

**THE USE OF MICROBE PLUS TO IMPROVE PHOSPHORUS AVAILABILITY
FROM ROCK PHOSPHATE UNDER OIL PALM (*Elaeis guineensis*, Jacq.)
NURSERY**

**A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of
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MASTER OF PHILOSOPHY

IN

SOIL SCIENCE

BY

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DECLARATION

I hereby declare that this submission is the result of my own original research except for the references from other people's work which have been duly cited and acknowledged accordingly, and that no part has been published by another person or has been presented for another award of a degree in this university or elsewhere.

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DEDICATION

This thesis is completely dedicated to my wife, Mrs. Mavis Oppong and our son,

Kwaku Gyedu Okumanyin Karikari Oppong.

God bless you.

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“Trust in the Lord always, lean not on your own understanding, in all your ways acknowledge Him and He shall direct your paths”. Prov. 3⁵⁻⁶. Glory be to God.

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TABLE OF CONTENTS

	Page
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	x
LIST OF FIGURES	xi
ABSTRACT	xii
CHAPTER ONE	1
INTRODUCTION	1
CHAPTER TWO	4
LITERATURE REVIEW	4
2.1 Role of mineral nutrients in the growth and development of oil palm.....	4
2.2 The use of fertilizers in oil palm production.....	5
2.3 Role of phosphorus in plant nutrition.....	6
2.4 Phosphate Fertilizers.....	7
2.4.1 The use of triple superphosphate (TSP) in oil palm production.....	7
2.4.2 Rock Phosphates (RPs).....	8
2.4.2.1 Reactivity RP's.....	9
2.4.2.2 Factors affecting rock phosphate dissolution.....	9
2.4.2.2.1 Rock phosphate properties affecting its dissolution.....	9
2.4.2.2.2 Soil physico-chemical properties.....	10
2.4.2.2.3 Influence of plant species.....	11
2.4.2.2.4 Organic manures.....	11

2.4.2.2.5 Green manuring.....	13
2.4.2.2.6 Partial acidulating of RP	13
2.4.2.2.8 Ion exchange	14
2.4.2.2.9 Mechanical activation	15
2.5 Chemical dissolution of RP.....	15
2.6 Phosphorus solubilizing bio-fertilizers	16
2.6.1 Phosphorus solubilizing microorganisms (PSM).....	17
2.6.2 Mechanisms of phosphorus solubilization.....	18
2.6.3 Interaction of Phosphorus Solubilizing Bacteria (PSB) with other microorganism ..	19
2.6.4 Effect of PSB on crop production	19
2.7 Effects of rock phosphate (RP) application on the soil.....	20
2.8 The response of oil palm to rock phosphate (RP) application	21
CHAPTER THREE	23
MATERIALS AND METHODS	23
3.1 Study area description	23
3.1.1 Study location	23
3.1.2 Climate of the study area.....	23
3.1.3 Soil type and properties.....	23
3.1.4 Soil sampling and preparation.....	23
3.2 Experimental design and treatments	25
3.3 Nursery practices.....	26
3.3.1 Pre-nursery	26
3.3.2 Main nursery	26
3.3.3 Cultural practices	26
3.5.3.1 Watering.....	26
3.3.3.2 Application of fertilizer treatments	26

3.3.3.3 Weed control	26
3.3.3.4 Pest and disease control	27
3.4 Data collection	27
3.4.1 Growth parameters	27
3.4.1.1 Plant height	27
3.4.1.2 Number of fronds	27
3.4.1.3 Butt of seedlings.....	27
3.4.1.4 Leaf area.....	28
3.4.2 Plant dry weight (Biomass yield).....	29
3.5 Laboratory analysis of soil and plant samples	29
3.5.1 Particle size analysis	29
3.5.2 Soil pH	30
3.5.3 Soil organic carbon	30
3.5.4 Total nitrogen	31
3.5.5 Available phosphorus.....	32
3.5.6 Extraction of the exchangeable bases	33
3.5.6.1 Determination of calcium and magnesium	34
3.5.6.2 Determination of calcium only.....	34
3.5.6.3 Exchangeable potassium and sodium determination	35
3.5.6.4 Exchangeable acidity	36
3.5.6.5 Effective cation exchange capacity (ECEC).....	36
3.6.1 Soil biological analysis	37
3.6.1.1 Soil microbial nitrogen.....	37
3.6.1.2 Soil microbial biomass phosphorus	37
3.7 Agro-economic analysis.....	39
3.8 Statistical analysis	39

CHAPTER FOUR	40
RESULTS	40
4.1 Effect of soil amendments on growth of oil palm seedlings.....	40
4.1.1 Number of fronds per seedling.....	40
4.1.2 Butt of seedlings.....	41
4.1.3 Seedling height.....	44
4.1.4 Leaf area and leaf area index of seedlings	46
4.1.5 Seedlings dry matter production	47
4.1.6 Nutrient concentration of fronds	50
4.2 Effects of soil amendments on soil pH and available P	53
4.2.1 Soil pH	53
4.2.2 Soil available phosphorus	56
4.3 Economic analysis.....	59
4.4 Relationship between growth parameters and P applied	60
 CHAPTER FIVE	 61
5.1 Effect of P fertilizers and microbe plus application rates on growth of oil palm seedlings	61
5.1.1 Number of fronds	61
5.1.2 Seedlings butt	61
5.1.3 Seedlings height	62
5.1.4 Leaf area (LA) and Leaf area index (LAI).....	63
5.1.5 Dry matter production	64
5.1.6 Frond nutrient concentration	65
5.2 Effect of P fertilizers and microbe plus rates on soil pH and soil available P	66
5.2.1 Soil pH dynamics	66
5.2.2 Available P dynamics.....	67
5.3 Economics of production	69

5.4 Relationship between growth parameters and P applied	70
CHAPTER SIX	71
CONCLUSION AND RECOMMENDATION	71
6.1 Conclusion	71
6.2 Recommendation.....	72
REFERENCES	73
APPENDICES	98

LIST OF TABLES

	Page
Table 3.1 Initial physical, chemical and microbial biomass (N, P and C) of the soil used.	24
Table 3.2 Treatments description.....	25
Table 4.1. Effect of P fertilizers and Microbe plus rates on number of fronds.....	41
Table 4.2. Effect of P fertilizers and Microbe plus rates on seedlings butt	42
Table 4.3 Effect of P fertilizers and Microbe plus rates on seedlings height.....	45
Table 4.4 Effect of P fertilizers and Microbe plus rates on Leaf Area (LA) and Leaf Area Index (LAI) of seedlings.....	47
Table 4.5 Effect of P fertilizers and MP rates on seedling dry matter production.....	49
Table 4.6. Effect of P fertilizers and Microbe plus rates on frond nutrient content.....	51
Table 4.7. Interaction effect of P fertilizers and Microbe plus rates on frond nutrient content	52
Table 4.8 Effect of P fertilizers and Microbe plus rates on soil pH of the medium	54
Table 4.9 Effect of P fertilizers and Microbe plus rates on soil available P.....	56
Table 4.10 Interaction effect of P fertilizers and Microbe plus rates on soil available P ...	58
Table 4.11: Cost of raising 1000 oil palm seedlings at nursery	59
Table 4.12: Correlation coeffecient of selected parameters.....	105

LIST OF FIGURES

Figure 4.1: Interaction effect of P fertilizers and Microbe plus on seedlings butt at 6 MAT.	43
Figure 4.2: Interaction effect of P fertilizers and Microbe plus on root biomass at 12 MAT.	50
Figure 4.3: Interaction effect of P fertilizers and Microbe plus on soil pH at 10 MAT.	55

ABSTRACT

Although there are several options open to process Rock Phosphate (RP) into a form that is more plant available, the options for small-holder farmers are limited. Practical alternative methods and technologies of rock phosphates have to be developed for the farm level. The use of Microbe plus to improve RP solubility under oil palm nursery was evaluated at Oil Palm Research Institute (OPRI) of Ghana, Kade – Kusi, in the Eastern Region from May 2013 to May 2014. The study consisted of 16 treatments replicated 3 times in a 4 by 4 factorial experiment arranged in Randomized Complete Block Design (RCBD). The factors tested were: P fertilizers (P_0 ; Triple superphosphate, TSP; Senegal rock phosphate, SRP; Togo rock phosphate, TRP) and Microbe plus rates (0, 50, 100, 150 %). Data collected included number of fronds/palm, butt, height, LA, LAI, dry matter, frond nutrient contents, soil pH and soil available P. The results showed that the P fertilizers and Microbe plus rates applied alone or their interactions had no significant ($p>0.05$) effect on frond numbers/palm, butt, height, LA, and LAI. Frond nutrient contents, dry matter produced and soil parameters on the other hand, were significantly different from each other ($p<0.05$). The highest pH of 5.1 and available P of 180.36 mg kg^{-1} was observed at 10 months after transplanting (MAT) following the application of TSP. The use of MP_{100} showed pH of 5.2 and available P of 142.55 mg kg^{-1} at 10 MAT. The combined applications of TSP + MP_{150} produced the highest dry weight (285.50 g) and available P (180.90 mg/kg) (which represented 42 and 285 % increase respectively over the control). Also, TSP + MP_{100} treatment recorded 47 % increase in dry weight over the control at 10 MAT. Soil pH produced by the combined applications was also within the critical range (4 – 6.5) for oil palms, except for TRP and MP_{50} and MP_{100} which was below the critical range. Besides, the combined application of Microbe plus with TSP or SRP proved to be better option in terms of vegetative growth than the TRP. In all, the

combined use of MP₁₀₀ and P fertilizers gave the highest vegetative and soil parameters measured. Economically, the most cost effective nutrient input was the use of TSP alone while the least cost effective options were MP₁₅₀ with TSP or SRP or TRP. The correlation matrix also showed a strong positive correlation ($r = 0.873$) between seedling height and butt; a weak positive correlation ($r = 0.325$) between soil available P and LAI, as well as, LA and LAI. ($r = 0.528$). Soil available P and biomass P correlated positively ($r = 0.339$), whereas, available P and biomass N correlated negatively ($r = -0.543$).

CHAPTER ONE

INTRODUCTION

Oil palm, *Elaeis guineensis* Jacq., is a perennial crop and the world's leading source of vegetable oil with a potential oil yield of 6 to 7 tons/ha (Cochard *et al.*, 2001; Oil World, 2008). Ghana currently has a total of 305,758 ha of oil palm of which more than 80 % are cultivated by private small-scale farmers (MoFA, 2013). Though 243,852 tons of oil palm is estimated to be produced, Ghana currently still has an unmet demand of 305,000 tons of palm oil (MoFA, 2014). To increase oil palm productivity, tropical soils often low in available phosphorus require addition of P fertilizer for optimum yield (Omoti, 1989). A crucial aspect of improving and maintaining soil fertility is the application of deficient nutrients (Imogie *et al.*, 2011). Sangakkara *et al.* (2003) reported N as most limiting plant nutrient for crop production, however, in sub-saharan Africa, phosphorus has been found to be a major limiting factor in crop production with an average consumption of about 1.5 kg of P₂O₅ per hectare (IAEA, 2002).

It is a common practice to supply phosphorus in a form of superphosphate, triple superphosphate and diammonium phosphate to oil palm seedlings (Imogie *et al.*, 2011) which according to Mutert *et al.* (1999) is the foundation on which healthy and vigorously growing transplantable seedlings can sustain fresh fruit bunches (ffb) yield up to 25 years. However, phosphorous fertilizer use is constrained by the availability and cost; contributing about 40 % of production cost (IFA, 2013). The high cost of TSP fertilizers has generated considerable interest in the use of alternatives as soil amendment (Akande *et al.*, 2005 and Uwumarongie-Ilori *et al.*, 2012). Cheaper and effective source such as rock phosphate (RP) is being recommended (Bationo *et al.*, 1990; Abedemi *et al.*, 2006; Imogie *et al.*, 2011; Panhwar *et al.*, 2011). In addition to being cheaper than inorganic P-

fertilizers, it creates less environmental pollution as it requires minimum processing and its dissolution results in slow release of P in the soil (Amapu *et al.*, 1990; Le Mare, 1991; Rajan *et al.*, 1996).

As stated by Grimme and Härdter (1991), Al toxicity is widespread in the humid tropics where soils are acidic and highly weathered which reduces root growth and root formation in young palms, but can be corrected by RP application. The search for alternative ways to enhance the breakdown of RP into plant-available P forms has led to an array of RP modification techniques. Over the last two decades, various innovative techniques to enhance RP solubility such as partial acidulation, heap leaching, thermal treatment, mechanical activation, as well as modification through biological processes have been investigated (Singh and Amberger, 1998; van Straaten, 2002). These approaches, however, involve additional costs (Panhwar *et al.*, 2011). Currently, there is increasing emphasis on application of P-solubilizing microorganisms for RP solubilization in soils (Rodriguez and Fraga, 1999; Whitelaw, 2000; Vassilev *et al.*, 2001; Arcand and Schneider, 2006). Although there are several options for enhancing RP solubility, the options for small-scale farmers are limited.

Microbe plus is a mixture of biological and conventional NPK fertilizers with comprehensive suite of bacteria and fungi which converts the nutrients into plant available form. Yet no studies have been done to evaluate the potential of microbe plus to solubilize RP in Ghana. The main objective of this study therefore was to enhance RP solubility through the use of microbe plus to improve growth of oil palm seedlings.

The specific objectives were to:

- i. assess the effects of MP and P fertilizers on soil available phosphorus and pH of soil.

- ii. evaluate the effects of MP and the P fertilizers on seedlings frond nutrient content
- iii. evaluate the effects of MP and P fertilizers applications on the growth of oil palm seedlings.
- iv. appraise the economic benefits of MP and the P fertilizers in oil palm nursery.

The above objectives were formulated to test the null hypothesis that the application of microbe plus on rock phosphate will improve rock phosphate solubility and eventually improve growth of oil palm seedlings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Role of mineral nutrients in the growth and development of oil palm

Oil palm is a consumptive plant of both macro and micro nutrients to support its growth and development (Adiwinganda, 2002). Jacquemard (1998) classified nutrients needed by the oil palm as major elements, present in high proportion and the minor elements, of which the level is low. The former are involved in the growth of the crop and the latter mainly involved in specific but essential physiological processes. According to Philips (2004), the benefit of micronutrients is not limited solely to the replenishment of the micronutrient itself but in addition, micronutrient acts as catalyst in the uptake and use of certain macronutrients.

The oil palm has a large requirement for nutrients (Soh, 1997) and its nutrient requirements is influenced by yield, age, soil type, cover crops and climate (Jacquemard *et al.*, 2002; Goh and Hardter, 2005; Uwumarongie-Ilori *et al.*, 2012). Goh and Hardter (2005) stated that, the mineral nutrient content of oil palm frond is affected by the nutrient status of the soil. Studying the relationship between fertilizer and photosynthesis, Breure (2002) observed that optimal nutrient concentration in the oil palm was essential for maximum photosynthesis. According to Adiwiganda (2002), factors to be considered in defining fertilizer requirement include the optimum production target, nutrient surplus from the soil, and the efficiency of production recovery due to fertilizer applied.

2.2 The use of fertilizers in oil palm production

Fertilizers are important input leading to increased yields (FAO, 2009). Studies have shown that oil palm is one of the most heavily fertilized tree crops grown in the tropics (von Uexkull and Fairhurst, 1991) due to its large requirement of nutrients to maintain its vegetative growth and yield (Nuerthey, 2000). Phosri *et al.* (2010) reported that large amounts of P fertilizers are applied in the nursery or during planting in the field. According to the authors, oil palm seedlings at the nursery receive about 41.9 g/palm (as P₂O₅) and 65 to 120 kg/ha/year (as triple superphosphate) on the field in major growing areas such as Malaysia.

High P levels have been shown to inhibit mycorrhizal formation and negate its benefits (Bolan *et al.*, 1984). Without mycorrhizal assistance, oil palm would not be able to take up enough P due to poorly developed root hairs (Phosri *et al.*, 2010). This is supported by results of experiments conducted by Blal and Gianinazzi-Pearson (1990). The authors showed that when micro propagated oil palm is planted into tropical acidic soils, the plants cannot grow well or efficiently use phosphate fertilizer unless AMF are present. Blal *et al.* (1990) reported that the coefficient of fertilizer utilization in micro propagated oil palms was increased 4–5 fold after mycorrhization, particularly when using rock phosphate. Thus, in the nursery situation, the interaction between P application and mycorrhizal inhibition may lead to unnecessary levels of P fertilization. Further work by Widiastuti and Tahardi (1993) showed that palm seedlings growing in sterilized soil in the nursery require much less phosphorus fertilizer if they are inoculated; such that they reach the stage suitable for transplanting 4 months earlier than the conventional method (Zakaria, 2006).

2.3 Role of phosphorus in plant nutrition

Phosphorus is absorbed by plants as the primary and secondary orthophosphate ions (H_2PO_4^- and HPO_4^{2-}), present in the soil solution (Robert, 2005). Root and stem development, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Khan *et al.*, 2009). However, the effectiveness of P fertilizers on crop performance depends not only on the characteristics of the P sources, but also on the chemical reactions between the P fertilizers and the soils to which they are applied and their physical factors (Zin *et al.*, 2008).

Phosphorus helps in the conversion of other nutrients into usable building blocks for growth and photosynthesis. It is also indispensable for cell differentiation and for the development of the tissues that form the growing points of the plants (IFA, 2013; Nutria-Facts, 2013). As stated by Rankine and Fairhurst, (1999), phosphorus is an essential element for plant growth and is particularly important for root growth during the establishment and early growth stages. Adebayo *et al.* (2006) observed that without addition of P, oil palm seedlings exhibited lower number of leaves, butt circumference and leaf nutrient content. Studies by Obigbesan *et al.* (2002) observed inhibited root growth as a result of low P supply.

Adequate P level has been found to increase plant water and nutrient use efficiency, and help plants to adapt to moisture stress (Nutria-Facts, 2013). Menon and Chien (1990) reported significant increase in leaf nutrient content of oil palm seedlings treated with different P sources, and indicated that doubling the application rate increased the nutrient content of the leaf more than the recommended rate. In P-deficient plants, shoot growth was found to be more affected than root growth due to assimilate partitioning towards the roots and this led to a decrease in the shoot : root dry matter ratio (Goh and Hårdter,

2003). The authors also observed a reduction in trunk diameter, bunch size and a pronounced pyramid shape of the palm due to the progressive depletion of soil P.

2.4 Phosphate fertilizers

Phosphate fertilizers are categorized as natural phosphates, either treated or processed, and also by-products of phosphates and chemical phosphates (Landscape-and-Garden, 2013). According to Benton and Jones (2003), phosphorous in fertilizer is expressed as phosphate or P_2O_5 and the main sources of P are: Normal superphosphate (0-20-0); Triple superphosphate (0-46-0); Monoammonium phosphate (11-48-0); Diammonium phosphate (18-46-0); Ammonium phosphates; Ammoniated superphosphates; Potassium phosphate (KH_2PO_4) and Rock phosphates (PR).

2.4.1 The use of triple superphosphate (TSP) in oil palm production

Bohra (2013) stated that TSP is used as a base fertilizer, and according to Menon and Chien (1990) and Komolafe (1997), it is a common practice to supply phosphorus in the form of triple superphosphate to growing seedlings, the use of which has been limited by high cost. According to Bohra (2013), TSP should be applied either during or immediately after planting, seeding for maximum effect. Excessive applications of soluble P (triple super phosphate, TSP) have been reported to induce Zn and Cu deficiencies on very sandy soils and peat soils in Indonesia and in Malaysia (Goh and Hårdter, 2003). Harjotedjo *et al.* (1996) reported significant responses on the number of frond, frond length and frond 3 petiole cross section at 12 months after planting in immature palm that received TSP; but at 24 months after planting, all P treatments gave significant effects on all growth parameters.

2.4.2 Rock Phosphates (RPs)

Rock phosphate (RP) is a globally accepted but imprecise term describing any naturally occurring geological material that contains one or more phosphate mineral suitable for commercial use (Notholt and Highley, 1986), which is the main raw material for preparing chemical phosphate fertilizers by treating mostly with sulphuric acid (H_2SO_4) (Brady, 1980; Das, 2005).

Kumari and Phogat (2008) categorized rock phosphates (RP) into four: Carbonate apatite [$3Ca_3(PO_4)_2 \cdot CaCO_3$], Fluoro apatite [$3Ca_3(PO_4)_2 \cdot CaF_2$], Hydroxy apatite [$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$], Sulpho apatite [$3Ca_3(PO_4)_2 \cdot CaSO_4$]. The apatites of igneous and metamorphic origin are generally regarded as less reactive because of their well-developed crystalline form. However, the apatites of sedimentary rock deposits are soft minerals possessing micro-crystalline structure and are of major commercial importance for direct application to the soil (Narayanasamy and Biswas, 1998). Approximately 75 % of the world's phosphate resources are obtained from sedimentary, marine phosphate rock deposits, 15-20 % from igneous and weathered deposits, and 1-2 % from biogenic resources, largely bird and bat guano accumulations (van Straaten, 2002).

According to Ghosal and Chakraborty (2012), different types of rock phosphates have differing mineralogical, chemical and textural characteristics. While there are more than 200 known phosphate minerals, the main mineral group of phosphates is the group of apatites (Kauwenbergh, 2010). Reactive apatite rock phosphates will dissolve to a greater extent more rapidly in suitable soils and environments than non-reactive apatites (Bolland, 2007).

2.4.2.1 Reactivity of rock phosphates (RP's)

Rock phosphates commonly have long-term residual effects and contribute to recapitalization of P in soils. However, direct application of RPs are not able to produce the desired short-term outcome and therefore must be modified before application to optimize the P utilization (Ghosal and Chakraborty, 2012; Khan and Sharif, 2012). The chemical reactivity or solubility of rock phosphates is a measure of its ability to release P for plant uptake (Ghosal and Chakraborty, 2012). According to Rajan *et al.* (1996), reactivity is the combination of RP properties that determines the rate of dissolution of the RP in a given soil under given field conditions. Studies by Zin *et al.* (2008), Ghosal and Chakraborty, (2012), and IFA, (2013) showed that the chemical and mineralogical features are key factors in determining the reactivity and subsequent agronomic effectiveness of a rock phosphate. Hammond *et al.* (1986) reported, however, that the effectiveness of a P source measured under actual field condition will vary with changes in climatic and agro-edaphic conditions.

2.4.2.2 Factors affecting rock phosphate dissolution

Several factors influence the direct application of rock phosphate as P fertilizer. According to Kumari and Phogat (2008), the availability of rock phosphate-P to plant largely depended on the properties of rock phosphate, soil characteristics, plant species and fertilizer management practices. Soil texture was one of the soil properties affecting the availability, accumulation and transport of applied P in soils (Zheng and MacLeod, 2005).

2.4.2.2.1 Rock phosphate properties affecting its dissolution

Rate of dissolution of rock phosphate in a given soil is determined by its chemical composition which includes apatite lattice composition, the type of accessory minerals and particle size (Kumari and Phogat, 2008). Studies have shown that increasing substitution

of CO_3^{2-} for PO_4^{3-} in the lattice structure increases the solubility of carbonate apatites. This occurs due to decreased \AA -dimension of the unit cell, and crystal instability on increased incorporation of planar CO_3^{2-} and F^{-1} for PO_4^{3-} tetrahedral (Lehr and McClellan, 1972; Chien, 1977).

According to Kumari and Phogat, (2008), rock phosphates are relatively insoluble materials, hence their geometric surface areas have an important bearing on their rate of dissolution in soil. Bagavathi Ammal *et al.* (2001) reported that the finer particles of rock phosphate (100 mm mesh) resulted in higher values of dissolution than relatively coarser particles (60 mm mesh). The authors explained that the finer the particle size, the greater the degree of contact between rock phosphate and soil, and therefore, greater the rate of dissolution (Bagavathi Ammal *et al.*, 2001).

2.4.2.2.2 Soil physico-chemical properties

The efficiency of rock phosphate is depended on its reaction and retention in the soil, chemical properties and type of soil to which it is applied (Chien *et al.*, 2010). Studies have shown that decreasing soil pH increases RP effectiveness (Prochnow *et al.*, 2006; Rivaie *et al.*, 2008; Chien *et al.*, 2010). According to Rajan *et al.* (1991) and Ghosal and Chakraborty (2012), the amount of rock phosphate-P dissolved decrease either exponentially or linearly with the increasing soil pH. Rajan *et al.* (1996) expressed dissolution of rock phosphate in acid soils as,



Capacity of the soil to retain P (Chien *et al.*, 1980; Hammond *et al.*, 1986; Babare *et al.*, 1997) and soil moisture (Kanabo and Gilkes, 1988; Bolland, 1994) are also important soil parameters affecting rock phosphate dissolution. According to Kanabo and Gilkes (1988), the dissolution of rock phosphate is considerably enhanced by the soil if it remains

sufficiently wet to allow the dissolved products to be transported away from the surface of the rock phosphate particles. Some organic acids (including oxalic, citric, tartaric, gluconic) have been reported to be produced in the soil as a result of microbial and chemical transformation of organic matter which have positive influence on RP dissolution (Rashid *et al.*, 2004; Kumari and Phogat, 2008; Khan and Sharif, 2012).

2.4.2.2.3 Influence of plant species

Kumari and Phogat (2008) reported that plants influence the rate of rock phosphates dissolution by the secretion of acid or alkali, and production of chelating organic acids (citric, malic and 2- ketogluconic acid). Plant species differ in their P uptake, demand and their ability to absorb soil solution P (Helyer, 1998; Baligar, 2001). Additionally, Plant species exhibit differences in their ability to access sparingly forms of P that are unavailable to other plants (Hocking *et al.*, 1997; Hasinger, 1998; Hocking, 2001).

According to Sale and Mkwunye (1993), the mechanism whereby high rooting density per se stimulates RP dissolution is probable related to the lowering of the concentration of Ca^{2+} and H_2PO_4^- in the solution surrounding the surface of the RP particles. Studies indicated that reactive RPs may have potential applications in alkaline soils when crop such as rapeseed (*Brassica napus*) which is organic-acid secreting is cultivated on it (Ae *et al.*, 1990; Hoffland, 1992; Adams and Pate, 1992; Montenegro and Zapata, 2002; Chien, 2003). According to Mnkeni *et al.* (2000) and Weil (2000), rapeseed is able to increase the solubilization, even from less reactive RP sources.

2.4.2.2.4 Organic manures

Agricultural wastes composted with rock phosphate are known to increase the solubility of rock phosphate (Amberger *et al.*, 1992; Van de Berghe, 1996; Akande *et al.*, 2011).

However, the dissolution of rock phosphate through composting varies with its characteristics and the kind of waste (Mahimairaja *et al.*, 1995). Composting of rock phosphate with organic manure/farm wastes produces organic and mineral acids as a result of their decomposition (Roy *et al.*, 1999; Goenadi *et al.*, 2000; Lakshminarayana, 2005). According to Rajan *et al.* (1997), the release of these acids creates a localized high acidity in the immediate vicinity of rock phosphate and some could lead to complexation of Ca.

Srikanth *et al.* (1999) and Manna *et al.* (2001) proposed that the application of phospho-compost at 10 Mg ha⁻¹ can give plant growth, dry matter accumulation, seed yield and P uptake of soybean crop equivalent to that when SSP is applied at 26.2 kg P ha⁻¹; while Manna *et al.* (2003) reported that application of phospho-compost at 5 Mg ha⁻¹ can give plant growth, dry matter accumulation, seed yield and N and P uptake by the plant equivalent to 25 kg N and 60 kg P₂O₅ ha⁻¹ application. Moreover, the continuous turnover of enriched phospho-compost can increase microbial biomass C and enzyme activity of the soil (Nazirkar *et al.*, 2004).

Jagdev and Singh (2001) found that the composting of Mussoorie rock phosphate at 12.5% with green lantana biomass and fresh dung biomass for 90 days increased the total P, water and citrate soluble-P contents. The results were found to be more pronounced when the compost was inoculated with *Aspergillus awamori*. Roy *et al.* (1999), Srikanth *et al.* (1999), Biswas and Narayanasamy (2002) also reported that the inoculation of compost with phosphorus solubilizing bacteria (PSB) increased the efficiency of compost in terms of P dissolution and release of soluble P from rock phosphates. In another study, Kole and Hajra (1998) found that P solubilizers isolated from carbon rich compost exhibited higher solubilizing ability than those isolated from the soil. These compost isolated strains are more efficient in biodegradation of wastes and rock phosphate solubilization (Kole and Hajra, 1998).

2.4.2.2.5 Green manuring

Decomposition process stimulated during the incorporation of green manure into the soil increases the P availability through the release of CO₂, which forms H₂CO₃ in the soil solution. The result is the dissolution of primary P-containing minerals (Pattanayak *et al.*, 2001; Sharma *et al.*, 2001; Cavigelli and Thien, 2003). According to Sharpley and Smith (1989), organic acids released during decomposition may help dissolving soil mineral P.

2.4.2.2.6 Partial acidulating of RP

Partially acidulated rock phosphates (PARP) are rock phosphates which have been acidulated with sulphuric or phosphoric acid with less than the stoichiometric quantity of acid needed for making SSP or TSP (Rajan and Watkinson, 1992). Chien and Menon (1995) also found that 40-50 % acidulation of less reactive rock phosphate with sulphuric acid or 20 % acidulation with phosphoric acid is appropriate for increasing efficiency of rock phosphate. Kato *et al.* (1995) reported that P recovery in the soil was 0.25 % for the North Carolina rock phosphate, whereas it ranged between 1.2-1.6 % for the corresponding 50 % PARP. The agronomic effectiveness of PARP has been found to be pronounced than rock phosphate (Owusu-Bennoah and Acquaye, 1996; Lewis *et al.*, 1997).

Rajan *et al.* (1994) compared the relative effectiveness of PARP prepared from 30 % acidulation of North Carolina rock phosphate with H₂SO₄ and H₃PO₄. H₂SO₄⁻ PARP was found inferior to H₃PO₄⁻ PARP containing similar amounts of water soluble P. This was attributed to the presence of CaSO₄ coatings. The performance of PARPs prepared from the mixture of HNO₃ + H₂SO₄, HNO₃ + HCl, HNO₃ + H₃PO₄ at 10 and 20 % acidulation was tried by Basak and De (1997). The authors found that the acid mixture used as

acidulants, $\text{HNO}_3 + \text{H}_3\text{PO}_4$ was the best. Further, Mandal *et al.* (1996) used rock phosphates acidulation with $\text{H}_2\text{PO}_4 + \text{HNO}_3$ in the ratios of 3:1, 1:1 and 1:3 on *Vigna mungo*. Dry matter yield and P uptake was found highest in PARP which was acidulated in the ratio 3:1.

2.4.2.2.7 Management factors

Management practices such as method of placement, timing of application and lime application influence the effectiveness of RP and water-soluble P-fertilizer (Hellums 1991; Chien and Menon 1995). According to Sale and Mokwunye (1993) and Chien and Menon (1995), the method of broadcasting and incorporating the RP in the soil increases the effectiveness of PR. Banding of RP is less effective. However, smaller amounts of placed P can give the same yield as a larger broadcast application (Eijk *et al.*, 2006).

Timing the application of RPs has been found to be important for their effective use. Cabala-Rosand and Wild (1982a) demonstrated that the effectiveness of low soluble RP was enhanced when applied directly on acid soils well in advance of crop planting. From their observation, it was expected that early application of RP would allow some time for dissolution to begin. Contrary to their findings, Hammond *et al.* (1986b) observed that when applied to high P-sorbing soils the effectiveness was actually reduced when the RP was applied too early. Chien *et al.* (1990) explained that in very acid soils (pH less than 5.5) with a high P retention capacity, the incorporation of RP close to planting time is recommended in order to minimize conversion of dissolved P to plant unavailable forms.

2.4.2.2.8 Ion exchange

This is based on the principle that ions released during the dissolution of RP, especially Ca^{2+} , can be sequestered by zeolites, which in turn furthers the dissolution of the RP (Lai and Eberl 1986; Chesworth *et al.*, 1987). A simplified reaction of the systems is: $\text{RP} +$

NH_4^+ -zeolite \Leftrightarrow Ca-zeolite + NH_4^+ + H_2PO_4^- . For this reaction to take place, zeolites have to be charged with NH_4^+ and then react with RP. The NH_4^+ charged zeolite will act as Ca^{2+} sink during the exchange, thereby releasing NH_4^+ and taking up Ca^{2+} ions. This will lower the activity of Ca^{2+} in the solution and more RP will dissolve (Lai and Eberl, 1986).

2.4.2.2.9 Mechanical activation

Gock and Jacob (1984) tested rotary-chamber vibrating mill for mechanically ‘activating’ sedimentary phosphate rock from Egypt. This technique did not only reduce the grain size of the RP considerably, it opened up defect sites in phosphate minerals and subsequently changes the solubility parameters of the RP as a function of milling time. Results from X-ray diffraction and infra-red test supported by citrate solubility tests over time provided evidence for mineralogical changes that enhance solubility of the RP (Gock and Jacob, 1984). Citric acid tests of mechanically activated Togo RP showed increased solubilities with increased energy inputs for grinding (Gock and Jacob, 1984). Mechanically activated Kodjari RP from Burkina Faso was tested in greenhouse experiments and resulted in significantly higher yields (Kantor and Schwertmann, 1990).

2.5 Chemical dissolution of RP

Wet blending of low-grade rock phosphate with half the quantity of oxalic acid has been found as good as any of the commercial P fertilizer (Singh and Ruhel, 1993) because oxalic acid not only solubilized insoluble P but also chelated Ca as calcium oxalate. Bagavathi Ammal *et al.* (2001) studied the rate of dissolution and efficiency of low grade Udaipur rock phosphate when mixed with elemental sulphur (S) in a ratio of 5:1 and tested on onion–black gram sequence grown on soil having pH of 7.7. The results showed

significant increase in available P, due to microbial oxidation of S leading to production of protons (H^+) which dissolved rock phosphate-P and increased available P content.

According to Rastogi *et al.* (1976) and Somani (1994), the rate of rock phosphate dissolution can also be altered by the addition of pyrite. Mishra *et al.* (2002) studied the P release characteristics of rock phosphate when mixed with iron pyrite (1:1). Mixing of iron pyrite with rock phosphate decreased the total P_2O_5 content of rock phosphate because of dilution and resulted in conversion of insoluble phosphate to water and citrate soluble phosphate. The water soluble P fraction which was nil in original rock, increased from 0.06 to 1.14 %, and the citrate soluble P fraction increased from 4.2 to 7.2 % when mixed with pyrite. Mixing pyrite with rock phosphate not only helps in raising the solubility of P, but also adds sulphur to the source (Mishra *et al.*, 2002).

2.6 Phosphorus solubilizing bio-fertilizers

Phosphorus solubilizing bio-fertilizers are carrier based preparations containing living or latent cells of micro-organisms like bacteria, fungi and actinomycetes which stimulate plant growth by providing hormones and vitamins through solubilization of insoluble phosphorus (Gaur and Sunita, 1999; Park *et al.*, 2010). When compared with chemical treatments (SSP or TSP), microbial solubilization of rock phosphate was observed to be an environmentally mild approach (Vassilev and Vassileva, 2003).

Kaushik and Garg (2004) reported that 30 kg P_2O_5 as SSP can safely be replaced with 30 kg P_2O_5 as Udaipur Rock Phosphate in the presence of blue green algae, vesicular arbuscular mycorrhizae and phosphorus solubilizing bacteria as inoculants. It not only increased paddy and wheat yield significantly but also improved soil health in terms of carbon buildup, residual soil N and available phosphorus. Similarly, Asewar *et al.* (2003) reported that application of 20 kg P_2O_5 as RP in combination with PSB (*Bacillus magaterium*) was superior to 40 kg P_2O_5 as RP alone. The combined inoculation of

Rhizobium and PSB strains have recorded even higher number of nodules, grain and dry matter yield, and P uptake in pea (Tyagi *et al.*, 2003) as compared to MRP+PSB (Mussoorie rock phosphate and Phosphorus solubilizing bacteria) when applied alone. In another study, the application of rock phosphate and PARP along with PSB not only improved yield, P concentration in plant biomass and soil but also showed considerable residual effect for next season crop (Sharma and Vyas, 2001).

In spite of increasing P availability and P uptake, use of PSB also increases plant biomass and N accumulation in the plant biomass (Dubey, 2001). P solubilizing effect can further be enhanced by combined application of PSB+RP+FYM (Sundaravadivel *et al.*, 2000; Shehana and Abraham, 2001). Additionally, 50 % of the costly super phosphate could be replaced by rock phosphate, a cheap source of P, when applied in conjunction with PSB (Sundara *et al.*, 2002).

2.6.1 Phosphorus solubilizing microorganisms

Bacteria have been identified to be more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Among the whole microbial population in soil, PSB constitute 1 to 50 %, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential (Chen *et al.*, 2006). Microorganisms involved in phosphorus acquisition include mycorrhizal fungi and PSMs (Fankem *et al.*, 2006). As indicated by Igual *et al.* (2001), ectorrhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia among the soil bacterial communities, have been described as effective phosphate solubilizers.

According to Whitelaw (2000), strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizers. Subbarao (1988) and Kucey *et al.* (1989) identified *Bacillus*

megaterium, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* as important strains. Studies by Duponnois *et al.* (2006) identified nematofungus *Arthrobotrys oligospora* as having the ability to solubilize rock phosphates.

2.6.2 Mechanisms of phosphorus solubilization

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000; Khiari and Parent, 2005). This supports the assertion by Sagoe *et al.* (1998) and Park *et al.* (2010) that, phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms. Furthermore, phosphate solubilization takes place through various microbial processes/mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996; Nahas, 1996).

According to Whitelaw (2000), phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Kpombrekou and Tabatabai, 1994; Stevenson, 2005). Studies by Goldstein (1995) and Deubel *et al.* (2000) showed that, PSB dissolve soil P through production of low molecular weight organic acids mainly gluconic and ketogluconic acids, in addition to lowering the pH of rhizosphere. Additionally, the pH of rhizosphere is lowered through biotical production of proton/bicarbonate release (anion/cation balance) and gaseous (O₂/CO₂) exchanges (Goldstein, 1995; Deubel *et al.*, 2000). Phosphorus solubilization ability of PSB has direct correlation with pH of the

medium (Khan *et al.*, 2009). In one study, Kim *et al.* (1997) used inorganic acids (e.g. hydrochloric acid) on phosphate solubilization but was less effective compared to organic acids at the same pH. According to Gyaneshwar *et al.* (1999), phosphate solubilization is induced by phosphate starvation in certain cases.

2.6.3 Interaction of PSB with other microorganisms

Symbiotic relationship between PSB and plants is synergistic in nature as bacteria provide soluble phosphate and plants supply root borne carbon compounds (mainly sugars), that can be metabolized for bacterial growth (Pérez *et al.*, 2007). The PSM along with other beneficial rhizospheric microflora enhance crop production. Simultaneous application of Rhizobium with PSM (Perveen *et al.*, 2002) or arbuscular mycorrhizae (AM) fungi (Zaidi *et al.*, 2003) has been shown to stimulate plant growth more than with their sole inoculation in certain situations when the soil is P deficient. Synergistic interactions on plant growth have been observed by co-inoculation of PSB with N₂ fixers such as Azospirillum (Belimov *et al.*, 1995) and Azotobacter (Kundu and Gaur, 1984), or with vesicular arbuscular mycorrhizae (Kim *et al.*, 1998).

2.6.4 Effect of PSB on crop production

Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of PSMs can increase crop yields up to 70 % (Verma, 1993). Combined inoculations of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (Goenadi *et al.*, 2000; Cabello *et al.*, 2005). Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB (Zaidi, 1999). Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance plant growth by improving biological nitrogen fixation

(Kucey *et al.*, 1989; Ponmurugan and Gopi, 2006). *Pseudomonas spp.* enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006).

Phosphate solubilizing bacteria enhanced the seedling length of *Cicera rietinum* (Sharma *et al.*, 2007), while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield (Yazdani *et al.*, 2009). Inoculation with PSB increased sugarcane yield by 12.6 % (Sundara *et al.*, 2002). Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhizae achieved the maximum grain weight (Mehrvarz *et al.*, 2008). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat, and dual inoculation without P fertilizer improved grain yield up to 20 % against sole P fertilization (Afzal and Bano, 2008). Mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley (Mehrvarz *et al.*, 2008). Rhizospheric microorganisms can interact positively in promoting plant growth, as well as N and P uptake. Seed yield of green gram was enhanced by 24 % following triple inoculation of *Brady rhizobium* + *Glomus fasciculatum* + *Bacillus subtilis* (Zaidi and Khan, 2006). Growth and phosphorus content in two alpine *Carex* species increased by inoculation with *Pseudomonas fortinii* (Bartholdy *et al.*, 2001). Integration of half dose of NP fertilizer with biofertilizer gives crop yield as with full rate of fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized (Jilani *et al.*, 2007).

2.7 Effects of rock phosphate (RP) application on the soil

Zin *et al.* (2005) reported that RP fertilizers have a higher content of calcium ranging from 24 – 33 %. This makes RP beneficial in increasing soil pH and cation exchange capacity

(CEC) resulting in yield increases of oil palm (Zin *et al.*, 2005). Their use has also been shown to have great potentials for liming due to their high content of calcium (Isenmila *et al.*, 2006). According to Imogie *et al.* (2011), in the acid soils of the humid tropics, reactive rock phosphate can be substituted profitably for soluble fertilizers; nevertheless, the effect of RP is limited in neutral or alkali soils. Its incorporation ensures a steady supply of P over a long period and also provides a high rooting density to crops (Bolan *et al.*, 1990).

2.8 The response of oil palm to rock phosphate (RP) application

Oil palm requires a constant supply of available P to maintain rapid growth and development and the major source of P is RP. Finely ground RP directly applied to soil in Malaysia for oil palm production was reported to have improved growth and yield (Zaharah *et al.*, 1997). Moreover, in Brazil, a single application of RP per ha of land deficient of P gave 100 % yield increase in oil palm over a period of 6 years (Hartley, 1988). This supports the role RP play in increasing oil palm yield (ton / ha) by 58 % in Indonesia in a second year following implementation of best management practices (Griffiths and Fairhust, 2002).

Foster *et al.* (1998) noted the promising effects of direct application of RP on mature oil palm. Application of RP increased fresh fruit bunch (FFB) and on acid sands, was found to be superior to single super phosphate (Imogie *et al.*, 2011). Its incorporation ensured a steady supply of P over a long period and also provided a high rooting density to crops (Bolan *et al.*, 1990). Akinrinde *et al.* (2006) reported significant increase in stem girth and leaf area on oil palm seedlings treated with different sources of RP on two different soil types after 6 months of growth under nursery conditions. The authors further stated that, application of P fertilizer, irrespective of source or rates significantly increased the stem thickness in the two soil types (Rhodic Paludalf and Plinthic Tropudalf) studied. The

results of the study showed that using oil palm seedlings as test crop, RP had higher availability and crop response than super phosphate in acid soils (Akinrinde *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area description

3.1.1 Study location

The experiment was carried out at the Agronomy Nursery of Oil Palm Research Institute, (OPRI), Kade – Kusi, in the Eastern Region of Ghana (latitude 0.6° 00' N and longitude 0.01° 45' W).

3.1.2 Climate of the study area

The study area falls within the semi-deciduous forest zone of Ghana and is characterized by bimodal rainfall distribution. The major rainfall season begins from March and ends in July whilst the minor season starts from September and ends in November. The mean annual rainfall is 1600 mm. Day temperatures range from a mean minimum of 20 °C to a mean maximum of 31 °C with relative humidity ranging from 95 % in the rainy season to 40 % in the dry season.

3.1.3 Soil type and properties

Top soil was used for pre and main nursery activities. The soil used was Ferric Plinthic Acrisol (FAO/UNESCO, 1990) with the Kokofu series being the dominant units (Asiamah and Senayah, 1991).

3.1.4 Soil sampling and preparation

Soil samples were taken prior to the first fertilizer application and subsequently after fertilizer application using a spot plan. Using an auger, random soil samples were taken

monthly from the polybags, mixed together and a composite sample taken for analysis. Moist soil samples from the field were used to determine soil microbial biomass. Soil samples for the physical and chemical analysis were air dried, crushed in a mortar with a pestle and passed through a 2 mm mesh sieve.

The initial physico-chemical and microbial properties of the soil used for the trial is presented in Table 3.1. The soil was loam with pH of 5.1 (within the critical range for oil palm). The organic carbon, microbial biomass, CEC and available P were low. The low amount of available P suggested P deficiency in the soil, hence, the need for fertilizer application. Moreover, as stated by Asomaning *et al.* (2006), low available P content seemed to suggest that considerable proportion of the P was fixed and unavailable to plants. The P₂O₅ content of the P fertilizers used for the trial as indicated on the manufacturer's bag was 46 %, 37 % and 33.5 % for TSP, TRP and SRP respectively, and was in the order of TSP > TRP > SRP.

Table 3.1 Initial physical, chemical and microbiological properties of the soil used

Property	Value
pH (1:2.5 H ₂ O)	5.1
Organic carbon (%)	0.76
Total nitrogen (%)	0.08
Exchangeable cations (cmol _c /kg)	
Ca	1.87
Mg	0.53
K	0.17
Na	0.05
Exch. Acidity (cmol _c /kg)	0.45
Available P (mg/kg)	4.70
Sand (%)	46.76
Silt (%)	31.44
Clay (%)	21.80
Texture	Loam
Microbial N (mg/kg)	2.80
Microbial P (mg/kg)	11.96

3.2 Experimental design and treatments

The experiment was a 4 × 4 factorial arranged in a randomized complete block design with three replicates. Each treatment plot had 20 seedlings planted in a 70 × 70 × 70 cm triangular planting design. The treatments were 4 levels of phosphorus and 4 levels of microbe plus as follows:

- Phosphorus sources were: Triple superphosphate (TSP), Togo Rock Phosphate (TRP), Senegal Rock Phosphate (SRP) and No P fertilizer (Po)
- Microbe Plus (MP) rates were: Zero % MP (MP₀), 50 % MP (MP₅₀), 100 % (MP₁₀₀) and 150 % MP (MP₁₅₀)

Table 3.2 Treatments description

Treatment	Amount applied/palm/month
T ₁	Absolute control (Po + MP ₀)
T ₂	TSP was applied at 6 g/palm/month. The standard practice recommended by OPRI of CSIR, Ghana.
T ₃	*SRP was applied at 8.2 g/palm/month.
T ₄	*TRP was applied at 7.5 g/palm/month.
T ₅	MP ₅₀ . 25 ml of MP was dissolved in 1 liter of water and 50 ml of the mixture was applied.
T ₆	TSP + MP ₅₀ . 6 g of TSP and 50 ml of MP ₅₀
T ₇	SRP + MP ₅₀ . 8.2 g of SRP and 50 ml of MP ₅₀
T ₈	TRP + MP ₅₀ . 7.5 g of TRP and 50 ml of MP ₅₀
T ₉	MP ₁₀₀ . 50 ml of MP was dissolved in 1 liter of water and 50 ml of the mixture was applied.
T ₁₀	TSP + MP ₁₀₀ . 6 g of TSP and 50 ml of MP ₁₀₀
T ₁₁	SRP + MP ₁₀₀ . 8.2 g of SRP and 50 ml of MP ₁₀₀
T ₁₂	TRP + MP ₁₀₀ . 7.5 g of TRP and 50 ml of MP ₁₀₀
T ₁₃	MP ₁₅₀ . 75.5 ml of MP was dissolved in 1 liter of water and 50 ml of the mixture was applied.
T ₁₄	TSP + MP ₁₅₀ . 6 g of TSP and 50 ml of MP ₁₅₀
T ₁₅	SRP + MP ₁₅₀ . 8.2 g of SRP and 50 ml MP ₁₅₀
T ₁₆	TRP + MP ₁₅₀ . 7.5 g of TRP and 50 ml of MP ₁₅₀

*based on P₂O₅ content equivalent to TSP.

All plants received a basal dressing of 6 g Urea (N) and 6 g Muriate of Potash (MOP) mixture/palm/month.

3.3 Nursery Practices

3.3.1 Pre-nursery

Mini polybags (black) of dimensions 10 × 19 cm were filled with top soil. The lower third of the bags were perforated to enhance drainage of excess water. Germinated oil palm seed nuts of *Dura* × *Pisifera* (D × P) were sown singly in the black polybags for four months.

3.3.2 Main nursery

Maxi polybags (black) of dimension 35 × 45 cm were filled with 10 kg topsoil and arranged in triangular planting design of distance 70 × 70 × 70 cm. Pre-nursery seedlings at four month or four leaf stage were transplanted into each of the maxi polybags and mulched with palm kernel shells.

3.3.3 Cultural practices

The following cultural practices were undertaken during the trial period:

3.5.3.1 Watering

Watering was done as and when necessary using the drip irrigation system after 3 days of no rain.

3.3.3.2 Application of fertilizer treatments

Fertilizers were applied monthly as specified in the various treatments.

3.3.3.3 Weed control

Weeding (hoeing in-between polybags and hand picking within the polybags) was carried out manually as and when necessary.

3.3.3.4 Pest and disease control

Prophylactic fungicide (diathane) and insecticide (actellic) were sprayed every two weeks and monthly respectively when necessary.

3.4 Data Collection

Data collection on vegetative growth was undertaken when seedlings attained five leaves at 5 MAT and continued monthly until seedlings reached 12 MAT. Data collection on soil parameters was done at 6 MAT, 8 MAT and 10 MAT.

3.4.1 Growth parameters

Five plants per treatment were randomly selected and tagged for data collection. Growth responses measured were seedling height, number of fronds per seedling, seedling butt, leaf area and leaf area index.

3.4.1.1 Plant height

This was measured as height from the soil level in the polybag to the top of the highest point of the seedling frond using a meter rule at monthly interval from 5 MAT to 12 MAT.

3.4.1.2 Number of fronds

This was determined by counting the number of fronds per seedling at a monthly interval from 5 MAT to 12 MAT.

3.4.1.3 Butt of seedlings

Seedling butt was measured using a vernier caliper to determine the diameter at two places on the butt (a position on the trunk of the young seedling where the leaf base fused) from the soil level. Measurements were taken at monthly intervals from 5 MAT to 12 MAT. The butt was determined by the formula (πd), where π was taken as 3.14 and d was the mean diameter measured on the seedlings' butt.

3.4.1.4 Leaf area

Leaf area (LA) was calculated after the plants were 12 months old and had developed leaflets on the third leaf from the top opened leaf. Three leaflets were taken from the centre of each side of the frond and the width and length were measured with a ruler. The means of the length and width of the leaflets obtained were put into the formula to estimate the LA.

Calculation:

$$\text{Leaf area (LA)} = b (n \times LW)$$

where;

n = number of leaflets

LW = mean of length x mid-width for a sample of the leaflets

b = the correction factor of 0.55 (Hardon *et al.*, 1969)

3.4.1.5 Leaf area index

Having estimated the leaf area (LA) of the palm, it was related to the plant density. Leaf area index (LAI) was thus estimated as:

$$\text{Leaf area index} = \frac{\text{Leaf area}}{\text{Plant density}}$$

where;

$$\text{Plant density} = \text{Ground area} \left(\frac{1}{2} \Delta^2 \sqrt{3} \right)$$

Δ = planting distance

3.4.2 Plant dry weight (Biomass yield)

Destructive sampling method was used to estimate the dry matter production of the leaf, root and stem. Five plants were sampled from each plot at the end of the experiment and each plant was divided into leaves, butt and roots. Dry weight of the fronds, butt and roots were recorded after oven drying at 70 °C to constant weight. Sub-samples of the leaves were milled to pass through 2 mm sieve. Nutrient concentrations (N, P, K, Mg and Ca) were then determined in the laboratory.

3.5 Laboratory analysis of soil and plant samples

The physical, chemical, microbial biomass N and P of the soil and plant nutrient analysis were determined in the laboratory of Soil Research Institute, Kwadaso - Kumasi.

3.5.1 Particle size analysis

The main soil physical property determined was particle size. It was determined using the hydrometer method (Bouyoucos, 1962). A 50 g soil sample was weighed in a graduated cylinder. The soil was saturated with distilled water and 100 ml of 10 % calgon (sodium hexametaphosphate) and stirred for 10 minutes. The suspension was transferred to a 1000 cm cylinder and filled with distilled water to the mark. Two drops of amyl alcohol were added. The suspension was mixed thoroughly with a long glass rod and the temperature recorded. The hydrometer was gently lowered into the suspension and the readings were taken at 30 seconds and 3 hours.

Calculation:

$$\% \text{ Silt} = \frac{F1}{M} \times 100$$

$$\% \text{ Clay} = F2 \times \frac{100}{M}$$

$$\% \text{ Sand} = 100 \% - (\% \text{ Silt} + \% \text{ Caly})$$

where;

F_1 = corrected first hydrometer reading at 30 seconds

F_2 = corrected second hydrometer reading at 3 hours

M = mass of dry soil (50 g)

The texture was determined by interpolating the sand, silt and clay percentages in a textural triangle.

3.5.2 Soil pH

Soil pH was determined in a 1:2.5 suspension of soil and water using a HI 9017 Micro Processor pH meter. A 20 g sample was weighed into a 100 ml bottle. To this 50 ml distilled water was added from a measuring cylinder and the bottle capped. The solution was shaken on a reciprocating shaker for two hours. After calibrating the pH meter with buffer solutions at pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.5.3 Soil organic carbon

Organic carbon was determined by the modified Walkley and Black procedure as described by Nelson and Sommers (1982). This procedure involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid. After reaction, the excess dichromate is titrated against ferrous sulphate. One gram of soil sample was weighed into an Erlenmeyer flask. A blank sample was included. Ten millilitres of 1.0 *N* (0.1667 *M*) potassium dichromate solution was added to the soil and the blank flask. To this, 20 ml of concentrated sulphuric acid was carefully added from a measuring cylinder, swirled and allowed to stand for 30 minutes in a fume chamber. Distilled water (250 ml) and a concentrated orthophosphoric acid (10 ml) were added and

allowed to cool. One millimetre of diphenylamine indicator was added and titrated with 1.0 M ferrous sulphate solution.

Calculation:

$$\% \text{ Organic Carbon (O.C)} = \frac{M \times 0.39 \times \text{m.c.f} (V_1 - V_2)}{S}$$

where;

M = molarity of ferrous sulphate solution

V_1 = ferrous sulphate solution required for blank (ml)

V_2 = ferrous sulphate solution required for sample (ml)

S = weight of air dry sample (g)

m.c.f = moisture correction factor $(100 + \% \text{ moisture}) / 100$

0.39 = $3 \times 0.001 \times 100 \% \times 1.3$ (3 = equivalent weight of C)

1.3 = a compensation factor for the incomplete combustion of the organic matter.

3.5.4 Total nitrogen

Total nitrogen was determined by the Kjeldahl digestion and distillation procedure as described in Soil Laboratory Staff (1984). A 0.5 g soil sample was transferred into a Kjeldahl digestion flask and 5 ml distilled water added to it. After 30 minutes, 5 ml concentrated sulphuric acid and selenium mixture were added and mixed carefully. The sample was placed on a Kjeldahl digestion apparatus for 3 hours until a clear digest was obtained. The digest was diluted with 50 ml distilled water and mixed well until no more sediment dissolved and allowed to cool. The volume of the solution was added to 100 ml distilled water and mixed well. A 25 ml aliquot of the solution was transferred to the reaction chamber and 10 ml of 40 % NaOH solution was added followed by distillation.

The distillate was collected in a flask containing 2 % boric acid. The distillate was titrated with 0.02 *N* HCl solution with bromocresol green as indicator. A blank distillation and titration were also carried out to take care of traces of nitrogen in the reagent as well as the water used.

Calculation:

$$\% \text{ N} = \frac{N \times (a-b) \times 1.4 \times \text{m.c.f}}{S}$$

where;

N = concentration on HCl used in titration

a = HCl used in sample titration (ml)

b = HCl used in blank titration (ml)

S = weight of air dry sample (g)

m.c.f = moisture correcting factor (100 + % moisture)/ 100

1.4 = 1.4 x 0.001 x 100 % (14 = atomic weight of nitrogen)

3.5.5 Available phosphorus

The readily acid-soluble forms of P were extracted with a HCl: NH₄F mixture (Bray's No. 1 method) as described by Bray and Kurtz (1945) and Olsen and Sommers (1982). Phosphorus in the extract was determined on a spectrophotometer by the blue ammonium molybdate method with ascorbic acid as a reducing agent. A 2 g soil sample was weighed into a 50 ml shaking bottle and 20 ml of extracting solution of Bray (0.03 *M* NH₄F and 0.025 *M* HCl) was added. The sample was shaken for one minute by hand and then immediately filtered through Whatman No 42 filter paper. One ml of the standard series, the blank and the extract, 2 ml boric acid and 3 ml of the colouring agent (ammonium molybdate and antimony tartarate solution) were pipetted into a test tube and homogenized.

The solution was allowed to stand for 15 minutes for the blue colour to develop to its maximum. The absorbance was measured on a Spectronic 21D Spectrophotometer at 660 nm wavelength. A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg was prepared from a 12 mg P/l stock solution by diluting 0, 10, 20, 30, 40 and 50 ml of 12 mg P/l in 100 ml volumetric flask and made to the volume with distilled water. Aliquots of 0, 1, 2, 4, 5, and 6 ml of the 100 mg P/l of the standard solution were put in 100 ml volumetric flask and made to the 100 ml mark with distilled water.

Calculation:

$$P \text{ (mg/kg)} = \frac{(a-b) \times 20 \times 6 \times m.c.f}{S}$$

where;

a = mg/l P in sample extract

b = mg/l P in blank

S = sample weight (g)

m.c.f = moisture correction factor

20 = extracting solution (ml)

6 = final sample solution (ml)

3.5.6 Extraction of the exchangeable bases

A 10 g sample was transferred into a leaching tube and leached with 250 ml of 1 M buffered ammonium acetate (NH₄OAc) solution at pH 7.0 (Black, 1982).

3.5.6.1 Determination of calcium and magnesium

For calcium and magnesium, a 25 ml portion of the extract was transferred into an Erlenmeyer flask and the volume made to 50 ml of distilled water. A 10 ml portion of hydroxylamine hydrochloride, 1 ml of 2 % potassium cyanide (from a burette), 1 ml 2 % potassium ferrocyanide, 10 ml ethanolamine buffer and 0.2 ml Eriochrome Black T solution were added. The solution was titrated with 0.01 *M* EDTA (ethylene diamine tetracetic acid) to a pure turquoise blue colour. A 20 ml 0.01 *M* magnesium chloride solution was also titrated with 0.001 *M* EDTA in the presence of 25 ml of 1 *M* ammonium acetate solution to provide a standard blue colour for the titration.

3.5.6.2 Determination of calcium only

A 25 ml portion of the extract was transferred into a 250 ml Erlenmeyer flask and the volume made to about 50 ml with distilled water. Hydroxylamine hydrochloride (1 ml), potassium cyanide (1 ml of 2 % solution) and potassium ferrocyanide (1 ml of 2 %) were added. After a few minutes, 4 ml of 8 *M* potassium hydroxide and a murexide indicator were added. The solution obtained was titrated with 0.01 *M* EDTA solution to a pure blue colour. Twenty millilitres of 0.01 *M* calcium chloride solution was titrated with 0.01 *M* EDTA in the presence of 25 ml 1 *M* ammonium acetate solution to provide a standard pure blue colour.

Calculation:

$$\text{Ca + Mg (or Ca) (cmol}_c\text{/kg soil)} = \frac{0.01 \times (V_a - V_b) \times 100}{0.1 W}$$

where;

W = weight in grams of oven-dry soil extracted

V_a = 0.01 *M* EDTA used in titration (ml)

V_b = 0.01 *M* EDTA used in blank titration (ml)

0.01= concentration of EDTA used

3.5.6.3 Exchangeable potassium and sodium determination

Potassium and sodium in the percolate were determined by flame photometry. A standard series of potassium and sodium were prepared by diluting both 1000 mg/l potassium and sodium solution to 100 mg/l which was done by taking a 25 ml portion of each into one 250 ml volumetric flask and made to volume with water. Portions of 0, 5, 10, 15, and 20 ml of the mg/l standard solutions were transferred into 200 ml volumetric flasks respectively. Hundred millilitres of 1 M NH₄OAC solution was added to each flask and made to volume with distilled water. The standard series obtained was 0, 2.5, 5.0, 7.5, 10.0 mg/l for potassium and sodium. Potassium and sodium were measured directly in the percolate by flame photometry at wavelengths of 766.5 and 589.0 nm respectively.

Calculation:

$$\text{Exchangeable K (cmol}_c\text{/kg soil)} = \frac{(a-b) \times 250 \times \text{m.c.f}}{10 \times 391 \times S}$$

$$\text{Exchangeable Na (cmol}_c\text{/kg soil)} = \frac{(a-b) \times 250 \times \text{m.c.f}}{10 \times 23 \times S}$$

where;

a = mg/l K or Na in the diluted sample percolate

b = mg/l K or Na in the diluted blank sample percolate

S = air dried sample weight of soil (g)

m.c.f = moisture correcting factor

3.5.6.4 Exchangeable acidity

Exchangeable acidity is defined as the sum of Al + H. The soil sample extracted with unbuffered 1 M KCl, and the sum of Al + H was determined by titration (Page *et al.*, 1982). Fifty grammes of soil sample was put in a 200 ml plastic bottle and 100 ml of 1 M KCl solution added. The bottle was capped and shaken for 2 hours and then filtered. Fifty millilitres portion of the filtrate was taken with a pipette into a 250 ml Erlenmeyer flask and 3 drops of phenolphthalein indicator solution added. The solution was titrated with 0.1 M NaOH until the colour just turned permanently pink. A blank was included in the titration.

Calculation:

$$\text{Exchangeable acidity (cmol/kg soil)} = \frac{(a-b) \times M \times 2 \times 100 \times \text{m.c.f}}{S}$$

where;

a = NaOH used to titrate with blank (ml)

b = NaOH used to titrate with sample (ml)

S = air dried soil sample weight (g)

2 = 100/50 (filtrate/pipetted volume)

m.c.f = moisture correction factor (100 + % moisture) / 100)

M = molarity of NaOH solution

3.5.6.5 Effective cation exchange capacity (ECEC)

Effective cation exchange capacity was calculated by the sum of exchangeable bases (Ca^{2+} , Mg^{2+} , K^+ , and Na^+) and exchangeable acidity ($\text{Al}^{3+} + \text{H}^+$).

3.6.1 Soil biological analysis

Microbial N and P were determined prior to the experiment on field moist samples soon after sampling using the auger.

3.6.1.1 Soil microbial nitrogen

The method of chloroform fumigation and extraction as described by Ladd and Amato (1989) was used to determine the microbial biomass. Ten gram field moist soil samples, after passing through a 4 mm mesh, were each put in 50 ml beakers and placed in large jars (1.0 L). A small beaker containing 10 ml of chloroform was put by one of the soil samples in the jar and the other sample without chloroform (control). The jars were covered and allowed to stand at room temperature for 5 days. Immediately after fumigation, a 100 ml 0.5 M K₂SO₄ solution (Tate *et al*, 1988; Joergenson and Brookes. 1990) was used to extract microbial carbon from the lysed microorganisms. The total nitrogen was determined by the Kjeldahl method in the same extract. For biomass N calculation, k-factor of 0.45 (Jenkinson, 1988; Ross and Tate, 1993) was used. The following equation was used to estimate the microbial nitrogen from the extracted carbon:

$$\text{Microbial N (mg/kg)} = E_c/k \quad (\text{Sparling and West, 1988})$$

where;

E_c = the extracted carbon produced following fumigation

k = the fraction of the killed biomass extracted as nitrogen under standardized conditions.

3.6.1.2 Soil microbial biomass phosphorus

Microbial P was determined by the chloroform fumigation extraction method allowing for correction of P sorption occurring during fumigation and extraction (Morel *et al.*, 1997; Gijsman *et al.*, 1997). The fumigated samples were extracted using the Bray-1 method. Correction for adsorption of P during fumigation was made by simultaneously

equilibrating unfumigated soil with a series of P containing standard solutions followed by extraction with the Bray-1 solution. The amount of chloroform released P was determined according to the relationship between P added (from standard solutions or microbial lysis) and P extracted by the Bray-1 solution (Oberson *et al.*, 1997). Phosphorus adsorption during equilibrium is described by the following equation according to Barrow and Shaw (1975) and adapted by Morel *et al.* (1997);

$$\text{Ext}_p = \text{Ext}_0 + b_1 \text{Pad}^{b_2}$$

where;

Ext_p = Pi concentration (mg/kg) extracted after equilibration with different amounts of P added.

Ext_0 = Pi concentration extracted without P addition.

b_1 , b_2 = coefficients estimated by non-linear regression of mean values of Ext_p against Pad .

Pad = amount of P added (0-20 mg/kg).

Chloroform released P corresponds to P addition and is calculated from the equation:

$$P_{\text{chl}} = [(\text{Ext}_{\text{chl}} - \text{Ext}_0)/b_1]^{1/b_2}$$

where;

P_{Chi} = chloroform released P (mg/kg).

Ext_{chi} = Pi concentration in extracts of fumigated samples.

The amount of microbial P is estimated by assuming a K_p factor of 0.4 (Brookes *et al.*, 1982; Mclaughlin *et al.*, 1986).

3.7 Agro-economic analysis

Records were kept on materials, equipment and labour used for the trials. The cost analysis was done by comparing the phosphorus sources and microbe plus treatments with the standard practice and the absolute control. Using prevailing market prices, the cost of production for 1000 oil palm seedlings was evaluated.

3.8 Statistical analysis

All agronomic and soil data obtained were subjected to analysis of variance (ANOVA) using Genstat statistical package (Genstat Discovery Edition, 2012) and separation of means was done by the use of least significance difference method at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Effect of soil amendments on growth of oil palm seedlings

4.1.1 Number of fronds per seedling

The effect of P fertilizers and microbe plus on seedling frond numbers is shown in Table 4.1. There was a gradual increase in frond numbers as growth progressed, however, at 9 MAT, about 10 % reduction in frond numbers was observed as a result of general pruning after which a steady rise was recorded. At 12 MAT, the growth pattern on both the P fertilizers and microbe plus treated plots were similar in that the frond numbers per seedling were relatively uniform and the effects were not significantly ($p>0.05$) different from one another. Similarly, microbe plus treated seedlings had no significant ($p>0.05$) effect on fronds produced. Frond numbers produced was in the order of $P_0 > TRP > SRP > TSP$ for P fertilizers and $MP_{150} > MP_0 > MP_{100} > MP_{50}$ for MP rates at 12 MAT.

Appendix I showed the combined effect of P fertilizers and microbe plus on frond numbers of seedlings. The combined use of P fertilizers and microbe plus increased frond numbers throughout the experimental period, except on 9 MAT due to pruning, yet the effects were not significantly ($p>0.05$) different from one another. It was however observed that frond numbers produced were relatively uniform at 12 MAT.

Table 4.1. Effect of P fertilizers and Microbe plus rates on number of fronds

P fertilizers	Number of fronds (No./plant)							
	5 MAT	6 MAT	7 MAT	8MAT	9 MAT	10 MAT	11 MAT	12 MAT
P ₀	5.83	6.83	7.00	7.50	6.33	7.50	9.00	9.50
TSP	5.58	6.92	7.00	7.12	6.25	7.42	8.75	9.25
SRP	5.75	6.92	7.00	7.42	6.58	7.75	8.75	9.33
TRP	5.67	7.00	7.00	7.33	6.50	7.58	9.00	9.42
Pr	0.80	0.95	1.00	0.72	0.33	0.68	0.75	0.81
Lsd (0.05)	0.53	0.59	0.54	0.62	0.40	0.58	0.66	0.55
Microbe plus								
MP ₀	5.67	6.83	7.00	7.58	6.50	7.75	9.00	9.33
MP ₅₀	5.75	7.08	7.08	7.33	6.42	7.50	8.83	9.25
MP ₁₀₀	5.75	6.92	7.08	7.42	6.25	7.58	8.92	9.25
MP ₁₅₀	5.67	6.83	7.08	7.08	6.50	7.42	8.75	9.67
Pr	0.98	0.80	0.75	0.43	0.55	0.68	0.88	0.36
Lsd (0.05)	0.53	0.59	0.53	0.62	0.40	0.58	0.66	0.55
Pr P fert.* MP	0.39	0.94	0.92	0.92	0.07	0.06	0.50	0.94
CV (%)	11.21	10.28	9.18	10.05	7.51	9.18	8.90	7.82

4.1.2 Butt of seedlings

The application of P fertilizers, irrespective of source, and microbe plus rates had no significant effect ($p>0.05$) on seedlings butt throughout the experimental period (Table 4.2). As expected, seedlings butt increased progressively from 5 MAT to 12 MAT. Though not significant, P₀ treated palms performed better than the P fertilizers on butt increment at 12 MAT. The increase in seedling butt on P₀ plots over the P fertilizers was 2

% over TSP, 4 % over SRP and 2 % over TRP. Similarly, MP₀ treated palms produced the higher butt than the MP rates with 6 % increase over MP₅₀, and 5 % increase over MP₁₀₀ and MP₁₅₀ at 12 MAT. The ranking of this parameter was in the order of P₀ > TSP > TRP > SRP for P fertilizers and MP₀ > MP₁₀₀ > MP₁₅₀ > MP₅₀ for microbe plus rates (Table 4.2).

Table 4.2. Effect of P fertilizers and Microbe plus rates on seedlings butt

P fertilizers	Seedling butt (cm)							
	5MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	12 MAT
P ₀	1.37	1.93	2.35	2.74	3.33	3.74	4.04	5.31
TSP	1.43	1.88	2.32	2.75	3.27	3.73	4.08	5.18
SRP	1.37	1.88	2.33	2.70	3.26	3.71	4.02	5.13
TRP	1.37	1.90	2.30	2.73	3.20	3.70	4.13	5.18
Pr	0.92	0.65	0.84	0.88	0.78	0.10	0.96	0.92
Lsd (0.05)	0.09	0.09	0.12	0.14	0.26	0.33	0.43	0.57
Microbe plus								
MP ₀	1.39	1.93	2.34	2.76	3.37	3.77	4.17	5.41
MP ₅₀	1.38	1.90	2.34	2.72	3.35	3.70	4.06	5.10
MP ₁₀₀	1.32	1.91	2.33	2.74	3.24	3.74	4.00	5.16
MP ₁₅₀	1.36	1.86	2.29	2.70	3.20	3.68	4.04	5.14
Pr	0.34	0.46	0.74	0.85	0.58	0.93	0.86	0.70
Lsd (0.05)	0.09	0.09	0.12	0.14	0.26	0.33	0.43	0.57
Pr P fert.* MP	0.09	0.01	0.17	0.12	0.93	0.77	0.72	0.89
CV (%)	7.6	5.9	6.0	6.2	9.5	10.5	12.6	13.1

The combined effect of P fertilizers and microbe plus application on seedlings butt is shown in Appendix II. The increase in butt was gradual and consistent from 5 MAT to 12 MAT, however, this progression did not result in significant ($p>0.05$) differences between treatments except at 6 MAT (Figure 4.1). TRP + MP₀ treated palms produced the highest seedling butt of 5.64 cm (11 % increase over the control), closely followed by SRP + MP₀ of 5.55 cm (9 % increase over the control) at 12 MAT. Contrarily, the conjoint use of TSP and MP turned to be the best in terms of butt growth, on the average, than SRP and MP at 12 MAT (Appendix II).

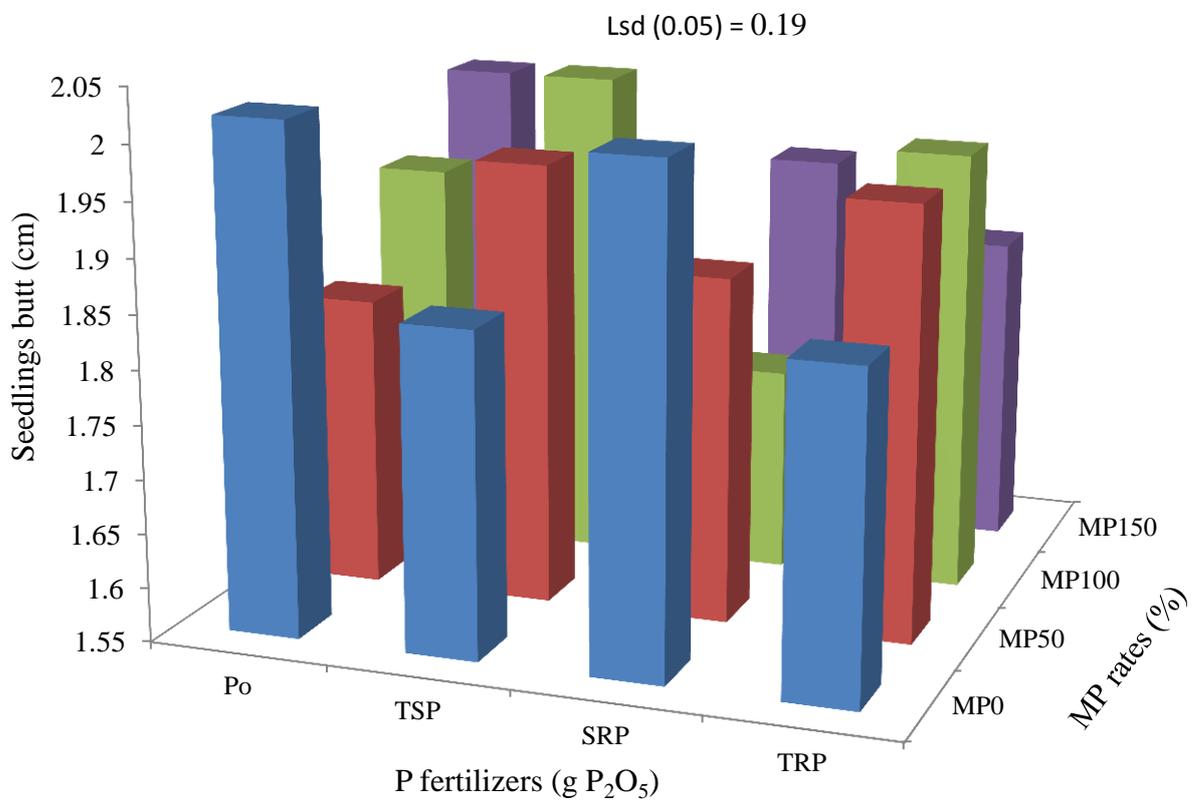


Figure 4.1: Interaction effect of P fertilizers and microbe plus on seedlings butt at 6 MAT.

4.1.3 Seedling height

The effect of P fertilizers and microbe plus application on seedlings height is shown in Table 4.3. There was a general progression in height from 5 MAT to 12 MAT, however, P fertilizers had no significant ($p>0.05$) effect on seedlings height throughout the experimental period. The highest height of 88.90 cm was recorded on P_0 plot at 12 MAT.

The combined effect of P fertilizers and microbe plus as shown in Appendix III was not significantly ($p>0.05$) different from one another irrespective of the treatments throughout the experiment. Yet, improvements in height attributed to the application of $SRP + MP_0$ recorded the highest height of 95.30 cm which was 12 % taller than the control. This was followed by $P_0 + MP_{100}$ (94.30 cm) which was 11 % taller than the control at 12 MAT. As indicated in Appendix III, the $P_0 + MP_0$ treated palms were taller than the other combinations. However, the least profitable combination on height growth turned out to be TRP with MP rates.

Table 4.3 Effect of P fertilizers and microbe plus rates on seedlings height

P fertilizers	Seedling height (cm)							
	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	12 MAT
P ₀	29.33	31.00	37.25	44.83	57.75	64.33	69.00	88.90
TSP	29.08	31.00	36.33	45.25	58.00	62.67	68.90	86.40
SRP	29.08	31.17	36.58	43.50	56.83	63.42	69.00	87.00
TRP	29.25	31.83	36.00	45.42	55.42	63.42	67.30	86.00
Pr	0.99	0.85	0.69	0.76	0.70	0.94	0.94	0.94
Lsd (0.05)	1.87	2.21	2.17	3.98	4.89	5.28	6.64	10.52
Microbe plus								
MP ₀	29.58	31.42	36.17	45.08	58.08	64.50	69.80	88.60
MP ₅₀	29.00	30.75	36.08	43.58	56.58	63.33	66.30	84.70
MP ₁₀₀	29.17	31.25	37.58	45.67	56.83	64.42	69.20	88.20
MP ₁₅₀	29.00	31.58	36.33	44.67	56.50	61.58	68.80	86.80
Pr	0.91	0.88	0.47	0.75	0.90	0.65	0.72	0.87
Lsd (0.05)	1.87	2.21	2.17	3.98	4.89	5.28	6.64	10.52
Pr P fert.* MP	0.72	0.51	0.28	0.58	0.97	0.92	0.92	0.94
CV (%)	7.7	8.5	7.1	10.7	10.3	10.0	11.6	14.5

4.1.4 Leaf area and leaf area index of seedlings

The results presented in Table 4.4 showed the effect of P fertilizers and microbe plus application on LA and LAI of seedlings. Phosphorus fertilizers had no significant ($p>0.05$) effect on LA and LAI of seedlings. Though not significant, SRP recorded the highest LA of 0.32 m^2 and contributed 7 % increase over the P_0 . This corresponded to a higher LAI of 0.69 with 21 % increase over the P_0 . The sole application of the different levels of microbe plus also had no significant ($p>0.05$) effect on LA and LAI measured. The highest LA of 0.32 m^2 was recorded by MP_{100} , which gave 7 % increase over the MP_0 , however, MP_{150} recorded the highest LAI of 0.66 with 2 % increase over the MP_0 .

Appendix IV showed the combined effect of P fertilizers and microbe plus application on LA and LAI of seedlings. The data recorded showed no significant ($p>0.05$) combined effect on LA and LAI. LA recorded by $P_0 + MP_{100}$ and $SRP + MP_0$ was higher than the other combinations (0.35 m^2) with 35% increase over the control and 25 % increase over the standard practice ($TSP + MP_0$). This was closely followed by $SRP + MP_{100}$ (0.34 m^2) with 31 % increase over the control. The least profitable combination in terms of LA turned out to be $TRP + MP_{50}$ which recorded 0.21 m^2 with 19 % and 25 % decrease compared to the control and $TSP + MP_0$. Moreover, LAI recorded by $P_0 + MP_{100}$, $SRP + MP_0$ and $SRP + MP_{100}$ was higher (0.75) compared to the other combinations and represented 32 % increase over the control and 26 % increase over the $TSP + MP_0$. This was followed by $TSP + MP_{150}$ (0.74) which also gave 30 % increase over the control. The lowest LAI of 0.45 was recorded by $TRP + MP_{50}$ which represented 37 % decrease compared to the control. It was however observed that the combination of TRP and MP recorded lower LA and LAI on the average compared to the sole application of MP and its interactions with TSP or SRP.

Table 4.4 Effect of P fertilizers and Microbe plus rates on Leaf Area (LA) and Leaf Area Index (LAI) of seedlings

	LA (m ²)	LAI
P fertilizers		
P ₀	0.30	0.57
TSP	0.30	0.65
SRP	0.32	0.69
TRP	0.27	0.59
Pr	0.49	0.14
Lsd (0.05)	0.07	0.12
Microbe plus		
MP ₀	0.30	0.65
MP ₅₀	0.27	0.59
MP ₁₀₀	0.32	0.61
MP ₁₅₀	0.30	0.66
Pr	0.50	0.64
Lsd (0.05)	0.07	0.12
Pr P fert.* MP	0.70	0.37
CV (%)	26.29	22.44

4.1.5 Seedlings dry matter production

The results presented in Table 4.5 showed dry matter produced by the application of P fertilizers and microbe plus. As shown, there was no significant ($p < 0.05$) effect on dry matter produced by the P fertilizers at the end of the experiment. The SRP treated seedlings produced the highest dry weight of roots (35.71 g) compared to the P fertilizers. Dry weight of butt produced by TSP was 23 % significant increase over the P₀. This corresponded to a

higher frond dry weight of 123.33 g which was 23 % higher than the P₀. With regard to roots dry weight under microbe plus rates, MP₀ treatment produced significantly (p<0.05) higher root biomass than the other rates. However, MP₁₅₀ treated seedlings produced higher dry weight of butt and fronds which were 34 % higher in butt dry weight and 42 % higher in frond dry weight over the MP₀. The trend of biomass produced under MP was MP₁₅₀ > MP₁₀₀ > MP₅₀ > MP₀.

The combined use of P fertilizers and microbe plus rates significantly (p<0.05) improved dry matter partitioning in roots at the end of the experiment (Figure 4.2). The control produced significantly (p<0.05) higher components partitioning of roots, however, TSP + MP₀ treated palms recorded the lowest dry matter partitioning to the roots (56 % decrease compared to the control), significantly lower than the other combinations. TSP + MP₁₀₀ gave 47 % increase in butt dry weight and 40 % increase in frond dry weight over the control respectively (Appendix V). The highest total dry matter (285.50 g) produced by TSP + MP₁₅₀ was 42 % higher than the control, whilst the lowest of 124.65 g produced by TSP + MP₀ indicated 38 % decrease over the control. Generally, it was observed that TRP and its interactions with microbe plus produced the lowest total dry matter on the average, as compared to microbe plus and its interactions with TSP or SRP. Notwithstanding, TSP and microbe plus rates produced the highest dry matter partitioning in butt and fronds at the end of the experiment (Appendix V).

Table 4.5 Effect of P fertilizers and MP rates on seedling dry matter production

P fertilizers	Dry weight (g/seedling)			
	Fronds	Butt	Roots	Total
P ₀	100.10	45.40	35.71	181.21
TSP	123.33	55.90	29.74	208.97
SRP	99.03	55.50	38.06	192.59
TRP	90.26	49.29	31.29	170.84
Lsd (0.05)	30.86	12.73	7.68	
Pr	0.18	0.28	0.12	
Microbe plus				
MP ₀	87.42	44.49	36.06	167.97
MP ₅₀	89.73	46.54	33.73	170.00
MP ₁₀₀	111.19	55.31	31.90	198.40
MP ₁₅₀	124.37	59.75	33.11	217.23
Pr	0.06	0.06	0.73	
Lsd (0.05)	30.86	12.73	7.68	
Pr P fert.* MP	0.07	0.08	0.03	
CV (%)	35.90	29.60	27.30	

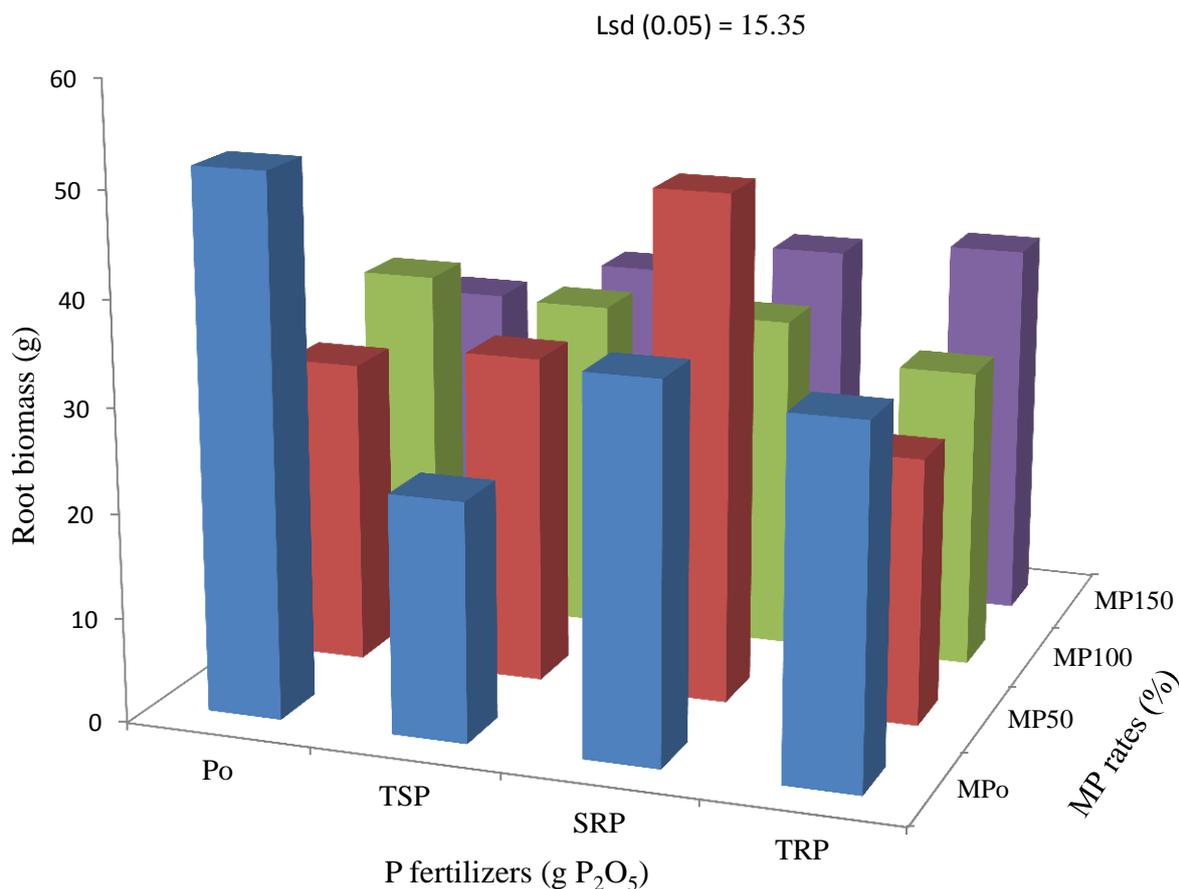


Figure 4.2: Interaction effect of P fertilizers and microbe plus on root biomass at 12 MAT.

4.1.6 Nutrient concentration of fronds

The percentage nutrient concentrations in seedlings fronds at 12 MAT is shown in Table 4.6. The use of both P fertilizers and microbe plus significantly ($p < 0.05$) influenced the nutrient concentrations of the fronds. In the case of P fertilizers, the P₀ treated palms recorded significantly ($p < 0.05$) higher and optimum N content (2.53 %) whilst TSP recorded optimum P content (0.23 %). K and Ca contents (0.47 and 0.36 % respectively) were significantly ($p < 0.05$) higher in MP₀ treated palms than all other treated palms. Similarly, Mg content (0.37 %) in MP₁₅₀ was significantly ($p < 0.05$) higher than the other treated palms.

Table 4.6. Effect of P fertilizers and microbe plus rates on frond nutrient content

P fertilizers	Nutrient concentration (%)				
	N	P	K	Ca	Mg
P ₀	2.53	0.16	0.42	0.28	0.28
TSP	2.29	0.23	0.41	0.23	0.35
SRP	2.10	0.20	0.41	0.31	0.27
TRP	2.30	0.15	0.40	0.26	0.28
Pr	<.001	<.001	0.07	<.001	<.001
Lsd (0.05)	0.02	0.01	0.02	0.01	0.01
Microbe plus					
MP ₀	2.48	0.16	0.47	0.36	0.27
MP ₅₀	2.25	0.18	0.40	0.26	0.24
MP ₁₀₀	2.26	0.21	0.39	0.24	0.29
MP ₁₅₀	2.23	0.20	0.39	0.21	0.37
Pr	<.001	<.001	<.001	<.001	<.001
Lsd (0.05)	0.02	0.01	0.02	0.01	0.01
Pr P fert.* MP	<.001	<.001	<.001	<.001	<.001
CV (%)	0.9	7.1	4.2	4.3	5.8

Table 4.7. Interaction effect of P fertilizers and Microbe plus rates on frond nutrient content

P fert. + MP	Nutrient concentration (%)				
	N	P	K	Ca	Mg
Po + MP ₀	3.17	0.16	0.47	0.32	0.23
Po + MP ₅₀	2.43	0.12	0.44	0.32	0.25
Po + MP ₁₀₀	2.39	0.19	0.40	0.32	0.25
Po + MP ₁₅₀	2.14	0.17	0.38	0.16	0.40
TSP + MP ₀	2.72	0.19	0.50	0.32	0.27
TSP + MP ₅₀	2.10	0.24	0.37	0.29	0.27
TSP + MP ₁₀₀	2.10	0.34	0.41	0.13	0.42
TSP + MP ₁₅₀	2.26	0.14	0.37	0.16	0.42
SRP + MP ₀	2.06	0.16	0.47	0.42	0.34
SRP + MP ₅₀	2.14	0.21	0.43	0.26	0.13
SRP + MP ₁₀₀	2.02	0.13	0.37	0.19	0.30
SRP + MP ₁₅₀	2.18	0.30	0.38	0.35	0.30
TRP + MP ₀	1.98	0.13	0.44	0.38	0.25
TRP + MP ₅₀	2.35	0.13	0.37	0.16	0.32
TRP + MP ₁₀₀	2.51	0.16	0.37	0.32	0.17
TRP + MP ₁₅₀	2.35	0.17	0.43	0.16	0.36
Pr	<.001	<.001	<.001	<.001	<.001
Lsd (0.05)	0.03	0.02	0.03	0.02	0.03
CV (%)	0.9	7.1	4.2	4.3	5.8

As presented in Table 4.7, the combined use of P fertilizers and microbe plus rates significantly ($p < 0.05$) influenced the concentrations of nutrients in seedlings fronds sampled. Based on the optimum nutrient levels (as 2.6 – 3.0 % N, 0.16 – 0.25 % P, 1.10 – 1.80 % K, 0.50 – 1.70 % Ca, 0.30 – 0.70 % Mg) and deficient nutrient levels (as % N < 2.50, % P < 0.15, % K < 1.00, % Ca < 0.30, % Mg < 0.20) in oil palm seedlings fronds reported by von Uexkull and Fairhurst (1999), N concentrations were optimum only in control ($P_0 + MP_0$) and TSP + MP_0 treated palms; P concentrations were in excess only in TSP + MP_{100} treated palms; Mg levels were optimum in SRP + MP_{150} , TRP + MP_0 , TSP + MP_{100} , TSP + MP_{150} and $P_0 + MP_{150}$ treated palms. P deficient contents were recorded in $P_0 + MP_{50}$, TSP + MP_{150} , SRP + MP_{100} , TRP + MP_0 and TRP + MP_{50} treated palms and indicated their negative interactions in terms of frond P content of seedlings. On the contrary, K and Ca contents were deficient in all the combinations at the end of the experiment (Table 4.7).

4.2 Effects of soil amendments on soil pH and soil available P

4.2.1 Soil pH

The application of P fertilizers and microbe plus alone significantly ($p < 0.05$) influenced the pH of the medium at 8 MAT (Table 4.8). However, variable pH values were recorded throughout the experiment. TSP treated plots had higher pH at 6 and 10 MAT, positively within the critical range for oil palms (4.0 – 6.5). TRP treated plots on the other hand recorded lower pH of 3.8 on 8 MAT, critically below the range for oil palms. The influence on soil pH following microbe plus application was higher in MP_{100} treated medium (6.0 at 6 MAT and 5.2 at 10 MAT) which was also within the range for oil palms. Besides, MP_{50} treated medium had lower pH (3.9) at 8 MAT critically below the range for oil palms (Table

4.8). The order of increasing pH was TRP < P₀ < SRP < TSP for P fertilizers and MP₁₅₀ < MP₅₀ < MP₀ < MP₁₀₀ for microbe plus rates.

Table 4.8 Effect of P fertilizers and Microbe plus rates on soil pH of the medium

P fertilizers	Soil pH		
	6 MAT	8 MAT	10 MAT
P ₀	5.6	4.9	5.0
TSP	5.9	4.8	5.1
SRP	5.8	4.6	5.0
TRP	5.8	3.8	4.9
Pr	0.89	<.001	0.22
Lsd (0.05)	0.78	0.40	0.17
Microbe plus			
MP ₀	5.8	4.6	5.1
MP ₅₀	5.4	3.9	5.0
MP ₁₀₀	6.0	4.7	5.2
MP ₁₅₀	6.0	4.9	5.0
Pr	0.42	<.001	0.01
Lsd (0.05)	0.78	0.40	0.17
Pr P fert.* MP	0.10	0.49	<.001
CV (%)	16.3	10.7	4.1

The combined use of P fertilizers and microbe plus rates as shown in Figure 4.3 were significantly ($p < 0.05$) influenced at 10 MAT. There was a considerable decrease in pH values recorded on 8 MAT upon the combined use of P fertilizers and microbe plus after which a steady rise was observed on the 10 MAT except in the control. With the exception of the

interaction between TRP and microbe plus which recorded averagely lower pH below the critical range for oil palm on 8 MAT, the combined application of P fertilizers and microbe plus gave pH values critically within the range suitable for oil palm nursery (Appendix VI). At 10 MAT, TSP + MP₁₀₀ gave significantly ($p < 0.05$) higher pH of 5.6, which represented 9 % positive increase over the control. From the results, the best positive combination with respect to pH turned out to be TSP and microbe plus rates and the least was TRP and microbe plus rates (Appendix VI).

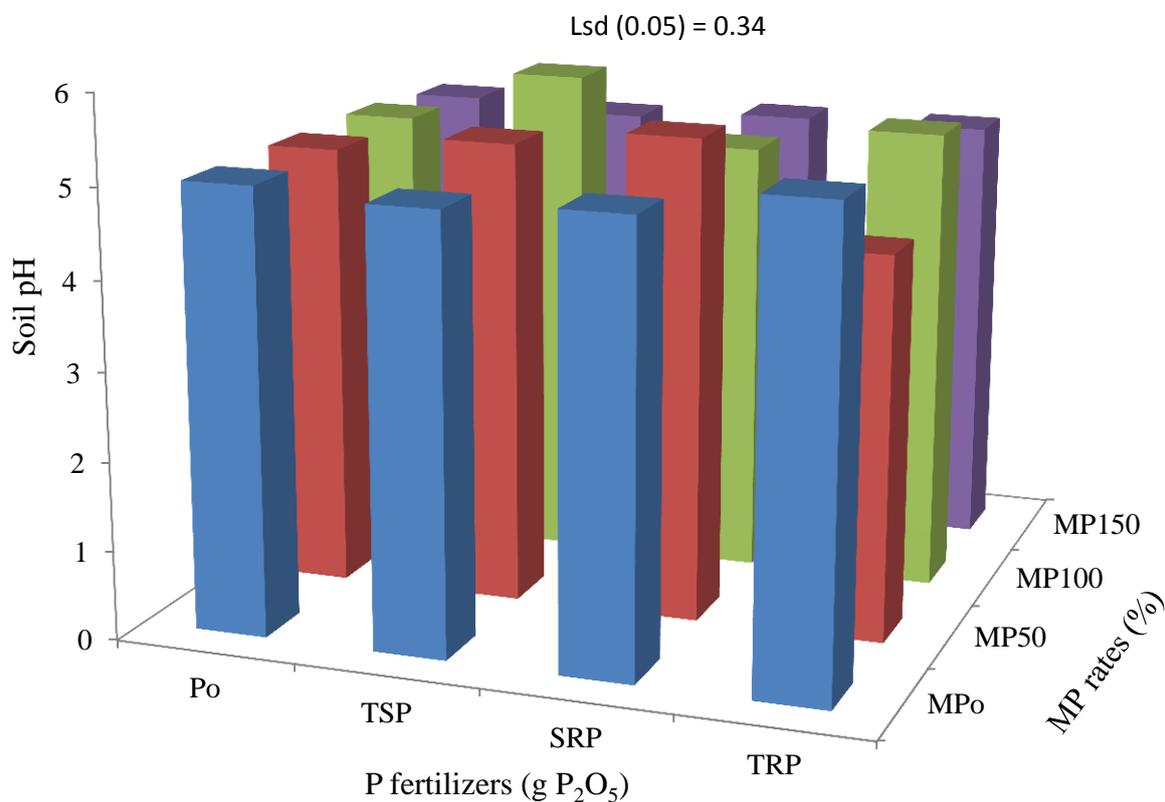


Figure 4.3: Interaction effect of P fertilizers and microbe plus on soil pH at 10 MAT.

4.2.2 Soil available phosphorus

Table 4.9 Effect of P fertilizers and microbe plus rates on soil available P

P fertilizers	Available P (mg/kg)		
	6 MAT	8 MAT	10 MAT
P ₀	55.83	81.65	97.53
TSP	156.48	173.98	180.36
SRP	156.37	113.12	161.34
TRP	149.59	61.13	107.25
Pr	<.001	<.001	<.001
Lsd (0.05)	1.20	1.04	1.12
Microbe plus			
MP ₀	142.39	120.60	138.56
MP ₅₀	138.50	96.80	136.45
MP ₁₀₀	125.71	101.65	142.55
MP ₁₅₀	167.49	110.82	128.92
Pr	<.001	<.001	<.001
Lsd (0.05)	1.20	1.04	1.12
Pr P fert.* MP	<.001	<.001	<.001
CV (%)	1.00	1.20	1.00

The results showed that P fertilizers and microbe plus rates had significant ($p < 0.05$) positive effects on P released into the soil (Table 4.9). The soil available P values recorded by TSP application increased steadily ($156.5 - 180.4 \text{ mg kg}^{-1}$) from 6 to 10 MAT unlike the erratic available P values recorded under SRP and TRP. Available P of $180.36 \text{ mg kg}^{-1}$ under by TSP at 10 MAT gave 85 % significant ($p < 0.05$) increase over the P_0 , 11 % over SRP and 41 % over TRP. The magnitude of available P released upon microbe plus application fluctuated. However, MP_{100} treated plots significantly ($p < 0.05$) recorded only 3 % increase over the MP_0 at 10 MAT. The order of increasing P released into the soil was $TSP > SRP > TRP > P_0$ for P fertilizers and $MP_{100} > MP_0 > MP_{50} > MP_{150}$ for microbe plus rates at 10 MAT.

Results presented in Table 4.10 showed that the combined application of P fertilizers and microbe plus rates significantly ($p < 0.05$) had positive effect on P released into the soil. As presented, the conjoint application of TSP and microbe plus rates consistently increased P release into the soil throughout the sampling months contrary to the other combinations where fluctuated P values were observed. The highest available P of 180.9 mg kg^{-1} recorded by $TSP + MP_{150}$ gave 285 % significant ($p < 0.05$) increase over the control at 10 MAT. The best positive combination in terms of P release into the soil was TSP and microbe plus rates, whereas TRP and microbe plus rates combined recorded the lowest P released into the soil. Generally, there was a consistent decrease in available P in the control plots from 6 to 10 MAT (Table 4.10).

Table 4.10 Intereaction effect of P fertilizers and microbe plus rates on soil available P
Available P (mg/kg)

P fert. + MP	Available P (mg/kg)		
	6 MAT	8 MAT	10 MAT
Po + MP ₀	66.81	95.50	47.04
Po + MP ₅₀	112.40	115.80	144.38
Po + MP ₁₀₀	48.71	45.20	105.32
Po + MP ₁₅₀	151.90	70.10	93.36
TSP + MP ₀	105.56	173.80	180.18
TSP + MP ₅₀	173.49	173.80	180.18
TSP + MP ₁₀₀	173.49	173.80	180.18
TSP + MP ₁₅₀	173.49	174.50	180.90
SRP + MP ₀	166.87	107.70	157.14
SRP + MP ₅₀	173.49	79.50	164.87
SRP + MP ₁₀₀	111.62	140.90	163.12
SRP + MP ₁₅₀	173.49	124.40	160.25
TRP + MP ₀	163.60	105.40	169.90
TRP + MP ₅₀	94.64	18.10	56.37
TRP + MP ₁₀₀	169.02	46.70	121.58
TRP + MP ₁₅₀	171.09	74.30	81.16
Pr	<.001	<.001	<.001
Lsd (0.05)	2.40	2.09	2.23
CV (%)	1.00	1.20	1.00

4.3 Economic analysis

Table 4.11: Cost of raising 1,000 oil palm seedlings at nursery

Expenditure Item	Cost (Ghc)	
	Without fertilizer application	With fertilizer application
A. Preparatory stage		
	100.00	100.00
1. cost of 1000 polybags		
2. 5 labour units @ 5.00 day ⁻¹ for soil collection	25.00	25.00
3. 5 labour units @ 5.00 day ⁻¹ for weighing soil (10 kg) into polybags	25.00	25.00
4. cost of water	30.00	45.00
5. cost of pesticides and fungicides	50.00	50.00
6. 5 labour units @ 5.00 day ⁻¹ for nursery and field layout	25.00	25.00
Sub-total (A)	255.00	270.00
B. Planting		
	500.00	500.00
1. 1000 seed nuts @ 0.50		
2. 10 labour units @ 5.00 day ⁻¹ for sowing and transplanting	50.00	50.00
Sub-total (B)	550.00	550.00
C. Cultural practices		
1. 2 labour units @ 5.00 day ⁻¹ for irrigation	10.00	10.00
2. 2 labour units @ 7.00 bi-weekly for weed control	14.00	14.00
3. 4 labour units @ 5.00 month ⁻¹ for pruning	20.00	20.00
4. 1 labour unit @ 7.00 for pest control	7.00	7.00
5. 5 labour units @ 10.00 month ⁻¹ for fertilizer application	50.00
Sub-total (C)	51.00	91.00
GRAND TOTAL	856.00	921.00

The economic analysis for the production of 1000 oil palm seedlings through the preparatory stage to the transplanting stage is presented in Table 13. The cost analysis as indicated generated Gh¢ 921.00 and Gh¢ 856.00 for raising seedlings with and without fertilizer inputs respectively, considering the various stages of the seedlings growth.

4.4 Relationship between growth parameters and P applied

The results of the correlation matrix for leaf number, butt, height, LA, LAI, available P, SMBC, total dry weight, applied P, N concentration (%) and P concentration (%) are presented in Table 4.14.

A strong positive correlation ($r = 0.87$) was observed between seedling height and butt. A weak positive correlation ($r = 0.33$) was found between soil available P and LAI, as well as, LA and LAI ($r = 0.53$). The correlation between soil available P and nutrient concentration in biomass was positive for biomass P and negative for biomass N.

CHAPTER FIVE

DISCUSSION

5.1 Effect of P fertilizers and microbe plus applications on growth of oil palm seedlings

5.1.1 Number of fronds

The growth pattern in seedlings treated with P fertilizers and microbe plus was similar in the number of fronds per seedling irrespective of the inorganic fertilizers added (Table 4.1). Also seedlings frond numbers produced were not affected by the complimentary use of P fertilizers and microbe plus. This may be due to poorly developed root hairs of seedlings as reported by Phosri *et al.* (2010), which inhibited the uptake and usage of the applied P fertilizers. Garcia *et al.* (2012) also found no significant ($p>0.05$) difference in frond numbers after the application of chemical fertilizers and compost and attributed it to low application rates. Furthermore, the lack of significance in frond numbers may be due to the natural growth habit of producing one frond per month, which may not be influenced by nutrient inputs. Earlier work by Mutert *et al.* (1999) and IICA (2006) reported 5 to 8 functional healthy fronds after 8 months in the nursery. Nonetheless, a similar observation was made in the current study. This according to Gomez *et al.* (2006) is indicative of high photosynthetic rate and therefore the greater possibility of synthesizing biomass.

5.1.2 Seedlings butt

Seedlings with no P fertilizer (P₀) and microbe plus (MP₀) produced larger butt than the treated seedlings (Table 4.2). This lack of significant effects may be due to the fact that P was not limiting in the soil and nutrients other than P influenced seedlings growth. This was contrary to the results of Abidemi *et al.* (2006) when the application of P fertilizers, irrespective of source or rate significantly ($p<0.05$) increased the butt of seedlings after 6

months of growth in the nursery. Akande *et al.* (2011) also reported significant increase in kenaf butt upon complementary use of rock phosphate and manure due to adequate nutrients available.

5.1.3 Seedlings height

As was evident from the study, seedlings height increased over the period following the application of P fertilizers and microbe plus, yet no significant ($p>0.05$) differences were observed (Table 4.3). This suggested that the applied amendments had no influence on seedlings height as indicated by Danso (2008). Perhaps, the inherent sluggish growth pattern of the seedlings could explain the lack of response observed. When P fertilizers were compared, SRP marginally increased seedling height more than the TSP. Thus the expectation that water soluble P fertilizer should have superior effect over rock phosphate was not supported by this result. A similar observation was reported by Abidemi *et al.* (2006). The observed lack of significant ($p>0.05$) effect of the complimentary use of P fertilizers and microbe plus on seedling height (Appendix III) could be attributed to the poorly developed root hairs (Phosri *et al.*, 2010), which inhibited adequate nutrient uptake for seedlings growth. Similarly, Geonadi (1998) using “Emas” biofertilizers, Rochels (2010) using compost and Garcia (2012) using phosphate solubilizing bacteria and compost on palm seedling reported lack of significance due to poor nutrient availability and uptake. Akande *et al.* (2011) also reported similarities in kenaf height upon complementary application of Ogun rock phosphate (ORP) with organic manure and urea. On the contrary, Sharma (2006) reported higher height in wheat when RP was inoculated with PSB (*Pseudomonas striata*). Blal *et al.* (1990) reported 4 to 5 fold increase in RP utilization in micro propagated oil palms after mycorrhization. In the current study, however, complementary use of P fertilizers and microbe plus had no superior effect on seedlings height (Appendix III).

Studies by Mutert *et al.* (1999) showed that after 8 months of nursery, healthy oil palm seedlings should be 0.8 to 1.0 m in height, whereas, TNUA (2013) reported 1 to 1.3 m for seedlings grown for 12 to 14 months in the nursery. Seedling heights averaging 0.8 to 1.0 m obtained in the present study were in agreement with the report of the above authors, which according to TNUA (2013) would maintain higher frond production, bear earlier, produce heavy bunches, give higher fruit/bunch ratio and higher oil to mesocarp in the first year of harvest.

5.1.4 Leaf area (LA) and Leaf area index (LAI)

The lack of significant effect observed showed that P and MP were not the major limiting nutrients in the growth medium. The superior effect of SRP on LA and LAI in relation to P fertilizers was contrary to the findings of Imogie *et al.* (2011) of superior benefits of conventional SSP due to quick solubility over Sokoto rock phosphaite (SRP) and Crystallizer super (CRS) which released nutrients slowly. The marginal increases in LAI following MP₁₅₀ application could be due to the release of more nutrients into the medium as a result of the higher rates. Complementary use of P₀ + MP₁₀₀, SRP + MP₀ and SRP + MP₁₀₀ gave the highest LA and LAI (Appendix IV), due to more nutrient released which also improved the nutrient use efficiency of the seedlings. Similar work by Bello *et al.* (2014) using P fertilizers amended with organic residues was attributed to the fact that organic fertilizers fortified with it enhanced the rate of nutrients released in the rhizosphere for quick absorption for plant growth. LA and LAI values were lower than 1.0 (Table 4.4). Dwarko (2001) reported that in oil palm seedlings, LA remained below 1.0 for some time since the total LA of the young seedling is negligible in relation to the land on which they stand. Studies by Tan and Hardon (1976) revealed significant correlation with many characteristics of the mature palm based on LA and LAIs measured at the later main nursery stage and concluded that nursery selection

based on LA and LAI could result in higher growth rates in the field and higher yields in then oil palm. Subronto *et al.* (1989) on the other hand reported that LA could be used as selection criteria in 9 months old seedlings as each was highly correlated with yield.

5.1.5 Dry matter production

The differences in seedlings dry matter produced among the various nutrient inputs could be attributed to the differences in their formulations. The superior effect of TSP fertilizer on total biomass produced could be ascribed to high solubility of phosphate in TSP (Imogie *et al.*, 2011). Additionally, higher biomass produced by MP₁₅₀ could be due to higher rate of MP applied unlike the observation made by Insemila and Omoti (2003) and Abidemi and Obigbesan (1999) that rates higher than the prescribed rates had no positive effect on RP utilization. The observed higher dry matter produced in TSP + MP₁₅₀ (Appendix V) could be associated with high solubilization and utilization in the metabolic processes of the seedlings especially when increased rates of MP corresponded to an increased biomass produced. This concurred with the results reported by Panhwar *et al.* (2011) in dry matter yields of aerobic rice when P fertilizers inoculated with PSB16 recorded significantly higher dry matter than PSB9 inoculated and the control. The mechanism for lower dry matter produced by the complementary use of TRP and MP rates in this study is not yet clear, perhaps due to antagonism.

The paramount importance of nutrient interactions due to synergistic effects as proposed by Cooke (1982) that could lead to increase yield potential, should be developed. In this study, the marginal increases in MP and its interactions with the P fertilizers or solely applied compared favourably with the standard practice (TSP + MP₀). Vernieri *et al.* (2005) asserted that biofertilizers worked to increase plant nutrient uptake and improve nutrient use efficiency; and that there were no advantages in the use of sole biofertilizers in the promotion

of plant growth (Albregts *et al.*, 1988). However, the selection of appropriate biofertilizer according to Fraser and Percival (2003) is critical as growth effect could vary widely based on the different active organisms used in the formulation of the products. Studies by Obigbesan *et al.* (2002) showed inhibited root growth as resulted from low P supplied, whereas, Goh and Hadter (2003) reported that in P-deficient plants, shoot growth was found to be more affected than root growth due to assimilate partitioning towards the root which led to a decrease in the shoot : root dry matter ratio. Contrarily, the results obtained in this study was not in support of the above authors' report as shoot : root growth of MP treated seedlings performed favourably with the P treatments, as well as, their interactions (Appendix V).

5.1.6 Frond nutrient concentration

Based on the optimum nutrient levels (as 2.6 – 3.0 % N, 0.16 – 0.25 % P, 1.10 – 1.80 % K, 0.50 – 1.70 % Ca, 0.30 – 0.70 % Mg) and deficient nutrient levels (as % N < 2.50, % P < 0.15, % K < 1.00, % Ca < 0.30, % Mg < 0.20) in oil palm seedlings fronds reported by von Uexkull and Fairhurst (1999), the deficient N, K, and Ca contents recorded showed that the application of P fertilizers and microbe plus rates had no positive effect on uptake of these nutrients (Table 4.6). The optimum P and Mg contents produced by TSP and MP₁₅₀ treated seedlings indicated their positive influence on the uptake of these nutrients and supported the finding of Lucas *et al.* (1979) and Menon and Chien (1990) when oil palm seedlings and maize plants were treated with different P fertilizers. The complementary application of P fertilizers and microbe plus (Table 4.7) showed optimum N levels only in the control and TSP + MP₀ treated seedlings. This could be due to available native soil nutrients in the control medium, whereas, the TSP with its high solubilization ensured high N availability in the vicinity of plant roots which contributed to high N use efficiency and a corresponded higher N content in the fronds. Contrarily, Dwivedi (1985) reported that high N use

efficiency of seedlings could maintain low level of nutrients in the tissues. The excess P content in TSP + MP₁₀₀ and optimum Mg levels in TSP with MP₁₀₀ and MP₁₅₀ rates treated seedlings supported Huang and Schnitzer (1986) and Orlov's (1995) observation that biofertilizers could enhance the uptake of P and Mg in plants; as accounted for high contribution of MP to this effect. Besides, the deficient K and Ca levels recorded in the complimentary use of P fertilizers and microbe plus (Table 4.7) supported the findings of Bah and Rahman (2004) who observed antagonistic effects between K and Mg in the plants and explained that the absorption of these elements by roots depended on their relative concentrations in the medium. Moreover, the K deficiency level recorded agreed with the findings of Fairhurst and Mutert (1999) that high level of N when K was low resulted in K deficiency because the increased seedling growth required more K. Thus K⁺ uptake increased as concentrations of Ca²⁺ and Mg²⁺ declined in the soil solution and vice versa (Havlin *et al.*, 1999).

5.2 Effect of P fertilizers and microbe plus rates on soil pH and soil available P

5.2.1 Soil pH dynamics

Soil pH measured at 10 MAT ranged from 4.9 to 5.1 for P fertilizers and 5.0 to 5.2 for microbe plus rates which indicated a slightly acidic medium (Table 4.8). This showed that the applied inputs gave pH values within the critical range reported by Bello *et al.* (2014) for oil palms (4.0 – 6.5). TSP recorded marginal increase in pH in relation to the other P fertilizers. RP with high Ca content (12 - 14 %) significantly increased pH in the studies conducted by Zin *et al.* (2005) and Insenmila *et al.* (2006). In contrast, however, the use of RP had no significant effect on pH.

The complimentary application of P fertilizers and microbe plus rates also gave pH values critically within the range for oil palm production, except at 8 MAT when TRP + MP₀, TRP + MP₅₀ and TRP +MP₁₀₀ recorded values below the range for oil palm. The mechanism for this negative influence may, perhaps, be due to antagonism rather than synergism. According to Anilkuma and Wahid (1989), the lowering of the pH following the application of fertilizers is a known phenomenon. Observation at 8 MAT could confirm when the pH values reduced and may be probably due to the H⁺ ions generated in the medium during the nitrification process. Notwithstanding, the slow release of RP reported by Rajan *et al.* (1996), together with the ineffectiveness of RP application in the short time (Geonadi *et al.*, 2000) could account for the decrease in pH as the 8 months duration of the study may not be adequate to achieve the full potential of RPs.

5.2.2 Available P dynamics

Available P in TSP amended soils was consistently higher than the available P in the other P fertilizers amended soils (156.5 – 180.5 mg kg⁻¹) because of its solubility (Table 4.9). This observation supports the findings of Khasawneh and Doll (1978) that the residual effects of soluble P fertilizers were greater than those of rock phosphates in the first 3 or 4 years after application. MP₁₀₀ recorded higher available P with an added advantage in relation to the other rates applied and could be the best rate in terms of P build-up. The variability in available P recorded was in agreement with the assertion of Roy *et al.* (2006) that available nutrients and their degree of availability and accessibility was not a static condition but an ever-changing and very dynamic process due to the various inorganic and biochemical processes that took place. Studies have shown that decreasing soil pH increases RP effectiveness (Prochnow *et al.*, 2010). According to Fankem *et al.* (2006), phosphate solubilization was the result of combined effect of pH decrease and organic acids produced.

Moreover, Ghosal and Chakraborty (2012) reported RP dissolution as linearly correlated with the reverse acidity of the soil. Contrarily, the results obtained at 8 MAT due to interactions did not correspond to these reports (Table 4.10). However, the lowest available P of 18.1 mg kg⁻¹ recorded by TRP + MP₅₀ could be due to low pH measured (3.7) in the same month (Appendix VI). In agreement, Khan *et al.* (2009) observed that phosphorus solubilization of PSB had direct correlation with pH; nevertheless, buffering capacity of the medium reduced the effectiveness of PSB in P released from tricalcium phosphates (Stephen and Jisha, 2009). Soil available P recorded at 10 MAT showed that, TSP either solely applied or combined with MP rates gave significantly ($p < 0.05$) higher available P. This clearly demonstrated the higher soluble nature of TSP and that more P was solubilized and made available in the rhizosphere. Contrarily, Gyaneshwar *et al.* (1999) stated that phosphate solubilization was induced by phosphate starvation (i.e. without P added). Besides, interactions between TRP and rates of MP yielded low available P than the other treatments. The observed antagonistic effect of MP on TRP could be due to immobilization. It was inferred therefore that interactions between SRP and any rate of MP would be superior (in terms of available P) to TRP. The superior effect of SRP was in line with Kaushik and Garg (2004) when RP amended with microbial inoculant improved soil available P; as resulted from the conversion of rock phosphate P to water-soluble form and greater efficiency of the dissolved P in terms of its availability to plants (Khanna *et al.*, 1983). Studies have indicated increased P availability from RP with length of incubation period (Sinclair *et al.*, 1986; Rajan *et al.*, 1987), which contributed to the superior effect of TSP over the RPs in this study. Yet, slow dissolution rates may also be an advantage over soluble fertilizers in soils with very low P-fixing capabilities, as P is less likely to be lost to leaching (Sanyal and De Datta 1991). Roy *et al.* (2006) explained that the amount of nutrients estimated to be available was not a measure of the total available pool of nutrient, but the proportion that correlated significantly

to crop response. The high available P observed in this study could be due to the fact that the study was conducted in polybags and therefore provided a concentrated environment which encouraged the build-up of P in the soil. This condition cannot be ruled out as there was a tendency for P build up in the soil and reduced leaching losses.

5.3 Economics of production

Farmers' decision on how much fertilizer to use for specific crops is linked with commodity prices. In general, higher fertilizer prices combined with lower crop prices cause farmers to use low rates of fertilizers (FAO, 2004). Table 4.13 revealed that the cost of fixed inputs for the production of 1000 polybag oil palm seedlings was Gh¢ 856.00 without the use of fertilizer and Gh¢ 921.00 with fertilizer application for a period of 8 months (5 MAT to 12 MAT). The comparison of different treatment combinations for their economic viabilities (Appendix VIII) revealed that the most cost effective combination was the application of standard practice and microbe plus at 50 % (i.e. TSP + MP₅₀), which accrued a net return on investment between Gh¢ 2 – 10 for a period of 8 months at nursery per palm. Contrary, the least profitable amendment options were MP₁₅₀ with TSP, TRP or SRP. The integrated applications of P fertilizers and MP could be expensive. However, Khan *et al.* (2009) asserted that PSB inoculants/biofertilizers hold great prospects for sustaining crop production with optimized P fertilization; and that of economic and physiological potential of crops, the entire dependence on organic sources of nutrients may not be adequate to attain the highest productivity (Anwar *et al.*, 2005).

5.4 Relationship between growth parameters and applied P

The correlation between seedling height and butt revealed that as much as 76.2 % of the variation in butt could be attributed to the variation in height. This is in agreement with the assertion by Arzai and Aliyu (2010) that the taller the plant, the wider the butt. Similarly, Lucas (1980) observed seedling height and girth to be highly and positively correlated with one another in a nursery study. Among the growth parameters assessed, only LAI correlated positively with available P ($r = 0.325$), suggesting that LAI could be a more responsive growth indicator for oil palm in soil with high available P. Perhaps, the observed relationship between available P and LAI could be due to the fact that P assimilated by plant facilitated cell division and enlargement (Nutri-Facts, 2013) leading to early root and shoot growth and consequently expansion of leaf area.

Mohd and Mohd (2004) asserted that LA directly affects LAI and this was confirmed by the positive relationship between LA and LAI. The observed weak correlation between available P and biomass P ($r = 0.339$) contrasted with the strong positive correlation ($r = 0.90$) reported by Chen *et al.* (2008) in ryegrass. The discrepancies in these results may be due to the inclusion of less reactive rock phosphate in the present study as opposed to the use of only soluble phosphates in the study by Chen *et al.* (2008).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Based on the outcome of the study to improve the solubility of rock phosphate using microbe plus, the following conclusions were drawn:

- i) The application of TSP, SRP and TRP at equivalent rates of 2.8 g P₂O₅ palm⁻¹ and microbe plus at rates of 50, 100 and 150 % had no significant effect on seedling frond numbers, butt, height, leaf area and leaf area index. However, root biomass and frond nutrient concentrations were significantly affected by the applied inputs. The similarities recorded in height, butt, frond numbers, leaf area and leaf area index were due to the inherent sluggish growth nature of the seedlings.
- ii) The application of TSP alone and TSP + MP₁₅₀ produced the highest soil available phosphorus at 12 MAT. This refuted the null hypothesis that the application of microbe plus would improve rock phosphate solubility and eventually increase soil available phosphorus.
- iii) In terms of frond nutrient concentrations, P and Mg contents were optimum in TSP and MP₁₅₀ treated palms. Complementary applications also produced optimum N content in TSP + MP₀, excess P content in TSP + MP₁₀₀ and optimum Mg content in TSP with MP₁₀₀ and MP₁₅₀.

iv) The comparison of the different nutrient inputs indicated that the cost effective input was the standard practice (TSP at 6 g/palm/month) recommended by OPRI, whilst the least cost effective input was MP₁₅₀ with TSP, SRP or TRP.

6.2 Recommendations

1. Microbe plus may be used as a substitute for the standard practice in the oil palm nursery.
2. The recommended monthly application of the fertilizers is necessary to boost the growth of oil palm seedlings.
3. Microbe plus can be used in combination with RP's in the absence of microbial inoculants to enhance solubility.
4. Field experiment is suggested to validate the effect of MP and these RP's on the performance of oil palm.
5. Additional studies with different application rates, both at nursery and at the field considering microbial biomass dynamics, are recommended.

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APPENDICES

Appendix I: Interaction effect of P fertilizers and Microbe plus rates on number of fronds

P fert. + MP	Number of fronds (No./plant)							
	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	12 MAT
Po + MP ₀	6.33	7.00	7.00	7.67	6.33	7.00	8.67	9.00
Po + MP ₅₀	5.67	7.00	7.00	8.00	7.00	7.67	8.67	9.00
Po + MP ₁₀₀	5.67	6.67	7.00	7.33	6.33	7.00	8.67	9.33
Po + MP ₁₅₀	5.67	6.67	7.33	7.67	6.33	7.33	8.67	9.33
TSP + MP ₀	5.33	6.67	7.00	7.33	6.33	7.67	8.33	8.67
TSP + MP ₅₀	5.67	7.00	7.00	7.00	6.33	7.67	8.67	9.67
TSP + MP ₁₀₀	6.00	7.33	7.33	7.33	6.00	7.00	8.67	9.33
TSP + MP ₁₅₀	5.33	6.67	7.00	7.00	6.33	7.00	8.67	9.67
SRP + MP ₀	5.33	6.67	7.33	7.67	6.33	7.00	8.67	9.00
SRP + MP ₅₀	6.67	7.33	7.33	7.67	6.67	7.33	8.67	9.33
SRP + MP ₁₀₀	5.67	6.67	7.00	7.67	7.00	7.00	8.67	9.33
SRP + MP ₁₅₀	6.33	7.00	7.00	7.00	6.33	7.67	8.33	9.00
TRP + MP ₀	5.67	7.00	7.00	7.67	7.00	7.67	8.67	9.00
TRP + MP ₅₀	6.00	7.00	7.00	7.00	6.33	7.33	8.67	9.33
TRP + MP ₁₀₀	5.67	7.00	7.00	7.33	6.67	7.00	8.33	9.00
TRP + MP ₁₅₀	5.33	7.00	7.00	7.33	7.00	7.67	8.33	9.33
Pr	0.39	0.94	0.92	0.92	0.07	0.06	0.50	0.94
Lsd (0.05)	1.94	2.16	1.95	2.25	1.47	2.11	2.40	1.99
CV (%)	11.21	10.28	9.18	10.05	7.51	9.18	8.90	7.82

Appendix II: Interaction effect of P fertilizers and Microbe plus rates on seedlings butt

P fert. + MP	Seedling butt (cm)							
	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	12 MAT
Po + MP ₀	1.47	2.02	2.39	2.72	3.45	3.54	3.79	5.10
Po + MP ₅₀	1.30	1.82	2.30	2.70	3.37	3.89	4.13	5.45
Po + MP ₁₀₀	1.30	1.91	2.36	2.93	3.38	3.93	4.11	5.45
Po + MP ₁₅₀	1.41	1.98	2.35	2.59	3.14	3.61	4.14	5.25
TSP + MP ₀	1.37	1.85	2.33	2.79	3.31	3.78	4.03	5.33
TSP + MP ₅₀	1.40	1.96	2.36	2.84	3.30	3.69	4.16	5.24
TSP + MP ₁₀₀	1.39	2.01	2.45	2.79	3.30	3.78	4.02	5.05
TSP + MP ₁₅₀	1.24	1.71	2.16	2.60	3.18	3.67	4.10	5.11
SRP + MP ₀	1.42	2.01	2.42	2.81	3.28	3.75	4.38	5.55
SRP + MP ₅₀	1.40	1.87	2.36	2.68	3.24	3.65	4.10	4.93
SRP + MP ₁₀₀	1.22	1.74	2.16	2.53	3.28	3.75	3.93	5.16
SRP + MP ₁₅₀	1.45	1.91	2.38	2.77	3.24	3.70	3.68	4.86
TRP + MP ₀	1.32	1.85	2.24	2.70	3.45	4.03	4.49	5.64
TRP + MP ₅₀	1.42	1.95	2.35	2.68	3.09	3.56	3.86	4.77
TRP + MP ₁₀₀	1.35	1.96	2.34	2.71	3.02	3.49	3.95	4.96
TRP + MP ₁₅₀	1.37	1.84	2.26	2.83	3.26	3.73	4.22	5.33
Pr	0.09	0.01	0.17	0.12	0.93	0.77	0.72	0.89
Lsd (0.05)	0.17	0.19	0.32	0.28	0.52	0.65	0.86	1.13
CV (%)	7.6	5.9	6.0	6.2	9.5	10.5	12.6	13.1

Appendix III: Interaction effect of P fertilizers and Microbe plus rates on seedlings height

P fert. + MP	Seedling height (cm)							
	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	12 MAT
Po + MP ₀	30.33	31.33	36.67	44.00	55.33	61.33	67.70	85.00
Po + MP ₅₀	27.67	30.00	36.00	43.33	59.00	66.00	67.33	87.30
Po + MP ₁₀₀	29.67	31.67	39.33	47.00	59.00	68.33	73.30	94.30
Po + MP ₁₅₀	29.67	31.00	37.00	45.00	57.67	61.67	69.70	89.00
TSP + MP ₀	29.33	32.33	37.33	46.67	59.67	65.00	71.00	86.70
TSP + MP ₅₀	28.67	30.00	35.00	44.33	56.67	62.00	65.70	84.70
TSP + MP ₁₀₀	29.67	31.67	38.00	46.00	58.00	63.00	68.00	86.70
TSP + MP ₁₅₀	28.67	29.67	35.00	44.00	57.67	60.67	71.00	87.70
SRP + MP ₀	30.33	32.33	37.33	47.00	60.67	66.33	73.30	95.30
SRP + MP ₅₀	28.67	31.00	37.67	43.33	55.33	63.67	70.30	84.00
SRP + MP ₁₀₀	28.67	30.33	34.67	40.00	55.33	61.33	66.70	88.30
SRP + MP ₁₅₀	28.67	31.00	36.67	43.67	56.00	62.33	65.70	80.30
TRP + MP ₀	28.33	29.67	33.33	42.67	56.67	65.33	67.30	87.30
TRP + MP ₅₀	31.00	31.67	35.67	43.33	55.33	61.67	64.00	82.70
TRP + MP ₁₀₀	28.67	31.33	38.33	49.67	55.00	65.00	69.00	83.70
TRP + MP ₁₅₀	29.00	34.67	36.67	46.00	54.67	61.67	69.00	90.30
Pr	0.72	0.51	0.28	0.58	0.97	0.92	0.92	0.94
Lsd (0.05)	3.74	4.42	4.35	7.96	9.78	10.55	13.29	21.03
CV (%)	7.7	8.5	7.1	10.7	10.3	10.0	11.6	14.5

Appendix IV: Interaction effect of P fertilizers and Microbe plus rates on LA and LAI of seedlings

	LA (m ²)	LAI
P fert. + MP		
P ₀ + MP ₀	0.26	0.57
P ₀ + MP ₅₀	0.26	0.57
P ₀ + MP ₁₀₀	0.35	0.75
P ₀ + MP ₁₅₀	0.32	0.71
TSP + MP ₀	0.28	0.60
TSP + MP ₅₀	0.30	0.64
TSP + MP ₁₀₀	0.30	0.64
TSP + MP ₁₅₀	0.33	0.74
SRP + MP ₀	0.35	0.75
SRP + MP ₅₀	0.31	0.69
SRP + MP ₁₀₀	0.34	0.75
SRP + MP ₁₅₀	0.27	0.58
TRP + MP ₀	0.30	0.66
TRP + MP ₅₀	0.21	0.45
TRP + MP ₁₀₀	0.28	0.62
TRP + MP ₁₅₀	0.28	0.61
Lsd (0.05)	0.13	0.23
Pr	0.70	0.37
CV (%)	26.29	22.44

Appendix V: Interaction effect of P fertilizers and MP rates on seedling dry matter production

P fert. + MP	Dry weight (g)			
	Fronds	Butt	Roots	Total
Po + MP ₀	106.70	42.70	51.60	201.00
Po + MP ₅₀	97.27	31.05	29.30	157.62
Po + MP ₁₀₀	118.40	49.80	33.95	202.15
Po + MP ₁₅₀	78.05	58.05	28.00	164.10
TSP + MP ₀	62.00	39.75	22.90	124.65
TSP + MP ₅₀	101.05	48.35	31.55	180.95
TSP + MP ₁₀₀	149.45	62.95	32.35	244.75
TSP + MP ₁₅₀	180.80	72.55	32.15	285.50
SRP + MP ₀	90.00	32.80	35.85	158.65
SRP + MP ₅₀	81.25	66.65	48.60	196.50
SRP + MP ₁₀₀	114.75	61.05	32.40	208.20
SRP + MP ₁₅₀	110.10	61.50	35.40	207.00
TRP + MP ₀	91.00	62.70	33.90	187.60
TRP + MP ₅₀	79.35	40.10	25.45	144.90
TRP + MP ₁₀₀	62.15	47.45	28.90	138.50
TRP + MP ₁₅₀	128.55	46.90	36.90	212.35
Lsd (0.05)	61.73	25.46	15.35	
Pr	0.07	0.08	0.03	
CV (%)	35.90	29.60	27.30	

Appendix VI: Interaction effect of P fertilizers and Microbe plus rates on soil pH of the medium

P fert. + MP	Soil pH		
	6 MAT	8 MAT	10 MAT
Po + MP ₀	5.7	5.2	5.0
Po + MP ₅₀	5.3	4.1	5.0
Po + MP ₁₀₀	5.5	5.4	5.0
Po + MP ₁₅₀	5.9	4.9	4.9
TSP + MP ₀	5.8	4.6	4.9
TSP + MP ₅₀	6.7	4.2	5.2
TSP + MP ₁₀₀	6.3	4.8	5.6
TSP + MP ₁₅₀	6.1	5.5	4.8
SRP + MP ₀	5.8	4.7	5.0
SRP + MP ₅₀	5.7	5.0	5.4
SRP + MP ₁₀₀	6.0	4.9	4.9
SRP + MP ₁₅₀	5.7	5.1	4.9
TRP + MP ₀	5.7	3.7	5.3
TRP + MP ₅₀	5.2	3.7	4.3
TRP + MP ₁₀₀	6.0	3.7	5.2
TRP + MP ₁₅₀	6.2	4.2	4.9
Pr	0.10	0.49	<.001
Lsd (0.05)	1.57	0.81	0.34
CV (%)	16.3	10.7	4.1

Appendix VII: Cost of fertilizers

Fertilizer	Cost (Ghc)
1. 50 kg of standard practice	75.00
2. 50 kg of TRP	60.00
3. 50 kg of SRP	60.00
4. 1 litter of microbe plus	20.00

Appendix VIII: Cost of fertilizer application monthly

Fertilizer	Unit cost (Ghc)/palm/month
1. 6 g of standard practice (TSP)	0.50
2. 7.5 g of TRP	0.75
3. 8.2 g of SRP	0.75
4. MP ₅₀	1.00
5. MP ₁₀₀	1.25
6. MP ₁₅₀	1.50
7. 6 g of standard practice + MP ₅₀	1.50
8. 7.5 g of TRP + MP ₅₀	1.75
9. 8.2 g of SRP + MP ₅₀	1.75
10. 6 g of standard practice + MP ₁₀₀	1.75
11. 7.5 g of TRP + MP ₁₀₀	2.00
12. 8.2 g of SRP + MP ₁₀₀	2.00
13. 6g of standard practice + MP ₁₅₀	2.00
14. 7.5 g of TRP + MP ₁₅₀	2.25
15. 8.2 g of SRP + MP ₁₅₀	2.25

Table 4.12: Correlation coefficient of selected parameters

	<i>Frond</i>					<i>Avail.</i>		<i>P</i>	Biomass	Biomass
	<i>No.</i>	<i>Butt</i>	<i>Height</i>	<i>LA</i>	<i>LAI</i>	<i>P</i>	<i>TDW</i>	<i>Applied</i>	N	P
Frond No.	1									
Butt	0.167	1								
Height	0.004	0.873*	1							
LA	0.065	0.032	0.027	1						
LAI	-0.056	0.102	0.068	0.528*	1					
Avail. P	-0.16	0.08	-0.004	0.162	0.325*	1				
TDW	-0.108	0.002	0.057	0.189	0.049	0.145	1			
P Applied	0.075	-0.185	-0.053	0.051	-0.036	-0.034	0.256	1		
Biomass N	0.03	-0.056	-0.044	-0.177	-0.28	-0.543*	-0.141	-0.339	1	
Biomass P	0.066	-0.13	-0.061	0.006	-0.008	0.339*	0.162	0.251	-0.183	1

(*) significant at $p < 0.05$, (LA) Leaf area, (LAI) Leaf area index, (TDW) Total dry weight