic

Sources (Pine bark and sawdust).

4.4.1 Seedling Emergence Responses to Nitrogen, Soil Texture and Organic Source

The results indicated that organic source increased the seedling emergence rate significantly ($p < 0.05$) with Pine bark producing the highest seedling emergence counts (Figure 4.8) in comparison to sawdust. Time factor also increased the seedling emergence counts significantly ($p < 0.05$) with the highest emergence counts being at 5 WACE and 4 WACE as these were not significantly different (Table 4.9). However, 3 WACE seedling emergence counts were not significantly different from 4 WACE counts, but were significantly different from the 2 WACE and 5 WACE counts.

Table 4.9 Effect of Time (weeks) on seedling emergence counts from Crop Science Department, July - August 2007

Time (weeks)	Mean (weekly counts)
2WACE	1.158 ^a
3WACE	1.985 ^b
4WACE	2.146 ^{bc}
5WACE	2.192 ^c
P value	<.001
SED	0.0933
LSD_{0.05}	0.1839
Significance	***

Means followed by the same letter are not significantly different at $P \leq 0.05$

*** Significant at $p < 0.001$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

The results further indicated that nitrogen*organic source interaction also significantly influenced the seedling emergence rate, with the highest seedling emergence counts being produced with Pine bark at nitrogen levels 2 g/pot, 4 g/pot and 8 g/pot, which were significantly different from the sawdust and no amendment organic source treatments at $p < 0.05$ across the nitrogen treatments (Figure 4.8). Conversely, emergence counts in no-amended media and media amended with sawdust declined with increasing nitrogen

application rates.

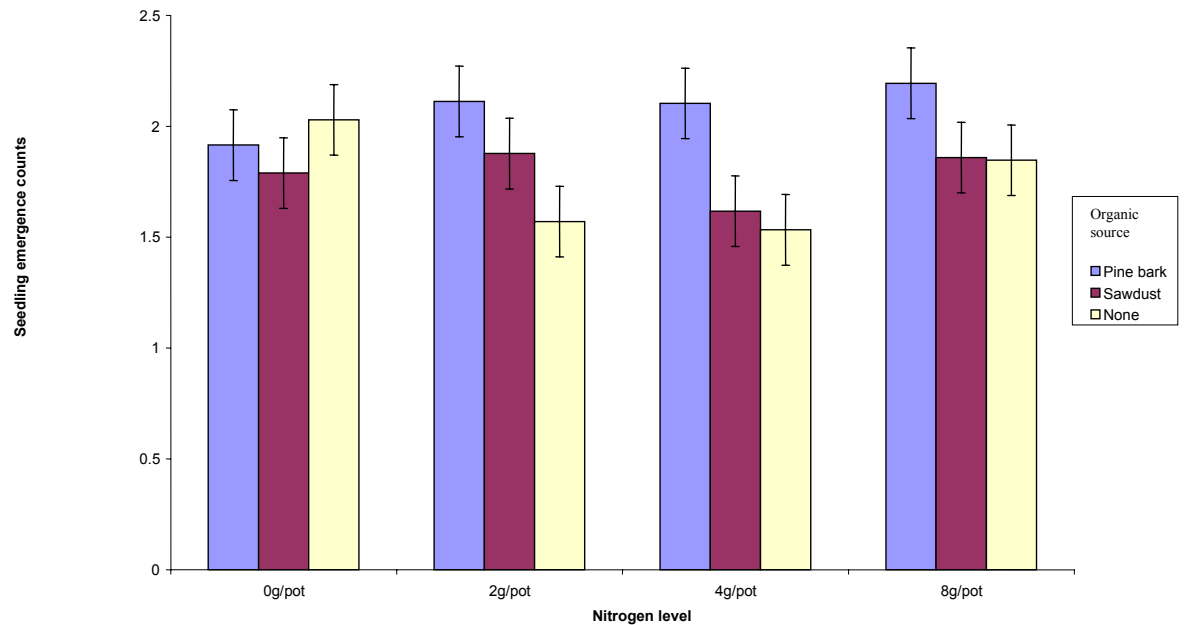


Figure 4.8 Nitrogen (g/pot) and Organic source effect on seedling emergence counts from Crop Science Department site, July - August 2007. Bars represent least significant difference at $\alpha = 0.05$.

4.4.2 Response of Stem-length to Nitrogen, Organic Matter and Texture

The results indicated that nitrogen and organic source increased the plant stem-length significantly ($p < 0.05$) (Figure 4.9a). Further observations indicated that Texture also influenced stem-length at $p < 0.05$. Similarly, Time factor increased the stem-length significantly at $p < 0.05$ (Table 4.10).

Table 4.10 Effect of Time (weeks) on stem length (mm) from Crop Science Department site, July - August 2007

Time (weeks)	Mean (mm)
2 WACE	159.3 ^{a#}
3 WACE	173.2 ^b
4 WACE	175.7 ^{bc}
5 WACE	181.0 ^{bc}
6 WACE	183.4 ^{bc}
7 WACE	181.1 ^{bc}
8 WACE	187.3 ^c
9 WACE	184.9 ^{bc}
P value	<.001
SED	6.52
LSD_{0.05}	12.81
Significance	***

Means followed by the same letter are not significantly different at $P \leq 0.05$

*** Significant at $p < 0.001$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

The results indicated the existence of interactions between nitrogen*organic source which increased the stem-length significantly ($p < 0.05$), and similarly the existence of a three-way interaction among nitrogen*organic source*texture which influenced stem-length significantly at $p < 0.05$ (Fig 4.9).

Sawdust as a source of organic matter significantly depressed stem length across both media textures and all nitrogen levels (Figure 4.9). Pine bark increased responses to nitrogen in sandy textures. In the clay texture, additions of pine bark significantly increased height when nitrogen levels were increased to between 4 and 8 grams/pot, otherwise not adding any organic amendment gave the highest stem length in clay as long as nitrogen was kept between 0 and 2 grams. Higher applications of nitrogen in clay reduced stem length.

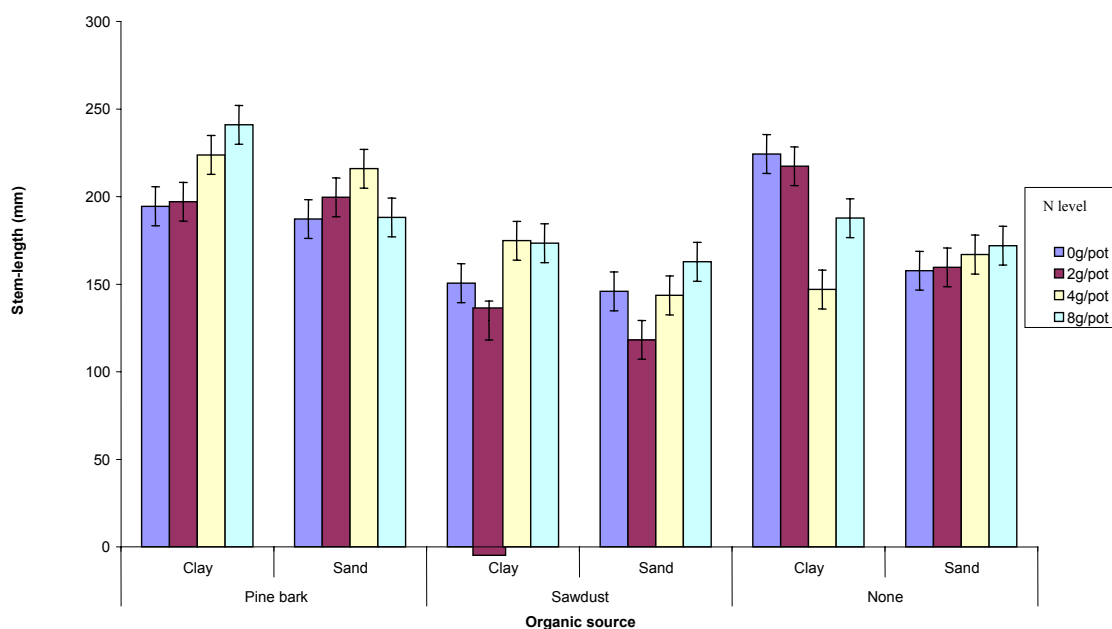


Figure 4.9 Effect of nitrogen (g/pot) organic source texture on stem-length (mm) from Crop Science Department site, July - August 2007. Bars represent significant difference at $\alpha = 0.05$.

4.4.3 Effect of Nitrogen, Texture and Organic Source on Plant Biomass Yield

Nitrogen and organic source increased both fresh and dry plant biomass as indicated by the results ($p < 0.05$) (Table 4.11). The highest plant biomasses were observed at the 4 g/pot and 8 g/pot nitrogen rates, which were not significantly different from each other at $p < 0.05$. The lowest biomasses were observed at the 0 g/pot nitrogen and the 2 g/pot nitrogen levels. The

organic source which produced the highest plant dry matter was pine bark.

Table 4.11 Effect of nitrogen (g/pot) and organic source as single factors on plant Biomass (g/pot) from Crop Science Department, July - August 2007

Plant Fresh weight (g/pot)				Plant Dry matter (g/pot)			
Nitrogen	Mean (g/pot)	Organic source	Mean (g/pot)	Nitrogen	Mean (g/pot)	Organic source	Mean (g/pot)
0g/pot	2.768 ^{a#}	Pine bark	4.950 ^a	0g/pot	0.6106 ^a	Pine bark	0.9968 ^a
2g/pot	3.034 ^{ab}	Sawdust	3.694 ^{ab}	2g/pot	0.6331 ^a	Sawdust	0.6976 ^b
4g/pot	4.477 ^{bc}	None	2.830 ^b	4g/pot	0.9144 ^{ab}	None	0.6559 ^b
8g/pot	5.020 ^c			8g/pot	0.9757 ^b		
P value	0.007		0.006		0.040		0.027
SED	0.722		0.625		0.1538		0.1332
LSD_{0.05}	1.454		1.259		0.3095		0.2681
Significance	***		***		*		*

Means followed by the same letter are not significantly different at $P \leq 0.05$

*** Significant at $p < 0.001$

* Significant at $p < 0.05$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

4.4.4 Effect of Nitrogen, Texture and Organic Source on Root Biomass Yield

The results for root biomass indicated that media texture only had the effect of increasing both fresh and dry root biomass at $p < 0.05$ (Table 4.12), with the largest biomass production being realized in the sandy soil whose treatment means were significantly different from those of clay soil ($p < 0.05$).

Table 4.12 Effect of media texture on root biomass (g/pot) from Crop Science Department site, July - August 2007

Root FW (g/pot)		Root DM (g/pot)	
Texture	Mean (g/pot)	Texture	Mean (g/pot)
Clay	10.69 ^{a#}	Clay	2.348 ^a
Sand	15.19 ^b	Sand	3.487 ^b
P value	0.011		0.017
SED	1.696		0.461
LSD_{0.05}	3.414		0.928
Significance	*		*

Means followed by the same letter are not significantly different at $P \leq 0.05$

* Significant at $p < 0.05$

SED Standard errors of differences between means

LSD_{0.05} Least significant differences of means (5% level)

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Effect of Population Density on Moringa Growth

Since an increase in the plant population density gave an increase in the biomass produced for both below ground (root) and above ground plant organs and low plant densities (12 346 plants/hectare) produced low root biomass, this suggests that the plants in low densities had adequate growth resources (moisture and nutrients), and roots did not have to expand in search of these resources. Studies done on other multi-purpose trees indicate that increases in plant population density results in an increase in the growth of the plants with resources being utilized when roots and stems entangle and when each plant competes with its neighbour (Squire, 1990).

Furthermore, the high root biomass produced at high plant densities (98 764 - 197 528 plants/hectare) suggest the development of a deep, extensive rooting system, capable of penetrating deeper into the soil profile thus improving soil nutrient absorption and the possibility of reaching deep soil moisture. An extensively developed rooting system results in plants which are more capable of withstanding adverse environmental conditions (dry spells, fluctuating water availability) and better able to anchor firmly into the soil. High plant biomass production implies more foliage which in turn improves radiation capture hence increased plant yields.

Therefore the most ideal density for improved initial seedling establishment are the high plant population densities from 98 764 plants/hectare to 197 528 plants/hectare which encourage robust, deep and extensive root development.

Since the study showed that lowest plant populations (12 346 plants/hectare) produced the highest stem diameters, the implications are that, if the aim is specifically seed and firewood, then low populations are most ideal as they result in faster and greater individual plant growth with larger stem diameters, and consequently higher seed yields.

5.2 Effects of pH, N, P and Time Interactions on Moringa Growth

Seedling germination and emergence occurred best in pH ranges 7.6 to 8.7. It is imperative that pH be maintained at these ranges for improved seedling production. The study clearly shows that lower pH levels of between pH 6.2 – 7.6 give lower emergence counts, especially with additions of phosphorus but such phosphorus effects do not occur at pH ranges of 7.6 to 8.6. Increased phosphorus reduced emergence at pH 6.2 and 7.6, not at pH 8.2 and 8.7.

The high seedling emergence which occurred in pH 8.7 might have been due to other favourable secondary effects resulting from the pH increase, such as reduction of aluminium or manganese toxicity, or increases in molybdenum or phosphorus availability for plant uptake, with phosphate availability being increased by the raised pH.

At the other end, when soil acidity was high most of the seeds did not emerge, and on exposing seeds most of the seeds were shrivelled and dead. Hence, pH levels below 4 should be avoided. At very low pH (below 4.0) levels, cell membranes are impaired and become more permeable, resulting in leakage of plant nutrients and direct root injury, thus hindering any further root development and plant growth (Archer, 1993). The direct injury to the rooting structures may have easily occurred to the protruding radicals as some of the shrivelled seeds had protrusions of radicles, some even had few roots developed. These results concur with other investigations on cotton in acidic soils which had revealed considerable root damage as a result of the high levels of aluminum concentrations, which was made available by the low pH levels (Adams and Lund, 1966).

Tolerance of Moringa to pH ranges between 7.6 and 8.7 at emergence apply with additions of phosphorus. However, ideal pH for further growth seems to be lower since results of this study indicate that as the level of pH continued to increase plant growth in terms of stem length was compromised, so much that at all levels of nitrogen supplementation, plant growth was adversely affected, that is it declined drastically. Therefore, pH levels for Moringa production should be maintained within ranges of pH 7.0.

Mengel and Kirkby (1982) in their studies, observed that most plant nutrients such as nitrogen, potassium, sulphur, calcium, magnesium, iron, copper zinc and molybdenum become readily available for plant uptake in pH range 7.0 – 7.5. In view of this result, phosphorus additions into the media at seed sowing should be avoided, but should instead, be ideally applied into the rooting zone 2-3 weeks after crop emergence to aid in robust root development.

Interactions between nitrogen*pH which had an influence on root biomass production, with the highest treatment mean being obtained at pH 8.7 and 4grams/pot nitrogen level implies that excessive amounts of nitrogen result in retarded or impaired plant growth and also, a waste of resources as the applied N does not result in corresponding biomass output. It

appears as if Moringa responds very well in terms of growth, to minute N additions which in a way, make it a fairly cheap MPTS to produce as it requires very few inputs of fertilizer. Stem length was highest at 4 grams/pot nitrogen between pH 6.2 and 7.6, whilst emergence and stem length were very low at pH 3.2. The negative response to application of 8 grams/pot nitrogen is supported other studies which indicate that Moringa lateral rooting is weak and responds negatively to high concentrations of applied inorganic fertilizers (Fuglie, 1999). Applications of 4 grams/pot nitrogen gave consistently high stem length throughout 7 weeks. Moringa roots were stunted at pH 3.2, with the highest root biomass at pH 8.7 and 4 grams/pot nitrogen. The highest above ground dry mass was at pH 6.2 and 7.6 at 4 grams/pot nitrogen, with 8 grams/pot nitrogen giving reduced biomass.

5.3 Effect of Media Texture, Organic Source and Seed Priming on Moringa

Growth

There is evidence in this study that texture plays an important role in germination and early establishment of Moringa seedlings with the clay soil texture, in comparison to sand texture, giving the best performance in terms of growth rate and biomass accumulation, of plant weights and root dry matter. This might be due to the physical properties of the clay which is said to retain a lot of moisture, does not leach out nutrients easily and its temperature does not fluctuate so rapidly and easily in relation to the ambient temperatures surrounding it. Clay soil is also fine grained and as such, if kept adequately moist at all times, will allow root penetration and expansion to occur easier and without much hindrance (Nyamangara, Mugwira and Mpofu, 2000). Since all other factors (moisture, fertilizer, organic matter and watering pattern/method) were held constant for all treatments, the properties of clay might have led to the better performance. The type of organic source used has also affected seedling germination and early establishment of Moringa, with well decomposed Organic Matter (OM) yielding the best results in terms of root fresh and dry weights and plant biomass, compared to pine bark and sawdust. This might be due to the fact that well decomposed OM is known to release its nutrients slowly into the soil making them readily available for plant uptake, it also improves the soil's properties by enhancing their water holding capacity, improved aeration, gives a good crumb structure to the soils (Nyamangara *et al*, 2000) and all these factors contribute to enhanced seed emergence and seedling establishment.

Comparison between pine bark and sawdust indicate that sawdust depressed growth across both clay and sandy media texture, even with additions of nitrogen and so use of sawdust

should be discouraged. When using sandy media texture, additions of pine bark increases responses to nitrogen application if pine bark is used in clayey soils, higher levels (4 – 8 grams) of nitrogen are necessary. Pine bark gave consistently greater emergence counts than sawdust and none across all N levels. Sawdust depressed stem length across all media textures and nitrogen levels. Pine bark increased responses to nitrogen in sandy textures. In clay texture, additions of pine bark increased height at 4 – 8 grams/pot, otherwise no organic matter gave highest mean when N was 0 – 2 grams/pot. Pine bark gave highest plant dry matter and sandy media texture gave highest root dry matter.

The use of 1% potassium nitrate and water-soaking as seed priming methods in comparison to oven-drying and dehulling with no pretreatment resulted in improved seedling performance as shown by the high root fresh weight yields. This might have been as a result of the effect of the nitrate which is known to be very beneficial in reviving seed (Basra, 2002) due to the presence of nitrate ions during imbibition providing additional substrate for amino acid and protein synthesis which enhances germination during priming (Khan, Tao, Knypl, Borkowsk and Powell, 1978). The alternate priming method (water soaking) is an affordable method for the smallholder farmer.

5.4 Conclusions

- From the observations of the study, osmotic priming of Moringa seed using 1% potassium nitrate and water priming greatly improves seed germination, emergence and initial establishment of the seedling, compared to oven drying and dehulling (no pre-treatment) the seed.
- Population density influences Moringa growth and biomass accumulation. High plant densities (197 528 plants/hectare) produce higher plant biomass yields and longer stems, but smaller stem diameters. Conversely, lower plant densities (12 346 plants/hectare) produce lower plant biomass yield per given area, but produce higher individual plant growth as indicated by the thicker stem diameters produced.
- For maximum germination and rapid initial seedling establishment, well decomposed organic matter, as opposed to pine bark or sawdust, combined with clay is the most ideal media mix, with the clay texture producing the best germination results as a sole seeding media compared to sand.
- Comparison between pine bark and sawdust indicate that where sandy media texture is used, pine bark is ideal regardless of nitrogen levels applied. If pine bark is used in clay,

additions of 4 – 8 grams nitrogen would overcome the negative effects it has in this media.

- There is interaction between pH, nitrogen and phosphorous which influences initial establishment and growth of Moringa, the interaction which yielded best results being 4 g/pot phosphorus at pH 7.6, whilst the pH and nitrogen levels which gave the best yields were the 4 g/pot nitrogen at pH ranges 6.2 to 7.6. Therefore for best yields use 4 g/plant nitrogen, 4 g/plant phosphorus at pH ranges around pH 7.

5.5 Recommendations

- Excessive additions of nitrogen and phosphorus should be avoided as they result in depressed establishment, growth and biomass yield of Moringa. A general recommendation of 4 g/plant nitrogen and 4 g/plant phosphorus within a pH range around pH 7.0 is suggested. However, it is highly recommended that phosphorus applications be done two – three weeks after crop emergence when the crop is able to make use of it for vigorous root development.
- For optimum biomass production and an extensive root development, high plant densities of up to 197 528 plants/hectare, are recommended, but for thicker stemmed trees with faster individual growth rates, low plant densities (12 346 plants/hectare) are most ideal.
- It is highly recommended that Moringa seedling production and establishment media mixtures make use of well decomposed organic matter and clay as essential components.
- These experiments on pH, N and P interactions, media texture and organic source need to be repeated.

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APPENDICES

APPENDIX A: ANOVA FOR POPULATION DENSITY EXPERIMENT ONE

Appendix 4A1: Analysis of variance for above ground plant dry-matter (g/plot) at crop science department field (Experiment 1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	51337.	25668.	3.96	
Block.*Units* stratum					
Ppn	4	79552.	19888.	3.07	0.037
Residual	23	149204.	6487.		
Total	29	280093.			

Appendix 4A2: Analysis of variance for above ground plant fresh weight (g/plot) at crop science department field (Experiment 1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	1722712.	861356.	3.85	
Block.*Units* stratum					
Ppn	4	2482040.	620510.	2.78	0.051
Residual	23	5139415.	223453.		

Total 29 9344167.

Appendix 4A3: Analysis of variance for below ground (root) plant fresh
Weight (g/plot) at crop science department field (Experiment
1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	21810572.	10905286.	5.24	
Block.*Units* stratum					
Ppn	4	30570908.	7642727.	3.67	0.019
Residual	23	47861751.	2080946.		
Total	29	100243230.			

Appendix 4A4: Analysis of variance for below ground (root) plant dry
Matter (g/plot) at crop science department field (Experiment
1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	799555.	399778.	5.40	
Block.*Units* stratum					
Ppn	4	1754091.	438523.	5.92	0.002
Residual	23	1704331.	74101.		
Total	29	42			

Appendix 4A5: Analysis of variance for leaf dry matter (g/plot) at crop
Science department field (Experiment 1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	35568.	17784.	14.71	
Block.*Units* stratum					
Ppn	4	69672.	17418.	14.41	<.001
Time	2	1977.	988.	0.82	0.445
Ppn.Time	8	1083.	135.	0.11	0.999
Residual	73	88243.	1209.		
Total	89	196543.			

Appendix 4A6: Analysis of variance for leaf fresh weight (g/plot) at
crop Science department field (Experiment 1)

Source of variation	df.	ss.	ms.	vr	F pr
Block stratum	2	919476.	459738.	14.52	
Block.*Units* stratum					
Ppn	4	1676478.	419119.	13.23	<.001
Time	2	50393.	25197.	0.80	0.455
Ppn.Time	8	38491.	4811.	0.15	0.996
Residual	73	2311845.	31669.		
Total	89	4996682.			

Appendix 4A7: Analysis of variance for stem diameter (mm) at crop
Science department field (Experiment 1)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum					
Popln	4	3393.072	848.268		
Residual	-2	0.056			
Block.*Units* stratum					
Popln	4	156.361	39.090	11.28	<.001
Residual	6183(1)	21427.942	3.466		
Total	6189(1)	24930.750			

Appendix 4A8: Analysis of variance for stem-length (mm) at crop science
Department field (Experiment 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Popln	4	636629.	159157.		
Residual	-2	156289.			
Block.*Units* stratum					
Popln	4	208098.	52024.	24.29	<.001
Residual	6184	13245860.	2142.		
Total	6190	13847108.			

**APPENDIX B: ANOVA FOR PH, PHOSPHORUS AND NITROGEN INTERACTION
EXPERIMENT TWO**

Appendix 4B1: Analysis of variance for seedling counts from crop
science Department site (Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	3.0884	1.5442	6.25	
Block.*Units* stratum					
pH	4	73.9834	18.4958	74.89	<.001
Phos	2	1.2479	0.6240	2.53	0.081
Time_wks	3	80.7040	26.9013	108.92	<.001
pH.Phos	8	28.2069	3.5259	14.28	<.001
pH.Time_wks	12	2.4352	0.2029	0.82	0.628
Phos.Time_wks	6	1.2188	0.2031	0.82	0.553
pH.Phos.Time_wks	24	2.4471	0.1020	0.41	0.994
Residual	478	118.0600	0.2470		
Total	539	311.3916			

Appendix 4B2: Analysis of variance for stem-length (mm) from crop
Science department site (Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	77547.	38774.	11.57	
Block.Nitrogen.pH.Phos.Time_wks.*Units* stratum					
Nitrogen	2	383191.	191596.	57.19	<.001
pH	4	383887.	959722.	286.47	<.001
Time_wks	6	78439.	13073.	3.90	<.001
Nitrogen.pH	8	376303.	47038.	14.04	<.001
Nitrogen.Time_wks	12	157271.	13106.	3.91	<.001
pH.Time_wks	24	71166.	2965.	0.89	0.624
Nitrogen.pH.Time_wks	48	158700.	3306.	0.99	0.500
Residual	838	2807412.	3350.		
Total	944	7948917.			

Appendix 4B3: Analysis of variance for below ground (root) plant fresh
weight (g/pot) at crop science department site
(Experiment 2)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	2	758.78	379.39	8.65	
Block.Nitrogen.pH.Phos stratum					
Nitrogen	2	783.19	391.60	8.93	<.001
pH	4	4734.72	1183.68	27.00	<.001
Nitrogen.pH	8	749.40	93.68	2.14	0.066
Residual	28	1227.53	43.84	0.92	

Block.Nitrogen.pH.Phos.*Units* stratum	89(1)	4235.59	47.59
Total	133(1)	12483.39	

Appendix 4B4: Analysis of variance for below ground (root) dry matter (g/pot) at Crop science department site (Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	10.296	5.148	1.79	
Block.Nitrogen.pH.Phos stratum					
Nitrogen	2	77.496	38.748	13.46	<.001
pH	4	404.038	101.010	35.09	<.001
Nitrogen.pH	8	85.031	10.629	3.69	0.005
Residual	28	80.610	2.879	0.71	
Block.Nitrogen.pH.Phos.*Units* stratum	90	364.171	4.046		
Total	134	1021.641			

Appendix 4B5: Analysis of variance for above ground plant fresh weight (g/pot) at crop science department site (Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	258.66	129.33	6.66	
Block.Nitrogen.Phos stratum					
Nitrogen	2	440.16	220.08	11.34	0.022
Residual	4	77.66	19.41	1.45	
Block.Nitrogen.Phos.*Units* stratum					
pH	4	697.80	174.45	13.00	<.001
Nitrogen.pH	8	248.09	31.01	2.31	0.025
Residual	114	1529.83	13.42		
Total	134	3252.21			

Appendix 4B6: Analysis of variance for above ground plant dry matter (g/pot) at crop science department site (Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0867	0.0434	0.08	
Block.Nitrogen.Phos stratum					
Nitrogen	2	36.6957	18.3478	33.58	0.003
Residual	4	2.1855	0.5464	0.99	
Block.Nitrogen.Phos.*Units* stratum					
pH	4	58.6865	14.6716	26.67	<.001
Nitrogen.pH	8	11.6136	1.4517	2.64	0.011
Residual	114	62.7023	0.5500		
Total	134	171.9703			

APPENDIX C: ANOVA FOR GERMINATION EXPERIMENT THREE

Appendix 4C1: Analysis of variance for below ground (root) plant dry
Matter (g/pot) from crop science department site (Experiment
3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.4397	0.2199	0.55	
Block.*Units* stratum					
OS	2	3.8197	1.9098	4.82	0.013
Pretreat	3	2.3517	0.7839	1.98	0.130
Texture	1	4.2978	4.2978	10.85	0.002
OS.Pretreat	6	0.5841	0.0974	0.25	0.959
OS.Texture	2	3.3929	1.6965	4.28	0.020
Pretreat.Texture	3	0.4284	0.1428	0.36	0.782
OS.Pretreat.Texture	6	1.4133	0.2356	0.59	0.733
Residual	46	18.2245	0.3962		
Total	71	34.9521			

Appendix 4C2: Analysis of variance for below ground (root) plant-fresh
Weight (g/pot) from crop science department site (Experiment
3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	5.307	2.654	1.41	
Block.*Units* stratum					
OS	2	13.979	6.990	3.71	0.032
Pretreat	3	16.121	5.374	2.85	0.048
Texture	1	17.980	17.980	9.53	0.003
OS.Pretreat	6	3.662	0.610	0.32	0.921
OS.Texture	2	11.184	5.592	2.96	0.062
Pretreat.Texture	3	1.317	0.439	0.23	0.873
OS.Pretreat.Texture	6	8.272	1.379	0.73	0.627
Residual	46	86.770	1.886		
Total	71	164.593			

Appendix 4C3: Analysis of variance for above ground plant dry
matter (g/pot) from crop science department site (Experiment
3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.01426	0.00713	0.38	
Block.*Units* stratum					
OS	2	0.03287	0.01643	0.87	0.424
Pretreat	3	0.14307	0.04769	2.54	0.068
Texture	1	0.04114	0.04114	2.19	0.146
OS.Pretreat	6	0.05215	0.00869	0.46	0.832
OS.Texture	2	0.10088	0.05044	2.68	0.079
Pretreat.Texture	3	0.01561	0.00520	0.28	0.842
OS.Pretreat.Texture	6	0.17507	0.02918	1.55	0.183
Residual	46	0.86466	0.01880		
Total	71	1.43970			

Appendix 4C4: Analysis of variance for above ground plant fresh
Weight (g/pot) from crop science department site (Experiment
3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.1729	0.5865	0.71	
Block.*Units* stratum					
OS	2	1.7079	0.8540	1.03	0.364
Pretreat	3	4.5645	1.5215	1.84	0.153
Texture	1	3.8721	3.8721	4.68	0.036
OS.Pretreat	6	1.5443	0.2574	0.31	0.928
OS.Texture	2	4.3416	2.1708	2.62	0.083
Pretreat.Texture	3	0.4412	0.1471	0.18	0.911
OS.Pretreat.Texture	6	6.1094	1.0182	1.23	0.308
Residual	46	38.0525	0.8272		
Total	71	61.8064			

APPENDIX D: ANOVA FOR NITROGEN USE EXPERIMENT FOUR

Appendix 4D1: Analysis of variance for seedling emergence counts from
Crop science department site, 2007 (Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	2.7603	1.3801	4.41	
Block.*Units* stratum					
Nitrogen	3	1.8296	0.6099	1.95	0.123
OS	2	6.4712	3.2356	10.34	<.001
Texture	1	0.4001	0.4001	1.28	0.260
Time	3	50.4153	16.8051	53.68	<.001
Nitrogen.OS	6	4.1689	0.6948	2.22	0.043
Nitrogen.Texture	3	1.8662	0.6221	1.99	0.117
OS.Texture	2	0.9213	0.4606	1.47	0.232
Nitrogen.Time	9	0.6653	0.0739	0.24	0.989
OS.Time	6	1.3836	0.2306	0.74	0.621
Texture.Time	3	0.0574	0.0191	0.06	0.980
Nitrogen.OS.Texture	6	2.8093	0.4682	1.50	0.182
Nitrogen.OS.Time	18	2.7414	0.1523	0.49	0.962
Nitrogen.Texture.Time	9	0.8570	0.0952	0.30	0.973
OS.Texture.Time	6	0.1128	0.0188	0.06	0.999
Nitrogen.OS.Texture.Time	18	2.6385	0.1466	0.47	0.969
Residual	190	59.4828	0.3131		
Total	287	139.5810			

Appendix 4D2: Analysis of variance for stem-length (mm) from crop
Science department site (Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	29728.	14864.	9.72	
Block.*Units* stratum					

Nitrogen	3	21991.	7330.	4.79	0.003
OS	2	302079.	151040.	98.72	<.001
Texture	1	59140.	59140.	38.66	<.001
Time_wks	7	40390.	5770.	3.77	<.001
Nitrogen.OS	6	92071.	15345.	10.03	<.001
Nitrogen.Texture	3	9640.	3213.	2.10	0.100
OS.Texture	2	6974.	3487.	2.28	0.104
Nitrogen.Time_wks	21	9739.	464.	0.30	0.999
OS.Time_wks	14	6430.	459.	0.30	0.994
Texture.Time_wks	7	4287.	612.	0.40	0.902
Nitrogen.OS.Texture	6	74932.	12489.	8.16	<.001
Nitrogen.OS.Time_wks	42	19418.	462.	0.30	1.000
Nitrogen.Texture.Time_wks	21	9370.	446.	0.29	0.999
OS.Texture.Time_wks	14	4686.	335.	0.22	0.999
Nitrogen.OS.Texture.Time_wks	42	14398.	343.	0.22	1.000
Residual	382	584427.	1530.		
Total	575	1289699.			

Appendix 4D3: Analysis of variance for above ground plant fresh weight (g/pot) from crop science department site (Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	5.261	2.631	0.56	
Block.*Units* stratum					
Nitrogen	3	64.729	21.576	4.60	0.007
OS	2	54.542	27.271	5.81	0.006
Texture	1	0.778	0.778	0.17	0.686
Nitrogen.OS	6	40.708	6.785	1.45	0.218
Nitrogen.Texture	3	24.852	8.284	1.76	0.167
OS.Texture	2	6.253	3.126	0.67	0.519
Nitrogen.OS.Texture	6	14.767	2.461	0.52	0.787
Residual	46	215.903	4.694		
Total	71	427.793			

Appendix 4D4: Analysis of variance for above ground plant dry matter Weight (g/pot) from crop science department site (Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.2873	0.1437	0.68	
Block.*Units* stratum					
Nitrogen	3	1.9186	0.6395	3.00	0.040
OS	2	1.6603	0.8302	3.90	0.027
Texture	1	0.0332	0.0332	0.16	0.695
Nitrogen.OS	6	2.0448	0.3408	1.60	0.168
Nitrogen.Texture	3	1.0956	0.3652	1.72	0.177
OS.Texture	2	0.2358	0.1179	0.55	0.578
Nitrogen.OS.Texture	6	0.3129	0.0522	0.25	0.959
Residual	46	9.7901	0.2128		
Total	71	17.3786			

Appendix 4D5: Analysis of variance for below ground (root) plant fresh Weight (g/pot) from crop science department site (Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Block stratum	2	84.49	42.25	0.82	
Block.*Units* stratum					
Nitrogen	3	127.30	42.43	0.82	0.490
OS	2	313.18	156.59	3.02	0.058
Texture	1	364.54	364.54	7.04	0.011
Nitrogen.OS	6	348.44	58.07	1.12	0.365
Nitrogen.Texture	3	78.45	26.15	0.50	0.681
OS.Texture	2	41.05	20.53	0.40	0.675
Nitrogen.OS.Texture	6	80.39	13.40	0.26	0.953
Residual	46	2382.49	51.79		
Total	71	3820.34			

Appendix 4D6: Analysis of variance for below ground (root) plant dry
Weight (g/pot) from crop science department site
(Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	4.246	2.123	0.56	
Block.*Units* stratum					
Nitrogen	3	8.179	2.726	0.71	0.549
OS	2	18.465	9.233	2.41	0.101
Texture	1	23.335	23.335	6.10	0.017
Nitrogen.OS	6	29.041	4.840	1.27	0.292
Nitrogen.Texture	3	10.283	3.428	0.90	0.450
OS.Texture	2	9.285	4.642	1.21	0.306
Nitrogen.OS.Texture	6	6.991	1.165	0.30	0.931
Residual	46	175.914	3.824		
Total	71	285.740			