EFFECT OF MICRO-ORGANISM ON ROCK PHOSPHATE SOLUBILIZATION IN SOYABEAN AND MAIZE PRODUCTION IN THE NORTHERN GUINEA SAVANNA

BY

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Dissertation submitted to The Postgraduate School in Partial Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy (Soil Science)

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DECLARATION

I hereby declare that the contents of this thesis are products of my imagination, formulation and execution. Extraneous sources of information have been duly acknowledged through references and citations. The subject matter herein has not been presented in any previous application for a higher degree at the Ahmadu Bello University or any other University.

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CERTIFICATION

This thesis entitled "Effect of micro-organism on rock phosphate solubilization in soyabean and maize production in the Northern quinea savanna", by Oluwatoyin Abimbola Babalola, meets the regulations governing the awards of the degree of DOCTOR OF PHILOSOPHY of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To Mama, who left everything else to be here and help make it a success.

Also to Fola, Tope, Tolu, and Yewande, you have been so patient, so supportive and so encouraging.

I appreciate you all.

ACKNOWLEDGEMENT

I wish to express my gratitude to Professor V.O. Chude and Dr. J.K. Adu for their guidance, patience, support and most of all their constructive critisisms and invaluable encouragement in the conduct of this study.

I am grateful to Professors J.J. Owonubi and A. Ogungbile, for their fatherly support and encouragment during the course of this study. I wish also to acknowledge the Farming system Research Programme, I.A.R., for funding the field trials.

My sincere gratitude to Messrs U.O. Bello, O.C. chigbu, V. Odigie, L. Lawal, Ilu and E. Ajide, for the technical assistance rendered.

I also wish to express my appreciation to Messrs Asuquo, Ajegena and Miss V. Madu for assistance rendered in Word Processing.

I am grateful to my colleagues, Dr. B.A. Raji and Mr. I.Y. Amapu for their advice and encouragement.

Finally and most importantly, I want to return all glory to the Almighty God, the El-Shaddai, the King of glory, without whom this work will not have been possible.

ABSTRACT

The role of micro-organisms in the solubilization of phosphates from Sokoto rock phosphate (SRP), Togo rock phosphate (TPR) and Single super phosphate (SSP) was studied. Phosphate solubilizing organisms were isolated from Samaru soils, screened and selected in a preliminary laboratory studies. Over 120 organisms were isolated, sixty one of them were able to solubilise P. Twenty organisms were observed to solubilise P to an appreciable extent,and thus further studied in the laboratory, out of which ten were selected for greenhouse studies. It was observed that fungi isolates could solubilize phosphate and consequently release 12-28.9 /ug P_2O_s/g of substrate, while the bacteria isolates released 10.5 - 25.9 ug P_2O_s/g of substrate.

The pattern of phosphorus release varied among the organisms and the length of immobilization also differed. While F_{21} was able to release a range of 18.3 - 60.8 ug P_2O_s/g of substrate in 6 weeks of incubation, another organism, F_{2U}, released 20.9 - 33.8 ug P_2O_s/g of substrate. Ten micro-organisms (5 bacteria and 5 fungi) were selected for further study in the greenhouse.

Maize and soyabean were used as test crops in the greenhouse study. Results from the greenhouse showed that SSP was superior to the rock phosphates in plant height, leaf area and dry matter yield in soyabean at the first cropping. Residual effect of rock phosphate was however, superior to SSP.TPR gave 20% and 3% superior values when cropped to soyabean and maize respectively. Soyabean

also responded to inoculation at first cropping and also at second cropping, the magnitude of response was however, more at the second cropping. The results obtained for maize showed that the inoculation of organisms resulted in retardation of maize growth at first cropping. Whereas, the organisms promoted maize growth at the second cropping. However in soyabean, inoculation of organisms to SSP, Togo rock and Sokoto rock resulted in crop response at both first and second croppings. Among the ten organisms tested in the green house, five were selected for field studies. The organisms were identified as *Rhizopus* sp, *Aspergillus niger, Penicillium* sp, *Pseudomonas striata* and *Bacillus megaterium.*

The results obtained in the field showed that Togo rock phosphate was superior to SSP in soyabean grain production. Togo rock phosphate produced yields of 2,132 kg/ha,compared to 1,931 Kg/ha, and 1,909 Kg/ha produced with SSP and SRP. *Rhizopus* sp and P. *striata* inoculation resulted in higher yields than the other organisms. These two organisms gave increases of 351 Kg/ha and 345 Kg/ha above uninoculated plots. Inoculation of *Rhizopus* sp, B. *megaterium* and P. *striata* in SSP was observed to enhance its performance. Togo rock phosphate was enhanced by *A. niger* and *Penicillium* sp while Sokoto rock phosphate was enhanced by inoculation with P. *striata.*

The maize crop on the other hand responded more to SSP than the rock phosphates. Yield differences of 975 Kg/ha and 1390Kg/ha were recorded between SSP and Togo rock phosphate and SSP and SRP respectively. However, only inoculation with *A. niger* and P.

striata favoured maize grain yield production. SSP alone was favoured by inoculation of micro-organisms. No significant response was recorded to rock phosphates in combination with inoculation.

Inoculation studies showed that P immobilization proceeds for 8 weeks after inoculation prior to substantial P release into the soil. A study ofthe effect of inoculants on mycorrhiza showed that inoculation influenced root infestation by mycorrhiza, and the subsequent development of mycorrhizal organism depended on the organism inoculated.

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CHAPTER 1

INTRODUCTION

The major soils of the Nigerian savanna are Alfisols, Entisols, and Inceptisols which are predominantly coarse textured, generally low in organic matter poorly structured and low in CEC. They are therefore low in natural fertility and their productivity declines quite rapidly under continuous cultivation.

The challenge that threatens the existence of farmers is nutrient mining; the gradual removal of soil nutrient without committed efforts to return adequate amounts of these nutrients into the soil. Phosphorus (P) is the second most limiting nutrient in the Nigerian savanna next to Nitrogen. The soils are inherently deficient in P. Response to other nutrients is often limited by the absence of P in the soils and thereby limit crop production to a very great extent (Lombin 1979). These soils however are of low P - fixing capacities and hence the application of large initial doses of basal phosphate fertilizers followed by periodic 'topping up' dressing will maintain the soil fertility and also improve crop yield (Baker *et al* 1977).

Presently, the most common forms of chemical fertilizers being used to correct P deficiency include compound fertilizer(N.P.K.), single super phosphate (SSP) and Triple super phosphate (TSP). The cost of these chemical fertilizers is in most cases far beyond the reach of the poor resource farmer. For example during the 1995 farming season the cost of SSP was N11,000 per tonne in the local market as against the official price of N3,000.

The use of imported phosphorus fertilizers is for the most

part socio-economically and politically unsustainable. The situation has been worsened by currency devaluations and ongoing structural reforms in Nigeria, including reduction in subsides on fertilizers and inconsistency in distribution.

In view of the above stated problems most farmers are likely to withdraw from the use of chemical fertilizers and this will result in drastic reduction in crop yields. It is therefore necessary to look inward for possible replacement and supplements of these chemical fertilizers.

The use of animal manure is limited by huge quantities required in order to satisfy nutritional needs of crops. Such quantities are not obtainable and where they are, transportation and handling cost will constitute a major constraint.

Studies have shown that the build up of soil P can be achieved more rapidly through the application of rock phosphate (Balasubramanian *et al*, 1978). It must be understood that phosphate rocks are able to serve both a productivity goal (Yield increment) and a sustainability goal (Mokwunye, 1994). It has also been suggested that once P level of the soil is built up by the application of an appropriate basal doses of phosphatic rock, small maintenance doses of the more expensive super phosphate can be applied annually to maintain high yields (Mokwunye 1979).

In the face of dwindling foreign exchange and rising prices of imports the obvious solution might be to utilize these local phosphorus sources as an amendment to build up the phosphorus fertility of the soils so that response to other nutrient elements

can be improved.

The dissolution of rock phosphate into forms readily taken up by plants is a primary concern in its direct application as a fertilizer. Various factors that influence the solubility of rock phosphate has been studied. These factors include pH (Joos and Black 1950, Bennet *et al*, 1957), solubility and plant growth in H^+ and Ca^{2+} saturated systems (Cook, 1935 and Graham, 1955). The solubility product principle was also applied to apatite (Clark, 1955, Chaverri and Black, 1966) and this led to studies of the effect of chemical and physical properties on dissolution of rock phosphate. These properties include surface area and chemical composition of phosphate rock (Willson and Ellis, 1984). The important components of rock phosphate being Co_3, Po_4, Ca and Fe or OH.

Studies of the effect of organic matter on rock phosphate dissolution (Martinez *et al* 1984), showed that, effect of several processes occurring at various levels of decomposition and humification of added organic matter may be involved in phosphate rock dissolution. Sinha (1972), indicated that fulvic acid and intermediate products of organic matter decomposition play a significant role in mobilizing insoluble phosphate.

Various microbiological studies have also attempted to ease the problem of rock phosphate dissolution. Numerous heterotrophic organisms have been found to produce large quantities of organic acids such as 2 - ketogluconic, lactic and glycolic acids which dissolve minerals containing potassium (Muller and Foster 1961)

Magnesium (Webley and Duff 1962) Calcium and Phosphorus (Webley and Duff 1962) and trace elements (Bromfield, 1959). Specifically among the organisms found capable of producing these acids, are bacteria (Yin, 1988) Actinomycetes (Mba and Odu, 1991) and Fungi (Salih *et al*, 1989). So far no information is available on the effect of microbes on the efficiency of indigenous Sokoto rock phosphate.

There is therefore no doubt that improving the dissolution of rock phosphate will help to improve its use as a direct phosphorus fertilizer, and this will positively affect crop yield.

The Sokoto rock phosphate occur within the dange formation (Kogbe 1976) and compares favourably with Togo rock phosphate which has 36.6% P_2O_5, (Nehikhare, 1987) The chemical analysis of Sokoto rock phosphate showed a range of 28 - 35.5 wt % of P_2O_5. There is need to evaluate the effect of micro-organisms in solubilisation of Sokoto rock phosphate so as to cut down on dependance on expensive imported P fertilizers.

To achieve this, the following objectives will have to be met:-

i To evaluate the role of micro-organisms in the solubilization of insoluble phosphates and rock phosphates.

ii. To select, culture and attempt to multiply specific micro-organisms responsible for any positive role in phosphate solubilization.

iii. To determine how such positive role in phosphate rock solubilization can be put into maximal use concerning insoluble phosphate utilization by plants.

iv. To compare the nutrient efficiency of such microbial innoculants with other mineral phosphatic fertilizers.

v. To verify the influence of Mycorrhizal fungi on such soil innoculants.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Rock Phosphate Forms and Occurrence

Rock phosphate deposits in Nigeria have been extensively reported by Nehikhare (1987) and Adegoke *et al* (1991). They indicated that phosphates have been discovered from lower tertiary state in the Dahomey basin, the Sokoto basin, the Anambra basin and along the Benin flank in Edo state.

The phosphate bearing beds in Ogun State belong to the Oshosun formation and out crop along an E - W belt, it overlies the Ewekoro formation. The formation is of Eocene age and it is partly marine and partly continental, the maximum thickness being 13 O.M. The formation is dominantly composed of pale, greenish - gray laminated glauconitic clays and shale and are stratigraphically equivalent to the phosphate beds at Hahotoe and Kpogame in Togo.

Two main types of phosphates are found by Adegoke *et al* (1991), the granular type of phosphates has 13.99 wt % P_2O_5 while the nodular type has 31.38 wt % P_2O_5. They also reported that the total reserve is estimated to be over 100,000 tonnes of phosphate rocks.

The Sokoto phosphate materials occur within the Dange formation and it is of upper paleocene age (Kogbe, 1976). The formation consist of bluish - grey shales with interbedded yellow lime stones, the thickness ranges from 22-45 O.M. Adegoke *et al* also identified two types of phosphates; nodules and pellets in clays, shales and siltstones and the primary phosphatic shale in

limestones and siltstones. Nehikhare (1987) reported that it compares favourably with Togo rock phosphate of 36.6% P_2O_5 Chemical analysis showed that the nodules have an average P_2O_5 value of 35.5 wt %, primary limestones has 21 wt % P_2O_5 and primary shale and siltstones 30 and 28 wt % P_2O_5.

The generalised geology of Anambra Basin and Benin Flank were also reported by Adegoke *et al* (1991). The phosphate occur in the middle Ameki formation in facies normally regarded as the lateral equivalent of the Oshosun formation. It is dominantly composed of greenish - gray, clayey sandstone and sandy claystone displaying rapid lateral facies changes. The formation is over 1,600 meters thick and is of lutheran age. Both the nodular and granular phosphates in shale occur at these localities.The nodules have P_2O_5 content averaging 32% in Imo State and 36.25% in Edo State.

2.2 Solubility of Rock Phosphate

The solubility of rock phosphate has been an area of focus by many workers. Chien and Black, (1975) observed that the solubility decreased with an increase in proportion of the phosphate rock dissolved, this they attributed to the existence of the solid apatite in a range of activity. Roy *et al*, (1989) concluded that the solubility of partially acidulated rock phosphate depend on the pressure of octacalcium phosphate, hydroxy apatite, fluoro apatite and fluorite mineral. Olivo and Elizade (1988) found the major component of rock phosphate to be apatite but some rocks has quite appreciable contents of quartz and calcite. Werner and Fritsch

(1989) correlated the release of P from rock phosphate with concentration of P and calcium in the soil as well as soil reaction.

Chaverri and Black (1966) treated rock phosphate with HCL (concentrated), and observed that the surface was altered by a process involving the reaction of dissolved OH ions with H ions of the acid. Marwaha and Sood (1966) suggested that the soil rich in Al^{3+} or having a higher degree of exchange acidity may be better adapted to the use of rock phosphate. They also observed that rock phosphate has a favourable effect on P - fixing capacity of the soil compared to water soluble source of P. Chien and Hammond, (1987) used various chemical methods to extract and determine the availability of P contained in rock phosphate and correlated the indexes with dry matter produced by plants fertilized with the minerals. Quito *et al* (1987) gave a mathematical expression of rock phosphate solubilization and concluded that for a long period of reaction apatite solubilization was more highly correlated with clay content and Free Al. Flach *et al*, (1987) however suggested that the shift in mass action equilibrium of alkaline rock phosphate brought about by high Ca absorption also favour their solubilization.

Considerations have also been given to the fineness of rock phosphate to enhance its fertilizer potential. Olivo and Elizaide (1988) suggested its use as source of fertilizer production or ground and apply directly to the soil. Bolland and Gilkes (1989) also indicated that finely powdered phosphate rock give better

results. Fassbender and Urich (1965) indicated that when finely grounded phosphate rock is used, it has fertilizer value similar to commercial phosphates.

Bollen *et al* (1988) however concluded that rock phosphate fertilizer is not economic substitutes for fertilizers containing water soluble P for most agricultural application. Mackey *et al*, (1986) enunciated percent Ca - saturation, P - sorption capacity and Ca - exchange capacity of soil as the most important parameters influencing the dissolution of rock phosphates. They however recommended 90 days as the completion time for rock phosphate dissolution.

2.3 Acidulation of Rock Phosphate

Marwaha (1983) postulated that; (i) raw rock phosphate has no fertilizer value (ii) acidulation at all levels improved effectiveness of rock phosphate significantly and (iii) samples acidulated with nitric acid were as effective as those prepared with phosphoric acid. He also observed that 50% acidulants was as effective as Super phosphate. He suggested also that band placement is best for partially acdulated rock phosphate.

Various acidulants have been reported for the acidulation of rock phosphate. Basak *et al*, (1988) acidulated rock phosphate with phosphoric and sulphuric acid to increase its fertilizer value, while Boronin *et al* (1988) defouorinated rock phosphate and found that it has the same effect on crop yield and quality as standard super phosphate on both irrigated and non-irrigated lands Nachtigal

et al (1987) suggested 2% citric acid as an enhancement of the dissolution of rock phosphate and its behaviour as fertilizer while Ashby *et al* (1966) on acidulation of phosphate rock observed that yield and P uptake increased with increasing acidulation. Sood and Marwaha (1983) observed maximum decomposition after 24 hours on acidulation of phosphate rock with concentrated HNO_3. Leo *et al*, (1987) showed that treating rock phosphate increased the release of *inorganic P in carbonate.*

2.4 Phosphorus Solubilizing Micro-organisms

Sackett *et al* (1980) was one of the earliest researchers who observed that some bacteria could dissolve insoluble phosphates. They mixed rock phosphate, bone - meal, tricalcium phosphate, dicalcium phosphate and calcium carbonate in agar plates and found that certain organisms gave clear zones around the colonies to dissolve the phosphates. Extensive work has been carried out ever since, to isolate phosphate solubilizing organisms.

Barea *et al*, (1976) isolated 108 phosphate dissolving bacteria from the rhizosphere of crop plants. They also correlated the dissolution of rock phosphates by these organisms to their growth factors such as IAR, gibberelins and cytokinins. They encountered *Bacillus Pseudomonas* and *Chromobacterium* more than other organisms. Salem *et al* (1990) made similar observations. Chonker and Subba - Rao (1967) observed that *Penicillium lilacium, Aspergillus* sp, *Aspergillus flavus, Aspergillus terraus* and *Aspergillus nidulans* will solubilize $Ca_3 PO_4$. Salih *et el*, (1989) found in an experiment

that *Aspergillus foetidus* significantly increased the availability of P in soil treated with rock phosphate. Louw, (1970) observed that most phosphate dissolving bacteria isolated from the rhizosphere and rhizosplanes of wheat and lupin were gram negative non sporing rod. Ali *et al*, (1987) selected and identified *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megaterium* as the most efficient P - dissolving bacteria. They also found *B brevis* to give the highest organic fraction of soil phosphorus while *Bacillus cereus* had more effect on soil pH and available organic P. Bajpai and Rao, (1971) isolated *Bacillus megaterium* var. *phosphaticum* over a wide range of pH (5.2 - 8.5), and found *Bacillus megaterium* to dissolve substantial amounts of phosphate. They also observed that each selected bacteria had a different optimum pH for maximum solubilization. Yin, (1988) observed the highest number of phosphate dissolving micro-organisms in chernozem and the lowest in alkaline soils. He also identified, *B megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *serratia* sp and *Bacillus firmus* as having various degrees of P - solubilizing capacities. However Molla *et al* (1984) observed that gram - negative short rod *Bacillus* and *Streptomyces* were active in solubilizing phosphate than *Aspergillus*, *Penicillium*, *Proteus Serratia*, *Pseudomonas* and *Micrococcus*.

Investigation of the behaviour of bacteria on the weathering of rock minerals (Wagner and Schwertz (1975) showed that only Eubacteria developed while actinomycetes occurred only sporadically and yeast were altogether absent.

2.5 Microbial Solubilization of Organic Phosphates

It is commonly accepted that micro-organisms will mineralize organic phosphorus together with N and S (Martin (1972). However, in a comprehensive review of organic phosphorus mineralization Ramirez - Matinez (1968) reported that the direct participation of soil organic phosphorus is not clearly established. Particular attention has been given to the mineralization of inositol polyphosphate which represent a major component of the organic P in soil. Greaves and Webley (1965) reported that 30 - 50% of bacterial isolates from the root surface of pasture grass examined could hydrolyse sodium phytate with the release of inorganic phosphate. Sexana (1964) found that the phytase activity of soil from plants grown in garden soil was higher than from plants grown under aseptic conditions. Szember (1964) however, showed that phytin could serve as a phosphorus source for radish and kale plants grown in aseptic sand culture and inoculation with micro-organisms having phytase activity. Organisms identified as solubilizer are *Bacillus megaterium, Serratia phosphaticum, Aspergillus* spp, *Penicillium* sp, *Aspergillus flavus, Actinomyces coelicolour, Bacillus cereus, Bacillus mycoides, Aerobacter aerogenes, Psendomonas fluorescens, Erwinia* sp *Streptomyces* sp, *Rhodotorula* and *Aspergillus awamori* (Menkina, 1959; Sen and Paul, 1957; Casida, 1959; Szember, 1960; Bottcher, 1962; Halstead, 1964 and Gaur, 1972).

2.6.1 Microbial Solubilization of Insoluble Inorganic Phosphates

The dissolution of tricalcium phosphate by *Bacillus megaterium* var *phosphaticum*, *Serratia phosphatoculum* and *Aspergillus* were reported by Menkina (1950) and Johnston (1954). Certain fungi (*Aspergillus terreus, Aspergillus niger* and *Sclerotium rolfsii*) were found to solubilize tricalcium phosphate (Rose 1957). The solubilization of calcium and iron phosphate in liquid medium by *Bacillus subtilis, Bacillus Mycoides, Bacillus megateruim* and *Bacillus mesentericum* with ammonium sulphate as nitrogen source was observed by Sen and Paul (1957).

Muromtsev (1958) found that micro-organisms could dissolve tricalcium phosphate in acidic, neutral and alkaline culture medium. Rock phosphate was dissolved to a lesser extent than tricalcuim phosphate. Pobrzanka and Golebiowska (1959) observed that bacteria which dissolved phosphate to a high degree in liquid medium were not active in sand or in soil, but soil and yeast extract assisted the process of solubilization in liquid medium.

Two bacterial strains of *Achromobacter* and *Pseudomonas* were efficient in solubilizing tricalcium phosphate (Myskow 1960). A few other workers reported the solubilization of tricalcium phosphate in liquid medium; gram-negative Bacilli, bacteria, fungi and actinomycetes (Medina De Wanerli, 1968); *B cereus varalbolactis, B mycoides, B mesentericum* and *B megaterium* (Merzari, 1968). *Penicillium* spp *Aspergillus* spp, *Cladosporium* spp, *Fusarium* spp and *Paecilomyces* spp (Sethi and Subba, Rao 1968; Subba Rao and Bajpai 1965) and some bacteria by Bhurat and Sen

(1968) and *Taha et al* (1969).

Bajpai and Sundara Rao (1971a) showed that solubilization of tricalcium phosphate occurs over a wide range of pH. The species of *Bacillus, Streptomyces, Aspergillus* and *Penicillium* were isolated from a laterite soil by Banik and Dey (1981) and were found to solubilise tricalcium phosphate. There was also a study of the periodic solubilization of ferric and aluminum phosphate in liquid medium (Gaur and Gaind 1983). Results showed that maximum solubilization of aluminum and iron phosphate by *Aspergillus awamori* was activated on the tenth and eleventh days respectively.

2.6.2 Phosphate Solubilization by Rhizosphere Micro-organisms

Plant root surfaces and soil in contact with roots comprise a site of intense microbial activity, and this environment under the influence of plant root is referred to as rhizosphere.

Micro-organisms are influenced in many ways by growing plants and the microbial processes are more rapid than in non-rhizosphere soils.

The increased availability of phosphorus to crop plants when phosphate solubilizing micro-organisms are present in the rhizosphere soil has been documented by various workers. The occurrence of phosphate solubilizing activities of bacteria in the rhizosphere have been reported by Urova (1956). Nowotny and Golebiowska (1956) found that the rhizosphere of lupin stimulated the growth of micro-organisms solubilizing tricalcium phosphate Swaby and Sperber (1958) observed dissolution of hydroxyapatite by several general of bacteria, streptomyces and fungi isolated from

rhizosphere soils of legumes. Katnelson and Bose, (1959) and Nikitin (1959) reported solubilization of tricalcium phosphate by bacteria isolated from the rhizosphere of wheat and maize plant respectively. Louw and Webley (1982) reported that the number of dicalcium phosphate dissolving bacteria were greater in the rhizosphere of oat plants than in the surrounding soil. Chonker and Subba Rao (1967) isolated a few fungi associated with root nodules of leguminous plants and found that *Penicillium lilacium* solubilized tricalcium phosphate. More micro-organisms mineralizing organic phosphorus compounds than micro-organisms dissolving inorganic phosphate were found in the wheat rhizosphere (Saric 1965). He also observed that the count of both groups increased up to maturity and then declined (Saric 1965).

Paul and Sundara Rao (1971) isolated phosphate solubilizing bacteria, *Bacillus megaterium, Bacillus subtilis, Bacillus brevis, Bacillus pulvifaciens, Bacillus pumilus* and others from the rhizosphere of berseem and green gram. Bardiya and Gaur (1974) isolated six cultures of yeasts and 21 cultures of fungi belonging to species of *Aspergillus, Penicillium curvularia* and *Trichoderma* from rhizosphere soils of cowpea; soybean, groundnut, black gram and green gram.

2.6.3 Microbial Solubilization of Rock Phosphate

The earlier evidence regarding rock phosphate solubilization indicated the involvement of mixed inoculants, soil fungi, streptomyces and a few gram-negative and gram positive bacteria.

Bromfield (1959) studied the solubility of one percent rock phosphate in liquid medium with various mixed inoculant and found that the solubility was closely related to the magnitude of the acidity produced. The solubility for a lower percentage of rock phosphate (0.01%) increased under alkaline conditions. Louw and Webley (1959) showed that many isolates which did not show any zone of solubilization on agar plates, released phosphates from rock phosphate when inoculated in liquid medium. A similar observation was made by Ahmad and Jha (1968). The effects of twenty - four - strains of fungi and three strains of streptomyces were examined for solubilization of rock phosphate (Meyer and Konig 1960). They showed that the solubilization of phosphorus from less soluble rock phosphate proceeds through the utilization by certain organisms of small amounts of initial phosphorus followed by secretion of organic acids by growing mycelia in dissolving phosphates. Konig (1961) showed that organic acids liberated by fungi dissolved phosphates and this was followed by the absorption of phosphate ions by the fungi mycelia which were ultimately released by autolysis of the organisms. This process was observed both in pure culture and in the soil.

Ahmad and Jha (1968) published results regarding the solubilization of hydroxyapatite and rock phosphate by gram-positive and gram-negative rods, cocci-shaped bacteria and fungi, (*Aspergillus, Penicillium* and *Rhizopus*) and species of *Norcadia* and *Micromonspora.* Bardiya and Gaur (1972) compared the tricalcium phosphate and rock phosphate dissolving capacity of 14 selected

isolates of rock phosphate solubilizing bacteria. The results showed that although ten isolates out of fourteen solubilized appreciable amounts of tricalcium phosphate only the tested strains of *Bacillus pulvifaciens* could solubilize about 5 to 10 % of applied $P_2 O_5$ of Singbhum or Mussoorie rock phosphate.

An interesting observation was made by Gaur *et al* (1973) regarding the presence of spore-forming bacteria and fungi in rock phosphate samples. The bacterial and fungi isolates obtained from rock phosphate were tested for solubilization or mineralization of tricalcium phosphate, Mussoorie, Jharmarkotra and Matoon rock phosphates, and organic phosphates viz lecithin and calcium phytate. The cultures isolated from alluvial soil for the breakdown of organic phosphate were compared for their solubilizing capacity in the case of inorganic and organic phosphate compounds. The bacteria and fungi isolated from soils as organic phosphate mineralizers showed a capability of solubilizing 12-20% of tricalcium phosphate but failed to solubilize any rock phosphate. Among the isolates from rock phosphates, *Aspergillus carbonum* proved the best culture since they solubilized all three types of phosphates tested (Gaur et al, 1973).

2.7 Mechamism of Phosphate Solubilization and Mineralization

Several mechanisms have been implicated in the process, the production of organic acids however seems to be the main cause of solubilization but other factors such as carbon-dioxide, hydrogen sulphide and alkalinity production have also been implicated.

A fall in pH during the growth of phosphate solubilizing micro-organisms in liquid media containing insoluble phosphate has often been reported (Gerreston, 1948; Bromfield, 1959; Louw and Webley, 1959; Tardieux and Roche, 1966; Chonker and Subba- Rao, 1967 and Ahmad and Jha, 1968). The studies have shown that during the solubilIzation of rock phosphate by fungi the pH of the culture broth was lowered from 7 to 3 (Gaur and Sachar 1980, Gaur and Gaind 1983).

Phosphate solubilizing micro-organisms were found to produce acetic, formic, lactic, gluconic, glyconic, 2 - ketogluconic, oxalic, succinic, malic, maleic and citric acids in liquid media from simple carbohydrates (Rose, 1967; Sperber, 1957; Swaby and Sperber, 1958; Louw and Webley, 1959 and Duff et al, 1963). Non volatile acids such as citric and lactic acids were also produced by *Bacillus megaterium, Bacillus circulans* and *Escherichia freundii* from glucose (Bajpai and Sundara Rao 1971c). Model experiment by Mishustin *et al* (1972) showed the solubilization of tricalcium phosphate in liquid medium by citric, glutamic and oxalic acids which also depended on pH and the formation of soluble Ca-complexes. Pareek and Gaur (1973) however reported that aliphatic acids were more effective in phosphate solubilization than phenolic acids.

Numerous studies have indicated that the availability of phosphate in soil was increased by addition of organic matter and humic substances (Dalton *et al*, 1952; Nielson *et al*, 1953; Gaur 1959). It appears that organic acid production from organic

substrates by heterotrophic micro-organisms may partly be responsible for the increased availability of phosphate.

2.8 Soil Inoculation of Rock Phosphate

Various workers have inoculated micro-organisms into the soil along with the application of rock phosphate to enhance its solubilization Bajpai and Rao (1971) showed that P was released in significant amount by *Bacillus megaterium* in treatments with farm yard manure, ammonium sulphate and super phosphate.
B circulans however caused more P to be released when apatite was used instead of super phosphate (Bajpai and Rao 1971). Mosse et al (1976), inoculated with vascular-arbuscular mycorrhiza endophytes and observed increased P uptake. They also observed that rock phosphate improved nodulation and nitrogen fixation of the mycorrhizal legume plants. Molla *et al* (1984), showed that mixed cultures were most effective in mineralization of organic phosphate followed by pure cultures of *Bacillus*.

Dry matter and P uptake responses to inoculation with *Aspergillus foetidus* were observed to be better in soil treated with rock phosphate than soil treated with triple super phosphate (Salih *et al* 1989). Jalali and Thareja (1985) also observed that inoculation with mycorrhizal fungus (Glomus sp) significantly increased root and shoot dry weights, total dry matter production and phosphorus uptake. Cardoso (1985) inoculated vascular-arbuscular mycorrhiza applied with rock phosphate, and observed increase in dry matter yields, seed production, number and weight

of nodules, P concentrations in shoot and total contents of N, P, K, Ca, Mg, S, Fe, Zn, Al and Na in soybean. However, soils inoculated with *B cereus* were observed to show 40% increase of P mobilization and 50% increase in dry matter over the control (Fernandez et al, 1985). Monib et al (1984) observed a successful colonization of the rhizosphere when they inoculated castor oil with symbiotic nitrogen fixer and a mixed culture of P-solubilizing organisms. They also observed increase in dry weight, P and N uptake of the plants. Bajpai and Rao (1971a) in their study postulated that *Bacillus circulans* may be useful as inoculant in Indian soils low in organic matter because it increased crop yield through the release of P from super phosphate and apatite.

2.9 Factors that Affect Solubilization of Insoluble Phosphates

Organisms responsible for solubilization of rock phosphates are mostly heterotrophs solubilizing insoluble phosphates by secreting organic acids, they therefore require carbon sources as energy. Rose (1957) showed that glucose or xylose was the best source of energy for fungi in liquid medium, whereas, Katnelson and Bose (1959) and Sobieszcsanki (1961) reported that either yeast or soil extract was essential for the proper growth of phosphorus solubilizing organisms in liquid or solid medium. Gaur and Sachar (1980) reported that by increasing the concentration of glucose from 1 to 3 percent in the medium, the rate and quantity of rock phosphate solubilized by *Aspergillus awamori* increased and also greater acidity was produced by glucose at higher concentration,

which is an important factor in phosphate dissolution.

Rock phosphate solubilization probably also depend on nature of rock and the organisms. The relative efficiency of the organisms for rock phosphate solubilization may depend on nature and quality of organic acids secreted in the medium (Gaur, 1990). Generally greater microbial solubilization was observed with High-grade rock phosphate. There is however lack of conclusive information on this point and therefore more work is needed.

Gaur(1990) observed significant improvement in the quantity of rock phosphate solubilization by various bacteria and fungi with increasing concentration of P_2O_5 up to 100 mg P_2O_5 added to 100 ml liquid medium. Gaur and Sachar (1980) found that the lower the quantity of phosphate added to the medium, the greater was the conversion to soluble form by fungus.

Finely ground rock phosphate of about 150 - 250 mesh is generally recommended for direct use as fertilizer in acidic soils and in neutral or alkaline when treated with efficient phosphate dissolving micro-organisms (Marwaha et al 1981).

The pH of the medium is known to affect the growth and activity of micro-organisms. The optimum pH range for maximum rock phosphate solubilization was studied (Taha *et al* 1967). The maintained pH value was observed to change appreciably due to sterilization of the medium. The selected efficient micro-organisms were capable of solubilizing rock phosphate over a wide range of pH from 5 - 7.8.

Temperature is a vital factor for the growth and activity of

micro-organisms. A range between 26°C and 30°C was found suitable for rock phosphate solubilization by strains of *Pseudomonas striata* although 25 - 30°C was more favourable (Gaur, 1990). Wani *et al* (1979) reported that 30°C was optimum for tricalcium phosphate solubilization by
P striata, Aspergillus awamori and *Penicillium digitatum*, whereas 35 - 40°C favoured phosphate solubilization by *Bacillus polymyxa*.

A significant increase in rock phosphate solubilization was observed in shake culture as compared to static culture with strains of *Penicillium striata* and *Aspergillus awamori* (Ahmad and Jha 1968) indicating that aeration may be necessary for increase in the level of solubilization.

Several investigations have shown that, under cultural conditions, phosphate solubilization progressively increases up to 10 - 15 days (Wani *et al* 1979, Gaur 1981, Gaur and Gaind 1983). Ortuno et al. (1978) reported that tricalcium phosphate solubilization started after 24 hours of incubation and reached a maximum on the 7th and 15th days with *Aspergillus niger* and *Pseudomonas fluorescence* respectively.

Finally, *Bacillus megaterium* var *phosphobacterium* solubilized increased amounts of phosphate from sodium humate and fulvic acid. Humic substances act as strong complex forming and chelating agents, better or comparable to synthetic cheating agents (Pareek and Gaur 1973). The chelation property of humic substance is of great use in increasing the efficiency of phosphatic fertilizers

which are rendered insoluble through fixation.

2.10 Interaction of Rhizobium with Phosphate Solubilizers

Bhatnagar *et al* (1979) studied the associative effect of inoculum of *Rhizobium* and *Bacillus megaterium* var. *phosphaticum* on the yield of mungbean and soybean. They observed a higher yield than that obtained by the use of *Rhizobium* alone as inoculant. Azcon *et al* (1978) studied the influence of mycorrhiza and phosphobacteria on nodulation and nitrogen fixation by *Medicago sativa*, and observed highest yield and maximum N and P absorption by plants inoculated with a whole culture of *Rhizobium* and 'phosphobacteria' together. Combined inoculation of *Rhizobium* and phosphate solubilizing *Pseudomonas striata* or *Bacillus polymyxa* with or without added chemical fertilizer on chickpea yield and nutrient content under greenhouse condition showed that single inoculation of *Rhizobium* increased nodulation and nitrogenase activity, the 'phosphate-solubilizers' increased the available P content of the soil (Alagawadi and Gaur 1988). Combined inoculation of *Rhizobium* and *P striata* or *B polymyxa* increased the above parameters and also the dry matter content, grain yield, nitrogen and phosphorus uptake significantly over the control (Alagawadi and Gaur 1988). The result indicated that by combined inoculation of *Rhizobium* and phosphate solubilizers' there is a possibility of saving 10kg N and replacing super phosphate entirely by rock phosphate and micro-organism inoculation.

2.10.1 Interaction of VA Mycorrhizae (VAM) and Phosphate Solubilizing Micro-organisms

The mycorrhizal plants, particularly in low phosphate soils are more efficient in phosphate uptake than non-mycorrhizal ones (Mosse *et al* 1976) and in such soils satisfactory nodulation and nitrogen fixation are dependant on an adequate supply of phosphorus (Schreven 1988). Hence there must be some synergistic interactions between *Rhizobium* and VAM as described by Crush (1974) and Mosse *et al* (1976).

Plants inoculated with VAM and 'Phosphate solubilizers'together were also able to mobilize significantly higher amounts of phosphorus than plants inoculated with these micro-organisms separately in phosphate deficient soils (Azcon *et al* 1976, Barea *et al* 1975). Azcon *et al* (1978) tried to differentiate the role of phosphate solubilizing micro-organisms, rhizobia cells and their cell-free supernatant on mycorrhizal infection and studied the influence of VAM and phosphate solubilizing micro-organisms on nodulation and nitrogen fixation by *Medicago sativa*. They observed positive interactions between phosphate solubilizing bacteria and VAM assayed on the formation of VA symbiosis.

2.10.2 Rock Phosphate Utilization In The Savanna

The soils of the Nigerian Savanna are inherently deficient in phosphorus, and low in P-fixing capacity (Bache and Rogers 1970, Jones and Bromfield, 1974; Mokwunye, 1977; Enwezor and Moore, 1966). Large amounts of phosphate fertilizers are therefore needed

to build up the soil solution concentration to a potentially sufficient level high enough to sustain good yield. Because of the low capital and energy imputs needed for production of ground rock, phosphate rock, and partially acidulated formulations they could be considered cheap source of phosphates for these phosphorus deficient soils.

A large volume of these fertilizer materials have accumulated over the years (Mclean and Wheeler, 1964; Mclean and Logan, 1970; Misra and Pander, 1968; Mokwunye, 1979). There is generally a low reactivity of West African phosphate rock and they are also erratic especially with areas of less than 700 mm rainfall (Prieri 1971, Jones, 1973). Increasing the solubility of phosphate rock will enhance phosphate availability to annual crops (Uyovbisere 1988).

In the Nigerian Savanna, sulphur acidulated Togo phosphate rock with phosphorus/sulphur (P/S) ratio of 1:0.7 compared favourably with single super phosphate on groundnut and maize(Bromfield, 1975; Mokwunye, 1979). However, this ratio of acidulation was observed to acidify the soil appreciably, resulting in increased loss of calcium when compared with single super phosphate (Mokwunye, 1979; Mokwunye, 1981). Uyovbisere and Lombin (1991), also acidulated rock phosphate at 50% and observed it to be agronomically as effective as commercial super phosphate. Continuous use of acidifying fertilizers on poorly buffered soils as those of Nigerian Savanna will create disastrous effects on the long run (Bache and Heath- Cote 1969, Jones and Stockinger 1976. Literature is however lacking in the area of microbial enhancement

of rock phosphate solubilization except for the work of Odu and Mba (1991), who working on the South eastern soils isolated some actinomycetes from earthworm cast that were capable of solubilizing Sokoto rock phosphates. There is therefore a need to do more work in this area.

2.10.3 Plant Uptake of Soil Phosphate

It is generally agreed that most, if not all of the soil phosphorus taken up by plants is in the form of orthophosphate ions in the soil solution (Walker, 1975). Although some organic and inorganic phosphate are present in soil solution, (Wild and Oke, 1966), plant generally preferred the $H_2PO_4^-$ ion to the HPO_4^{2-} ion (Walker, 1975).

The average orthophosphate concentration in the soil solution of around 10^{-6} M is near the limit at which plants can absorb adequate concentration (Walker, 1975), critical concentration however vary for different crops. Rorison (1968), using solution cultures found that *Urtica dioica* which only grows in reasonably fertile soils thrive only at 10^{-5} M of orthophosphate or above, where as *Rumex acetosa* which has a broad edaphic tolerance survived over a wide range of concentrations.

The concentrations seem to be related to the inherently different growth rates and metabolism of different plant species because of the concentration inside the plant root is usually over 1000 times greater than in the soil solution (Walker 1975). Values of 10^{-2} M in the cortical cells and 10^{-1} M in the xylem have been

suggested by Bieleski (1973). An average crop removing 10 or more kg $P_2 O_5$/ha/yr requires hundreds of times as much phosphorus as can be found at any one time in the soil solution within the rooting depth (Walker, 1975). Thus the most vital factor affecting plant uptake of soil phosphate is maintenance of the soil solution P at the root surface.

The efficiency of several crop species in utilizing rock phosphate was studied by Baron and Diest (1989). They found the relative effectiveness 1 - 0 increase in the order of rice, cowpea (*vigna unguiculata*), maize, finger millet (*Eleusine coracana*) velvet bean (*mucuna pruriens*) and rape. Studies by Flach *et al*, (1987) to compare rock phosphate mobilizing capacities of various crops showed that the increase is in the order maize, *Penisetum americanum* and *Eleucin coracana* corresponding with the order of increasing quantities of calcium adsorbed. Sarojini and Mathur (1990), observed a significant effect on the yield of wheat grown when urea and rock phosphate were added. Sale and Mokwunye (1993), explained that the effectiveness of a given phosphate rock is determined by the extent to which the P uptake rate required for satisfactory plant growth is maintained by the dissolution rate of the phosphate rock. Mokwunye (1994) however reported that West African phosphate rocks are not very reactive.

Deductions from Literature Review

i The maintenance of soil fertility has become a huge problem

particularly in Nigerian Savanna. The initial fertile soil when cropped for 2 - 3 years becomes depleted of most of its mineral elements especially nitrogen and phosphorus which are the limiting nutrients in the West African Savanna soils.

ii These elements and others - such as potassium, sulphur and zinc can be supplied into the soils as chemical fertilizers.

iii The chemical fertilizers are however not easily obtainable to the farmers as a result of poor distribution network and high prices.

iv The rock phosphate is a mineral rock containing apatite and some impurities. The apatite are inorganic forms of phosphorus which are not directly available to the plants unless they are first brought to a solubilized form.

v Attempts have been made to acidulate rock phosphate; such acidulated forms, however render the soil acidic after it has been used continuously for a few years. Such acidic soils will then develop other problems associated with poorly buffered soils such as the savanna soils.

vi Various micro-organisms have been identified as solubilizers of rock phosphate. Such organisms include, bacteria, fungi and actinomycete. These have been studied in many parts of the world including India, Russia, Australia, Canada, United States etc.

vii Such literatures are lacking in the savanna particularly the Nigerian savanna.

viii Proper study and selection of indigenous micro-organisms capable of solubilizing rock phosphate may enable us to put such

organisms into maximal use in the area of rock phosphate solubilization and therefore making it available to the farmers as biofertilizers at a much lower cost and damage to soil than the chemical fertilizers.

ix Deposits of rock phosphate have been discovered in at least 4 or 5 locations of the country. If mined this can be harnessed into a more economical phosphorus use in Agriculture.

x Maize and soyabean are cereal and legume respectively grown widely in the savanna. They both have high phosphorus needs and are relevant as test crops in studying rock phosphate solubilization by micro-organisms.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Study Area

The experiments were conducted at Samaru (lat. 11°1'N, 7°38'E) in the Northern Guinea Savanna Zone of Nigeria (Keay, 1959). The mean annual rainfall for the period of study (1993 - 1995) was 946 mm falling entirely in the months of April to October (Samaru Meteorological Station). The soil is predominantly Alfisol derived from loess, overlying undifferentiated basement complex (Moberg and Esu 1989), The chemical and physical characteristics of the soil are given in table 8 and the parameters used in the classification of the soil appear in appendix 2.

3.2 Experiments

The study consisted of preliminary laboratory work to isolate, screen and select phosphate solubilizing micro-organisms, Greenhouse studies to further screen the selected organisms and field studies to test the selected organisms under field conditions.

3.2.1 Laboratory Studies

The laboratory studies consisted of microbiological studies of selected phosphate solubilizing micro-organisms and incubation studies to further characterize the phosphate solubilizing organisms.

3.2.1.1 Micorbological Studies

Various microbiological methods were used to isolate, screen and select phosphate solubilizing micro-organisms present in soils collected from five locations at Samaru.

3.2.1.1.1 The Laboratory Isolation of Phosphate Solubilizing Micro-organisms

Micro-organisms were isolated from freshly cultivated soils, taken from five different locations at Samaru, Latitude 11° 1'N and longitude 7° 38'E.

3.2.1.1.1.1 Culture Media

The phosphate precipitated medium (Louw and Webley 1959), was employed. For the isolation of bacteria 10% fungocide powder was *added to the medium while for the isolation of fungi and* actinomycete 10% streptomycin was added to the medium. The organisms were preserved on glucose-peptone yeast extract slants, (described below).

3.2.1.1.1.2 Phosphate Precipitated Medium (Louw and Webly 1959)

Carrot infusion was obtained by boiling 1 kg of finely chopped carrots in 1 liter of tap water in an autoclave for 2 hours and then filtered. One gram of asparagine, 1.0 g glucose and 20.0 g of agar were weighed and these were dissolved in one liter of the carrot infusion prepared above. The mixture was dissolved by heating to boiling on the hot plate. It was then autoclaved for 15 mins at 121 psi. Ten percent solution of $KHPO_4$ (solution A) and 10% of $Cacl_2$ (solution B) were sterilized separately by boiling in the autoclave. 0.5 ml of solution A and lml of solution B were added

quickly and aseptically to the molten agar medium at 50°C.

3.2.1.1.1.3 Yeast Extract Agar

The glucose-peptone-yeast extract slant was used for preserving the organisms over a long period of time. One gram of glucose, 0.5g of yeast extract, 0.5g of peptone and 15g of agar were weighed and dissolved in 1 liter of tap water by heating to boiling on the hot plate.

Fifteen milliliters of this medium was dispensed in each Bojour or McCartney bottles and autoclaved at 121 psi for 15 minutes and the bottles were laid slanted on the table.

3.2.1.1.1.4 Carrot Infusion Broth

One liter of carrot infusion was obtained as described above. 1.0g of asparagine and 1.0g of glucose were weighed, dissolved in 1 liter of carrot infusion and dispensed into conical flasks, then autoclaved at 121 psi for 15 mins, and allowed to cool. The broth was inoculated from pure cultures of isolated phosphate solubilizing organisms.

3.2.1.1.2 Isolation of Bacteria

Suspension of soil samples were made to 10^{-3} and 10^{-4}. One millimeter of these dilutions were taken into sterile petridishes and 15 mls of phosphate precipitated agar medium was poured onto it under aseptic conditions. The molten agar already contained 10% fungocide. The inoculum - medium mixture was homogenized by swirling the petridishes, to ensure complete dispersal of the inoculum. Each dilutions of soil samples were prepared in triplicates and the plates were left to set. They were then

incubated at 25°C for 10 - 14 days. The bacteria which gave zones of clearing around their colonies were subcultured on plates of phosphate precipitated medium. Many subcultures were made to get pure cultures. The organisms were given code numbers and descriptions of their morphological appearance made. They were finally placed on prepared glucose-peptone-yeast extract slants.

3.2.1.1.2 Isolation on Fungi and Actinomycete

As in bacteria, soil samples were made to 10^{-3} and 10^{-4} serial dilutions. One millimeter of these dilutions were introduced into sterile petridishes and 15 mls of molten phosphate precipitated agar was added into the petridishes. The agar already contained 10% streptomycin. The plates were homogenized by swirling and left to set and they were then incubated at 25°C for 10 - 14 days. Subcultures of organisms having clear zones around their colonies were made on already prepared phosphate precipitated agar plates until the pure cultures were obtained. The pure cultures were given code numbers, described and placed on glucose-peptone-yeast extract slants.

3.2.1.2 Screening of Phosphate Solubilizing Micro-organisms

Acid washed sand was used for this screening. Twenty grams of acid-washed-sand were weighed in petridishes and sterilized in the oven at 250°C for 2 hours. The petridishes were left to cool and 1% (0.2g) Togo rock phosphate were added to each petridishes.

The petridishes were then inoculated with phosphate solubilizing micro-organisms contained in broth medium. The

petridishes were incubated at 30°C for 6 weeks. Samples of sand were taken weekly from each petridish for extractable P analysis using the Bray No 1 method (Bray and Kurtz 1975). P in solution was determined by ascorbic acid method.

3.2.2 Chemical and Physical Analysis of Soils

3.2.2.1 Particle Size Distribution

To ensure complete dispersal of the soil aggregates, the soil samples were dispersed in 5% calgon (sodium hexametaphosphate) solution by shaking for 6 hours in a reciprocating shaker. The particle size distribution was then determined by the hydrometer method as described by Day (1965).

3.2.2.2 Soil pH

Soil pH was determined in both water and 1m $cacl_2$ solutions using a 1:2.5 soil/water or $Cacl_2$ solution ratio (Peech 1965). The pH was then read with a glass electrode on a pye unicam 290 MK pH meter upon equilibration.

3.2.2.3 Organic Carbon

The organic carbon was determined by the Walkley - Black dichromate wet oxidation method as described by Nelson and Summers (1982). Concentrated H_2SO_4 was added to act as catalyst.

3.2.2.4 Exchangeable Bases

The exchangeable bases were extracted with neutral (pH 7) ammonium acetate (NH_4OAC) solution by shaking on a reciprocating shaker for 2 hours and centrifuging at 10,000 rpm for 20 minutes (IITA, 1979). Potassium and sodium were read from the undiluted

extracts on a Gallenkemp flame analyser. Calcium and magnesium in solution were read on atomic absorption spectrophotometer.

3.2.2.5 Cation Exchange Capacity (CEC)

The CEC was determined by the neutral (pH 7) ammonium saturation method (Chapman 1965) by repeated leaching.

3.2.2.6 Total Nitrogen

The total Nitrogen was determined in both soil and plant samples by the micro-kjeldahl method described by Bremner (1965).

3.2.2.7 Available Phosphorus

Available phosphorus was extracted by Bray No 1 method (Bray and Kutz 1945). Phosphorus in solution was then determined by the ascorbic acid method (Murphy and Riley, 1962).

3.2.2.8 Total Phosphorus and other Elements (Tissue Analysis)

The total P in the plant samples were determined by both the wet digestion method using perchloric, nitric and suphuric acids as compiled by Juo (1974) and the dry ashing method using Nitric acid, 1N hydrochloric acid and 0.1N hydrochloric. The P content was determined by the vanado-molybdate method the Ca and Na were determined on the flame analyser and the Mg, K, Zn, Mn, Fe and Cu were read on the atomic absorption spectrophotometer. The plant samples collected on the field were analysed for P, using the wet digestion method while those collected in the green house were analysed using the dry ashing method.

3.2.3 Microscopic and Bhochemical Characterization of Micro-organisms

3.2.3.1 Morphological Characterization

The cultural and morphological characteristics of the isolates were studied. The bacterial isolates were identified using the manual of Bucchanon et al. (1974) and the Baggey's manual. While the fungi were identified using mostly Alexopoulus (1962).

3.2.3.2 Biochemical Characterization

Various biochemical tests were carried out using the conventional microbiological methods for characterizing bacterial isolates. Such methods included catalase test (Gangnon et al. 1959); Gelatin hydrolysis (Frazier 1962); Methyl red test, Nitrate reduction test, oxygen relationship (Hugh and Leifson, 1953) amylase activity citrate utilization, carbohydrate metabolism and voges-proskauer test.

3.2.4 Incubation Studies

Five organisms,which were finally selected for field exprimentation, were further studied regarding their solubilizing pattern and levels of acid production. Purified acid washed sand (pH 7) was weighed into petridishes (30g) and sterilized in the oven at 250°C for 2 hours 0.3g of Togo rock phosphate was weighed into each of the petridishes. The petridishes were then inoculated with 15mls broth cultures of the five organism, uninoculated petridishes served as control. The petridishes were incubated at 30°C for 12 weeks, at weekly intervals one petridish of each of the

five organisms and control was taken out for analysis.

The analysis carried out for this study included pH; of samples were taken as 1:2.5 ratio of sand solution in water and 0.01M $Cacl_2$; extractable P using the Bray - 1 method and total titratable acidity by the method described by Pearson (1976).

3.2.5 Mycorrhizal Studies

The method described by Debaud *et al* (1981) and Dexheimer *et al* (1979) were employed. The plants, freshly harvested in the greenhouse were taken to the laboratory where white healthy root hairs were removed, heated in 0.05% tryphan-blue for 10 mins at 90°C and viewed under the microscope.

3.3 GREENHOUSE STUDIES

Three greenhouse studies were carried out. The first study was aimed at further studying the micro-organisms selected from the preliminary screening in the laboratory. The second was to study the residual effects of the microbial solubilization. The third greenhouse experiment was to study the effect of the micro-organisms on mycorrhizal plant.

3.3.1 Soil Samples (Green house I)

Soil sample was collected from experimental field of the Institute for Agricultural Research, Samaru.

The soil was thoroughly mixed in the laboratory, air-dried and sieved through a 2 mm sieve. About 5kg soil was weighed into

each pot measuring 20.5 x 22 cm. A small portion of the soil was stored in plastic bottles for chemical analysis.

3.3.1.2 Test Crop

The soils were seeded to maize (Hybrid) and soybean (Samsoy 2). The choice of these crops was based on their importance in the savanna cropping system. Maize is an important cereal, it has high requirement for soil fertility and any fertilizer recommendation for maize may be satisfactory for a number of other crops. Soybean was recently introduced into the savanna, cropping system and it is rapidly gaining ground, like all other legumes. It has high need of phosphorus and its inclusion will also enhance the study of inoculant-Rhizobia interaction.

3.3.1.3 Treatments

(a) Maintreatments

The study consisted of four main treatments:

Treatment 1 (T_1) = Togo rock phosphate

Treatment 2 (T_2) = Sokoto rock phosphate

Treatment 3 (T_3) = Single super phosphate

Treatment 4 (T_4) = No source of P

a. Treatment 1 (T_1) received its P from Togo rock phosphate, T_2 received its P from Sokoto rock phosphate, T_3 from Single super phosphate while T_4 received no P at all. The fertilizer calculation for P was based on the recommended rate of 60kg P_2O_5/ha for maize and 40kg/ha P_2O_5 for soybean.

b. Sub-treatment

Ten organisms were selected from the preliminary laboratory studies. These were inoculated into all the main treatments in a randomized block design, and replicated 3 times.

3.3.1.4 Other Fertilizer Amendments

All treatments (T_1, T_2, T_3 and T_4) received the same amount of Nitrogen, Potassium and Zinc, The calculation was based on recommended rate for maize and soyabean.

The recommended rate for Nitrogen is 150kgN/ha for maize and 20kgN /ha for soybean. For potassium, 60kg K_2O/ha for maize and 20kg K_2O/ha for soybean and for Zinc 5kg $ZnSO_4$/ha for maize.

3.3.1.5 Pot Experiment

The soil sample was collected and prepared as described in section. 3.3.1. Each plastic pot was perforated in 4 places at the bottom prior to weighing of soil samples. The pots were labelled, adequately watered for 2 days after which they were inoculated with their corresponding organisms from broth culture, and then left to incubate for three days. All pots received 0.85g of N, 0.25g of K_2O and 0.033g of Zn for maize and 0.109g of N and 0.083g of K_2O for soybean. The nutrient materials were supplied from Urea, Muriate of potash and zinc sulphate.

The main treatments were applied to corresponding pots. The pots for single super phosphate received 0.833g of SSP for maize and 0.556g for soybean. Those for Togo rock phosphate received 0.409g of the rock for maize and 0.273g for soybean, while the pots for Sokoto rock phosphate received 0.422g of the rock for maize and

0.281g for soybean. These were based on the amount of P_2O_5 contained in the various fertilizer materials.

Six seeds of maize (*Zea mays*, Hybrid AG KADUNA) obtained from Pioneer seeds and soyabean (*Glycine max*, Var. Samsoy 2) obtained from IAR were planted in each pot and later thinned to four plants per pot one week after planting, and allowed to grow for additional 7 weeks before harvesting.

3.3.1.6 Sampling and Parameters Measured

The percentage germination was taken after one week of planting, subsequently, plant height, leaf breath, leaf length and number of leaves were taken every week.

The experiments were terminated after 8 weeks of growth, the shoots and roots were taken for analysis.

3.3.1.7 Plant Analysis

The shoots of the plant were dried in the oven at 60°C for 48hrs and ground, the total P was determined using dry ashing method (3.2.2.8).

3.3.1.8 Output:

Dry matter

P uptake

Plant height

Leaf area

Soil extractable P

3.3.1.9 Statistical Analysis

The randomized complete block design was used. Analysis

include:

a) analysis of variance

b) linear correlation analysis.

3.3.2 Residual Effect (Greenhouse II)

A second cropping of maize and soybean was carried out on the same soil used in 3.3.1 above.

3.3.2.1 Experiment

The soil used for 3.3.1 was prepared for this experiment. After the plants and roots had been harvested, the soil was poured on a clean paper and all plant and root residue were picked before pouring it back into the same pot and watered.

The pots were cropped to the same crop that were planted in them during the first cropping.

3.3.2.2 Fertilizer Application

Phosphorus sources were not applied in this experiment since the objectives was to evaluate the residual effect of the initial P sources Only N, K and Zn were applied.

3.3.2.5 Soil Sample Analysis

The soil samples were taken for the determination of available P.

3.3.2.6 Out Put

As stated in 3.3.1.8

3.3.2.6 Statistical Analysis

As stated in 3.3.1.9

3.3.2.6

Selection of 5 micro-organisms for field study.

3.3.3 Mycorrhizal Studies (Greenhouse III)

This study was aimed at studying the effect of the phosphate solubilizing micro-organisms on mycorrhizal fungi of soyabean. The organisms selected in section 3. 3. 2. 6 above were put through this test.

3.3.3.1 Experiment

Soil samples were collected and prepared as described in 3.3.1. Three kilograms of soil were weighted into plastic pots of 5kg capacity and watered.

The 5 organisms were inoculated into soils contained in the pot from broth cultures. The same fertilizer were applied into all pots. The fertilizers include N (Urea) (Muriate of Potash) and phosphorus (Togo rock phosphate). The calculations were based on recommended rates of the three nutrients stated in section 3.3.1.4.

The pots were left to incubate for one week, while watering every day before it was seeded to soybean.

The crops were left to grow for 8 weeks before harvesting. The white healthy root hairs were treated as described in 3.2.5.

3.4 FIELD TRIALS

3.4.1 Site: The experiment was laid out on one of the Institute for Agricultural Research Station, Samaru experimental plots.

3.4.2 Duration: The trial was carried out in one location for three cropping seasons for maize, while two cropping season for soyabean.

3.4.3 Test crops: The test crops used were soyabean and maize.

3.4.4 Plot size: Six rows of 6m long by 4m wide represented a plot or treatment and it was replicated three times.

3.4.6 Soil Sampling

Composite soil samples were taken from the field at 0 - 15 cm and 15 - 30 cm depths of each replicates for routine analysis. Soil samples were also taken at the same depth after harvest during each season.

3.4.7 Experimental Design

The experimental design was a split plot in randomized complete block design. Main plots were four phosphorus sources viz: Togo rock phosphate, Sokoto rock phosphate, single super phosphate and No P source (Zero P). The sub treatments were five organisms selected after greenhouse study and control (zero organisms).

The subplots were randomized within the main plots, each treatment was replicated 3 times for each crop.

3.4.8 Land Preparation

The land was prepared mechanically by the Estate Department of Institute of Agricultural Research. It was ploughed as soon as the rains established, harrowed and then ridged at 75 cm between

ridges.

Each plot of 6 ridges by 4m were marked out using wooden pegs. The herbicides Gramoxone and Galex were sprayed to reduce effect of weed during the season.

3.4.9 Fertilizer Application

The plots were inoculated with their corresponding micro-organisms from broth cultures. The broth were applied in drills on top of the ridges. The phosphorus treatments were also applied. That is; Togo rock phosphate, Sokoto rock phosphate, single super phosphate and zero P. The three P sources were applied based on the recommended rate of 60kg P_2O_5 g/ha for maize and 40kg P_2O_5/ha for soybean. Other fertilizers were also applied as blanket application. About 150kg N/ha for maize and 20kg N/ha for soybean was applied as Urea, while 60kg K_2O/ha for maize and 20kg K_2O/ha for soybean was applied as muriate of potash. Zinc sulphate was applied at 5Kg Zinc Sulphate /ha for maize.

In the case of muriate of potash and Zinc Sulphate application was made once before planting. But the urea was applied in split dose, the first dose was applied at planting and the second dose at 3 - 4 weeks after planting.

3.4.10 Planting Method

Maize (Hybrid) and soybean (Samsoy 2)were planted after being dressed with Aldrex T.

For maize, one seed each was planted 75 cm between and 25 cm

within row-spacing giving a plant population of 53,000 plants/ha. for soybean the seeds were planted along the drills and later thinned to one seed per hole.

Weeds were controlled by manual hoe weeding in conventional and manual tillage system.

3.4.11 Plant Sampling

At 50% silking and flowering maize ear leaf and soybean leaves were taken from ten stands in two cultural rows in each plots. The plant height and leaf area were also taken. Samples were dried at 60°C for 48 hours, weighed ground and stored for tissue analysis.

3.4.12 Harvesting

At maturity the maize and soybean were harvested. For maize, cobs were dehusked, weighed before shelling and the stalk were also weighed. The net plots were the two middle rows for each plot.

The soybean were cut and laid in heap to reduce shattering, they were then beaten and winnowed on the field. The grains were taken to the laboratory for weighing.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 LABORATORY STUDIES

4.1 Isolation of Phosphate Solubilizing Organisms (PSM)

The soil is one of the most dynamic sites of biological interactions in nature. It contains a vast array of micro-organisms. Over one hundred and twenty micro-organisms were encountered in the cause of this study. However, only sixty one of those micro-organisms encountered solubilized phosphates, nineteen of them were fungi and forty two were bacteria. The levels to which they solubilize insoluble phosphates are shown in tables 1a and b.

Phosphate solubilizing organisms were mostly encountered in the rhizosphere, very few were encountered in the rhizoplane. The phosphate solubilizing efficiencies of those encountered in the rhizosplane appeared to be higher than the efficiencies of those encountered in the rhizosphere (tables 1a and b). Katnelson and Bose (1959) observed that the capacity of bacterial isolates to solubilize phosphate depends upon their zone of origin. And Gaur (1981) attributes the productiveness of the rhizosphere for Phosphate solubilizing micro-organisms (PSM) to the favourable influence exerted by root exudates.

4.1.2 Fungi Isolates

Nineteen fungi isolates were found to solubilize insoluble phosphates and the values ranged from 12-28.9ug P_2O_5 per gram of soil (table 1a). More than half of the fungi isolated were able to solubilize as much as 20µg

Table 1a: Phosphate solubilizing fungi isolated from soils

Code No.	Source	P_2O_5 Solubilized (μg/g soil).
F_1	Rhizosphere	27.9
F_2	Rhizosphere	14.6
F_3	Rhizosphere	23.5
F_4	Rhizosphere	25.6
F_5	Rhizosphere	20.6
F_6	Rhizosphere	12.0
F_7	Rhizosphere	26.8
F_8	Rhizosphere	18.5
F_{10}	Rhizosphere/Rhizoplane	21.0
F_{11}	Rhizosplane	16.5
F_{12}	Rhizosphere	21.6
F_{13}	Rhizosphere	25.6
F_{14}	Rhizosphere	23.1
F_{15}	Rhizosplane	12.0
F_{16}	Rhizosplane	21.1
F_{18}	Rhizosphere	15.3
F_{19}	Rhizosphere	10.1
F_{20}	Rhizosphere	28.9
F_{21}	Rhizosphere	24.8
	Mean	20.5

Table 1b: Phosphate solubilizing bacteria isolated from the Samaru soils

Isolate No.	Sources of isolation	Gram stain	P_2O_5 solubilized ($\mu g/g$)
B_1	Rhizosphere	Gram positive rod	24.9
B_2	Rhizosphere	Gram negative rod	11.7
B_3	Rhizosphere	Gram positive cocci	14.6
B_4	Rizosphere/ Rhizosplane	Gram negative cocci	11.6
B_5	Rhizosphere	Gram positive rods	23.3
B_6	Rhizosplane	Gram positive rods	19.8
B_7	Rhizosphere	Gram positive rods	13.3
B_8	Rhizosplane	Gram positive cocci	11.8
B_9	Rhizosphere	Gram positive rods	11.2
B_{10}	Rhizosphere	Gram negative cocci	13.3
B_{11}	Rhizosphere	Gram negative cocci	12.2
B_{12}	Rhizosplane	Gram positive rod	15.0
B_{13}	Rhizosphere	Gram positive rod	25.9
B_{14}	Rhizosphere	Gram positive cocci	21.2
B_{15}	Rhizosphere	Gram negative rod	14.6
B_{16}	Rhizosplane	Gram positive cocci	15.9
B_{17}	Rhizosphere	Gram positive cocci	24.0
B_{18}	Rhizosphere	Gram negative rod	25.9
B_{19}	Rhizosphere/ Rhizosplane	Gram positive cocci	10.5
B_{20}	Rhizosplane	Gram positive rod	13.5
B_{21}	Rhizosphere	Gram negative cocci	15.6

B_{22}	Rhizosphere	Gram negative cocci	15.3
B_{23}	Rhizosphere	Gram positive rod	13.4
B_{24}	Rhizosphere	Gram positive rod	14.6
B_{25}	Rhizosphere	Gram negative rod	17.7
B_{26}	Rhizosphere	Gram negative rod	11.3
B_{28}	Rhizosphere	Gram positive cocci	17.6
B_{29}	Rhizosplane	Gram positive cocci	18.5
B_{30}	Rhizosphere	Gram negative cocci	16.0
B_{31}	Rhizosphere	Gram negative cocci	19.3
B_{33}	Rhizosphere	Gram positive cocci	16.0
B_{34}	Rhizosphere	Gram positive rod	16.2
B_{35}	Rhizosplane	Gram negative cocci	18.5
B_{36}	Rhizosplane	Gram positive rod	12.3
B_{37}	Rhizosplane	Gram positive rod	17.7
B_{38}	Rhizosphere	Gram negative rod	16.0
B_{39}	Rhizosphere	Gram negative cocci	13.0
B_{40}	Rhizosphere	Gram negative rod	18.0
B_{41}	Rhizosphere	Gram positive rod	17.7
B_{42}	Rhizosphere	Gram positive cocci	12.8
B_{43}	Rhizosphere/ Rhizoplane	Gram negative rod	20.3
B_{44}	Rhizoplane	Gram positive cocci	13.3
	Mean		16.3

P_2O_5/g soil individually, with overall average of 20.5kg indicating that they were good solubilizers of insoluble phosphates (table 1a). Although the organic acids excreted by the organisms were not measured, Konig (1961) showed that organic acid liberated by fungi dissolved phosphates and this was followed by the absorption of phosphate ions by the mycelia which were ultimately released by autolysis of the organisms.

4.1.3 Bacteria Isolates

Forty-two bacteria isolates were found to solubilize insoluble phosphates, their solubilizing capacity ranged between 10.5-25.9 ug P_2O_5 per gram soil with a mean of 16.3ug/g (table 1b). Only about seven of the bacteria isolates could solubilize up to 20 ug P_2O_5 g^{-1} soil. The low solubilizing capacity of bacteria relative to fungi had been observed also by Muromtseve (1988), Sperber (1958) and Medina De Wanerli (1968). The bacterial isolated were mostly rods and cocci either gram positive or gram negative in reaction (table 1b). Ahmad and Jha (1968) also published results regarding the solubilization of hydroxyapatite and rock phosphate by gram positive and negative rods and cocci shaped bacteria.

4.2 Screening of Isolates

Nineteen microorganisms, comprising eleven fungi and eight bacteria that showed appreciable levels of phosphate solubilization were screened in the laboratory. The amounts of P_2O_5 released as a result of solubilization, and the pattern of release were considered in this screening. The results are presented in figures 1 and 2. The relationship between P release and number of weeks was non linear. Generally, an initial release of P_2O_5 occurred immediately after inoculation and this was followed by a drop in the second week (Fig. 1 and 2). Pattern of P release differed from one organism to another; most of the organisms seemed to show one or two peaks of P release in the 6 weeks of incubation. The drop in the level of P_2O_5 released witnessed at the 3rd to 5th weeks of incubation can be attributed to immobilization of P by some organisms. (Benians and Barber, 1974). The assimilation of phosphorus into microbial nucleic acids, phospholipids or other protoplasmic substances leads to the accumulation of non-utilizable form of the element (Alexander 1977). In the case under study inoculation of organisms on the applied raw rock phosphates in acid washed sand led to initial increase in microbial population and activity and this might have put a great demand on the phosphate supply. Consequently, in a phosphorus deficient acid

washed sand the microbial assimilation of available phosphate as it was being solubilized might have further depressed the amount of phosphorus in the medium.

The fungi organisms seem to show two peaks of P release (fig. 1), most of them gave the first peak at the second week, and the second peak at the fourth week. Fungi 3, 10, and 13 showed some consistencies in the amount of P solubilized. They gave lowest releases of15.9,15.9 and 18.3 and highest releases of 43.1, 40.9 and 41.4μg/g respectively. Organism F_{21} showed an initial high amount of P_2O_5 release (60.7 ug/g), the level of P release gradually dropped after that, however at the 5th week there was another increase in P release. Organism F_{20} on the other hand, gave two clear peaks of P release (fig. 1) and the amounts of P released did not show much variation. The lowest was 20.8 and the highest was 33.78 μg/g. It showed some consistency when compared with the other organisms. The organisms discussed above were selected for further studies, due to their consistency in amount of P released and pattern of release. Organisms F_4 and F_{12} did not show any high level of P release until the 4th week and by the 5th week there were high drops in the P released and they did not seem to release any more high levels of P after that. Organisms F_5, F_8, F_{14} and F_{16} on the other hand gave low levels of P releases throughout the six weeks of incubation and were therefore

not used for further studies.

The bacterial organisms unlike the fungi, generally showed one peak throughout the six weeks of incubation, some of the organisms however gave indications of higher activity after the sixth week (fig. 2). Initially, organisms B_1, B_5, B_{18} and B_{43} gave high levels of P releases as 59.5, 59.5, 63 and 59μg/g soil respectively (fig. 2). However, they all showed immediate drop in level of P_2O_5 at the second week, while B_{18} did not release as much P_2O_5 again throughout the experiment. Organism B_1 was able to release as much as in the 6th week (55 μg/g). B_5 and B_{20} like B_{18} did not release any more high levels of P after the initial drop (fig. 2). B_5 differs from other bacteria in its pattern of solubilization. It released low levels of P_2O_5 but with small variation in the range (28.0-35μg/g). It seemed similar to F_{20} in its consistency in level of P releases.

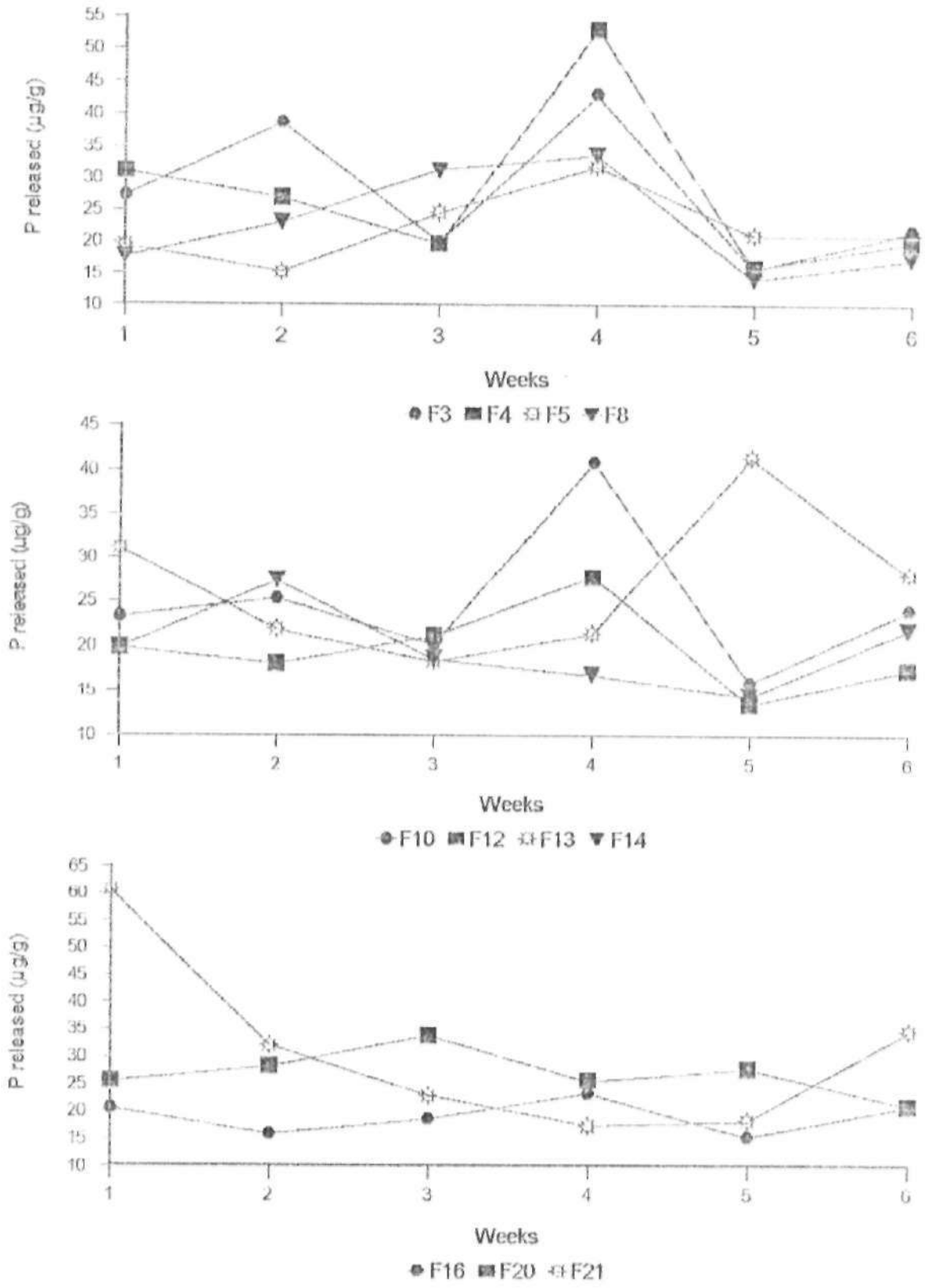


Figure 1 Amount and pattern of phosphorus released by the fungal isolates.

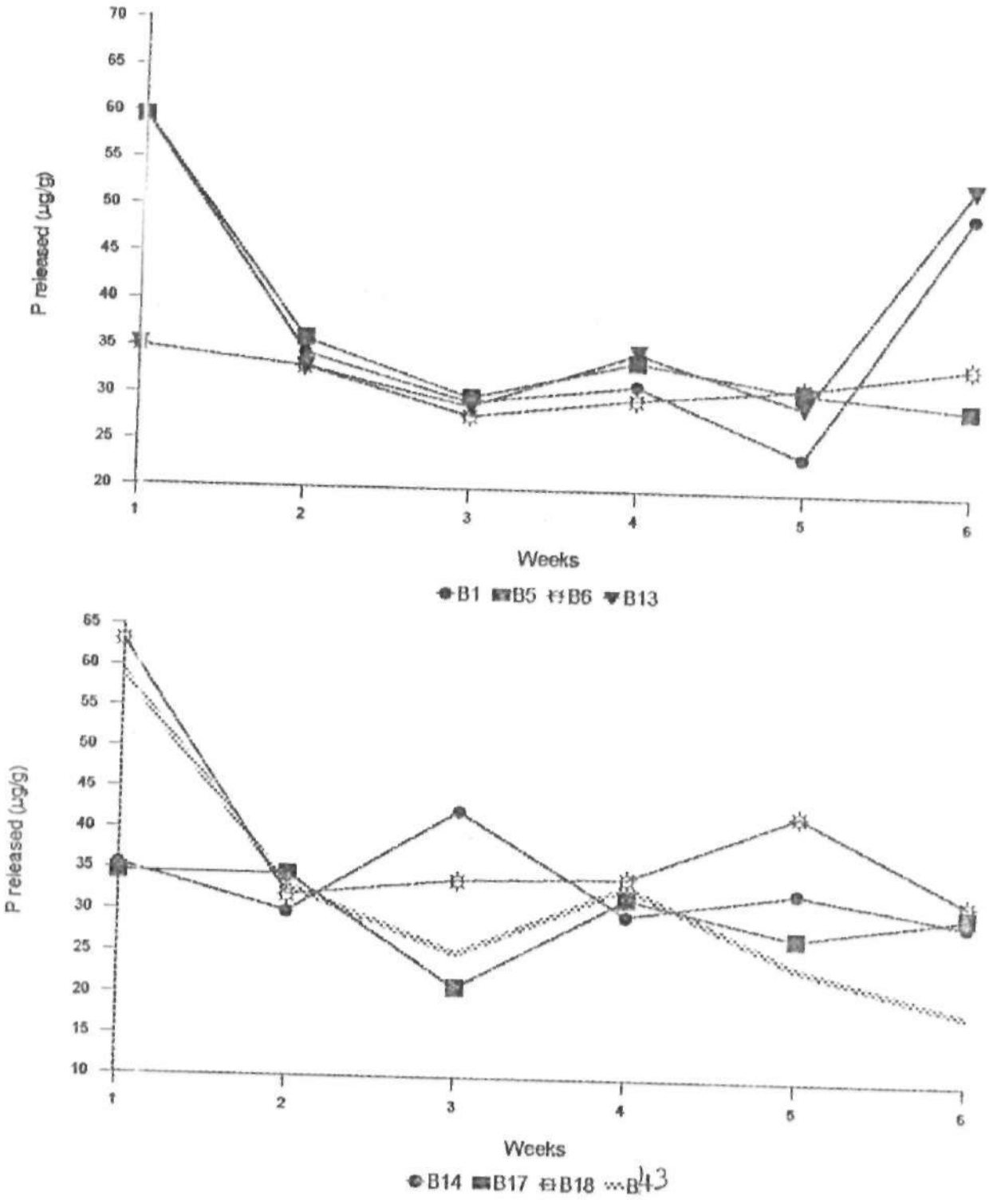


Figure 2: Amount and pattern of phosphorus released by the bacterial isolates.

Although B_{13} gave initial low level of P released it was however able to release higher amounts of P at the 6th week (53μg/g),but its period of immobilization seems to be longer than the others. B_{14} and B_{17} did not show much rock phosphate sulubilization and their pattern of solubilization are similar to those already discussed above. They showed a lot of reduction in P immobilization (26 and 21μg/g) compared to B_6 (7μg/g) and they did not appear to increase level of P release during the 6weeks of incubation (fig. 2). Konig (1960) showed that solubilization of phosphates from less soluble rock phosphate, proceed through the utilization of small amounts of initial phosphorus available in the medium by certain organisms, followed by secretion of organic acid by growing mycelia for dissolving phosphates.

Organisms B_1, B_6, B_{13}, B_{18} and B_{11} were selected for greenhouse studies, based on the amount of P_2O_5 solubilized and the length and pattern of immobilization of the organisms as has been discussed above.

The organisms selected from this screening experiment were given new code names in order to bring about a uniform nomenclature. They were no longer called bacteria or fungi but organism. This was aimed at reducing the combersomeness in labelling of pots in the green house, the new names are shown on table 2.

Table 2: Organism selected for greenhouse studies.

Organisms	New names	Mean P released (µg/g)
B_1	Organism 6	38.2
B_6	" 10	31.9
B_{13}	" 7	35.8
B_{18}	" 8	39.6
B_{44}	" 9	31.9
F_3	" 1	40.0
F_{10}	" 2	37.0
F_{13}	" 3	33
F_{20}	" 4	46.9
F_{21}	" 5	51.0

4.2 Green House Studies

4.2.1 Soil Sample

The soil test parameters of the soil potted for greenhouse studies are shown in table 3. It is a silt loam soil with pH value of 5.8, total nitrogen was 1.5gkg^{-1}, organic carbon was 5.5gkg^{-1}, CEC was 9.0 cmol and the available P was 6.0µg/g.

The values presented above are characteristic to savanna soils. They are mainly Alfisols generally low in organic matter due to poor vegetation, removal of crop residues from the farm and grazing by animals. The soils

Table 3: Soil test values of surface soil used for the greenhouse studies.

Soil Parameter		Values
% Clay		7.6
% Silt		40
% Sand		52.4
Textural Class		Silt loam
pH/H_2O 1:2.5		5.8
$pH/0.01mCaCl_2$ 1:2.5		4.7
Organic carbon (g kg^{-1})		5.5
Total nitrogen (g kg^{-1}		1.5
Exchangeable bases (cmol (±) Kg^{-1})		
	Ca	6.6
	Mg	2.9
	K	0.34
	Na	0.26
CEC (Cmol±Kg^{-1})		9.0
Available P (µg/g soil)		6.0

are also poorly structured and poorly buffered and low in CEC. They are therefore low in natural fertility and their productivity declines quite rapidly under continuous cultivation (Lombin, 1987). The soils are highly deficient in N and P and response to these two elements has been most pronounced in cereals. The mean available P in soils are in the range of 4.1-6.0 ppm (Singh *et al*, 1983).

4.2.2 Response of Soyabean to treatments

4.2.2.1 Visual observation

Observation of seedlings at first cropping did not reveal any evidence of phosphorus deficiency as typified by purple coloration in all the P sources. However, by the 6th week all the pots supplied with SSP had flowered while only 3 out of the ten pots supplied with Togorock showed flowers. Sokoto rock on the other hand did not show any flower by the 6th week. Phosphorus uptake in Soyabean as observed by FAO (1977) is relatively constant throughout the growing season, however peak absorption normally takes place during the early stages of seed development. This observation by FAO (1977) tends to explain the reason for the inability of pots supplied with rock phosphates to flower early, as rock phosphates could not supply the high demand of plants for P at this stage of development.

The pots that showed flowers among the pots supplied with Togorock were those inoculated with organisms 1, 3 and 10 suggesting that these organisms were efficient at solubilizing Togo rock phosphate but not Sokoto rock phosphate.

During the second cropping, there was no visual symptoms of P deficiency on the test crops, normal flower development by all the potted plants were observed. This change in crop performance is attributed to the

availability of P in all treatments during second cropping, suggesting that enough time had been allowed for solubilization to take place, and subsequent release of P from the rock phosphates for plant use.

4.2.2.2 Effect of P Sources:

Response of soyabean to the different sources of P applied are presented in table 4. Minimal responses were observed at the first cropping. By the second cropping, however, the responses of crops to P increased. Higher values of the parameters were observed in second cropping due to differences in the length of experiments. The first crop was harvested after 6 weeks while the second crop was terminated after 8 weeks of growth. This inconsistency in lenght of experiment was due to aphid attack on the soyabean seedling experienced during the first cropping.

Table 4a: Effect of 4 different P sources on mean yield components of soyabean in the greenhouse

	First Cropping						Second Cropping					Soil parameters		
P-Sources	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter yield (g/pot)	Nodule weight (mg/pot)	P-conc % P	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter Yield (g/pot)	Nodule weight (mg/pot)	P conc. (% P)	Residual P µg/g Soil	pH of Soil
SSP	19.8a	18	2.8	3.0a	37	0.31	23.5a	33	9.9a	19b	125.8b	0.16	36.2	5.1
TPR	18.8a	18	2.8	3.0a	24	0.25	22.8a	32	7.76	21.7a	209.1	0.18	43.5	4.5
SPR	17.2b	16	2.4	2.0b	21	0.30	20.8b	31	9.6a	24.48a	233.9	0.15	37.0	4.8
Control	16.2b	14	2.1	2.0b	23	0.25	20.1b	28	7.16	20.1b	179.1	0.14	30.0	6.0
LSD (0.05)	1.74	NS	NS	0.91	NS	NS	1.81	NS	1.72	4.48	NS	NS	NS	NS
(0.01)	2.74	NS	NS	NS	NS	NS	2.86	NS	2.71	7.06	NS	NS	NS	NS

Neans not followed by the same letter are statistically significant at 0.05 by DMRT.

4.2.2.3 Plant Height

Significant differences were observed in crop response to P source at the first and second croppings. At both croppings SSP and Togo rock were similar but significantly different (P=0.05) from Sokoto rock and control which were at par (table 4a). The above observation suggests that Togo rock is more reactive than Sokoto rock and so able to release some P into the soil for crop growth. However, as earlier observed, soyabean P uptake at initial stages of development is minimal and so it is not suprising that Togo rock is able to release enough P to meet the P requirements at this stage.

4.2.2.4 Leaf Area

Similar to observation on plant height, SSP and Togo rock gave similar values of leaf area (18cm^2) at the first cropping, while Sokoto rock and control gave lower values of 16cm^2 and 14cm^2. Similarly, SSP and Togo rock were superior to Sokoto rock and control in the area of leaf produced at the second cropping (table 4a). The area of plant leaf is a function of photosynthetic activities of the plant and thus it is expected that treatments that will promote bigger leaves formation will also ultimately promote higher yield production.

4.2.2.5 Root Weight

The weights of roots were not significantly different at the first cropping, however SSP and Togo rock gave

similar root weights (2.8g) which were superior to Sokoto rock phosphate (2.4g) and control (2.1g) as shown in table 4a. The second cropping, however gave significant difference in the root weight produced. SSP and Sokoto rock gave significantly different (P=0.05) value from Togo rock and the Control, suggesting that by the second cropping Sokoto rock had released enough P to promote increased root growth. A good supply of phosphorus has been associated with increased growth of roots which proliferate extensively in the area of treated soil (Tisdale, 1984).

4.2.2.6 Dry Matter Yield

Significant differences were recorded in dry matter yields at both croppings (table 4a). SSP and Togo rock were comparable and significantly different (P=0.05) from Sokoto rock and Control, giving an increase of 1g at the first cropping. On the other hand, the second cropping showed the two rock phosphates (Togo and Sokoto rock) as being significantly different (P=0.05) from SSP and the Control, an increase of 4.5g was recorded between SSP and Sokoto rock. Among the rock phosphates, Sokoto rock was superior to Togo rock by an increase of 2.8g (table 4a). The superiority of the rock phosphates over SSP as observed above is an indication of residual effectiveness of rock phosphates. The water soluble SSP dissolves more easily in the soil solution, the released phosphate ions are quickly

transformed by reactions with soil constituents into less soluble forms. On the other hand the non water soluble rock phosphates release P slowly and its reactivity is influenced by the reactivity of the rock, the physical form of the rock and different soil factors (Visker *et al*, 1994).

4.2.2.7 Nodule Weight

There were no significant difference in nodule weights, SSP promoted increased nodule formation at the first cropping. It gave increased nodule weight of 13mg above Togo rock (table 4a). The second cropping however showed Sokoto rock phosphate as superior to other sources at enhancing nodule formation. It gave increases of 24.8mg and 108mg above Togo rock and SSP. This observation suggests superior residual effectiveness of Sokotorock over Togo rock.

4.2.2.8 P Concentration

SSP enhanced higher P concentration in the plant tissue at the first cropping (Table 4a). Among the rock phosphates, however, Sokoto rock gave higher P of 0.05% over SSP in the plant tissue. Contrary to this the second cropping showed that more P was taken up from soils supplied with Togo rock (table 4a).

4.2.2.9 Residual P

At the end of both cropping, higher P levels was recorded in soils supplied with rock phosphates. However,

the differences among the levels of residual P from various sources were not significant (table 4a). Phosphate rock required time to undergo reactions in the soil and this reactions are influenced by chemical and mineralogical composition of the rock phosphates. However, the pH of the soil and the Ca and P concentrations in the soil solution are equally important in solubilization of rock phosphates (Khasawneh and Doll, 1978). Observation on the pH of the soil shows that the soils supplied with rock phosphates recorded lower pH values at the end of experiment (table 4a).

4.2.2.10 Crop Response to P

Three types of response were observed: (1) At the first cropping, the magnitude of response was SSP > Togo rock > Sokoto rock > Control.

(2) At second cropping, the magnitude of response was Sokoto rock > Togo rock > SSP > Control

(3) The residual P of soil, the magnitude of response was Togo rock > Sokoto > SSP > Control. Table 4b and 4c show the correlatin matrix of soyabean. P source correlated significantly with the root weight of first cropping, further coroborating the observations made about root growth as influenced by good phosphorus supply. At the second cropping, however, P sources correlated significantly with leaf area, dry matter yield and nodule weight as shown in table 4b. Also indicating the

efficiency of rock phosphate at this second cropping.

4.2.3 Effects of Organisms

Crops did not respond to inoculation significantly at the first cropping, however at the second cropping, significant response were recorded to inoculation in some of the parameters taken. The results are presented on table 5.

Table 4b: Correlation Matrix at First and second Croppings of Soyabean

First cropping

	LA	PH	DMY	ROW	NW	P-upt
Leaf Area (LA)	-					
Plant Height (PH)	-0.144	-				
Dry matter yield (DMY)	0.2364*	-0.1230	-			
Root Dry Weight (RDW)	0.2833*	-0.0758	0.8286**			
Weight of Nodules (NW)	-0.0595	-0.0656	0.3050**	0.2666*	-	
Puptake (P-upt)	0.0656	-0.0318	-0.0818	0.0171	-0.0549	-

Significant level: * = P 0.05, ** = P 0.01

Second cropping

	LA	PH	DMY	ROW	NW	P-upt	Re
Plant Height (PH)	-						
Leaf Area (LA)	0.6066**	-					
Dry Matter Yield (DMY)	-0.1223	-0.2309	-				
Root Dry Weight (RDW)	-0.1988	-0.3228**	0.1810	-			
Wt. of Nodules (NW)	-0.606	-0.1903	0.4390**	0.0184	-		
P-Uptake (P-upt)	-0.0720	-0.0178	-0.1149	-0.0462	0.0037	-	
Residual P (Res P)	-0.2827*	-0.1468	-0.0664	0.1329	-0.0551	0.0802	-

Signficant level: * = P 0.05, ** = P 0.01

Table 5: Effect of micro-organisms on the yield components of soyabean in the greenhouse

	First Cropping						Second Cropping						Soil Parameters	
Organisms	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter yield (g/pot)	Nodule weight (mg/pot)	P-conc % P	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter Yield (g/pot)	Nodule weight (mg/pot)	P conc. (% P)	Residual P µg/g Soil	pH of Soil
1	192	19	2.9	3.0	6.0	0.29	201[c]	30	8.9	20	182.2[b]	0.13	32.5	4.2
2	195	21	3.0	3.0	21.0	0.28	223[b]	31	8.2	21	179.7[b]	0.15	37.3	4.5
3	185	18	3.2	3.0	48.0	0.27	221[c]	30	8.6	23	236[a]	0.15	38.4	5.1
4	193	17	2.3	3.0	50.0	0.32	268a	32	8.8	20	186.9[b]	0.18	29.4	5.4
5	194	16	3.0	3.0	26	0.25	231[b]	35	8.8	23	153.2[b]	0.22	43.0	4.5
6	183	18	2.5	2.0	61	0.31	227[b]	33	8.3	20	162.0[b]	0.16	47.1	5.4
7	176	16	2.2	2.0	32	0.25	208[c]	32	8.4	20	133.5[b]	0.17	51.4	5.3
8	184	17	2.6	2.0	30	0.31	227[b]	34	9.6	22	174.6[b]	0.17	29.7	4.5
9	189	18	2.5	2.0	12	0.25	222[b]	29	8.4	25	228.4[a]	0.14	41.8	4.3
10	187	15	2.8	3.0	3.0	0.28	214[b]	32	9.1	27	259.7[a]	0.18	39.6	5.6
Control	172	18	2.4	2.0	0.0	0.24	202	24	8.5	20	189.7[b]	0.16	28.9	5.8
LSD (0.05)	NS	NS	NS	NS	NS	NS	21.8 28.9	NS	NS	NS	65.31 103.35	NS	NS	

Means not followed by the same letter are statistically significant at 0.05 by DMRT.

4.2.3.1 Plant Height

At the first cropping organisms 2, 5, 4 and 1 gave high values of plant height of 195, 194, 193 and 192cm respectively (table 5). Organisms 2 gave an increase above Control of 23cm, and it gave increase over organism 6 (lowest among inoculants) of 12cm, all the inoculants however gave higher values than Control. Similarly, at the second cropping, significant differences were observed in plant height, organism 4 was significantly different from organisms 2, 3, 5, 6, 8, 9 and 10 which were at par but also significantly different (P=0.05) from organisms 1, 7 and Control (table 5). An outstanding performance of organism 4 is observed at the second cropping. It increased above Control by 66cm as a result of enough time between inoculation and planting thus allowing for solubilization to take place and the subsequent release of P.

4.2.3.2 Leaf Area

The leaf areas were not significantly different at both croppings, however at first cropping organism 2 gave highest value of leaf area while at second cropping organism 5 produced the biggest leaves, it increased above control by 11cm^2 (table 5)

Higher photosynthetic rates especially at the upper leaves of soyabean is essential for good pod filling (Stephenson and Wilson, 1978). Bigger leaves produced by these organisms will thus increase the photosynthetic rates and ultimately improve pod yield.

4.2.3.3 Root weight

There were no significant differences due to difference of inoculants on root weights (table 5). However, organisms 3, 2 and 5 gave high values of root weight at the first cropping, while at the second cropping most of the organisms produced more root weight than control. Organism 8 was observed to give an increase of 1.1g above Control.

4.2.3.4 Dry Matter Yield

The dry matter yeilds did not show significant differences, however organisms 1, 2, 3, 4, 5, and 10 were superior in dry matter production at first cropping, they gave an increase of 1g above the other organism and control (table 5). At the second cropping, organisms 9 and 10 gave an increase of 5g and 7g over control.

4.2.3.5 Nodule Weight

Organisms 3 and 4 gave the best nodulations of 48 and 50mg at the first cropping while control did not produce any nodule. However, in the second cropping, there were no significant differences in the performance of organisms over Control (table 5). Organisms 3, 9 and 10 increased nodule weight by 47mg, 38.7mg and 70mg respectively over control.

4.2.3.6 P-concentration

Organisms 4, 6 and 8 gave higher values of P concentration in plant tissue at the first cropping. They gave increased P of 0.08, 0.07 and 0.07% respectively (table 5). At the second cropping only organism 5 gave much increase of 0.06% over

control.

4.2.3.7 Residual P

All the organisms inoculated were superior to control in the amount of residual P recorded in the potted soils. However, organisms 5, 6, 7 and 9 proved much superior in their residual P. They gave increases of 14.1, 18.2, 22.5 and 12.9 µg/g respectively (table 5). The residual effect of these micro-organism can also be related to the observed reduction of pH of soils. All the inoculated soils gave lower pH than the uninoculated soil (Table 5).

4.2.3.8 Crop Response to Organisms

The crop responded to inoculation at both croppings, however, the magnitude of response was more at the second cropping. The organisms were not consistent in their performance and so it was difficult to rate each of the organisms. However, crop responded to inoculation of the organism and the magnitude is inoculation > uninoculation.

The inconsistency of the organisms is also indicated on the correlation matrix table (tables 4b). Organism did not correlate significantly with all parameters observed. The first cropping however showed significant correlation between organism and root weight.

4.2.4 Yield Parameters as Influenced by Inoculation on P sources

The influence of inoculation combined with P sources on soyabean seedlings are presented in figures 3-7. The differences in the parameters taken were not significant.

However, the influence of treatment on soyabean is highlighted between croppings.

4.2.4.1 Leaf Area

During the first cropping, SSP produced higher values of leaf area at certain inoculations than the rock phosphates (Fig. 3). However, it gave an increase of 6.57g above control when inoculated with organism 2. Togo rock phosphates gave higher values of leave area than SSP at most inoculation, however at control SSP was superior to Togo rock.

At the second cropping, organism 5 enhanced the performance of SSP above control by 6.77gm, however, at other inoculations SSP was similar to or less than the rock phosphates in performance. Contrary to observation at the first cropping, Sokoto rock appeared to be superior to Togorock at certain inoculations. It gave increases of 6.4 and 4.9g above Togo rock when inoculated with organisms 6 and 8.

4.2.4.2 Dry Matter Yield

At the first cropping, SSP was enhanced in its dry matter production especially at inoculations with organisms 1, 2, 4, 5 and 6. (Fig. 4). Most of the organisms however, enhanced SSP to produce more dry matter than control. Organism 2 brought about an increase of 3.47g above control. Togo rock gave higher dry matter than SSP when inoculated with organism 3 and suprisingly at Control.

The second cropping was quite different from first cropping as the rock phosphates were quite superior in their production

of dry matter to SSP at all inoculations and control. Among the rock phosphates, Sokoto rock proved superior to Togo rock in its performance, at inoculation with organism 5 it gave a difference of 9.45g. However, it appears that uninoculation of Togo rock enhances its production of dry matter yield than inoculation with most of the other organisms with the exception of organism 10.

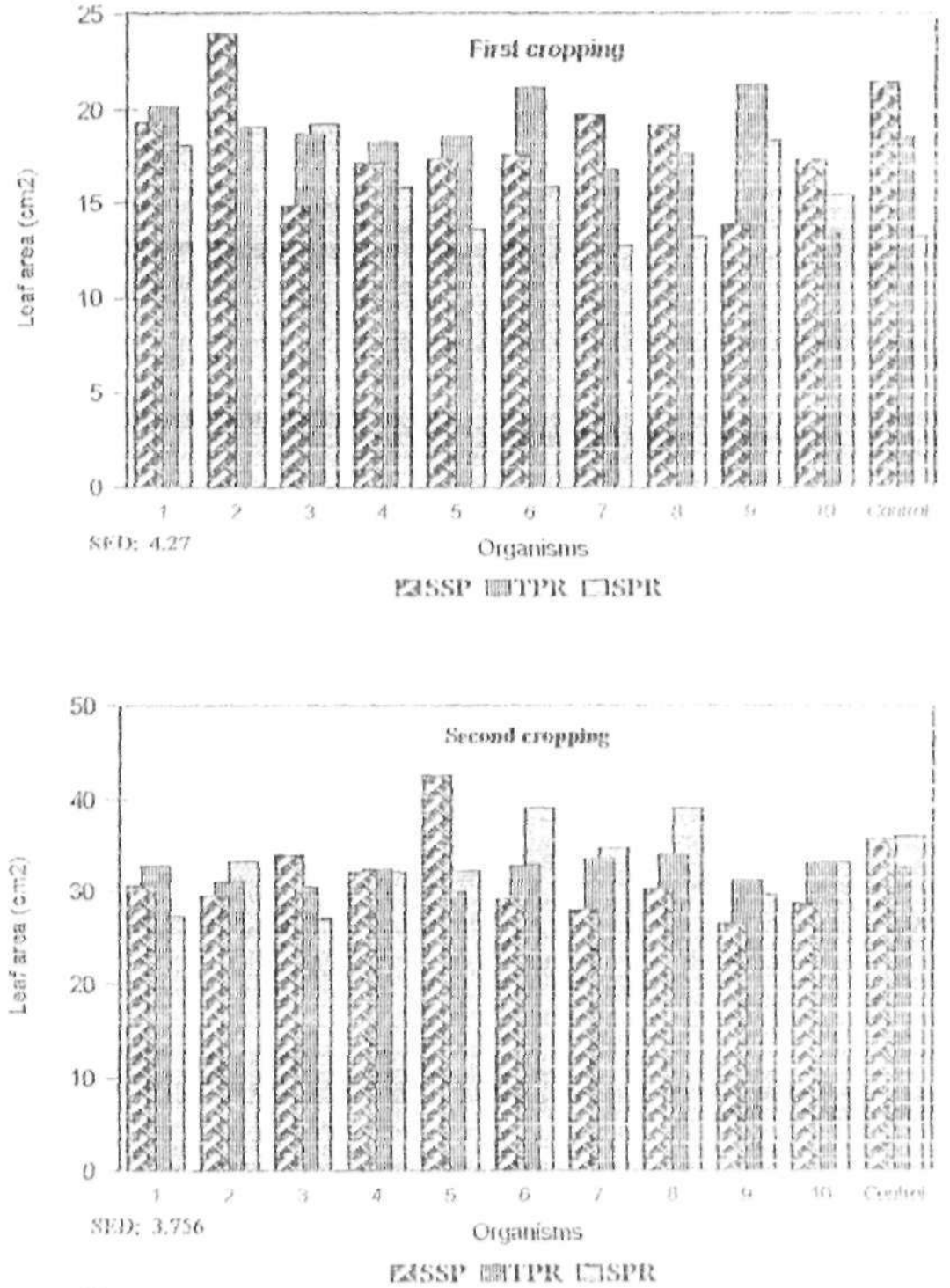


Figure 3: Effect of P sources and organisms on soyabean leaf area in the greenhouse.

4.2.4.3 Nodule Weight

Nodulation at the first cropping was poor (fig. 5), and SSP gave superior values of nodule weight at inoculation with organisms 3 and 4 and these gave increases of 104mg and 142mg above Togo rock at the same inoculations. Togo rock was improved upon by inoculation with organism 2 while inoculation with organisms 6 and 7 improved the performance of Sokoto rock.

Unlike the first cropping, nodulation at second cropping was good. The rock phosphate gave higher nodule weights than SSP at all inoculations (fig. 5). The organisms appeared to be selective in performance among the rock phosphates. Organism 9 enhanced an increase of 172.3mg in Sokoto rock above Togo rock, while organism 10 brought about an increase of 128.9mg in Togorock above Sokoto rock at same inoculations.

4.2.4.4 P-concentration

At first cropping, SSP produced more P in plant-tissue at most inoculations than the rock phosphate (fig. 6). However effect of inoculation on SSP was not superior to uninoculated SSP in P concentration. At the second cropping the rock phosphate became more prominent in their release of P to plant tissue. At some of the inoculations either of the rock phosphate released more P than SSP. Togo rock was quite superior in its releases of P at inoculation with organisms 4, 5, 8 and 9. These organisms brought about increases of 0.08, 0.13, 0.06 and 0.10% P in Togorock above SSP.

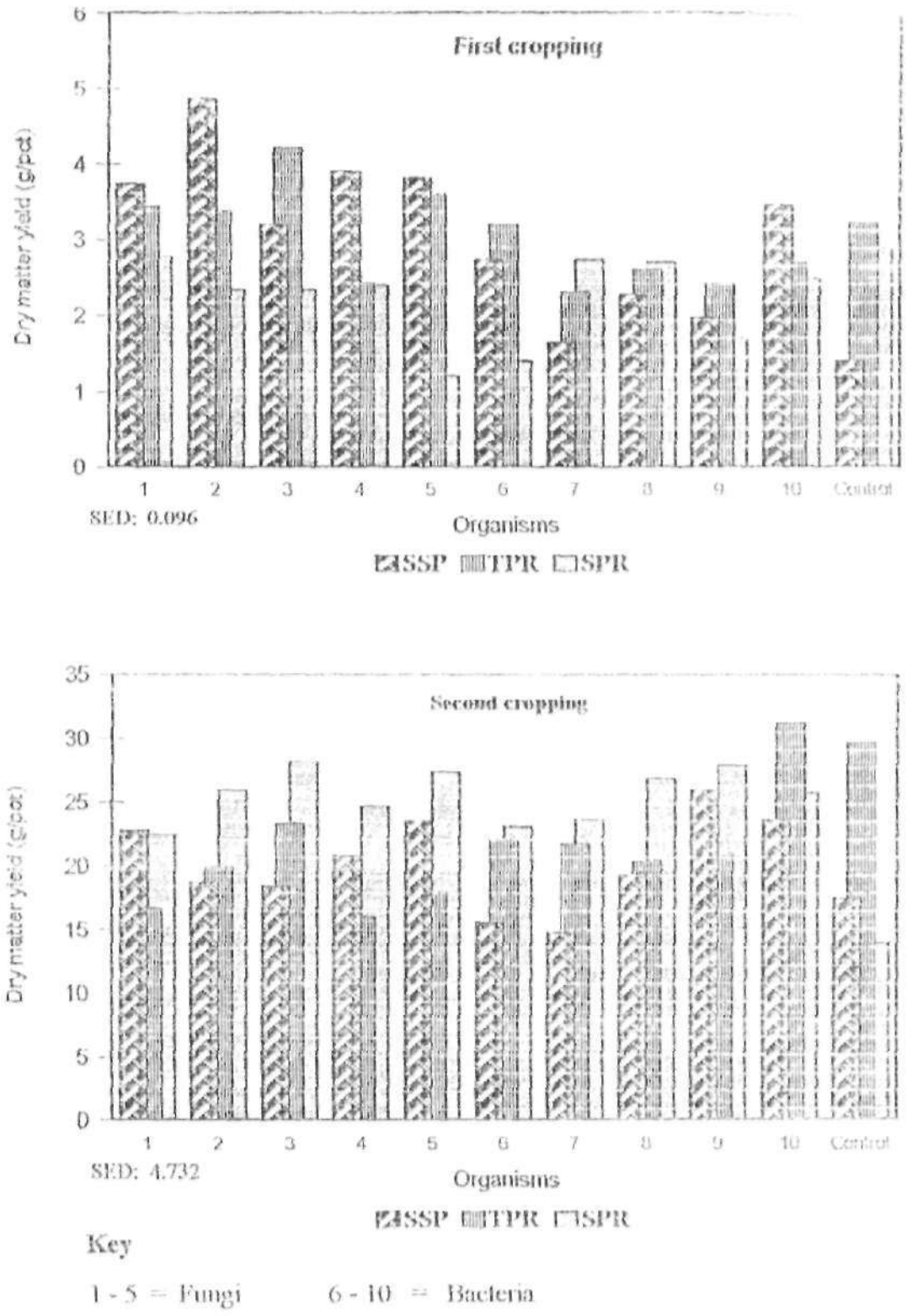


Figure 4: Effect of P sources and organisms on soyabean dry matter yield in the greenhouse.

4.2.4.5 Residual P

The rock phosphates gave superior values of residual P at most inoculations (fig. 7). Among the rock phosphates Togorock seemed to have higher residual values than Sokoto rock at most inoculations. Inoculations with organisms 4, 5 and 7 were observed to increase the residual P in Togo rock by 40.8µg/g, 10.7µg/g, 47.6µg/g above Sokoto rock. This suggests that inoculation enhances residual values of Togo rock than Sokoto rock.

4.2.4.6 Crop response to combined effects of P source and Inoculation

The results presented above suggest that certain microbial inoculations promote the performance of SSP at the first cropping. Gaind and Gaur (1991) attributed this observation to reduction in soil fixation of SSP as a result of inoculation. However, at the second cropping, the rock phosphates showed more prominent performance than the first cropping and they were superior to SSP in performance for all the parameters considered. This observation suggests the need to allow soils inoculated and suppied with rock phosphate to incubate for some time before cultivation. The residual values of rock phosphate was superior to SSP, however among the rock phosphates Togo rock showed higher residual efficiency than Sokoto rock.

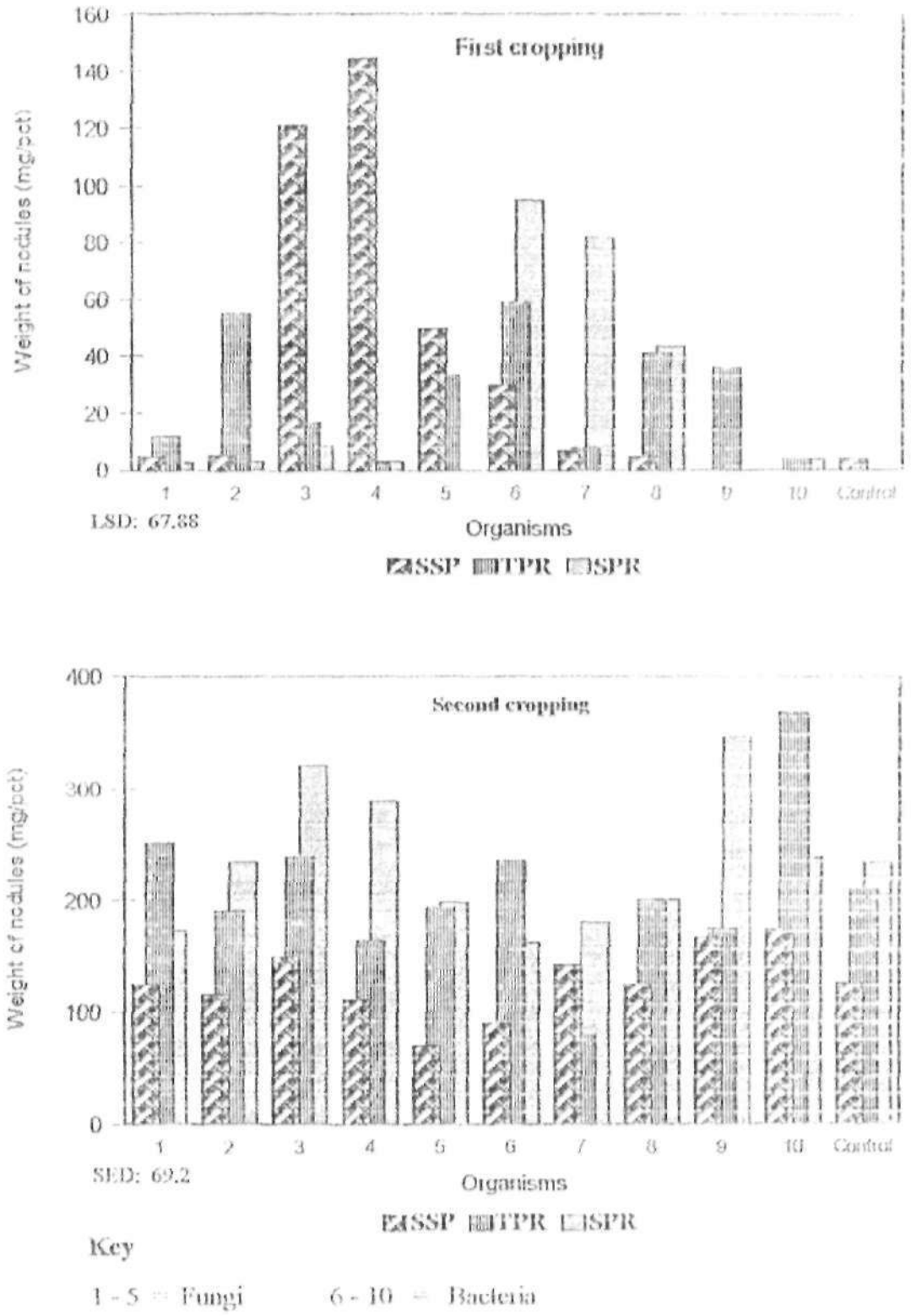


Figure 5: Effect of P sources and organisms on soyabean nodules in the greenhouse.

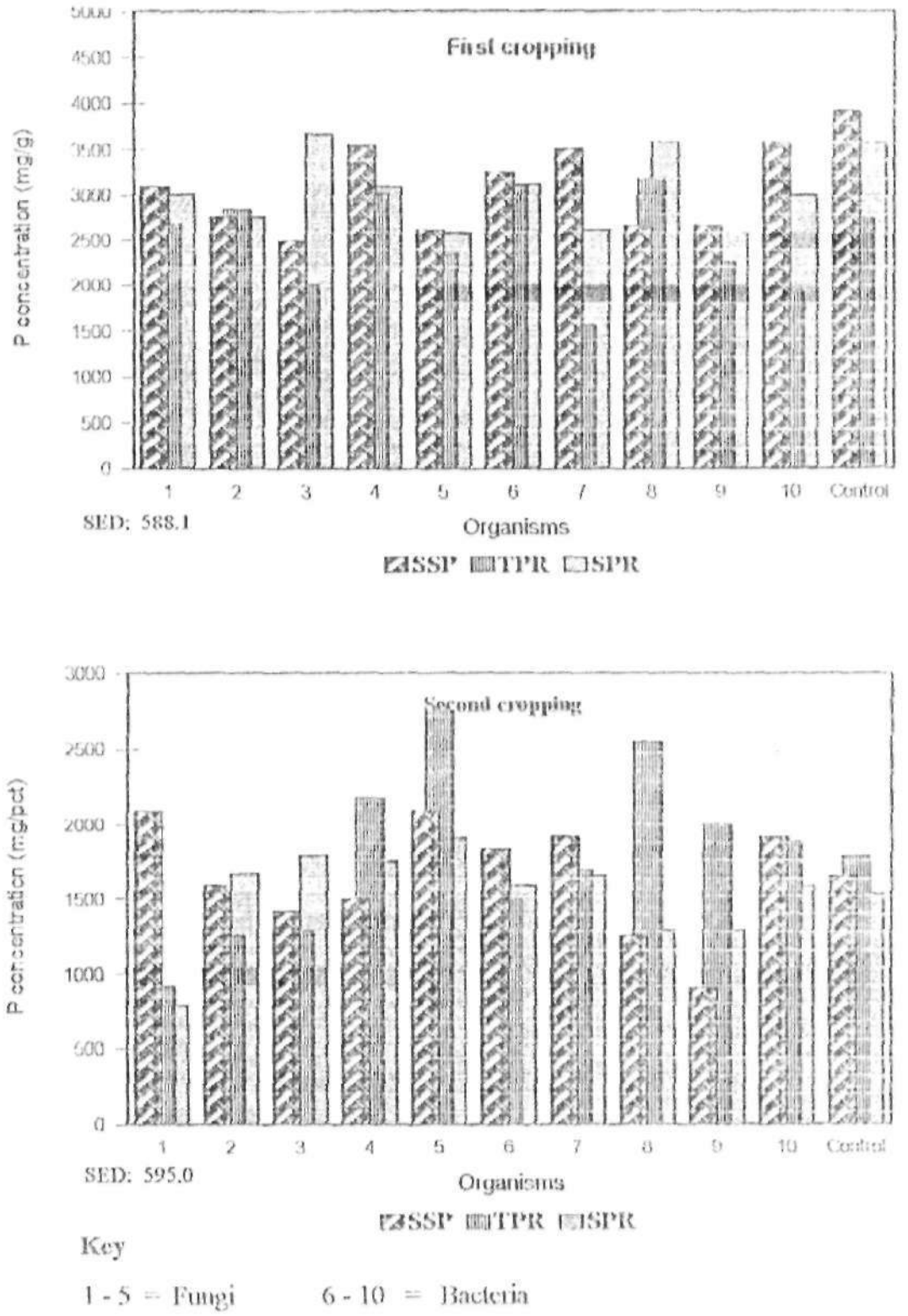


Figure 6: Effect of P sources and organisms on soyabean P concentration in the greenhouse.

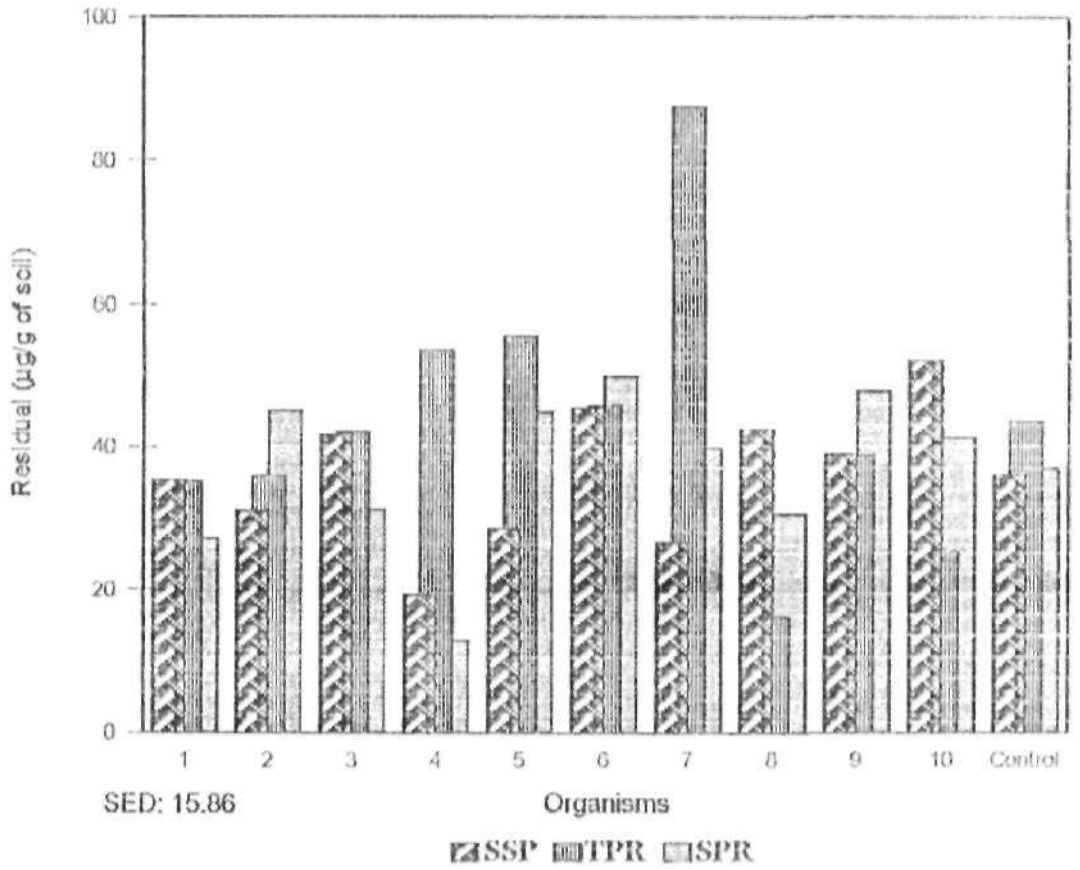


Figure 7: Effect of P sources and organisms on residual P of greenhouse soil cropped to soyabean.

Three types of magnitude of response were observed viz: (1) At the first cropping SSP + Inoculation > Rock Phosphates + Inoculation.

(2) At the second cropping Togo rock + Inoculation > Sokoto rock + Inoculation > SSP + Inoculation.

(3) Residual values Togo rock + Inoculation > Sokoto rock + Inoculation > SSP + Inoculation.

4.2.3 Response of Maize to Treatment

4.2.3.1 Visual Observations

The seedlings supplied with SSP responded positively to treatments since no purple coloration was observed throughout their growth period at the first cropping. Whereas, seedlings supplied with rock phosphates showed purple coloration up to the 5th week after planting. However, at the second cropping, seedlings supplied with rock phosphates showed initial purple coloration which disappeared by the third week while those supplied with SSP showed P deficiency throughout the period of growth.

Immediately after the application of rock phosphates, P releases were slow from both chemical and microbiological processes, therefore enough P was not available to meet plant's phosphorus need. Whereas SSP is water soluble and thus P releases were faster and plant phosphorus need was easily met by SSP at the first cropping. However, at the second cropping most of the SSP had been utilized at the first cropping and was not available to seedlings at the second cropping. Whereas the rock

phosphates which had hitherto been mostly unavailable was slowly being released and accumulated through solubilization of the rock phosphates. Also the chemical and microbilogical factors which were needed to enhance solubilization of the rocks such as soil pH and microbial immobilization had been effected in the soil by this time.

4.2.3.2 Effect of P Sources

Table 6 shows that P influenced maize in most of the parameters taken significantly at first and second croppings.

4.2.3.2.1 Plant Height

At the first cropping, SSP and Control gave similar results and the plant heights were significantly different (P=0.05) from the rock phosphates. SSP gave increases of 104cm and 91cm (table 6a) above Togo rock and Sokoto rock. Whereas at the second cropping the rock phosphates influenced plant height significantly different (P=0.05) from SSP and Control. Togo rock and Sokoto rock gave differences of 50cm and 62cm above SSP (table 6a). Among the rock phosphates, Sokoto rock produce higher value of plant height giving an increase of 12cm over Togo rock. Above observation suggests that P released from rock phosphate was able to meet crop demands at the second cropping while P from SSP could not.

4.2.3.2.2 Leaf Area

Observations on leaf area were similar to plant height, SSP and Control also influenced leaf area significantly different (P=0.05) from the rock phosphates at the first cropping. SSP gave increases of 40cm^2 and 43cm^2 above Togo rock and Sokoto

Table 6a: Effect of 4 different P sources on the mean yield components of maize in the greenhouse

	First Cropping					Second Cropping					Soil Parameters	
P-sources	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter yield (g/pot)	P-conc % P	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter Yield (g/pot)	P conc. (% P)	Residual P µg/g Soil	pH of Soil
SSP	4.61a	123a	10.5	21a	0.021	35.1b	1036	4.3b	8.01b	0.072	47.8	5.0
TPR	37.5b	83b	9.2	15c	0.046	40.1a	121.1a	7.24a	13.91a	0.069	49.3	4.7
SPR	37.0b	80b	9.4	17b	0.032	41.3a	123.6a	6.72a	12.67a	0.064	43.3	4.8
Control	44.8a	123.9	9.1	15c	0.029	34.8b	122.1a	4.0b	4.26c	0.069	18.7	5.8
LSD (0.05)	6.458	24.33	1.75	2.38	NS	33.63	20.62	1.86	3.55	NS	NS	NS
(0.01)	10.182	38.36	NS	3.75	NS	5.301	32.52	2.94	5.60	NS	NS	NS

Means not followed by the letter are statistically significant at 0.05 by DMRT.

Table 6b: Correlation Matrix at the first and second Cropping of Maize

First cropping

	PH	LA	DMY	RDW
Plant Height (PH)	-			
Leaf Area (LA)	0.3686***	-		
Dry Matter Yield (DMY)	0.2255*	0.3907**	-	
Root Dry Weight (RDW)	-0.0588	0.2350*	0.4565**	
	0.0275	-0.1297		

Significant level: * = P 0.05, ** = P 0.01

Second cropping

	PH	LA	DMY	RDW	P-upt	Res-P
Plant Height (PH)	-					
Leaf Area (LA)	0.7850*	-				
Dry Matter Yield (DMY)	0.4237**	0.3939**	-			
Root Dry Weight (RDW)	0.2632*	0.1719	0.4945**	-		
P uptake (Pupt)	-0.0252	0.0007	-0.0591	0.0213	-	
Residual P (Res-P)	-0.4372**	-0.3319**	-0.1798	-0.0359	0.1181	-

Significant level: * = P 0.05, ** = P 0.01.

rock (table 6a). While at the second cropping, the rock phosphates again were significantly different (P=0.05) from SSP and Control. This also suggesting increases in P availability from rock phosphates at the second cropping whereas P from SSP and Control were being depleted.

4.2.3.2.3 Root Weight

The influence of P source on root weight was not statistically significant at the first cropping, SSP gave increases of 1.3g and 11g over Togo and Sokoto rocks in its value of root weight (table 6a). Contrary to first cropping, significant levels were recorded at the second cropping. Togo rock and Sokoto rock gave significant differences (P=0.05) in values of root weight from SSP and Control. Togo rock and Sokoto rock gave increases of 2.9g and 2.4g over SSP. This observation suggests increases in P release from rock phosphates at the second cropping. A good supply of P is associated with increased root growth (Tisdale, 1984).

4.2.3.2.4 Dry Matter Yield

At first cropping, SSP influenced dry matter yield significantly different (P=0.05) from Sokoto rock which was also significantly different from Togo rock and Control (table 6a). Whereas, during the second cropping the two rock phosphates influenced dry matter yield significantly different (P=0.05) from SSP, which was also significantly different (P=0.05) from Control. Contrary to observations on plant height and leaf area, it is observed that Control was not superior to the rock

phosphates at the first cropping, and at the second cropping, rock phosphate gave superior dry matter yield than SSP and Control. Togorock and Sokotorock gave increase of 5.9g and 4.7g over SSP at second cropping.

4.2.3.2.5 P-concentration

Although P sources did not appear to influence P-uptake singificantly at both croppings, Togorock gave higher P-concentration (0.015%) than SSP at the first cropping, while SSP gave slightly higher P-concentration (0.003 and 0.008µg/g) than the rock phosphates (table 6a). The result seems to suggest that the P-concentration in the plant tissue may not correlate positively with other parameters taken.

4.2.3.2.6 Residual P

Togorock gave highest residual P in the cropped soil, it gave an increase of 1.5µg/g above SSP and 6.0µg/g above Sokoto rock. However, Sokotorock gave an increase of 24.6µg/g above Control (table 6a). Above observation suggests that all the P sources have residual values. However, agronomic effectiveness of rock phosphates relative to that of water soluble P such as SSP increased with time. The pH of the soils at various P sources show that application of P fertilizers reduces the pH of the soil (table 6a). However, application of rock phosphates seemed to have more acidifying effects than SSP.

4.2.3.2.7 Crop Response to P

The crop responded significantly to SSP relative to the rock phosphates at the first cropping. However, crop responded

significantly to rock phosphates relative to SSP at the second cropping. The magnitude of response at the Second Cropping is *Togorock > Sokotorock > SSP > Control*. The magnitude of residual value is:-

Togo rock phosphates > Sokoto rock > SSP > Control.

The correlation matrix (tables 6b and 6c) show that P source correlated significantly and positively with leaf area and dry matter yield but negatively with plant height at the first cropping. At the second cropping P source also correlated positively and significantly with plant height, dry matter yield, root weight and residual P.

4.2.3.3 Effect of Organisms

The effects of organisms on some yield parameters are shown in table 7. The organisms did not influence any of the parameters significantly at the first cropping, whereas, at the second cropping, the organisms had significant influence on most of the parameters.

4.2.3.3.1 Plant Height

Most of the organisms gave lower values of plant height than control at the first cropping, only organism 3 seemed to perform as well as control. Whereas at second cropping, all the organisms had significantly different (P=0.05) influence on plant height. Organisms 1, 2, 3, 6, 5, 7, and 8 gave significantly different values from organisms 4, 9, and 10. Which were also significantly different from Control (table 7).

This observation suggests that the organisms depleted the P in soils through immobilization at the first cropping while at the second cropping they released P into soil for crop use.

4.2.3.3.2 Leaf Area

All the organisms gave low values of leaf area relative to control at the first cropping. However, by the second croping, most of the organisms gave higher values than the control (table 7). All the other organisms also show significant difference (P=0.05) relative to organism 10. Organisms 2, 3, 8 and 9 in particular gave higher values of 12cm^2, 13cm^2, 18cm^2, and 7cm^2 than value obtained at control. This result suggests that the organisms were dormant in their P solubilization and release during the first cropping, whereas at the second cropping rock phosphate solubilization occured and consequently, P was released for crop use.

4.2.3.3.3 Root Weight

Only organisms 3, 4, 5 and 6 gave higher values of root weight than control at the first cropping (table 7), the other organisms gave comparable or lower values than Control. Whereas at the second cropping, all the organisms gave significantly different (P=0.05) values than the Control, organisms 2, 3, 4 and 10 gave differences of 2.6g, 2.4g, 2.5g and 3.0g above the Control.

Table 7: Effects of Organisms on Yield components of maize in the greenhouse

	First Cropping					Second Cropping					Soil Parameters	
Organism	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter yield (g/pot)	P-conc % P	Plant Height (cm)	Leaf Area (cm^2)	Root weight (g/pot)	Dry matter Yield (g/pot)	P conc. (% P)	Residual P µg/g Soil	pH of Soil
1	35.9	95	9.0	16.6	0.054	43.3a	114a	6.8a	13.3a	0.064	38.2	4.4
2	37.0	83	9.3	16.5	0.048	41.2a	136a	7.2a	12.5a	0.069	39.2	4.9
3	43.6	77	9.7	19.9	0.040	45.0a	136a	7.0a	13.6a	0.066	39.2	5.8
4	39.8	101	10.5	19.4	0.042	36.0b	121a	7.1a	14.6a	0.067	46.5	5.4
5	37.6	91	11.7	17.1	0.066	40.2a	116a	6.9a	14.8a	0.065	54.1	4.7
6	42.0	105	10.3	17.5	0.042	40.4a	127a	5.9a	12.3a	0.070	54.9	5.2
7	41.4	102	8.9	18.2	0.035	41.0a	124a	5.8a	10.4a	0.063	50.6	5.2
8	39.6	101	8.9	17.0	0.066	42.1a	141a	6.3a	10.8a	0.072	48.3	4.8
9	38.2	96	9.9	18.2	0.048	36.0b	130a	5.5a	11.2a	0.070	42.9	4.9
10	37.1	85	9.4	16.7	0.043	37.5b	97b	7.6a	11.1a	0.080	40.5	5.1
Control	43.1	113	9.2	19.8	0.044	24.8c	123a	4.6b	2.1b	0.055	0.5	5.6
LSD (0.05)	NS	NS	NS	NS	NS	4.06	24.92	2.25	4.29	NS	NS	NS
(0.01)	-	-	-	-	-	5.35	32.85	3.60	6.85	NS	NS	NS

Means not followed by the letter are statistically significant at 0.05 by DMRT.

As observed earlier P availability enhances good root growth in plants and thus due to immobilization the organisms did not promote good root growth at the first cropping. Whereas at the second cropping, due to microbial actions at the end of immobilization rock phosphates were solubilized and P released.

4.2.3.3.4 Dry Matter Yield

All the organisms, except organism 10 gave lower dry matter yields than control at the first cropping, whereas at the second cropping all the organisms gave significantly different (P=0.05) values than the control (table 7). Most of the organisms gave as much difference as 10g over control. The above result again corroborate the earlier observations on Microbial P immobilization at the first cropping, thus depleting the potted soils of P, while at the second cropping immobilization had been satisfied and microbial solubilization now leads to P release in to soil to meet crop needs (Alexander, 1977).

4.2.3.3.5 P-concentrations

Organisms 1, 2, 5, 8 and 9 gave high releases of P at the first cropping than control, the other organisms gave similar or lower values than control (table 7). However, at the second cropping all the organisms gave higher values than the control, suggesting that all the organisms effected higher release of P into the soil for plant use at this second cropping than they did at the first cropping. Organisms 7, 8, 9 and 10 in particular gave high differences of 0.015%, 0.017%, 0.015% and

0.025% P over control.

4.2.3.3.6 Residual P

The inoculated pots gave higher residual values than uninoculated Pots. Organisms 5, 6, 7, 8 and 9 in particular, gave higher values of 23.6µg/g, 24.4µg/g, 20.1µg/g and 17.8µg/g than control (table 7). It is suggested that inoculated soils have higher residual effectiveness than uninoculated soils. pH of soil used also indicate that there is reduction in pH of inoculated soils relative to uninoculated soil. This acidic soil condition suggests a favourable condition for continual chemical dissolution of phosphates and microbial solubilization especially by fungi which are capable of solubilization at such low pH.

4.2.3.3.7 Crop Response to Organism

At the first cropping, rather than enhancing crop growth, the organisms were observed to retard the growth of plants as they gave values lower than uninoculated soils (table 7). Whereas at the second cropping, the organisms promoted plant growth relative to control. The organisms also increased the residual values of P in the soil above control. Therefore, it is indicated that rather than releasing P, the organisms were taking P from the soils during the first cropping. However, having met their need of P, releases were made into the soil at the second cropping. The above observations suggest that there is need to allow for an incubation period between the time of soil inoculation and planting.

Magnitude of response at the second cropping is

Organisms 8 and 5 > 2 and 3 > 1 and 9 > 4 and 10 > 7 and 6 > Control.

The correlation matrix tables (table 6b and c) seem to corroborate the above observations. There were no significant correlations of organism with all the parameters taken at first cropping. While at the second cropping, organisms correlated significantly with plant height, leaf area, dry matter yield and root weight. However, the correlation was negative in plant height, leaf area and dry matter yield.

4.2.3.4 Yield parameters as influenced by Inoculation on P Sources

For all the parameters taken, specific organisms were observed to influence different sources at both cropping as shown in fig 8 - 11. The values obtained did not show significant differences in all parameters.

4.2.3.4.1 Leaf Area

SSP gave superior values of leaf area at the first cropping. It also gave higher values at inoculation with organisms 1, 6, 8 and 9 than when not inoculated (control) as shown in fig 8. It is therefore, suggested that SSP benefited from inoculation at this first cropping than the rock phosphates. However, at the second cropping, the rock phosphates gave superior values than SSP at most inoculations, and low values were recorded by all P *sources at uninoculated control (fig. 8). Suggesting* that inoculation is quite essential for P release at this cropping. When inoculated with organism 8, SSP, Togo rock and Sokoto rock

gave increased value of 114.5cm², 103.6cm² and 110.4cm² over control.

4.2.3.4.2 Dry Matter Yield

SSP gave higher dry matter yield at all the inoculations. Values obtained at inoculation were comparable or lower than control suggesting that inoculation was not effective on SSP in the production of dry matter. Contrary to the first cropping, rock phosphates gave higher yield than SSP at all inoculation during the second cropping (fig. 9). All P sources gave higher values when inoculated relative to uninoculated pots. Inoculation with organism 3 brought about a difference of 9.1g, 11.5g and 13g in SSP, Togo rock and Sokoto rock over uninoculated control. It is suggested that inoculation is relevant in the second cropping.

4.2.3.4.3 P-Concentration

Sokoto rock gave higher values of P in plant tissue at most inoculations during the first cropping (fig. 10). It was particularly promoted in its P released by organism 6 causing an increase of 193% and 187% above SSP and Togo rock. Whereas, at the second cropping, SSP appeared to enhance higher P uptake than the rock phosphates. Organisms 4, 5, 8, 9 and 10 seemed to enhance the efficiency of SSP above uninoculated SSP pot. Their effects produced increases of 83mg, 83mg, 166mg and 143mg/g above control (fig. 9). Togo rock phosphate released more P than SSP at control at both croppings. These observations are

inconsistent with earlier observations on other parameters and P-concentration may not correlate significantly with P source.

4.2.3.4.4 Residual P

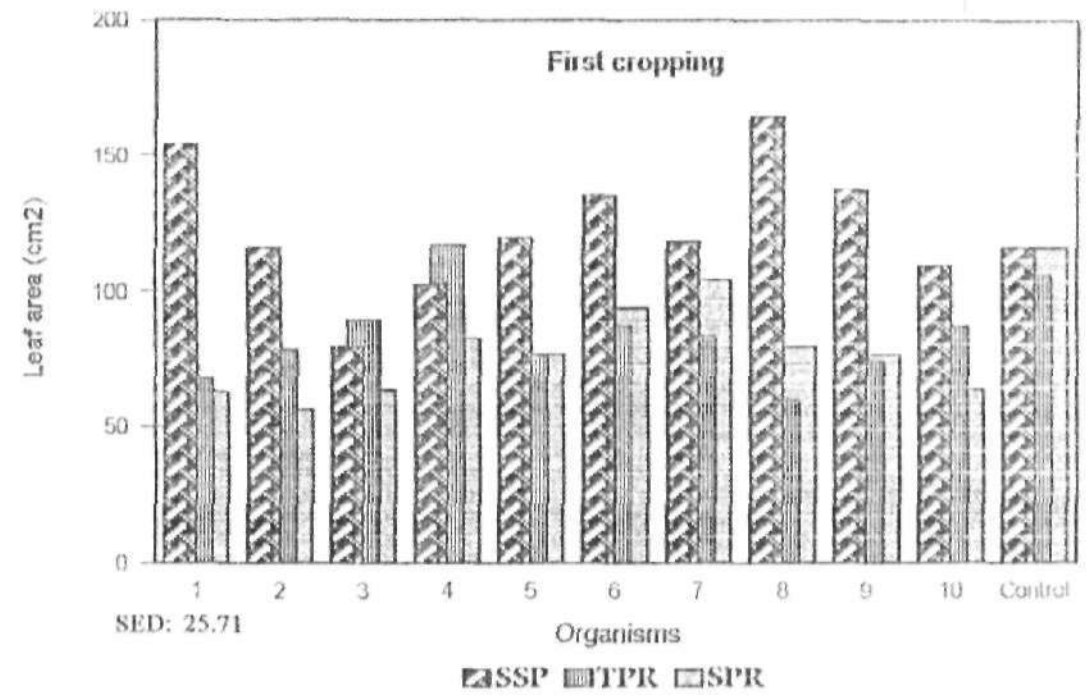
Similar to observations made in soyabean, all the P sources gave higher residual values at inoculation than at Control, and all the P sources seemed to have high residual values (fig. 11). However, the rock phosphates showed higher efficiencies in their residual values than SSP. Inoculation with organisms 1, 2, 4 and 7 particularly promoted the residual values of the rock phosphates above SSP.

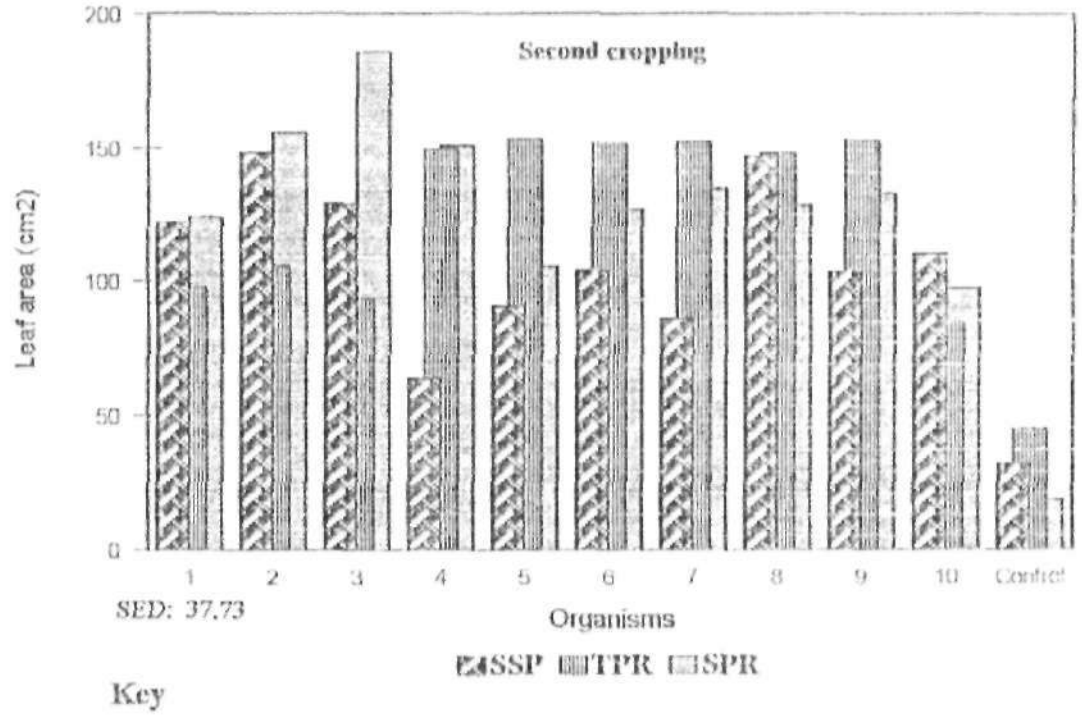
4.2.3.4.5 Crop Response to combined effect of P source and Inoculation

The first cropping seems to highlight the efficiency of SSP at most inoculations than the rock phosphates, while at the second cropping the rock phosphates were more effective at promoting growth parameters than SSP. Thus, 2 types of magnitude were observed:

(1) At first cropping: SSP + Inoculation > Rock phosphates + Inoculations. (2) At second cropping and residual P Togo rock + Inoculation > Sokoto rock + Inoculation > SSP + Inoculation.

The response of organisms to SSP rather than rock phosphates at first cropping may be associated with the reactivity of SSP and consequent availability of P which enabled the organisms to meet its P needs faster than when inoculated on rock phosphates which are less reactive. By the second cropping, however, more P have been released from rock phosphates and thus immobilization does not predominate.

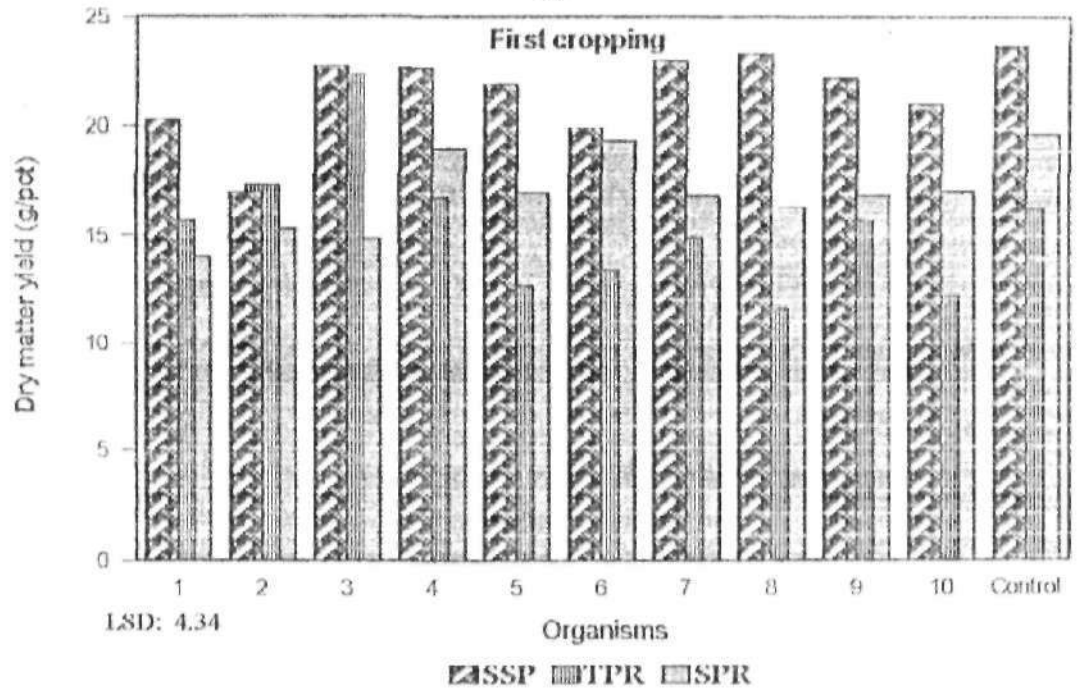


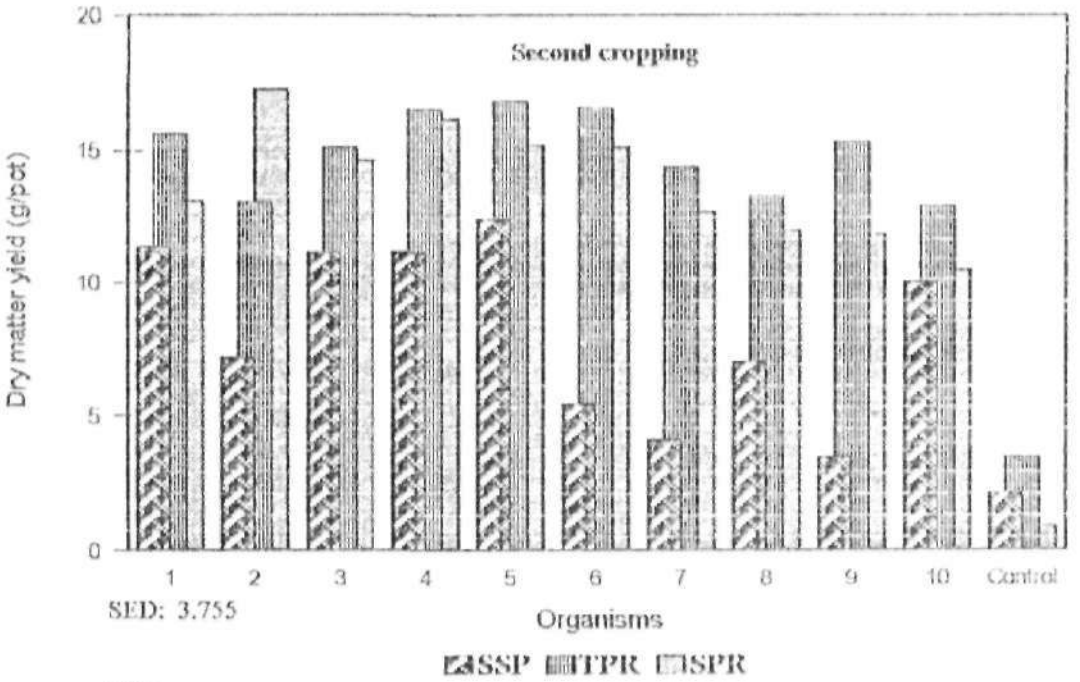


Key

1 - 5 = Fungi 6 - 10 = Bacteria

Figure 8: Effect of P sources and organisms on maize leaf area in the greenhouse.

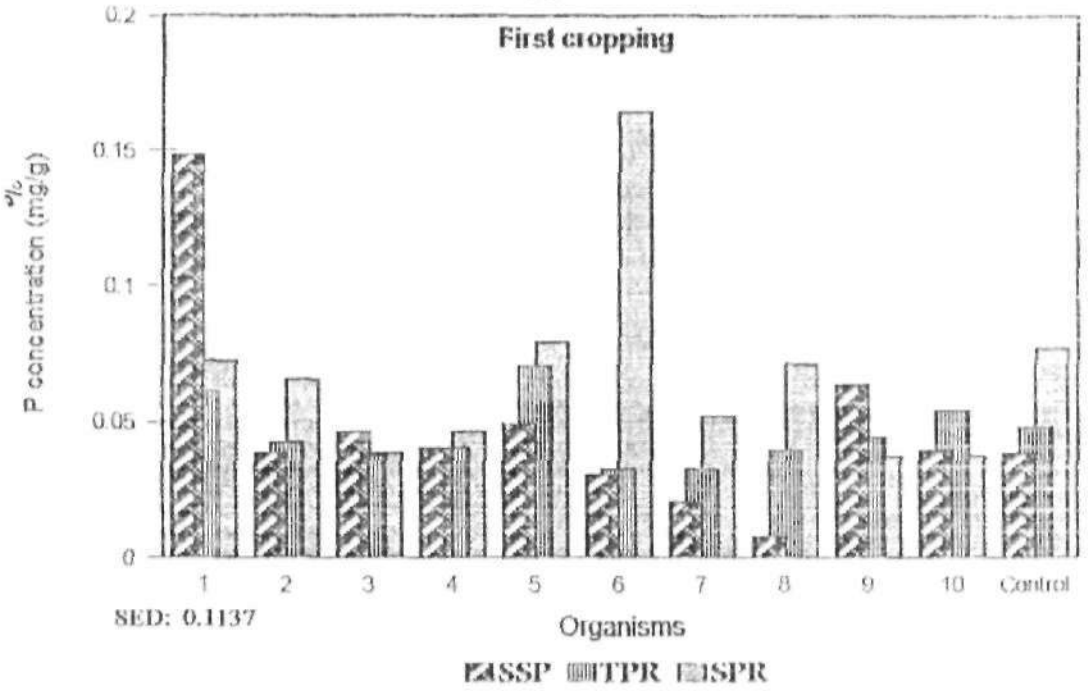


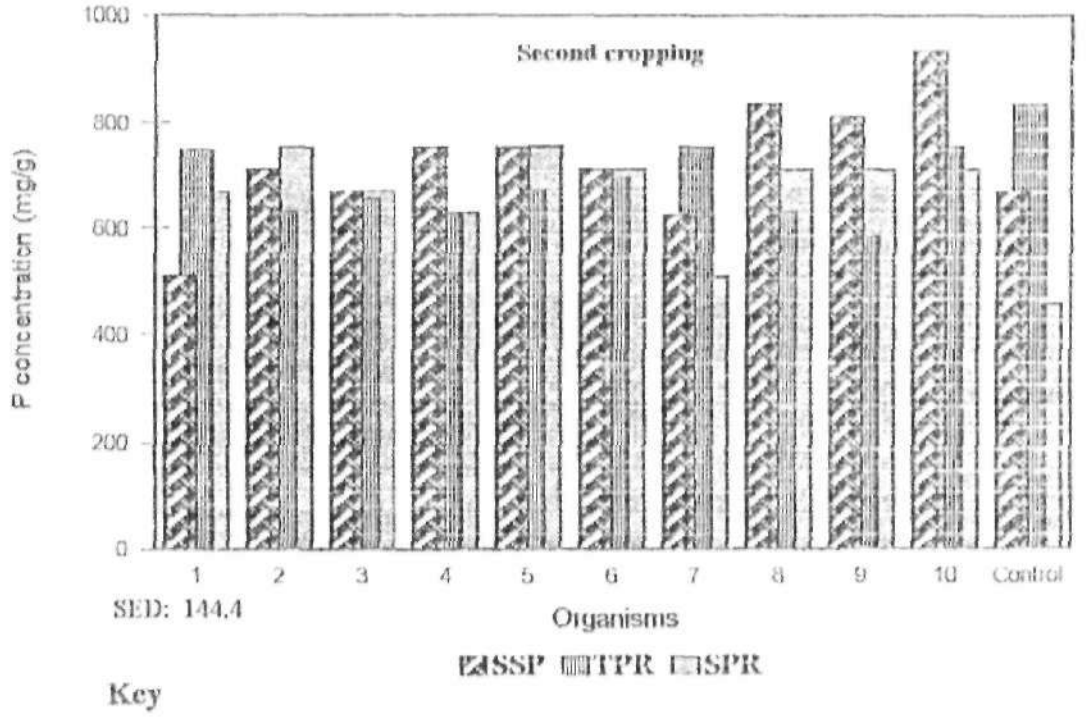


Key

1 - 5 = Fungi 6 - 10 = Bacteria

Figure 9: Effect of P sources and organisms on maize dry matter yield in the greenhouse.

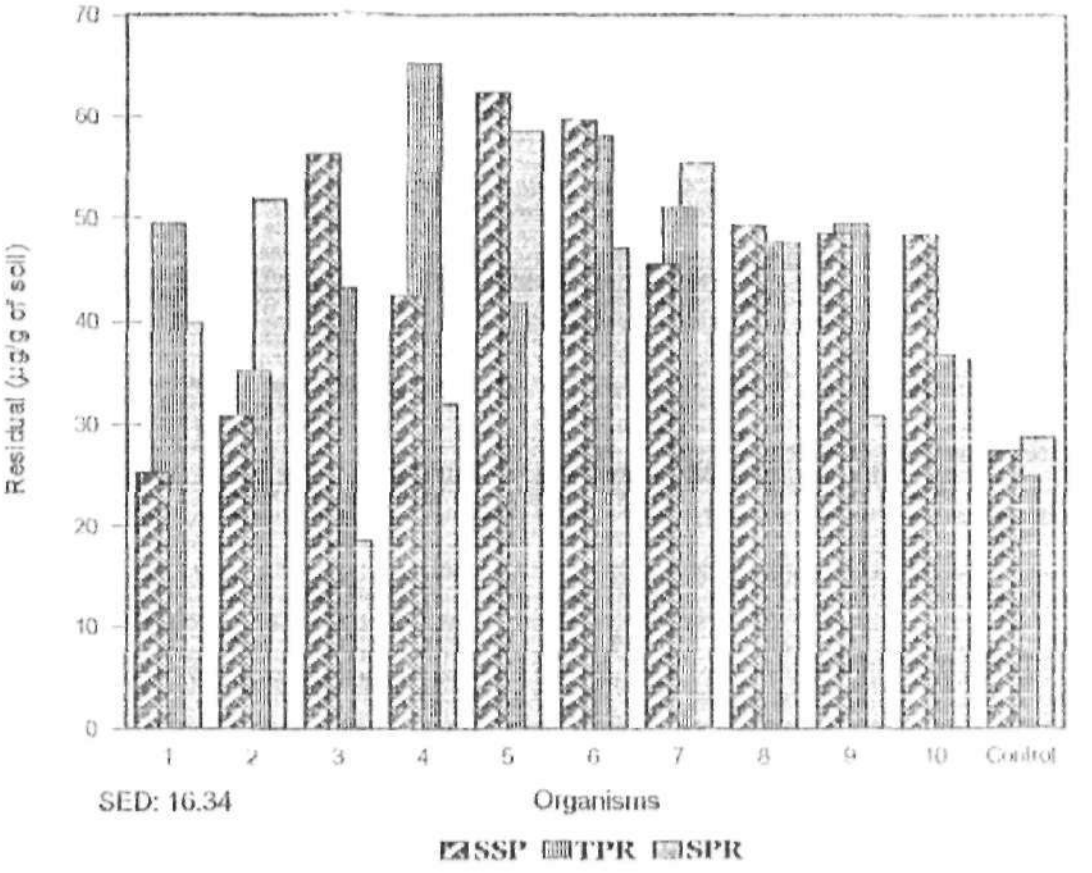




Key

1 - 5 = Fungi 6 - 10 = Bacteria

Figure 10: Effect of P sources and organisms on maize P concentration in the greenhouse.



Figu e 11: Effect of P sources and organisms on maize residual P of greenhouse soil cropped to *maize*.

4.2.3.5 General Discussion on Greenhouse Study

Soil

The pH of the initial soil which was 5-8, was reduced by the various treatments. Application of the various P sources reduced it to 5.1, 4.5 and 4.8 by SSP, Togo rock and Sokoto rock when cropped to soayabean (table 4a). Similar results were also observed when cropped to maize indicating that processes leading to the release of P from the P sources also lead to acidification of the soil. The inoculation of micro-organisms also led to reduction in soil pH. All the organisms gave lower pH than control and also initial pH to a minimum pH of 4.3 by organism 1 and maximum was 5.6 by organism 10 (table 5). This reduction in pH of soil by phosphate solubililizing organisms has been associated with production of organic acids (Gerreston, 1948). The ability of organisms to reduce the pH of the medium is one of the criteria used in selecting efficient phosphate solubilizing organisms (Gaur, 1990).

Soyabean

SSP influenced soyabean response to P significantly at the first cropping while at the second cropping the rock phosphate gave significantly (P=0.05) different influence relative to SSP and control. This is attributed to the higher reactivity of SSP which made it available at the first cropping whereas at the second cropping, most of the P were exhausted from the soil. Contrary to SSP at the second cropping enough time was allowed for solubilization of rock phosphates and thus making it

available for crop use at this time.

Soyabean crop was observed to respond to micro-organism inoculation at both croppings. However, at the second cropping, the magnitude of response increased. The organisms were however inconsistent in their performance and so they could not be rated. The low response of crop to inoculation at the first cropping was attributed to immobilization whereas, at the second cropping the organisms had satisfied their need for P and thus releases rather than assimilation now predominates at second cropping, such that crop benefited from such P release.

Soyabean also responded to the combined effect of P source and inoculation. Inoculation promoted the efficiency of all the P sources including SSP. The crop responded to inoculated SSP pots than inoculated rock phosphates at the first croping, whereas at the second cropping, inoculated rock phosphates gave better results. This was attributed to both chemical and microbiological processes both of which were uptimal at the second cropping.

Maize

There were significant responses to SSP relative to rock phosphates at the first cropping, while at the second cropping, responses to rock phosphates were significant. Among the rock phosphates, Togo rock appeared to be efficient in its agronomic efficiency than Sokoto rock. The efficiency of rock phosphate relative to SSP was due to its residual value which increased with time (Visker *et al*, 1994).

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Maize crop appeared to respond negatively to inoculation at the first cropping i.e. inoculation actually retarded the growth of maize at the first cropping. Whereas, at the second cropping, maize responded significantly and positively to inoculation. At the first cropping, the organisms rather than releasing P, assimilated P from the soil and so brought about P deficiency. While at the second cropping immobilization appeared to have been satisified and P was released into the soils.

Inoculation was observed to favour all the P sources. Maize responded more to inoculation of SSP at the first cropping while at the second cropping, maize responded more to inoculation of rock phosphates. This again was attributed to optimal chemical and micribiological processes at the second cropping.

4.3 FIELD TRIALS

4.3.1 Soil Chemical Characteristics

The soil used is a Typic haplustalf (U.S. DA 1990). Details of its characterization and classification are presented in appendix 2 and 3. The initial soil test values showed in Table 8 indicated that it is a loamy soil, slightly acidic in reaction, low in organic matter, nitrogen and CEC. Its phosphorus level falls within the medium fertility class.

It has long been established that the Guinea savanna soils are characteristically low in organic matter, CEC and also deficient in N and P. (Goldsworthy, 1967a, 1967b). The rather high value of P observed in this experiment is not a characteristic feature of savanna soil and thus may be attributed to annual application of phosphorus fertilizers on the plot. One feature of phosphate fertilizers is that only a small fraction of the applied fertilizer is generally taken up by the crop in the year of application. Part of what is not taken up goes to increase the labile pool of P in the soil. Part is converted, in slow reactions to less soluble phosphorus forms. The slowly soluble P forms continue to supply P to crops during the subsequent cropping seasons (Mokwunye, 1994).

The slightly acidic nature of the soil will enhance the release of P from the phosphate fertilizers applied. This is because the presence of H^+ ions in the soil results in the rapid conversion of Po^{1}_{4} to H_2Po_4 and HPo^{2}_{4} (Khasawneh and Doll, 1978). Inorganic phosphate solubilization by micro-organisms is a

consequence of organic acids produced by efficient phosphate solubilizing bacteria and fungi (Gerreston, 1948). The most efficient ones change the pH of the medium from initial 7.0 to 3.0-3.5 (Gaur, 1990).

The low organic matter content of this soil however, indicates that low microbial activities occur within the soil. These organisms are heterotrophs and solubilize insoluble phosphates by secreting organic acids., The role of carbon sources in this contest is therefore very important and limiting.

4.3.2 Climatic Factors

Samaru weather data between 1993 - 1995 are shown in appendix 4. In 1994 the rainfall was inconsistent, although heavy rains were experienced in May and June. The distribution was erratic and the rains did not actually establish until mid July. The 1993 and 1995 rains were more consistent and established by the middle of June. The relative humidity and soil temperature were normal during the cropping seasons.

Table 8: The initial and final soil test values of soil used for field studies

	Initial values		Final values	
Soil Parameters	0-15cm	15-30cm	0-15	15-30
% Clay	20.3	25	20.3	21
% Silt	46.7	44.7	45.3	46.7
% Sand	33	30.3	34.3	34.3
Textural Class	Loam	Loam	Loam	Loam
pH/H_2O (1:2.5)	4.4	4.5	4.3	3.9
pH/0.0/m$CaCl_2$(1:2.5)	4.0	4.0	3.8	3.7
Organic carbon (gkg^{-1})	5.9	5.8	7.8	7.1
Total N (gKg^{-1})	0.8	0.7	0.9	0.7
Exchangeable Bases ($Cml\pm Kg^{-1}$)	3.1	3.0	2.5	3.2
Ca	0.29	0.32	0.33	0.43
Mg	0.29	0.32	0.33	0.43
K	0.22	0.12	0.22	0.17
Na	0.07	0.06	0.07	0.03
CEC	6.1	6.5	6.2	7.0
Available P (µg/g)	16.4	12.2	20.0	20.4

4.3.3 *Organisms selected for Field Trials*

Based on the observations from greenhouse studies, five organisms were selected for the field trials. They include 3 fungi and two bacteria. Their selection was based on their effectiveness at solubilizing rock phosphates which was subsequently indicated on crop growth parameters taken. (Table 5 and 7). The final pH of the soil was also considered in this selection. This has been one of the criteria for selecting an efficient phosphate solubilizer as a fall in pH of the medium is an indication of organic acid production (Gaur, 1990).

The five organisms selected were characterized and identified using Baggey's manual. Details of the biochemical characteristics studied and used in the identification of the bacteria species are presented in appendix 1. The fungi were identified using mainly Alexopoulos (1962). The order and species names (where possible) of the organisms are presented in table 9.

Table 9: Organisms selected for field trial

Organism	Nomenclature
Organism 1	*Rhizopus* sp
Organism 2	*Aspergillus niger*
Organism 5	*Penicillium* sp
Organism 8	*Pseudomonas striata*
Organism 9	*Bacillus magaterium*

4.3.4 Effects of Treatments on Soyabean

4.3.4.1 Effect of P sources

The effects of P sources are shown on table 10 - 12. There were no significant differences in growth and yield parameter (Table 10).

4.4.4.1.1 Plant Height

The effect of SSP on plant height though not statistically significantly different from the rock phosphates, resulted in taller plants during the 1993 and 1994 seasons (table 10). This is expected as SSP is readily water soluble and has high reactivity. Thus plants will respond to its application than the rock phosphates which were not water soluble and are less reactive. The control was observed to be superior to the rock phosphates in 1993. However in 1994 rock phosphate were superior to control. The superior performance of control over the rock phosphates in 1993 may be attributed to certain experimental errors.

4.3.4.4.2 Leaf Area

In 1993, Togo rock phosphate produced bigger leaf area than SSP, while SSP showed superiority over Sokoto rock phosphate and Control (Table 10). However, in 1994 SSP was superior in its production of leaf than the rock phosphates. Size of the leaf is a function of the photosynthetic activity of the plant and thus it can be inferred that application of SSP particularly at the 1994 season enhanced the photosynthetic activities of soyabean more than when rock phosphates were

applied.

4.3.4.4.3 Dry Matter Yield

More dry matter were produced by all P sources in 1993 than 1994. In 1993, the rock phosphates produced more dry matter than SSP, however the situation was reversed in 1994. The production of bigger leaves by TPR in 1993 discussed above may be an indication of more dry matter yield also produced by Togorock phosphate in 1993. This may be due to water logging experienced in some parts of the field. The 1994 trial showed consistency as taller plants and bigger leaves were produced and these led subsequently to more dry matter than the rock phosphates. Combined analysis for both 1993 and 1994 seasons showed that SSP was superior in its production of dry matter than the rock phosphate (Table 10).

Table 10: Effect of P sources on growth parameters of soyabean from 1993 - 1994

	1993			1994			Combined Analysis
P-sources	Plant Height (cm)	Leaf Area (cm^2)	Dry matter Yield (g/plant)	Plant Height (cm)	Leaf Area (cm^2)	Dry matter Yield (g/plant)	Dry matter yield (g/plant)
SSP	46	63	38	57.0	53	30	29.4
TPR	39	67	40	51	48	21	25.5
SPR	39	56	41	51	50	24	27.7
Control	40	51	33	43	48	21	22.6
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS

Yield Parameters: The yield parameters were not significantly different as shown in table 11.

4.3.4.4.4 Nodule Numbers

More nodules were produced in 1993 than in 1994.The use of SSP resulted in the production of more nodules in both years and in combined analysis than the rock phosphates. Among the rock phosphates, Sokoto rock phosphates produced more nodules than Togo rock phosphates for both years while Togo rock was not superior to control (table 11). It thus indicates that the type of P applied may have direct effect on the nodulation of the soyabean and if this is the case indirectly, P source may influence N-fixation.

4.3.4.4.5 Nodule Weight

Weight of the nodules when dried also followed the trend discussed above. SSP produce bigger nodules in both 1993 and 1994 and when combined, being followed by Sokoto rock phosphate (Table 11) which produced only 50% of the SSP production. It again indicates that SSP enhances more nodulation and consequently more nitrogen fixation in soyabean than rock phosphates. Among the rock phosphates however, it appears as if Sokoto rock phosphate would promote more Nitrogen fixation than Togo rock phosphate.

4.3.4.4.6 Number of Pods

SSP produced more pods at flowering in 1993 and 1994. It was followed by Sokoto rock phosphate which produced about 13% less than SSP in 1993 and 7.3% less in 1994.

4.3.4.4.7 Grain Yield

Togo rock phosphate gave more yield than SSP and Sokoto rock in both years and when combined. This is surprising in view of the above discussions which indicated that Togo rock has been inferior to SSP and Sokotorock in it performance in other parameters. This superiority of Togorock (12.9%) above SSP in grain production may be due to its production of bigger leaves as shown by leaf area produced in Table 10. Stephenson and Wilson (1978) observed that photosynthesis is a factor of pod filling in soyabean and any factor tht will restrict photosynthesis may severely limit pod filling. Other factors may also be responsible for this superiority of Togo rock in grain production. However, many research results (Batiano *et al* 1986, Chien and Mokwunye, 1986) show that leguminous crops are more efficient in using phosphate rocks than in cereals. SSP showed superiority in this experiment over Sokoto rock.

Nutrient Uptake:

4.3.4.4.8 N and P-concentration

The P concentration in plant tissue was not significantly different in 1993. SSP however, produced slightly more P in plant tissue than the rock phosphates (table 12). The 1994 result showed significant difference (P=0.05) and contrary to observation in 1993. Togo rock phosphate was superior significantly to both SSP and Sokoto rock phosphate. The superiority of Togo rock phosphate was also confirmed by the combined analysis of both years in

Table 11: Effect of different sources of P on yield components of soyabean (1993 - 1994).

	1993					1994					Combined analysis	
P-sources	No of Nodules	Weight of Nodules (g/plant)	No. of Pods	Grain Yield (kg/ha)	Wt of 1000 seeds (g/plot)	No of Nodules	Weight of Nodules (g/plot)	No of Pods	Grain Yield (kg/Ha)	Weight of 1000-seed (g/plot)	Nodule weight (g/plant)	Grain Yield (kg/Ha)
SSP	50	0.6	62	1,937	120	30	0.2-2	41	2018	123	0.41	1,931
TPR	28	0.4	53	2,187	121	9	0.08	35	2103	132	0.24	2,132
SPR	48	0.5	54	1,420	116	15	0.12	38	1682	128	0.33	1,909
Control	28	0.3	52	1,777	124	11	0.1	32	1805	132	0.20	1,429
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

which Togorock was significantly different by about 30% (0.09% P) from both SSP and Sokoto rock (table 12).

This enhanced uptake of P from Togorock when compared with other P sources may also be partly responsible for the superiority of Togorock in the production of grain.

SSP enhanced more uptake of Nitrogen in the plant tissue than the rock phosphates. This tends to support the earlier observation made about N-fixation.

4.3.4.4.9 Ca, Mg and K Concentration

Contrary to expection, plants were able to take more Ca from SSP and Control than from rock phosphates in both 1993 and 1994 (Table 12). This may be attributed to soil factor. Graham (1955) observed that soil colloidal systems have a high selectivity for Ca^{2+} relative to H^{+} to cause greater dissolution of rock phosphate by providing a sink for the release Ca^{2+}.

The control showed more Mg in plant tissue than the treatments in 1993 while in 1994 SSP, Togo rock phosphate and control enhanced more Mg uptake than Sokoto rock. In 1993, more K was taken up from SSP than from the rock phosphates. The 1994 experiment however, gave more K in plant tissue from the rock phosphates. Both Ca and Mg are constitutents of the rock phosphate and one would expect that more of these elements will be taken up from soil supplied with rock phosphates than SSP. However, as explained above, soil colloids may react with these two elements and thus taking them away from soil-plant interphase.

4.3.4.4.10 Zn, Mn, Fe and Cu Concentration

Sokoto rock phosphate enhanced more Zn uptake in 1993 and 1994 than other P sources. This is expected as Zn is known to form impurities in rock phosphate and thus constitutes part of the rock. Togo rock in its own case did not show superiority over SSP in 1993. It however did in 1994. This may also be attributed to soil factors.

Manganese uptake was more enhanced from no -P control than when P was supplied to the soil in both years. This tend to suggest that application of P to the soil reduces the uptake of Mn as a result of nutrient imbalance. Iron Oxides also form part of the impurities found on rock phosphate (Lehr and McClellan, 1972). Results however, showed that the control enhanced more Fe in the plant tissue in 1994 than when P was supplied. In 1994, Sokoto rock enhanced Fe uptake more than any other P source. This inconsistency in the Fe uptake by plants from rock phosphates may be as a result of soil coloidal reaction and thereby Fe being occluded in the structures of the soil minerals.

The 1993 samples showed enough Cu in the plant tissue for analysis while in 1994 only traces of Cu were observed. The 1993 data however, showed that Sokoto rock phosphate enhanced more Cu uptake than other P sources.

The uptake of Zn, Mn Fe and Cu from Sokoto rock more than from Togo rock may also suggest that more of these impurities are present in Sokoto rock than in Togo rock.

4.3.4.4.11 Crop Response to P-source

SSP appeared to be superior to the rock phosphate as indicated by crop response in many of the parameters. However, leaf area, grain yield and P uptake data show that plants responded to Togo rock phosphate more than SSP. The magnitude of response is Togo rock > Single Super > Sokoto rock.

Correlation matrix (table 16) showed that P source were not statistically significantly correlated with all the parameters considered. However, dry matter yield correlated significantly with both grain yield and nodule weight, and nodule weight also correlated with grain yield.

Table 12: Effect of P sources on mean nutrient concentrations of soyabean from 1993 - 1994

	1993								1994								combined analysis
	%				Mg/kg				%					Mg/kg			
P-source	P	Ca	Mg	K	Zn	Mn	Fe	Cu	P	Ca	Mg	K	N	Zn	Mn	Fe	%P
			%		mg/kg	mg/kg											
SSP	0.22	1.16	0.38	1.9	387	129	368	7.8	0.39b	0.99	0.45	2.9	3.3	115	252	157	.30b
TPR	0.21	1.10	0.36	1.72	325	120	361	6.78	0.61a	0.89	0.37	3.3	3.2	205	346	222	41a
SPR	0.21	1.25	0.38	1.88	427	149	466	8.86	0.4b	1.04	0.46	3.0	3.2	295	411	234	.30b
Control	0.19	1.18	0.41	1.68	366	170	484	8.46	0.42b	0.89	0.46	2.4	3.2	257	437	231	.21b
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NN	0.2960	NS	NS	NS	NS	NS	NS	NS	.1135

Means not followed by the same letter are statistically significant at 0.05 by DMRT.

4.3.4.2 Effect of Organisms

The effects of organisms on growth parameters, yield components and nutrient concentration of maize are presented on tables 13-15.

4.3.4.2.1 Plant Height

There was significant difference in plant height in 1993 (table 13). The plots inoculated with *A. niger* were significantly different from the control. However, this trend was not maintained in the 1994 trial. In 1993, *A niger* was superior to other organisms while in 1994 *P striata* and Control gave higher values of plant height. The superiority of inoculation over control in the first year of experiment is attributed to microbial ability to solubilize rock phosphate and consequently release of P for crop use. The superiority of *A niger* and *P striata* over other organisms (in the 1993 and 1994 trials respectively) has also been observed by Gaur (1990). He observed that *A niger* and *A. awamori* gave higher grain yield and straw yield when inoculated along with rock phosphate application. (Gaur 1990) also observed that *P. striata* was superior when SSP and rock phosphate were mixed together. The higher values recorded in control over some of the inoculations in 1994 might be attributed to residual effects as the same plots used in 1993 were also used in 1994. It might also be a result of experimental error.

Table 13: Effect of organisms on growth parameters of soyabean (1993 - 1994).

Treatment	1993 Plant Height	1993 Leaf Area (cm^2)	1993 Dry matter Yield (g/plot)	1994 Plant Height (cm)	1994 Leaf Area (cm^2)	1994 Dry matter Yield (g/plot)	combined analysis Dry Matter Yield (g/plant)
Rhizopus sp	40.4a	60.0	43	50	53	18	30.6a
Aspergillus niger	44.0a	62.0	37	49	52	14	25.4a
Penicillium sp.	40.1a	59.0	35	49	50	10	22.5a
Pseudomonas striata	41.9a	65.0	42	51	54	16	29.1a
Bacillus mangaterum	41.7a	53.0	41	48	45	16	28.5a
Control	39.35b	57.2	31.0	51	49	12	21.7b
LSD (0.05)	3.63	NS	NS	NS	NS	NS	8.225

Means not followed by the same letter are statistically significant at 0.05 by DMRT.

Table 14: Effect of organisms on yield parameters of soyabean (1993 - 1994).

	1993					1994					Combined Analysis	
Organism	No of Nodules	Weight of Nodules (g/plant)	No of pods	grain yield (kg/Ha)	Weight of 1000-seed (kg/plot	No of Nodules	Weight of Nodules (g/plant)	No of Pods	Grain yield (kg/Ha)	Weight of 1000-seed (g/plot)	Nodule Weight (g/plant)	Grain Yield (kg/Ha)
Rhizopus sp	59	0.51	71a	2,014	121	23	1.7	42	1,949	133	0.35	2,053
Aspergillus niger	32	0.46	61a	1951	121	13	1.1	40	1,897	130	0.29	1,780
Penicillium sp	43	0.5	56a	1602	117	12	1.0	26	2,056	133	0.30	1,739
Pseudomonas striata	23	0.4	61a	2,094	121	19	1.7	40	2,083	127	0.28	2,047
Bacillus mengalerium	41	0.41	52a	1,632	119	20	1.5	37	1,577	117	0.28	1,639
Control	34	0.32	32b	1,694	122	10	1.0	33	1,710	132	0.26	1,702
LSD (0.05)	NS	NS	29.18	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means not followed by the same letter are statistically significant at 0.05 by DMRT.

Table 15: Effect of organisms on nutrient concentration of soyabean (1993 - 1994)

	1993								1994								
	%				Mg/kg				%					Mg/kg			Combined analysis
Organisms	P	Ca	Mg	K	Zn	Mn	Fe	Cu	P	Ca	Mg	K	N	Zn	Mn	Fe	%P
	---%---				---(mg/kg)---				---%---					---(mg/kg)---			
Rhizopus sp	0.22a	1.16	0.37	1.85	419	147	417	7.99c	0.46	0.96	0.44	2.55	3.4	284	327	222	0.34
Aspergillus niger	0.22a	1.33	0.35	1.72	317	163	490	11.70a	0.43	0.86	0.44	3.71	3.3	158	406	192	0.82
Penicillium sp	0.18b	1.05	0.41	1.76	364	143	436	7.60c	0.41	0.90	0.42	3.15	3.0	180	378	187	0.29
Pseudomonas striata	0.22a	1.07	0.38	1.88	378	141	312	6.79c	0.51	1.09	0.45	2.55	3.4	239	396	274	0.36
Bacillus mengaterium	0.22a	1.22	0.39	1.77	381	143	443	6.70c	0.48	0.40	0.41	2.52	3.2	215	342	221	0.34
Control	0.21a	1.22	0.39	1.8	299	136	421	7.10c	0.44	1.00	0.45	3.10	3.1	231	168	0.32	
LSD (0.05)	0.2093	NS	NS	NS	NS	NS	NS	2.46	NS	NS	NS	NS	NS	NS	NS	NS	NS
(0.01)	-	-	-	-	-	-	-	3.81						-			

Means not followed by the same letter are statistically significant at 0.05 by DMRT

4.3.4.2.2 Leaf Area

The leaf areas were not significantly different in both 1993 and 1994 (table 13). However, *P striata* gave higher values among the inoculants followed by *Rhizopos* sp in both years. While *B. megaterium* gave the least values in both years. The differences between the leaf area of *P striata* and *B magaterium* were 12cm' and 11cm' in 1993 and 1994 respectively. This indicates that *P. striata* promoted higher photosynthetic activities than other organisms and Control.

4.3.4.2.3 Dry Matter Yield

There were no significant differences in dry matter yield for both years. However, when the data were combined the inoculated plots were observed to be significantly different from Control (table 13). Among the inoculants *Rhizopus* sp gave highest value of dry matter yield in 1993 and 1994 and this superiority was also maintained when the data were combined. *Rhizopus* sp produced 8.9g of dry matter than the control. The effort of *Rhizopus* at solubilizing rock phosphate has also been recognized by Ahmad and Jha (1968).

Yield parameters:

4.3.4.2.4 Nodule Numbers

The nodule numbers were not significantly different in 1993 and 1994 as shown in table 14. However, most of the inoculants gave higher yield than control for both years. Among the inoculants, *Rhizopus* sp was superior in nodule formation than the other organisms. It produced more than 100% of nodules

produced by *P striata* which gave the least nodules in 1993. The superiority of inoculation over control inidicates the enhancement of nodulation by the phosphate rock solubilizers and also a positive interaction of the root nodule *Rhizobium* and phosphate solubilizers. White (1953) observed that the inoculation of phosphate solubilizers in legumes stimulated the multiplication of rhizobia. It is condusive to the development of mobile forms which are essentially required to migrate towards the root system.

4.3.4.2.5 Nodule Weight

Observations made on the nodule weight were consistent with the nodule number (table 14). Although no significant differences occurred in nodule weight, *Rhizopus* sp gave highest values of 0.51 and 42g/plant in 1993 and 1994 respectively. Weights of nodule produced in inoculated plots were superior to uninoculated plots in both years. This corrobates the above observation of positive interaction between phosphate solubilizers and rhizobia.

4.3.4.2.6 Number of Pods

The numbers of pods were significantly different in 1993. Inoculated plots were significantly different from control (table 14). However, the 1994 trial did not show significant differences. *Rhizopus* sp produced highest number of pods among the inoculants. It produce 39 nodules more than control and about 73% more than *B. megaterium* in 1993. Pod formation is a result of other previous developmental stage such as nodule formation,

flowering etc. and as has been observed above. *Rhizopus* sp. enhances more nodule formation than other organisms.

Rhizopus sp also gave superior value to other treatments when the data were combined.

4.3.4.2.7 Grain Yield

The grain yield was not significantly different in 1993 and 1994 (table 14). However, *Rhizopus* sp. and *P. striata* gave superior yield among the inoculants in 1993 while in 1994, *P. striata* and *Penicillium* sp. gave superior yield. In both years, all inoculants gave higher yield than control with the exception of *B. megaterium*.

The combined analysis of both years however, showed *Rhizopus* sp and *P. striata* as superior to other treatments. *P. striata* and *Rhizopus* sp were different from control by 345kg and 351kg/Ha respectively (table 14). It was observed earlier that *P. striata* and *Rhizopus* sp produced higher leaf areas than other treatment and therefore enhanced more photosynthesis. Photosynthesis is a factor of pod filling in soyabean and any factor that will enhance photosynthesis will also enhance pod filling (Stephenson and Wilson, 1978). *P. striata* was also observed by Satter and Gaur (1989) to enhance grain yield.

4.3.4.2.8 Thousand Seed Weight

There was no significant differences in weight of 1000-seed in 1993 and 1994. However, *Rhizopus* sp. maintained its superior performance in both years (table 14).

Nutrient Concentrations: The concentrations of various nutrients in plant tissue are shown in table 15.

4.3.4.2.9 P Concentration

The 1993 trial showed that other organisms and control were significantly different from *Penicillium* sp. The 1994 and combined data showed the same trend as *Penicillium* sp and *P. striata* were observed to be superior to other treatments in 1993, 1994 and when the data were combined (table 15). This superior uptake of P from soil inoculated with *P. striata* and *Rhizopus* sp may be partly responsible for the higher grain yield produced by these organisms. The low P-uptake observed in plots inoculated with *Penicillium* is however not consistent with grain yield as *Penicillium* was also observed to produce high grain yield in 1994. The inconsistency in the result may be attributed to soil factor.

4.3.4.2.10 Ca, Mg, K and N

There were no significant differences in the uptake of these nutrients. Ca was taken more from plots inoculated with *A. niger* in 1993 while in 1994, plots inoculated with *P. striata* (table 15) supplied more Ca to plant tissue. *Penicillium* enhanced Mg uptake in 1993 but in 1994, *P. striata* enhanced Mg uptake. *P. striata* and *Rhizopus* sp enhanced more K uptake in 1993 while in 1994 *A. niger* gave more K to the plant tissue. However, the inoculated plots did not show superiority over uninoculated plots

in the uptake of Ca, K and Mg. Some micro-organisms are known to solubilize these elements (Muller and Foster, 1961; Webley *et al*, 1960). However, organisms being tested in this experiment may not be efficient at mineralizing these elements.

The uptake of N into the plant tissue was also slightly enhanced by inoculation with *Rhizopus* sp and *P. striata*. This may be an indication of positive interaction between these organisms and nodule rhizobia observed earlier in the discussion.

4.3.4.2.11 Zn, Mn, Fe and Cu

There was no significant difference in Zn uptake, however, *Rhizopus* sp promoted more uptake of Zn in both 1993 and 1994, and uptake from inoculated plots was more than from control (table 15). *A. niger* promoted more Mn uptake in 1993 and 1994 and uptake was higher from inoculated plots than control in both years. *A. niger* also promoted higher Fe uptake in 1993 while in 1994, *P. striata* promoted higher Fe uptake. The inoculated plots were however not superior to control in all cases. Cu showed significant differences in 1993. *A. niger* gave significant difference from *Rhizopus* sp, which was also significantly different from other treatments (table 15).

The observation above indicated that specific organisms promoted the uptake of specific nutrients. *A. niger*, however, appears superior to other organisms in enhancing the uptake of these micro-nutrients. Bromfield (1959) observed that some organisms mineralize micronutrients. The promotion of Zn uptake by *Rhizopus* sp. is essential in avoiding P-Zn imbalance and

consequent induced deficiency of other nutrients (Langin *et al* 1964). This promotion of P uptake by *Rhizopus* may also contribute to superior yield qualities of the organism.

4.3.4.2.12 Crop response to organism

The response of crop to the organisms was mostly consistent. When leaf area, dry matter yield, nodule weight, number of pods, grain yield and Zn uptake were considered, plants were consistent in their response to *Rhizopus* sp., *P. striata*, *A niger*, *Penicillium* sp.

The magnitude of response observed is *Rhizopus* > *P striata*, > *A niger* > *Penicillium* > *B. magaterium*.

Correlation matrix shown in table 16 indicates non statistically significant correlation between organisms and the yield parameters considered. Nodule weight however, correlated significantly with grain yield as has already been observed in discussion.

4.3.4.3 Yield parameters as influenced by inoculation on P source

Response of crops to the combined influence of inoculation and P sources is presented in fig. 12-15.

4.3.4.3.1 Dry Matter Yield

The crop responded to SSP combined with inoculation more than control in 1993 and 1994. *Rhizopus* sp. seem to promote crop responce to SSP in the 2 years and when data were combined (fig. 12). Results showed about 20% increase in dry matter yield due to inoculation with *Rhizopus* in 1993 and 50.6% increase in 1994.

Togorock phosphate was enhanced in its dry matter yield when

inoculated with *A. niger* as shown in 1993 and combined data (Fig. 12). The magnitude of enhancement due to inoculation with *A. niger* is 27.6%.

Table 16: Correlation matrix between treatments and variables in soyabean

	P concentration	Dry matter Yield	Grain Yield	Nodule weight	Org.	Org.^{2}	P source	P source^2
P concentration	-							
Dry matter yield	-0.3194	-						
Grain Yield	-0.0659	0.2265*	-					
Nodule Weight	-0.2387	0.2533**	0.3323**	-				
Organism	0.0399	0.1078	-0.0230	0.037	-			
Organism^2	0.0435	0.1028	-0.0319	0.0240	0.9792**	-		
P source	-0.0450	0.1208	0.1070	0.1377	0.0260	0.02	-	
P source^2	-0.0712	0.1073	0.0668	0.1140	0.0335	0.0271	0.9831	-

Significant level: * = p 0.05, *** = P 0.01

Similarly, Sokoto rock phosphate was influenced particularly by *P. striata* in both 1993 and 1994 seasons and when the data were combined. Magnitude of enhancement was 49.4%, 120% and 64.5% in 1993, 1994 and combined data respectively.

The above data seem to suggest selective activities of the micro-organism depending on the P source. Many factors may be responsible for this. The most obvious factor however, is pH. Fungi organisms are known to tolerate lower pH and thus may be found more active with SSP which is more acidic than the rock phosphate. It appears however, that Sokoto rock benefits more from inoculation than any other P source as the difference between DMY at inoculation and zero inoculation is very much as discussed above.

4.3.4.3.2 Weight of Nodules

The 1993 and 1994 data showed that SSP produced more nodules when inoculated (fig. 13). In 1993 it was enhanced by all the inoculants above control. However, by 1994, *Rhizopus* sp. was oberved to promote response to SSP more than any other organism. The combined data also show *Rhizopus* as enhancing the use of SSP. Contrary to expectation at zero-inoculation, Sokoto rock was oberved to produce more nodules than SSP in 1993. This was however, reversed in 1994.

Togo rock phosphate seemed to be promoted in its nodule production by A. *niger* in 1993 and when combined. Combined analysis also indicates that Sokoto rock is enhanced by *P. striata*. These observations are consistent with the results

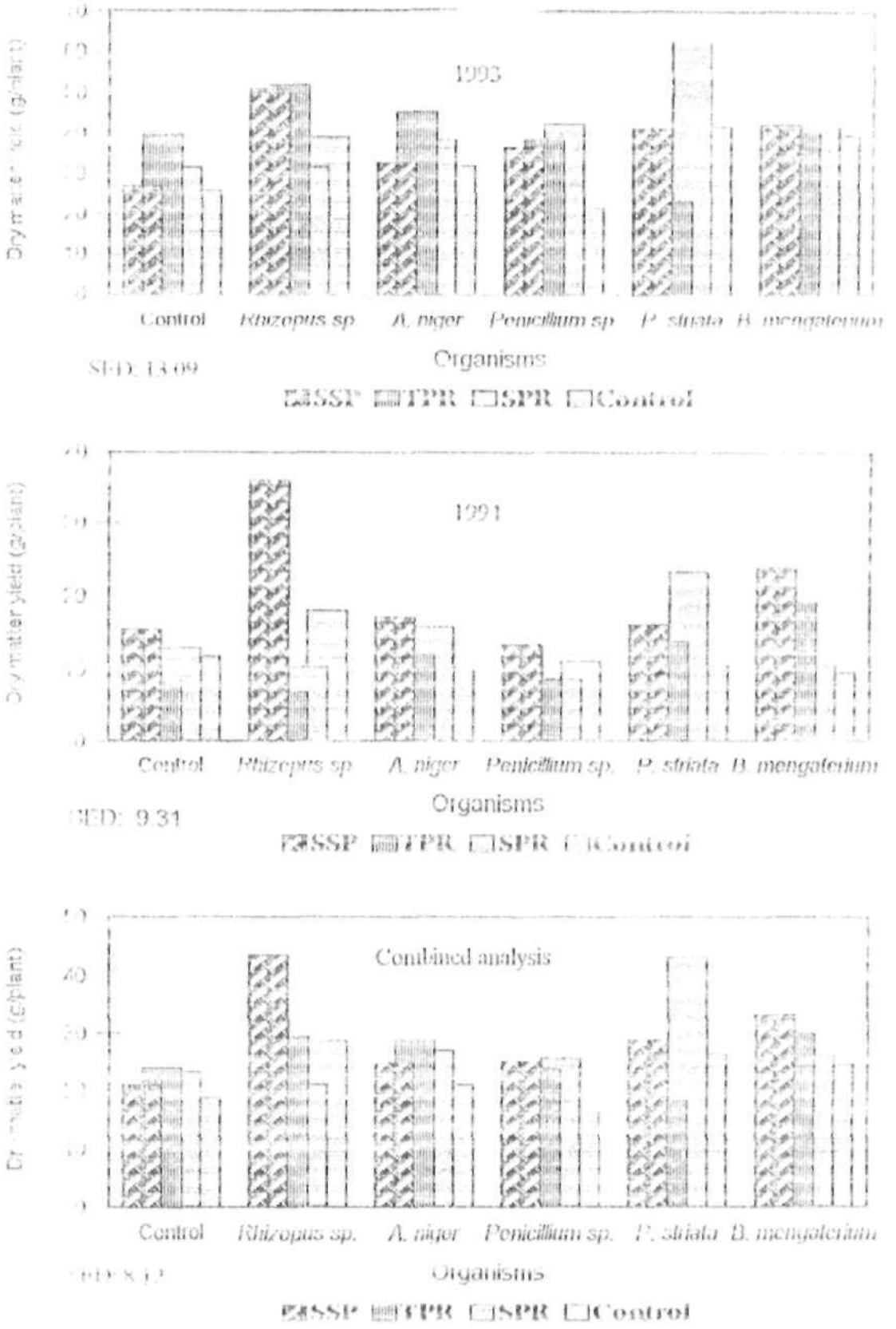


Figure 42. Effect of P sources and organisms on soyabean dry matter yield at 1993 and 1994

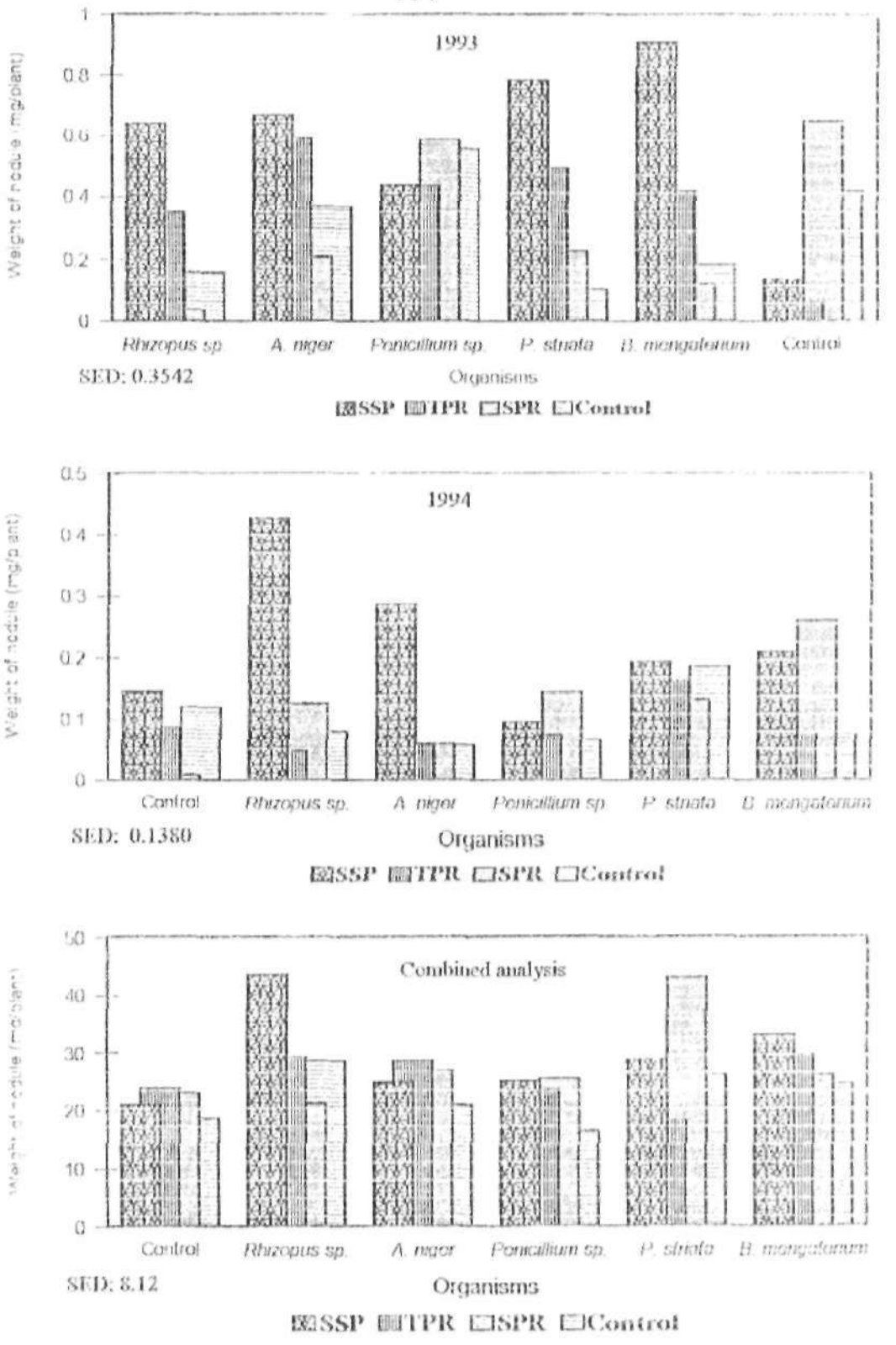


Figure 13: Effect of P sources and organisms on soyabean nodule weight in 1993 and 1994.

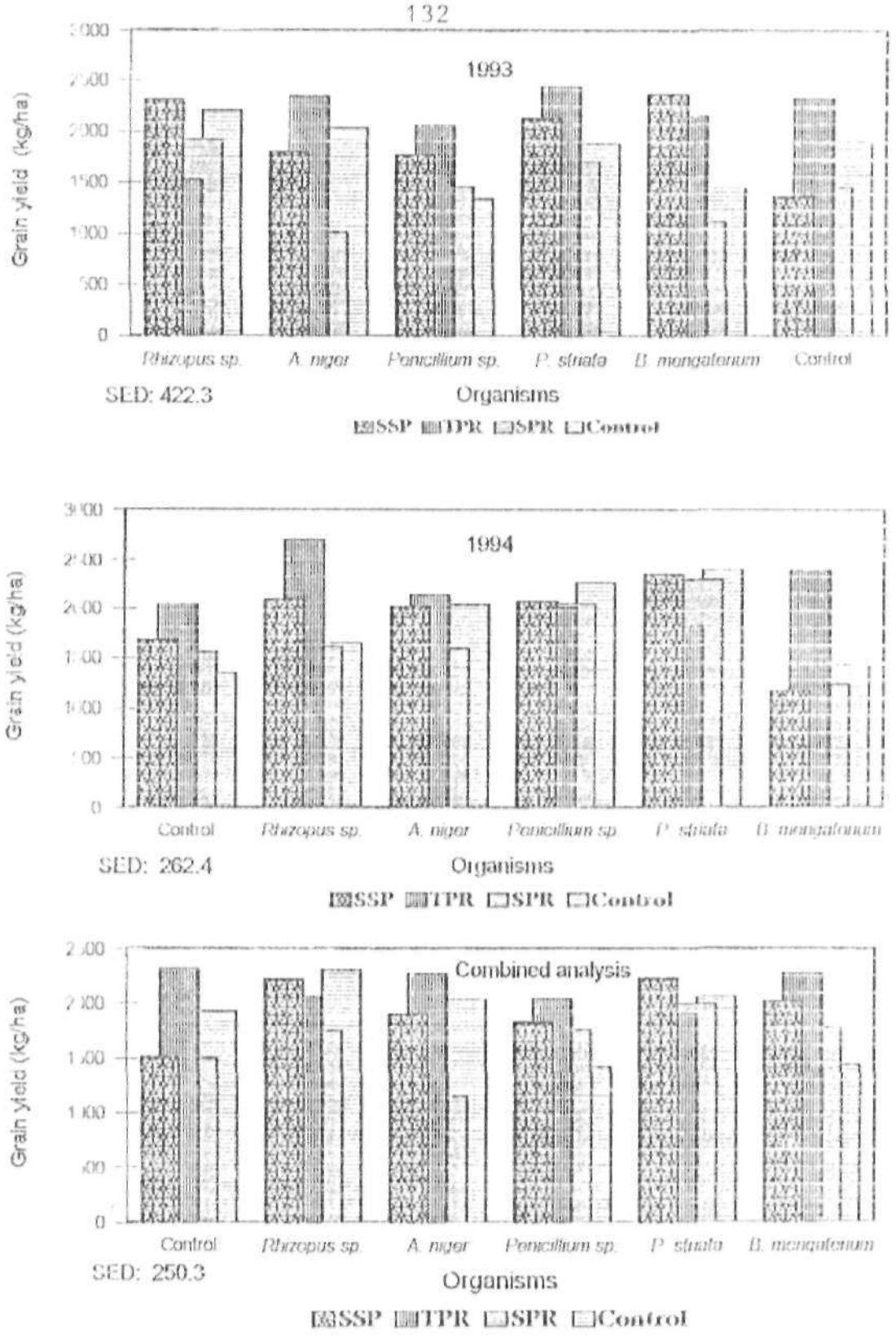


Figure 14: Effect of P sources and organisms on soyabean grain yield in 1993 and 1994.

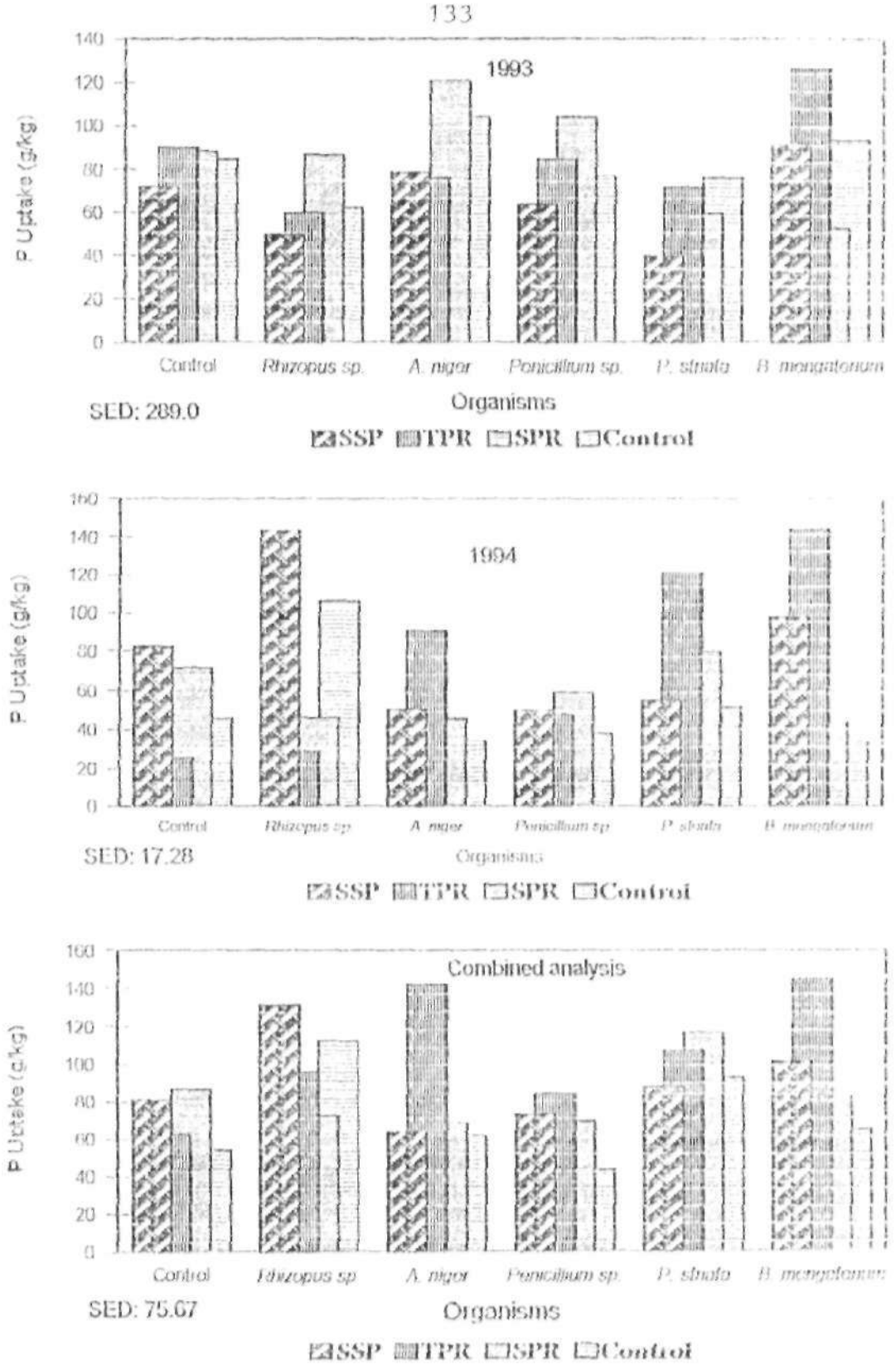


Figure 15: Effect of P and organisms on soyabean P uptake in 1993 and 1994.

observed in dry matter yield. The positive interaction of phosphates sources and dry matter yield (Fig. 12). The positive interraction of phosphate solubilizers and nodule rhizobia is also suggested by the better nodulation of inoculated plots than control in both years and when data was combined.

4.3.4.3.3 Grain Yield

SSP was enhanced in it grain yield by inoculation in 1993, 1994 and when the data were combined. *Rhizopus* promoted grain production in SSP supplied plots in both years (fig. 14). However SSP produced more grains at all inoculations than control. It increased in its grain production by 950.2kg/Ha, 862.5kg/Ha and 997.2 kg/Ha when inoculated with *Rhizopus* sp, *P. striata* and *B. megaterium* respectively.

Togo rock phosphate produced high grain yields at all inoculations and zero inoculation in 1993. However in 1994, it produced high yields also at control, and inoculation with *Rhizopus* sp., *A. niger*, *Penicillium* sp and *B. megaterium*. (fig. 14). Sokoto rock phosphate appeared to produce high grain yield at inoculation with *P. striata*. It appears from the above observations that Togo rock phosphate can be used as P fertilizer directly without inoculation. This observation is not consistent with already established facts. It may however, be attributed to soil factors. The favourable effect of inoculants on SSP may be attributed to the water solubility of SSP thus supplying both crop and micro-organisms with P and consequently removing the lag caused by immobilization. Similar observation was made by

Bhatnagar *et al* (1979) in a pot experiment. They reported that mixtures of *Rhizobium* and *B megaterium* with super phosphate gave significantly better results.

4.3.4.3.4 P-uptake

Rhizopus sp promoted the P-uptake in SSP supplied plot in 1994 and combined data. However 1993 data showed that *B. megaterium* promoted P-uptake in SSP supplied plots than any other organisms. The rock phosphates, contrary to expectation, showed greater uptake of P than SSP (fig. 15). Togo rock was enhanced by *Penicillium* sp and *B. megaterium* in 1993 and *P. striata* and *B. megaterium* in 1994 while combined analysis shows that *A. niger* and *B. megaterium* promote P-uptake from Togo rock (fig. 15). SSP in its own case was enhanced by *A. niger* and *Rhizopus* in 1993, while in 1994 *Penicillium* sp promotes P-uptake from it. The superiority of Togo rock in releasing P into the crop plant tissue may be partly responsible for its high yielding qualities.

4.3.4.3.5 Soyabean Response to Inoculation on P source

Increased yield were observed when SSP was applied accompanied by inoculation with organisms (Fig.14) *Rhizopus* sp enhanced SSP in dry matter yield (Fig.12) and nodulation while grain yield and P-uptake were promoted when SSP was accompanied by inoculation with *Rhizopus* sp, *B. megeterium* and *P. striata* (Fig.13 and 14). The crop responded to SSP in all parameters observed when inoculated than at control. Togo rock on the other hand was enhanced by *A niger* and *penicillium* sp and so, crop responded to it at inoculation with this organism. Sokoto rock

was enhanced by *P. striata* and crop response to Sokoto rock was promoted at inoculation by the organism. However, response of the crop to SSP at inoculation promoted higher yields than other treatments.

4.3.5 Effect of Treatments on Maize

4.3.5.1 Effects of P sources

The effects of the different types of P supplied to the maize crop are presented in tables 17-19.

Growth Parameters

The growth parameters did not show significant difference (table 17). However, SSP was observed to give higher values than the rock phosphates in all the parameters taken.

4.3.5.1.1 Plant Height

SSP gave higher plants than the rock phosphates in all the three years. It was superior to Togorock by 10%, 9% and 11% in 1993, 1994 and 1995. Among the rock phosphates, Togo rock phosphate gave higher values than Sokoto rock difference being 4cm in 1993, 9cm in 1994 and 1cm in favour of Sokoto rock in 1995. The control gave higher value than the rock phosphates in 1995. This anomaly is propably due to deficiency of other elements or experimental error.

4.3.5.1.2 Leaf Area

SSP gave higher values of leaf area in the three years of experiment (table 17). It differs from the rock phosphates by 10cm^2 in 1993, 37cm^2 in 1994 and 47cm^2 in 1995. Togo rock again gave higher values than Sokoto rock phosphate, differences being 7cm^2 in 1994 and 30cm^2 in 1995. The control was at par with Togo

rock phosphate but superior to Sokoto rock in 1993 and 1994, deficiency of other elements may be responsible for this observation. The area of leaves produce is a function of photosynthetic activities of the plant and these will consequently affect yield.

Table 17: Effect of P sources on growth parameters of maize from 1993 - 1995

P Sources	1993			1994			1995			Combined analysis (1993 and 1995)
	Plant Height (cm)	Leaf Area (cm^2)	Dry Matter Yield (g/plant)	Plant Height (cm)	Leaf Area (cm^2)	Dry Matter Yield (g/plant)	Plant Height (cm^2)	Leaf Area (cm^2)	Dry matter Yield (g/plot)	Dry Matter Yield g/plot)
SSP	175	469	24.0	150	322	17	197	576	37	30.5
TPR	159	454	25.0	137	285	15	177	529	33	28.6
SPR	155	459	16.0	128	278	14	178	499	30	27.0
Control	157	451	23.0	130	269	14	187	529	34	28.3
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 18: Effect of P sources on yield components of maize (1993 - 1995).

	1993			1994				1995				Combined (1993&1995)
P Source	Grain Yield (Kg/Ha)	Weight of 1000-seed (g/plot)	Shelling percent (%)	Grain Yield (Kg/Ha)	Weight of 1000-seed (g/plot)	Shelling Percent (%)	Stover Yield (kg/Ha)	Grain Yield (Kg/Ha)	1000-seed weight (g/plot)	Shelling percent (%)	Stover Yield (kg/Ha)	Grain Yield (kg/Ha)
SSP	4,239	193	89	1,931	171	97	3,052	5,668	226	80	5414	4846
TPR	3,347	210	84	1,522	156	94	2,722	4,647	208	80	4696	3871
SPR	2,593	207	55	1,073	167	96	2,227	4,616	201	79	3187	3456
Control	3,255	201	83	1,164	166	88	2468	4,398	205	79	3255	3735
LSD	NS	NS	-	NS	NS	-	NS	NS	NS	-	NS	NS

Table 19 Effect of P sources on nutrient concentration of maize from 1993 to 1995

	1993								1994							1995								Combined Analysis (1993&1995)
P Source	P	Ca	Mg	K	Zn	Mn	Fe	Cu	P	Ca	Mg	K	Zn	Mn	Fe	P	Ca	Mg	K	N	Zn	Mn	Fe	%P
	%				(Mg/kg)				%				(Mg/kg)			%					(Mg/kg)			
SSP	0.20	0.54	0.20	2.2	177	81	335	5	0.29	0.46	0.18	2.6	213	80	247	0.31	0.44	0.18	2.1	2.7	136.0	71	286	0.38
TPR	0.19	0.49	0.21	2.5	151	93	329	8	0.81	0.52	0.17	2.5	300	91	225	0.27	0.51	0.17	2.0	2.8	117	64	215	0.33
SPR	0.20	0.51	0.19	2.4	155	84	312	7	0.27	0.55	0.18	2.4	255	82	256	0.31	0.54	0.18	2.3	2.8	119	42	251	0.25
Control	0.20	0.52	0.21	2.2	149	90	261	6	0.29	0.44	0.16	2.2	342	89	212	0.27	0.42	0.16	2.1	2.8	121	64	223	0.29
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

4.3.5.1.3 Dry Matter Yield (DMY)

SSP gave slightly lower DMY in 1993 than Togo rock. However in 1994 and 1995 it gave superior values to the rock phosphates (table 17). Togo rock was superior in DMY to Sokoto rock and Control in the three years, however Sokoto rock was not superior to Control. Combined analysis of the three years showed that SSP was superior to other treatments. Togo rock and control gave similar values and were superior to Sokoto rock.

Yield Parameters

The yield parameters taken are presented in table 18. Significant differences were observed in all parameters. However, there were differences in values obtained among the treatments.

4.3.5.1.4 Grain Yield

SSP gave higher yields of 889kg/Ha more than Togo rock and 1646kg/Ha more than Sokoto rock in 1993 (table 18). Similarly, in 1994 SSP was superior to other treatments. It gave 409kg/Ha more than Togo rock and 858kg/Ha more than Sokoto rock. The 1995 data also show SSP to be superior to the rock phosphates giving Togo rock a difference of 1021kg/Ha and it was superior to Sokoto rock by 1052kg/Ha.

Among the rock phosphates, Togo rock was superior to Sokoto rock by 754kg/Ha, 449kg/Ha and 31kg/Ha in 1993, 1994 and 1995 respectively. The Togo rock was observed to have superior yield to the control (92kg/Ha, 358kg/Ha and 249kg/Ha in 1993, 1994 and 1995). On the other hand control was observed to give higher

yield than Sokoto rock in 1993 (662kg/Ha) and in 1994 (91kg/Ha), while Sokoto rock gave higher yield than control in 1995 (218kg/Ha). Combined analysis also showed SSP as superior in yield followed by Togo rock than control. The superiority of SSP over rock phosphate has long been established (Mokwunye, 1979, Lombin, 1989, Uyobvisere, 1989) and it is due to its water solubility and thus superior reactivity over rock phosphate which is not reactive in the soil. There is an observed consistent superiority of Togo rock phosphate over Sokoto rock as indicated by all parameters taken so far. This phenomenon was explained by Mokwunye (1994). In comparing the citrate solubility of West African rock phosphates, he ranged the solubility of Togo rock as 2.5-3.2% P_2O_5 while for Sokoto rock it is 3.2-3.7% P_2O_5. He mentioned that the higher the range, the more reactive is the rock. However Chien (1977) found that the solubility of phosphate rock in neutral ammonium was directly related to carbonate substitution.

The superior performance of control over Sokoto rock may be attributed to method of application of the rock. Drilling along ridges was employed and this can reduce phosphate dissolution as a result of overlapping diffusion zones around the closely spaced phosphate rock particles (Barrow, 1990). The practice of broadcasting and incorprating in the surface soil has been recommended as it exposes phosphate rock particles to larger volume of soil.

The yield recorded in 1993 was smaller when compared with the

two other seasons (1993 and 1995). This is due to change in varieties as a result of problems encountered due to late establishment of rains (discussed earlier). An early maturing variety (TZEE-YBC5) was planted in 1994. The variety was shorter in canopy and small grained and it matures at 75-80 days after planting.

4.3.5.1.5 Thousand Seed Weight

SSP gave superior seed weight in 1994 and 1995, while in 1993 the rock phosphate gave higher 1000-seed weight than SSP (Table 18). Togo rock and Sokoto rock appeared to be at par in the weight of seeds produced in the three years. They were however, superior to control in all the three years.

4.3.5.1.6 Shelling Percent

SSP gave higher shelling percent in 1993 and in 1994, while in 1995 it equalled Togo rock in performance (table 18). In 1993, Sokoto rock gave very low shelling percent (55%). Apart from this the difference in shelling percent for all the treatments was insignificant.

4.3.5.1.7 Stover Yield

Stover yield was taken from 1994 and 1995, SSP showed superior straw yield in both years, followed by Togo rock. The differences in straw yield between SSP and Togo rock were 330kg/Ha in 1994 and 718kg/Ha in 1995. Control again gave higher straw yield than Sokoto rock. No straw yield was recorded for 1993 due to theft from the field.

Nutrient Concentrations

The nutrient concentrations in plant tissue are presented in table 19. There were no significant differences in nutrient response to P source.

4.3.5.1.8 Phosphorus Concentration

There were no clear difference in tissue P concentration in all the three years. However, combined analysis shows in table 19 that maize plants were able to take more P from SSP (0.38% P). Among the rock phosphates, Togo rock gave more P to the plants (0.33% P) while control again produced higher P (0.29% P) in plant tissue than Sokoto rock (0.25% P). The pattern observed in tissue-P concentration was also observed in grain yield and most of the parameters discussed above (table 17-18). It therefore, indicates that P-uptake may be a consequence of some of the results discussed earlier.

4.3.5.1.9 Ca, Mg, K and N

SSP produced highest Ca (0.54%) in plant tissue in 1993 while Sokoto rock produce highest Ca in plant tissue in 1994 and 1995 (0.55 and 0.54%). Togo rock was consistent in the amount of Ca produced in the 3 years (it ranged between (0.49-05/mg/g). The P sources did not show significant differences in amount of Mg produced in plant tissues. The differences observed were small and inconsistent. However, the rock phosphates produced higher potassium in the plant tissue in 1993 and 1995. In 1994, SSP produced more K (0.26Mg/g) while Togo rock and Sokoto rock produced 0.25Mg/g and 0.24Mg/g in plant tissues (table 19).

There is a general higher performance of rock phosphate over SSP observed in the plant uptake of Ca, Mg and K. This may be attributed to the presence of these elements in rock phosphates. Mokwunye (1994) gave the empirical formular of Togo rock as $Ca_{0.85}$, $Na_{0.11}$, $Mg_{0.01}$ $(PO_4)_{5.55}$ $(CO_3)_{0.45}$, $F_{2.18}$ while Sokoto rock is $Ca_{0.85}$, $Na_{0.11}$, $Mg_{0.05}$ $(PO_4)_{5.57}$ $(CO_3)_{0.43}$ $F_{2.10}$. It is therefore obvious that Ca is present in rock phosphates in large amounts. Its mineralization, like in the case of P is however slow. K also may be found on the phosphate rock either as part of the mineral component or as impurities. N uptake was least in SSP, while all other P sources were at par. This may also be attributed to certain soil factors such as inadequate supply of K, which may affect the efficient use of N.

4.3.5.1.10 Zn, Mn, Fe and Cu

The plants took highest Zn from SSP in 1993 and 1995 while in 1994 the untreated control produced more Zn in the plant than all P sources (table 19). Togorock phosphates produced highest Mn in plants in 1993 and 1994 (93 and 91Mg/g), while in 1995 SSP produced the most Mn (71Mg/g) in the plants. Sokoto rock phosphate gave lower Mn in the plants than control in all three years. Cu could only be analysed in 1993 as only traces of it were observed in plant tissue in 1994 and 1995 and therefore could not be measured. The rock phosphates gave higher Cu in the plant tissue than SSP. This may be due to the presence of Cu in rock phosphates as impurities. Similarly, Zn, Mn and Fe have also been observed as impurities in rock phosphate (Smith and

Lehr, 1966). Plants needs of these elements are in small amounts and thus only small amounts are taken up accordingly by the plants.

4.3.5.1.11 Maize Response to P

Observations on growth parameters, yield components and P-uptake showed that crop responded to SSP more than the rock phosphates. Among the rock phosphates however, Togo rock phosphate was superior to Sokoto rock phosphate. This is attributed to higher reactivity of Togo rock than Sokoto rock. It was also observed that method of application (drilling along the ridges) adversely affected response of crop to Sokoto rock so much that untreated control gave better yields than Sokoto rock. The magnitude of performance observed is: SSP > Togo rock > Control > Sokoto rock.

Table 23 shows that P source is not statistically significantly corrlelated with the parameters taken. However, P concentration correlated significantly with dry matter yield while grain yield correlated highly significantly with dry matter yeild.

4.3.5.2 Effects of Organisms

The effects of organisms on growth parameters, yield components and nutrient concentrations are presented in tables 20-22.

Growth Parameters

There were significant differences in leaf area and dry matter yield in 1993 and 1995 as shown in table 20.

4.3.5.2.1 Plant Height

Pseudomonas striata and *Aspergillus niger* gave the highest plants in 1993 and 1995 while in 1994 *B megaterium* gave the highest plants (table 20). This difference in performance of the organisms in 1994 may be attributed to differences in the maize varieties used. AG Kaduna which is an hybrid was planted in 1993 and 1995 while an extra early maturing variety (TZEE-YBC5)was used in 1994. *Bcillus megaterium* gave lower values than the control in 1993 due to its poor adaptability to field conditions and inability to compete effectively with the soil indigenous organisms.

4.3.5.2.2 Leaf Area

The response of plant to organism inoculation in their production of leaves was similar to plant height (table 20). *P. striata, A. niger, Rhizopus* sp. and *Penicillium* sp produced significantly different values of leaf area (P = 0.05) from *B. megaterium* and control. This inability of *B. megaterium* to perform in this first year of trial may be attributed to poor adaptability to field conditions and inability to compete effectively with other organisms. However in 1994 the organism (*B. megaterium*) was able to compete more effectively and thus gave a higher value of leaf area (next to *Penicillium* sp) among the inoculants. By 1995 *B. megaterium* was able to produce high values of leaf area than all other organsms. It increased over *Rhizopus* by 11cm^{2} (table 20). However, in 1994 and 1995 the inoculated plots gave higher values than control. Increase in

leaf area due to inoculation in 1994 and 1995 was 11cm^2 and 82cm^2 respectively. It therefore indicates that inoculants may not perform effectively in the first year of application. However as the seasons increase and inoculation continues, they are able to adapt and compete more effectively with other soil factors and thus become more effective in their solubilization of P. Table 23 showed the microbial count of phosphate solubilizers in the soil. More phosphate solubilizers were observed in inoculated soils and cultivation of the soil further increased the numbers. However, they adapt poorly immediately after inoculation.

4.3.5.2.3 Dry Matter Yield (DMY)

P. striata produced increased dry matter yield above control in 1993 while in 1994 all the inoculants produced higher dry matter above control but there were no differences in dry matter yield among the inoculants (table 20). The 1995 result showed significant differences in dry matter yield. The other organisms and control were significantly different from *Penicillium* sp and *Aspergillus niger*. *Rhizopus* sp and *B. megaterium* produced the highest value of dry matter among the inoculants in 1995. Combined analysis also showed that *P. striata* was superior to other organisms in their production of dry matter.

Yield Parameters

The yield parameters are presented on table 21. Although there were no significant differences in all the parameters taken, there were differences in response to individual organisms as indicated by values of each parameters measured.

4.3.5.2.4 Grain Yield

A. niger and *P. striata* gave higher yields than control in 1993 (table 21). The increases in yield over control were 462kg/Ha and 150kg/Ha when inoculated with *A. niger* and *P. striata*. The other organisms (*Rhizopus* sp., *Penicillium* sp and *B. megaterium*) gave lower yields than control due to poor adaptation in this first year of trial. The 1994 trial was an improvement over 1993 as almost all the organisms gave higher yields than control. Only *Penicillium* sp gave lower yield than control while *A. niger* and *P. striata* were superior to control by increased yield of 557kg/Ha and 276kg/Ha. There is also an indication of continued improvement by the organisms as they become better adapted to field conditions. Contrary to the two previous years the 1995 trial showed control as superior to all the organisms (table 21). It gave increased yield of 289kg/Ha above *Penicillin* sp which is the highest grain yielder among the inoculants. By 1995 the experiment was laid out on a different plot and thus organisms have not been adapted to this plot and so rendering it less efficient than as observed in the previous years. Combined analysis also showed control giving increased yield of 29kg/Ha over *P. striata*.

Table 20: Effect of Organisms on growth parameters of maize (1993 to 1995)

Organisms	1993 Plant Height (cm)	1993 Leaf Area (cm^2)	1993 Dry Matter Yield (g/plant)	1994 Plant Height (cm)	1994 Leaf Area (cm^2)	1994 Dry matter Yield (g/plant)	1995 Plant Height (cm^2)	1995 Leaf Area (cm^2)	1995 Dry matter Yield (g/plot)	Combined analysis 1993 and 1995 Dry Matter Yield g/plot)
Rhizopus sp	162	462a	18	291	137	15	187	554	36a	27
Aspergillus niger	168	502a	20	292	134	15	187	531	32b	28
Penicillium sp	160	439a	18	307	141	15	180	537	31b	28
Pseudomonas striata	169	514a	21	291	136	15	185	530	33a	30
Bacillus megalerum	148	401b	18	281	139	16	189	565	35a	27
Control	162	429b	20	269	131	14	180	482	33a	30
LSD 0.05	NS	75.95	NS	NS	NS	NS	NS	NS	3.15	

Means not followed by the same letter are statistically significant at 0.05 by DMRT.

Table 21: Effect of organisms on Yield components of maize (1993 to 1995)

Organism	1993 Grain Yield (Kg/Ha)	1993 Weight of 1000-seed (g/plot)	1993 Shelling percent (%)	1994 Grain Yield (Kg/Ha)	1994 Weight of 1000-seed (g/plot)	1994 Shelling percent (%)	1994 Stover Yield (kg/Ha)	1995 Grain Yield (Kg/Ha)	1995 1000-seed weight (g/plot)	1995 Shelling percent (%)	1995 Stover Yield (kg/Ha)	Combined (1993&1995) Grain Yield (kg/Ha)
Rhizopus sp	3,172	213	75	1,721	173	2,898	98	4,748	205	3,224	80	3,8406
Aspergillus niger	3,842	213	81	2,078	168	2,578	95	4,354	214	3,282	78	4,062
Penicillium sp	3,091	196	95	1,431	156	2,526	99	4,999	207	3,308	79	3,862
Pseudomonas striata	3,530	216	60	1,797	152	2,839	97	4,853	210	3,376	80	4,118
Bacillus megaterum	3,288	196	81	1,735	169	2,658	97	4,810	208	3,328	79	3,907
Control	3,380	182	83	1,521	172	2,499	95	5,288	219	3,494	80	4,147
LSP 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS

Table 21: Effect of organisms on Yield components of maize (1993 to 1995)

	1993			1994				1995				Combined (1993& 1995)
Organism	Grain Yield (Kg/Ha)	Weight of 1000-seed (g/plot)	Shelling percent (%)	Grain Yield (Kg/Ha)	Weight of 1000-seed (g/plot)	Stover Yield (kg/Ha)	Shelling Percent (%)	Grain Yield (Kg/Ha)	1000-seed weight (g/plot)	Stover Yield (Kg/Ha)	Shelling Percent (%)	Grain Yield (kg/Ha)
Rhizopus sp	3,172	213	75	1,721	173	2,898	98	4,748	205	3,224	80	3,8406
Aspergillus niger	3,842	213	81	2,078	168	2,578	95	4,354	214	3,282	78	4,062
Penicillium sp	3,091	196	95	1,431	156	2,526	99	4,999	207	3,308	79	3,862
Pseudomonas striata	3,530	216	60	1,797	152	2,839	97	4,853	210	3,376	80	4,118
Bacillus megaterum	3,288	196	81	1,735	169	2,658	97	4,810	208	3,328	79	3,907
Control	3,380	182	83	1,521	172	2,499	95	5,288	219	3,494	80	4,147
LSP 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS

4.3.5.2.5 Weight of 1000-Seeds

P. striata gave the highest value for 1000-seed weight in 1993 being closely followed by *Rhizopus* sp and *A. niger*. While in 1994, *Rhizopus* sp gave highest seed weight of 173g/plot, it was only slightly different from control which gave 172g/pot (table 21). This is contrary to the higher yield produced by control in 1995, the 1000-seed weight increased above inoculants by only 5g relative to yield difference of 289 Kg/Ha). This tend to indicate that although uninoculated plots produced more grain than inoculated plots, the grains produced by the inoculated plots were bigger than uninoculated plots.

4.3.5.2.6 Shelling Percentage

Penicillium sp gave highest percentage of shelling (95%) in 1993 while *P. striata* gave lowest of 60%. The 1994 trial similarly showed the inoculated plots as having higher shelling percent than control, while in the 1995 trial shelling percentages were comparable for all the treatments.

4.3.5.2.7 Stover Yield

Stover yields were taken in 1994 and 1995, *P. striata* and *Rhizopus* sp gave highest straw yields of 2839 and 2898kg/Ha in 1994, and they increased above control by 340kg/Ha and 399kg/Ha (table 21). On the other hand, in 1995 control was superior to treatments in straw yields. However, it gave an increase of 118kg/Ha over *P. striata* which produced highest straw among the inoculants.

Nutrient Concentrations

The concentrations of various elements as observed in the plant tissue are presented in table 22. No significant difference was observed in all the elements taken.

4.3.5.2.7 P Concentration

In 1993, *A. niger* and *B. megaterium* supplied more P into the plant tissue than control and other inoculants. Similarly, in 1994, plants took more P from *A. niger* and *B. megaterium* than control and other inoculations. P concentrations produce by both organisms increased above control by 0.02 and 0.03% P (table 22). Contrary to this however, plants took more P from the untreated plots in 1995 giving an increase of 0.02% P over *Penicillium* sp and *P. striata* which produced highest P in plant tissue among the inoculants.

Table 22: Effect of organisms on nutrient concentration of maize (1993 to 1995).

analysis	1993								1994								1995								combined analysis (1993&95)
Organisms	P	Ca	Mg	K	Zn	Mn	Fe	Cu	P	Ca	Mg	K	Zn	Mn	Fe	P	Ca	Mg	K	N	Zn	Mn	Fe	%P
		%			(Mg/kg)					%			(Mg/kg)				%				(Mg/kg)			
Rhizopus sp	0.20	0.60	0.24	2.2	156	87	276	7.0	0.29	0.53	0.15	2.4	261	92	235	0.25	0.41	0.16	2.4	2.8	133	49	305	0.27
A. niger	0.21	0.56	0.23	2.4	169	86	340	5.0	0.31	0.49	0.18	2.6	265	98	240	0.27	0.37	0.19	2.1	2.8	131	48	215	0.42
Penicillium sp	0.19	0.43	0.16	2.2	156	80	269	6.0	0.26	0.39	0.14	2.3	238	87	228	0.28	0.36	0.18	2.0	27	115	84	227	0.29
P. striata	0.20	0.53	0.21	2.6	129	94	333	7.0	0.27	0.59	0.18	2.3	278	80	208	0.28	0.43	0.19	2.0	2.8	125	41	242	0.24
B.mengaterum	0.21	0.50	0.20	2.5	179	96	372	7.0	0.32	0.46	0.15	2.7	317	96	247	0.26	0.45	0.19	2.0	2.7	110	54	221	0.23
Control	0.20	0.48	0.19	2.3	157	79	267	7.0	0.29	0.51	0.14	2.4	307	80	252	0.30	0.39	0.15	2.2	2.8	125	86	254	0.26
LSD 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

This observation is consistent with grain yield produced by the various treatments (table 21). This implied that P-uptake may be correlated linearly with grain yield. The observation made in 1995 tend to suggest that the inoculated organisms did not cause as much release of P as Control thereby leading to higher grain yield in Control than in the plots inoculated.

4.3.5.2.8 Ca, Mg, K and N

More Ca was taken up into plant tissue from plots inoculated than from uninoculated plots in 1993. Only *Penicillum* sp gave less Ca in plant tissue than Control (table 22). Similarly, in 1994 and 1995 *Rhizopus* and *P. striata* produced more Ca in plant tissue than Control. While in 1995 *B. megaterium* also produced more Ca in plant tissue than Control.

Observation on uptake of Magnesium was more consistent than in calcium. The three year trial showed that all the organisms with the exception of *Penicillium* sp produced higher amount of Mg in the plant tissue than Control. *A. niger P. striata* and *B. megaterium* enhanced more uptake of K into the plant tissue in the Control in 1993 while in 1994, *Rizopus* sp, *A. niger* and *B. megaterium* enhanced more K uptake than control. 1995 data on the other hand show control as superior to inoculated plots in the uptake of K.

All the elements discussed above are constituents of rock phosphates. Their release from the rock is however slow as a result of slow releases of the elements from rock phosphates.

Some micro-organisms have been observed to solubilize these elements (Muller and Foster, 1961, Webley and Duff, 1960). Some of these organisms appear to show some efficiency at solubilizing these elements, prominent among the organisms are *P. striata, A. niger* and *B. megaterium*.

The inoculated plots gave comparable amount of N in the plant tissue as the control (table 22). It does not appear that the organisms have any influence on N uptake of the plants.

4.3.5.2.9 Zn, Mn, Fe and Cu

A. niger and *B. megaterium* enhanced more Zn uptake than Control in 1993 while in 1994 only *B. megaterium* enhanced higher Zn uptake than uninoculated plots. The 1995 trial show *Rhizopus* sp and *A. niger* as enhancing more uptake than Control (table 22). Zn is particularly important in the cultivation of cereals as its deficiency has been reported in certain parts of the savanna. Thus any practices that will enhance the release of Zn should be encouraged. The P-Zn imbalance has also been reported in certain crops which could result in the induced deficiency of either of the nutrients when not appropriately combined.

Manganese uptake was enhanced by the inoculated organisms in 1993 and 1994. However, in 1995, control enhanced more uptake of manganese than the inoculants. The inability of the organisms to enhance the uptake of Mn in 1995 can be attributed to poor adaptability to soil condition in the 1995 trial discussed earlier.

More Fe was taken up from inoculated plots in 1993 than from

control. Contrary to this, in 1994 Control enhanced more Fe uptake than inoculants. Similar to the 1994 experiment, Control gave superior release of Fe than most of the inoculants (table 22). In 1995, only *Rhizopus* sp enhanced high uptake of Fe than Control.

The nutrients discussed above (Zn, Mn, Fe and Cu) are required by the plants in small quantities and thus most soil are able to supply enough of the nutrients to meet plant needs except in the case of Zn mentioned above where 5-10kg Zn/ha has been recommended to ensure good yield in Cereal.

Table 23: Microbial counts of phosphate solubilizing micro-organisms in inoculated and uninoculated soils

	Bacterial (10^6)	Fungi (^{6})
Uncultivated		
Uninoculated	33	5
Inoculated	101	15
Cultivated		
Uninoculated	52	5
Inoculated	132	20

4.3.5.3 Maize Response to Organisms

The 1993 and 1994 trials indicated that crop responded to inoculation with *A. niger* and P. striata. The organisms gave higher yield with these treatments than in control (table 20-21). However, in 1995, due to poor adaptation to the field condition and ineffective competition with other soil micro-organisms, uninoculated control gave higher yield than the treatments. Therefore, the magnitude of response as observed in combined analysis is Control > *P. striata* > *A. niger* > *B. megaterium* > *Penicillium* sp > *Rhizopus* sp.

Table 24 however, shows that the correlation between organisms and the parameters shown were not statistically significant. Significant correlation however, was observed between grain yield and dry matter yield and between P concentration and dry matter yield.

Maize Yield Parameters as Influenced by Inoculation on P Source

Response of crop to the combined influence of inoculation and P sources are presented in figures 16-18.

4.3.5.3.1 Dry Matter Yield

In 1993, Togo rock produced higher values of dry matter at all inoculations than SSP and Sokoto rock phosphate, No-P control also gave higher values of dry matter than SSP and Sokoto rock. (Fig. 16) However, in 1994, SSP produced high yield of dry matter. At inoculation with *B. megaterium* and *A. niger*, it gave higher dry matter yields than at zero inoculation. Togo rock phosphate was promoted in its

performance by inoculation with *Rhizopus* sp and *Penicillium* sp. in 1994, while Sokoto rock was at par with Control. In 1995, an increase in performance of SSP was observed when inoculated with *Rhizopus* sp. over uninoculated SSP plots. This observed increased due to inoculation was very small (2g). Combined analysis however, shows that crop response to SSP was not influenced by inoculation.

Table 24: Correlation matrix between treatments and variables in soyabean

	P concentration	Dry matter Yield	Grain Yield	Nodule weight	Org.	Org.^{2}	P source	P source^2
P concentration in plant	-							
Dry Mattery Yield	0.1576*	-						
Grain Yield	0.0942	0.2944*	-					
Organisms^1	-0.0418	-0.0395	-0.0180	-				
Organisms^2	-0.0620	-0.0331	-0.0021	0.9793**	-			
P Source	0.0254	-0.0177	0.0370	-0.0083	0.0061	-		
P Source^2	0.0166	-0.0392	0.0022	-0.0055	-0.0047	0.9843**	-	

Significant level: * = p 0.05, ** = P 0.01

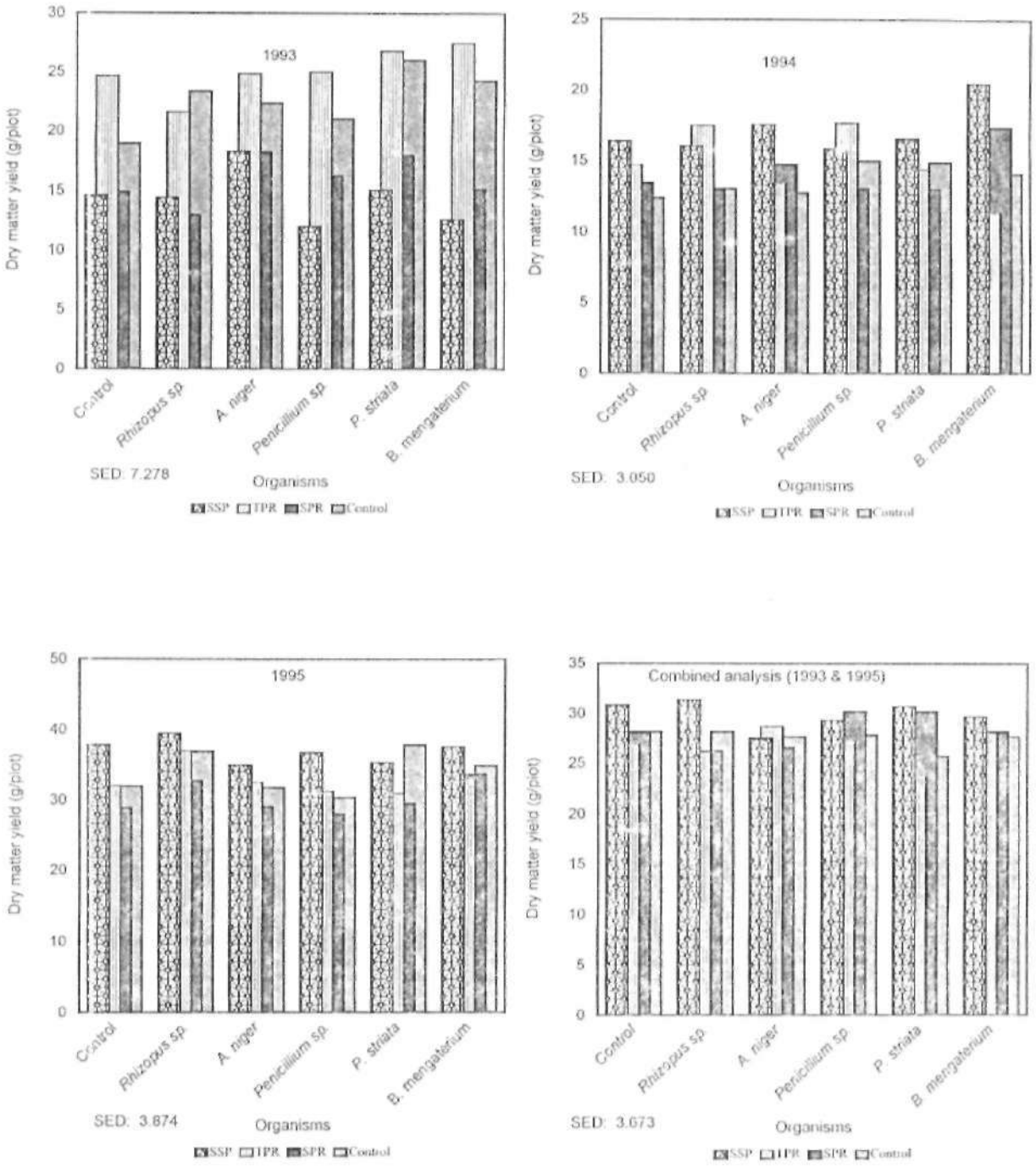


Figure 16: Effect of P sources and organisms on maize dry matter yield in 1993 and 1994.

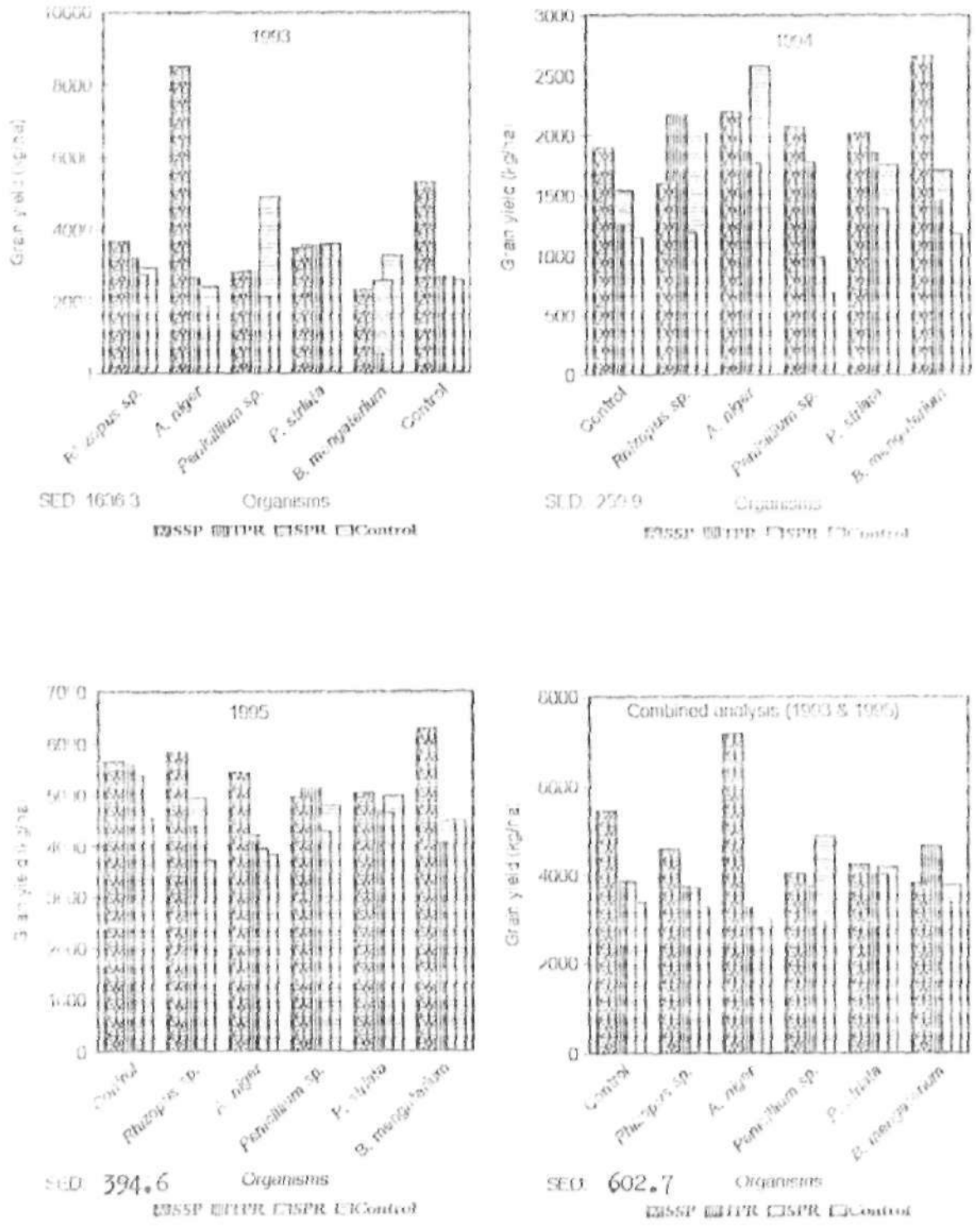


Figure 17: Effect of P sources and organisms on maize grain yield in 1993 , 1994 and 1995.

4.3.5.2.2 Grain Yield

In 1993, inoculation with *A. niger* produced an increase of 3233kg/Ha above uninoculated plots supplied with SSP while Togo rock, Sokoto rock phosphates produced very low yield (Fig. 17). Plots not supplied with P but inoculated with *Penicillium* sp gave an increase of 2274kg/Ha above zero inoculation no P control. The above trend was repeated in 1994 when *A niger, Penicillium* sp, *P. striata* and *B. megaterium* inoculated on SSP. They gave higher yields than at zero inoculation (Fig. 17). However, *B. megaterium* gave outstanding yield increase of 764kg/Ha on SSP above zero-inoculation. *B. megaterium* also produced an increase of 627kg/Ha when inoculated on SSP above uninoculated SSP plots. *A niger* was again observed in 1994 to enhance yield of no-P plots by 1424.3kg/Ha. Combined analysis showed that *A. niger* enhanced yield production in SSP plots by an increase of 1738kg/Ha above uninoculated SSP plots (Fig. 17).

There is an indication of enhance performance of SSP by inoculation with some organisms, particularly *A. niger* and *B. megaterium*. Of particular interest however, is yield promotion of plots not supplied with P by certain organisms particularly *A. niger* and *Penicillium* sp. The organisms are suspected to solubilize the native soil P which were otherwise unavailable to the crops.

4.3.5.2.3 P-uptake

In 1993, Togo rock phosphates and no-P control gave high P-uptake than other treatments (Fig. 18). SSP was enhanced by *A.*

niger in plant uptake of P. In 1994, however, SSP gave high P-uptake at most inoculations and control, Sokoto rock phosphate and Togo rock phosphate were enhanced to produce as much P-uptake as SSP by *A. niger* and *Penicillium* sp. *Rhizopus* sp was observed to promote P-uptake in SSP in 1995 by 18g/kg while the No-P control increased by 8g/kg above uninoculated no-P plots. Combined analysis however showed that SSP was enhanced by inoculation with *A. niger* causing an increase of 101g/kg while No P Control was promoted by inoculation with *Penicillium* sp to provide 54g/kg increase than uninoculated no-P control (Fig. 18). These observations on P-uptake again supported the observations made concerning correlation of P-uptake and grain yield as the organisms that support higher grain production in the various P sources also supported their P-uptake.

4.3.5.3.4 Crop Response to Combined effects of P Sources and Inoculation

Maize crops do not appear to respond to inoculation of rock phosphates with micro-organisms (table 20-22). However, maize crops responded to combine effect of SSP and certain organisms particularly *A. niger* and *B. megaterium*. Gaind and Gaur (1991) attributed increase in the efficiency of SSP as a result of inoculation to the reduction in phosphate fixation by soil fraction as a result of inoculation. This may also partly explain the response of maize crop to plots not supplied with P but inoculated with *A. niger* or *Penicillium*.

4.3.5.4 General Discussion of Field Trials

Soil

The initial and the final values of selected chemical properties of the soil employed for this trial are given in table 8. The CEC of the soil increased at the end of the field trials. This may be attributed to increases in some of the exchangeable bases (Ca, Mg and K) as shwon in table 8. These bases form part of the constituents of the rock phosphates and so releases from the rock phosphate structure will increase their levels in the soil. The available P of the soil increased above the initial levels at both depths (0-15 and 15-30cm)(table 8). This increase in P level is as a result of residual effectiveness of phosphate fertilizers. A feature of rock phosphates in particular is that their agronomic effectiveness relative to that of water soluble fertilizer improves appreciably with time (Mokwunye, 1994). The organic carbon and total nitrogen of the soil were also improved upon at the end of the experiment (fig. 8). One cannot overlook the role of micro-organism in residue decomposition and the increase in carbon and Nitrogen level may be attributed to this process. The final soil was slightly acidic than the initial soil. This may be as a result of both the chemical processes and microbiological solubilization of rock phosphates taking place in the soil concurrently. The presence of H^+ ions in the soil results in the rapid conversion of PO_4^{3-} to $H_2PO_4^-$ (Khasawneh and Doll, 1978). The ability of the soil to provide the H^+ ions needed for the

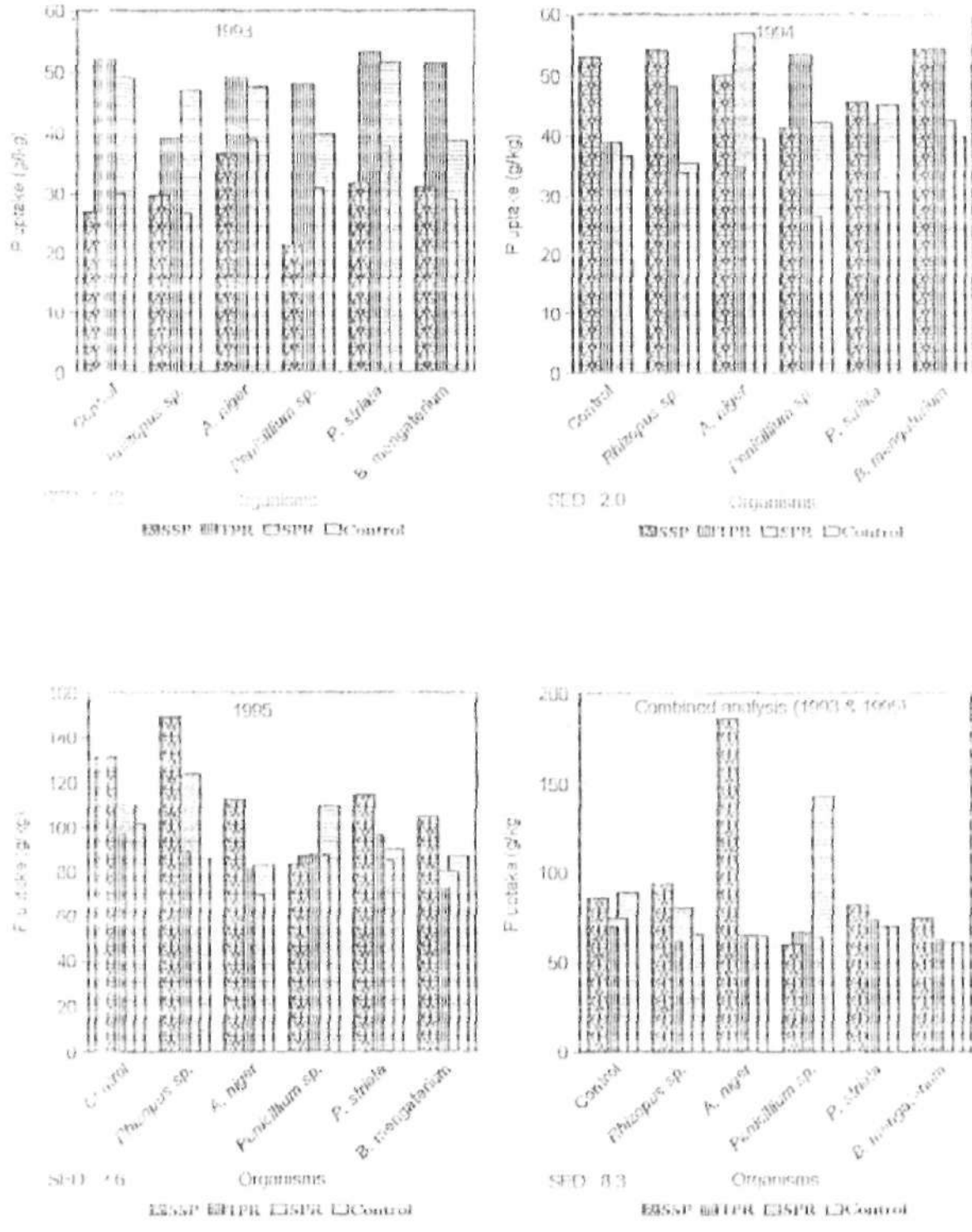


Figure 1[illegible] Effect of P sources and organisms on maize P uptake in 1993,1994 and 1995.

neutralization reaction is essential to the effectiveness of phosphate rock as a supplier of P to crops.

In microbiological solubilization of phosphate rocks, a fall in pH during growth of phosphate solubilizing micro-organisms has often been reported (Gerreston, 1948; Muromtsev, 1958; Sperber and Webley, 1959; Chhonker and Subba Rao, 1967; Gaur, 1972 and Kundu and Gaur, 1981). Various workers have attributed this fall in pH of the medium to the production of organic acids by micro-organisms involved (Rose, 1957; Sperber, 1957; 1958; Louw and Webley, 1959; Duff *et al*, 1963; Tahad *et al*, 1970 and Bajpai and Sundara Rao, 1971). Other workers have however attributed the ability of these micro-organisms to reduce pH to their acid and Neutral phosphatase production (Casida, 1959; Chhonker and Tarafdar, 1984; Salih *et al*, 1989).

Soyabean

Togorock phosphate gave high yield when applied to soyabean than SSP. Other researchers (Batiano *et al*, 1986; Mokwunye, 1986) have also observed this phenomenum of efficient utilization of rock phosphate by legumes. Soyabean also produced high yields when plots were inoculated with micro-organisms particularly *Rhizopus* sp and *P. striata*. The response of soyabean to the combined effect of organisms and P source were however selective. Different organisms promoted different P sources. SSP was promoted in its performance by *Rhizopus* sp, *B. megaterium* and *P. striata*. While Togo rock was promoted in its performance by *A. niger* and *Penicillium* sp and Sokoto rock

was enhanced by *P. striata*. The organism inoculated were also observed to interact positively with the nodule bacteria (*Rhizobium*) of soyabean. There was no observed problem of organisms adaption to field conditions. This is probably due to effect of soyabean rhizobia and mycorrhizal organisms.

Maize

SSP gave higher grain and straw yield than the rock phosphates. However, among the rock phosphates, Togo rock was superior to Sokoto rock. The method of application (drilling along the ridges) might have significantly affected the performance of Sokoto rock such that the No-P control gave superior yield than Sokoto rock. However, the organisms were not able to adapt to field conditions immediately after inoculation and they competed with other soil micro-organisms ineffectively, thus leading to low yields of plots inoculated, at the first year of inoculation. The effectiveness of the organisms was observed to increase as yearly inoculation on the same plot continued.

Inoculation do not appear to improve the effectiveness of rock phosphates when applied to maize. However, SSP and zero P plots appeared to benefit from inoculation with micro-organisms more than rock phosphates. SSP was observed to be enhanced by inoculation with *A. niger* and *B. megaterium* while plots not supplied with P benefited from inoculation with *A. niger* and *Penicillium* sp. Gaind and Gaur (1983) attributed this phenomenum to reduction in phosphate fixation due to

inoculation. However, the non improvement of rock phosphates by inoculation on our soils is not suprising as it has been observed that responses to inoculation in general were positive on highly fertile soils such as those of chermozem type rather than on poor soils (Cooper, 1959, Menkina 1963 and Mishustin *et al*, 1972). The soil used also contain a high amount of initial P (Table 8). This will also partly explain the reason for such low response.

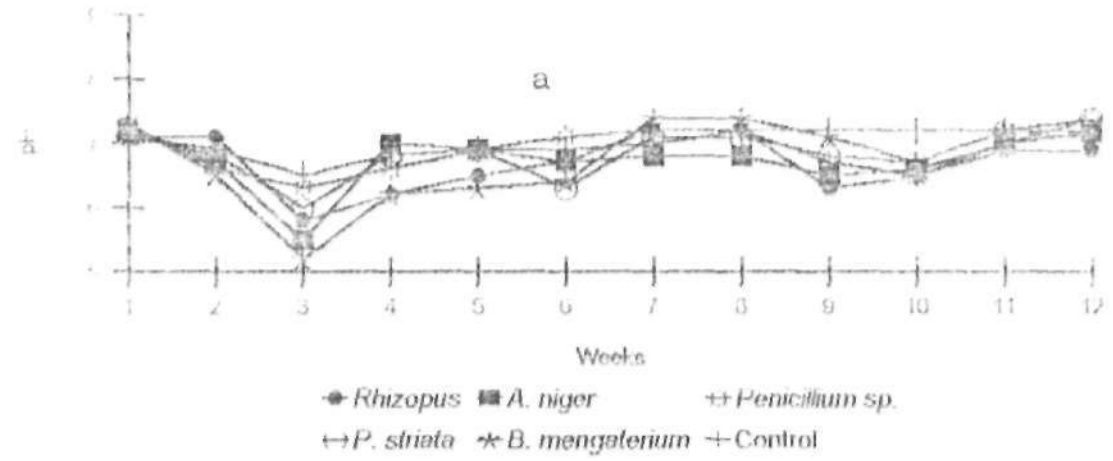
4.4 Incubation Studies

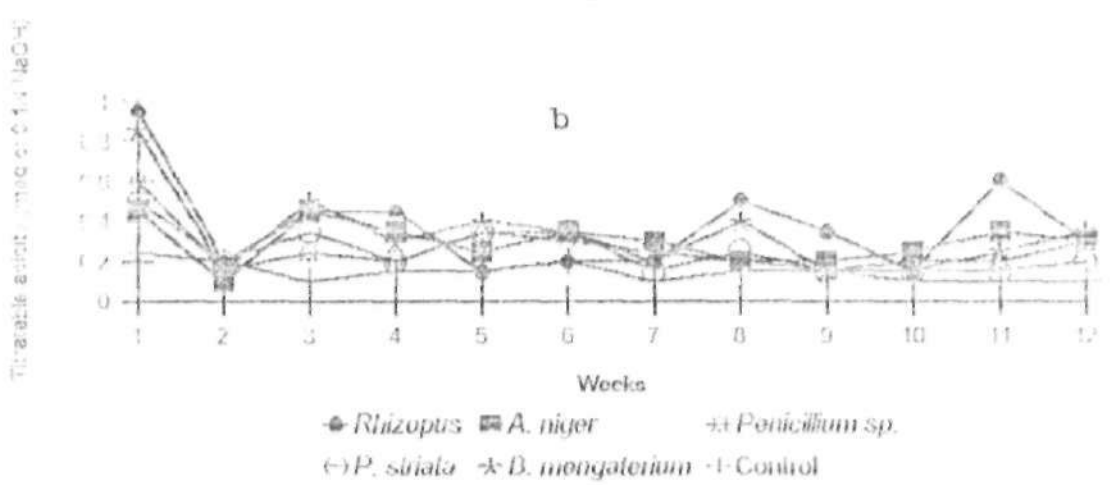
Togo rock phosphate was applied to Acid washed sand and inoculated with the organism studied on the field (*Rhizopus sp, A. nier, Penicillium* sp, *P. striata* and *B. megaterium*) and then incubated for 12 weeks. The patterns of changes in pH, titratable acidity and available P were observed and shown in figure 19.

PH

Generally, the pH of the soil decreased sharply from 7 between 2nd and 3rd weeks of incubation (Fig. 19a). This decrease was accompanied by a rise after the third week, and there was gradual rise up to the 9th week. A few organisms however, became stable after the 3rd week. *B. megaterium, A niger* and *Rhizopus* sp achieved very low pH of 5.2, 5.5 and 5.8 respectively. These general drop in pH is attributed to the production of organic acids (Gerrestron, 1948). The inoculated control remained almost stable in pH throughout the period of incubation.

Inorganic phosphate solubilization takes place as a result of the production of organic acids by efficient phosphate solubilizing micro-organisms, resulting in lowering of the pH of the medium (Gaur 1990).





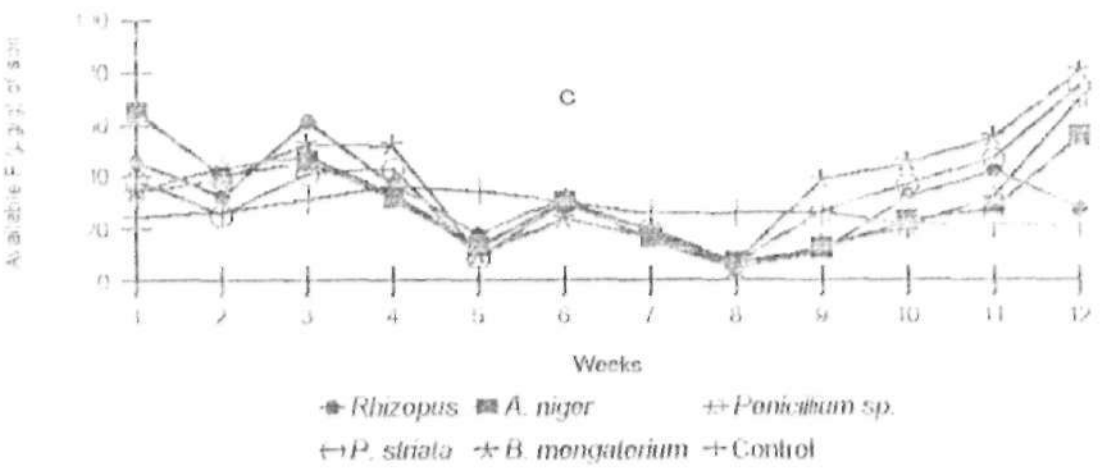


Figure 19: Effect of incubation period on pH, titratable acidity and available P.

Gaur and Sachar (1980) showed that during the solubilization of rock phosphates and tricalcium by fungi, the pH of the culture broth was lowered from 7 to 3. Salih *et al* (1989) however concluded that the nature of organic acids produced by the fungi is more important in releasing P from insoluble phosphate than the total acidity.

Titratable Acidity

Concurrent to the drop in pH at the third week, the titratable acidity of all the organisms also increased (fig. 19b). *B. megaterium*, *A niger* and *Rhizopus* sp produced the highest amount of acid (0.5, 0.45 and 0.45 Meg of 0.1N NaOH). Although *Rhizopus* produced fluctuating amount of pH throughout the lenght of incubation, *A. niger* and *B. megaterium* remained relatively stable after the initial increase.

Generally, all the organisms produced high amounts of acid at the first week of solubilization while the control showed very little increase and its acidity remained low throughout the period of incubation (fig. 19b). Titratable acidity is an estimation of the amount of acid in the medium. Pareek and Gaur (1973) showed the release of phosphate from tricalcium phosphate and rock phosphates by organic acids. He reported that aliphatic acids were more effective in solubilization of phosphate than phenolic acids. Asea *et al* (1988) also showed that inorganic P solubilization by *Penicillium* sp was related to the ability of the micro-organisms to reduce pH particularly in the presence of ammonium N.

On the other hand, Salih *et al* (1989) observed that pH of inoculated soils remain unchanged at different sampling periods due to high buffering capacity of calcareous soils. However, available P increased, suggesting that solubilization may not be related to changes in soil pH. Other workers have also suggested that the phosphate solubilizing property of soil micro-organism is related to their acid and neutral phosphatase production (Casida 1959, Chonker and Tarafdar, 1984).

Available Phosporus

The available P of the medium gave an initial increase at the first week (fig. 19c), in all the organisms. The organisms then showed a sharp drop in available P at the 5th week and they remained low in available P until after the 8th week, after which another increases were observed. The control however, remained low and stable throughout the period of incubation. Goswami and Sen (1962) in a similar experiment observed that maximum solubilization of phosphate under condition of culture occured within a week. Banik *et al*, (1981) also observed that available P increased to a maximum at 30 days of crop growth and declined at 120 days after which there was further increase.

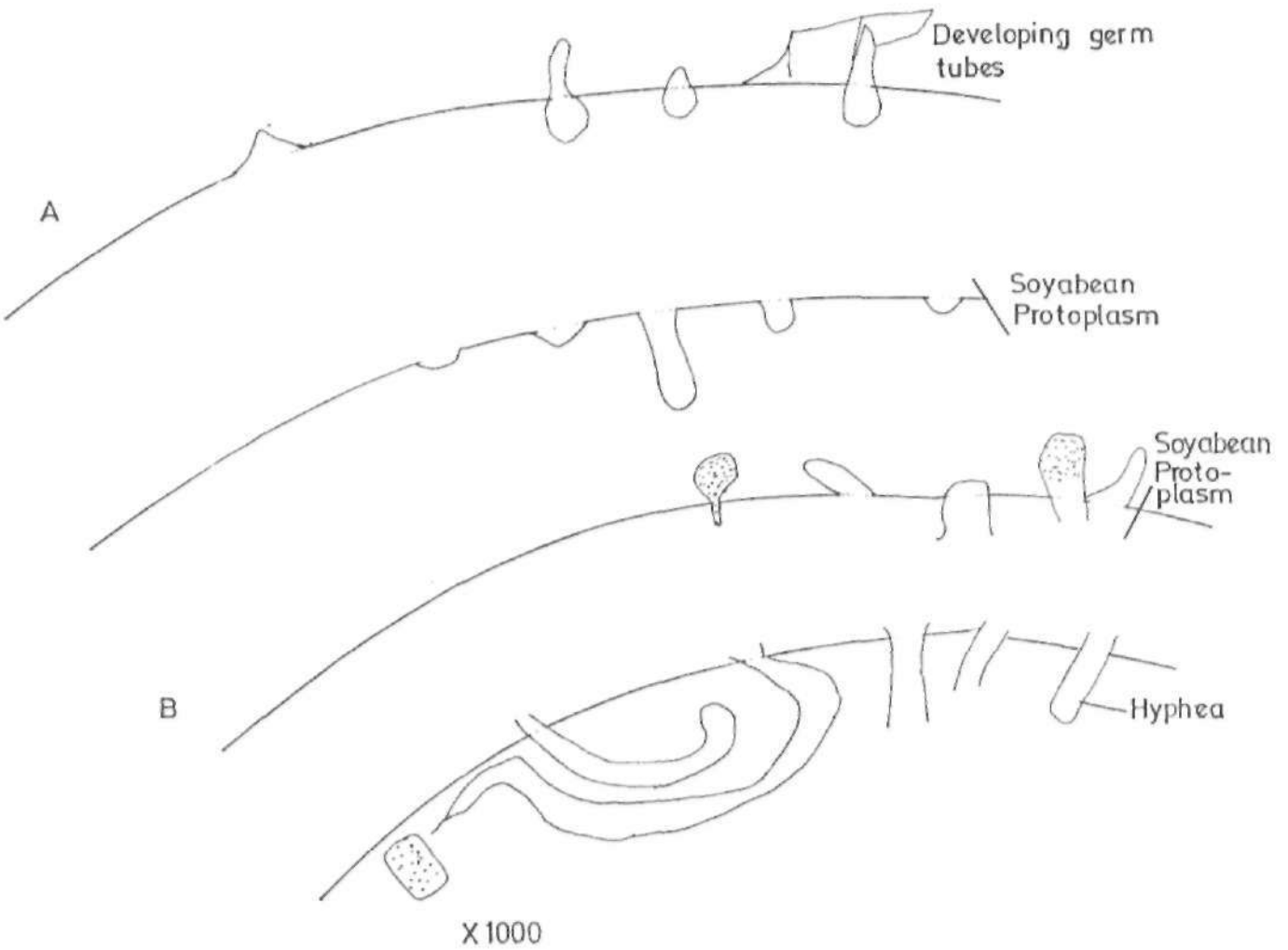
The reduction in P observed after the first week and subsequently may be attributed to microbial assimilation of the released P through immobilization (fig. 19c). It appeared that microbial need of P was fully satisfied after the 8th week and then appreciable increases in level of P released were observed thereafter.

Microbial growth requires the presence of available forms of P as the element is essential for cell synthesis. The development of the microfora is governed by the quantity of utilizable phosphorus compounds in its habitat (Alexander, 1977). Thus at the initial stages of solubilization as a result of low amounts of P in the medium immobilization prodominates. However, as P increases in the medium the net effect of mineralization will change from immobilization to P release.

4.5 EFFECT OF PHOSPHATE SOLUBILIZING ORGANISM ON MYCORRHIZAE

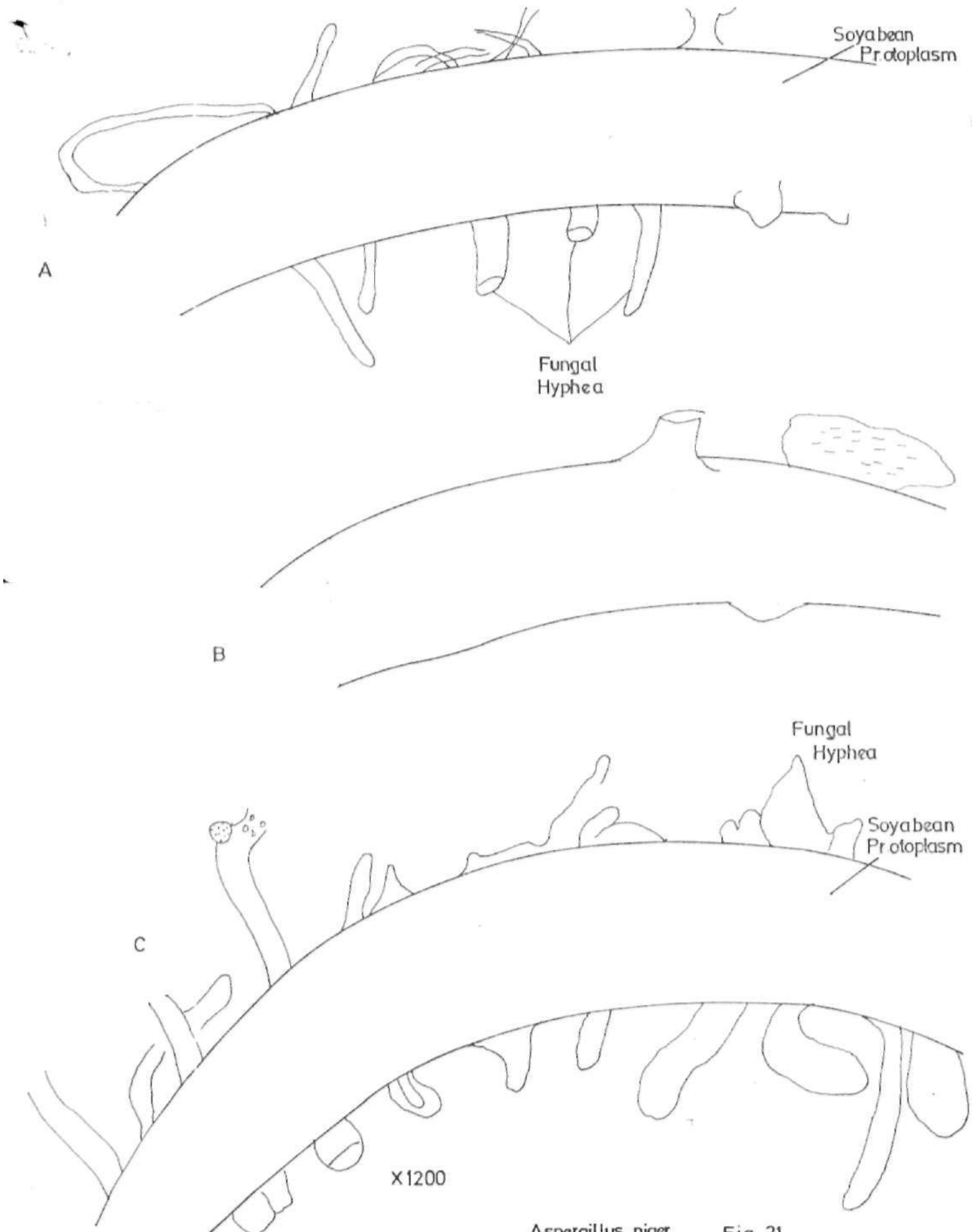
Details of the interractions between phosphate solubilizing micro-organisms (PSM) inoculated on the field and mycorrhizae are shown in figures 20-25. Although the soil was not inoculated with mycorrhizae, it was observed that phosphate solubilizing micro-organisms (PSM) had great influence over the infection of plant roots by mycorrhiza organisms present in the soil.

The control gave very low level of infestation of the plant root by mycorrhizae (fig. 25). The roots inoculated with *Rhizopus* sp and *P. striata* were well infected. (Figs. 20 and 23). The fungal infection was however still at an early stage of development. The germ tubes were establishing and hyphae elongation in most cases have not begun. The roots inoculated with *A. niger* and *Penicillium* sp gave an intermediate stage of infection (fig. 21 and 22). The hyphae were well developed, spores had been formed and released. Most of the hyphae, however, still occured singly.



Rhizopus sp

Fig. 20



Aspergillus niger Fig. 21

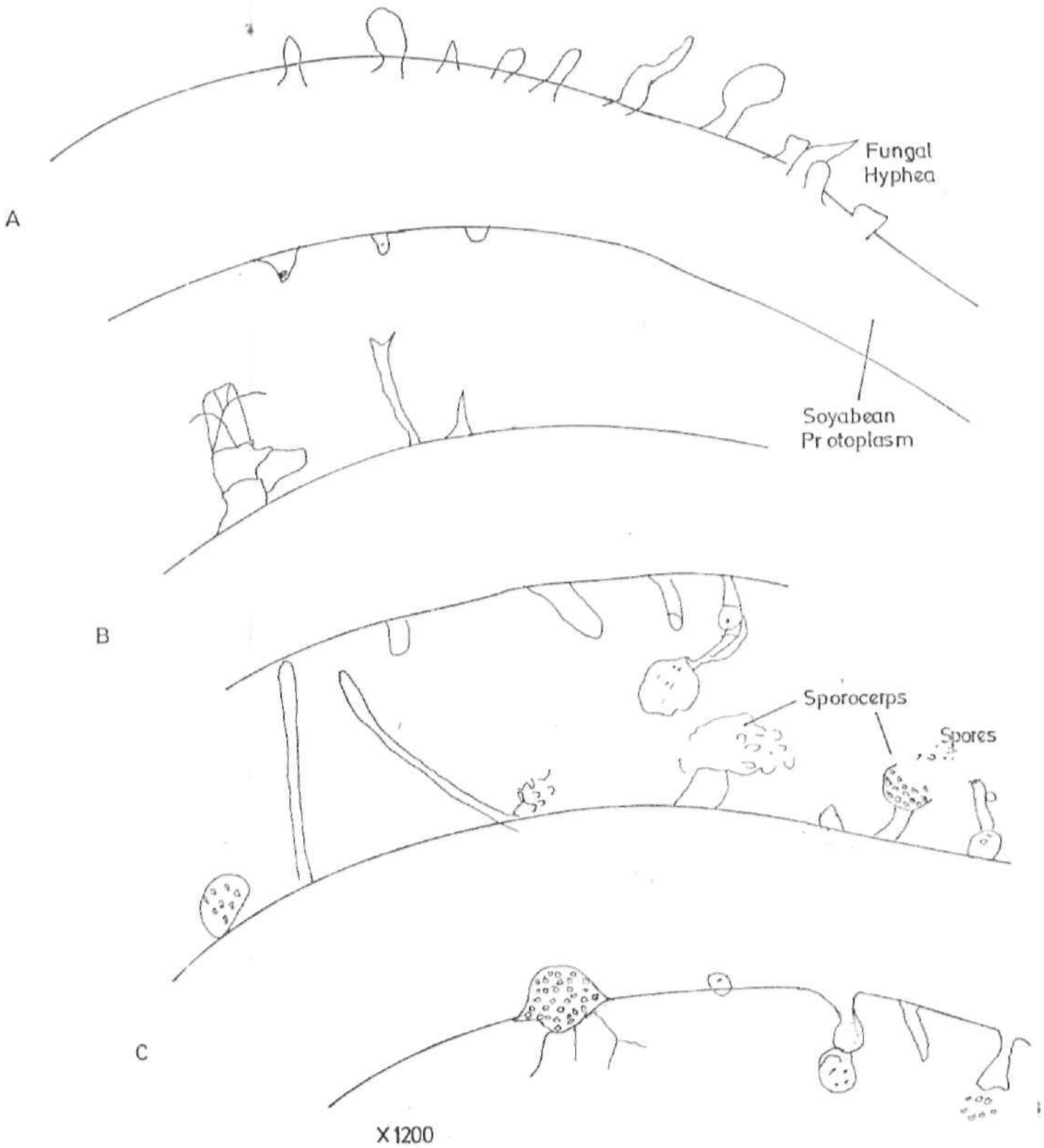
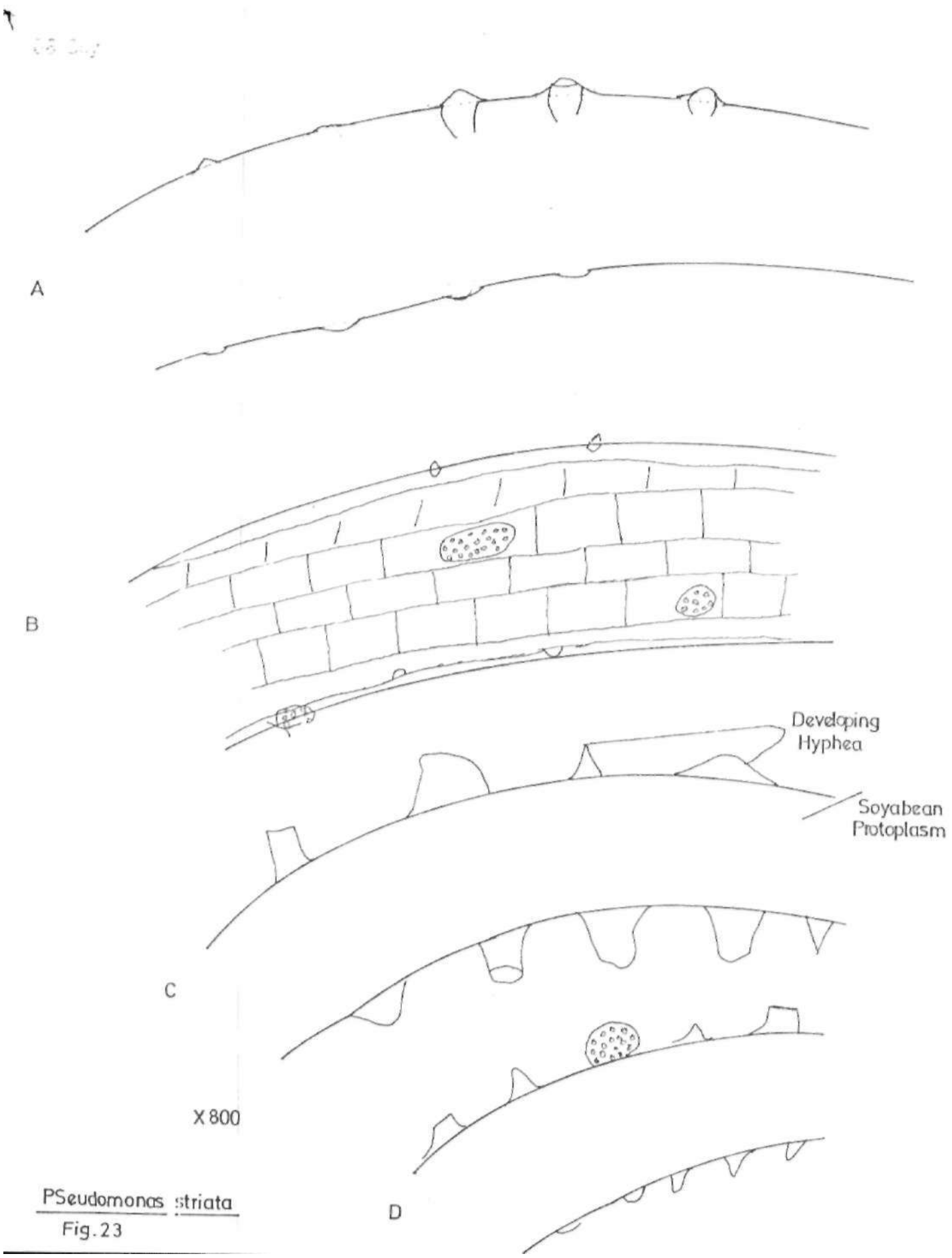


Fig. 22 Penicillium sp

The roots inoculated with *B. megaterium* in its own case gave a very advanced stage of development (fig. 24). The mycorrhizal fungi had formed rhizomorphs on the plant root and most of the hyphae were observed to bear sporocarps. All the samples described above were taken on the same day from pots cropped to soyabean, the soils of which were inoculated with PSM.

The mycorrhizae organisms were observed to be at various stages of development, depending on the organism inoculated. Thus it can be concluded that inoculation of PSM influenced the infection of plant roots by mycorrhizae. The subsequent development of the mycorrhizae after infection, depended on the particular organism that was inoculated.

Barea *et al* (1976) and Sattar and Gaur (1987) have tried to attribute this influence to the production of substances like auxins, gibberellins and Cytokinins.



PSeudomonas striata

Fig. 23

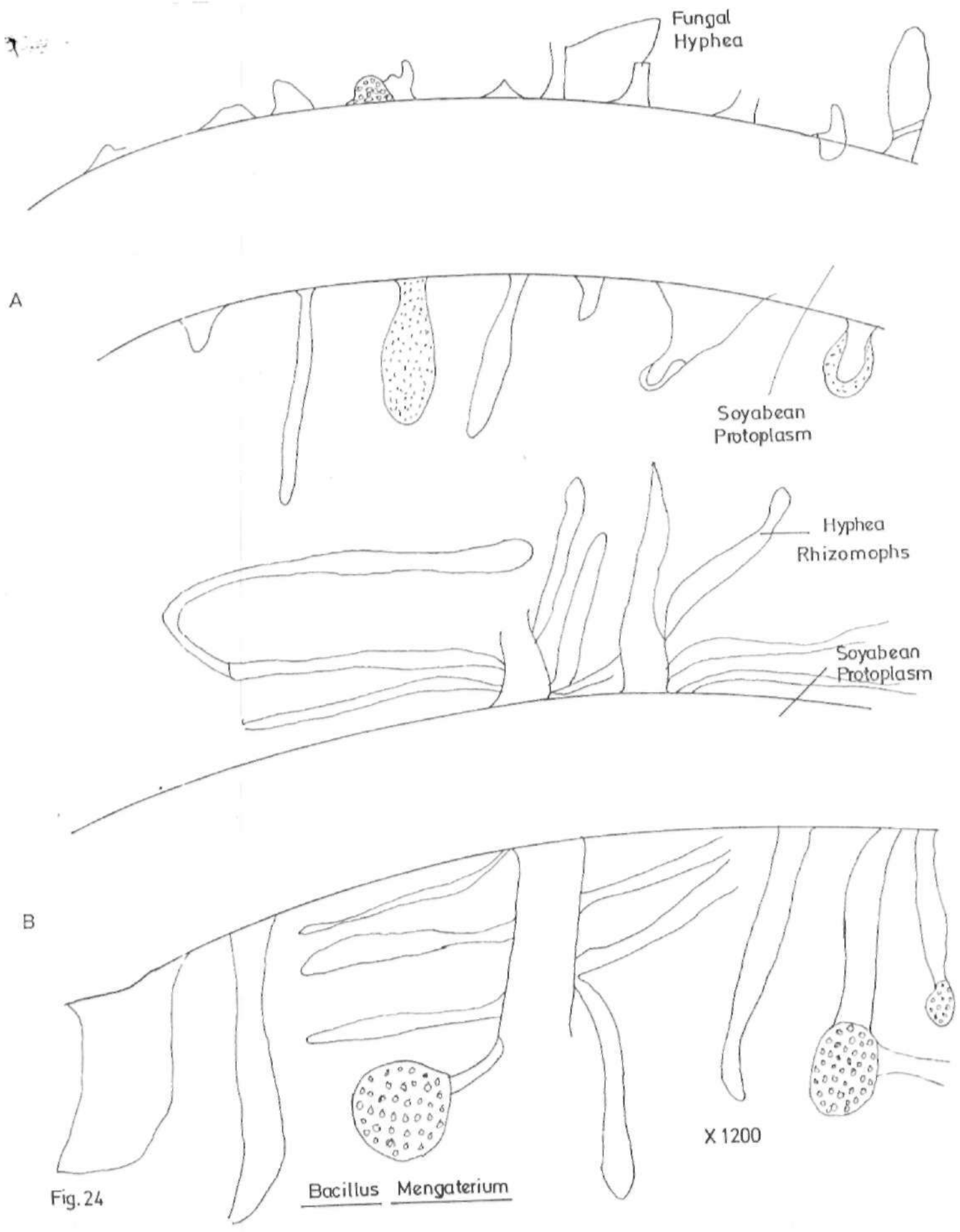

Fungal
Hyphea
A
Soyabean
Protoplasm
Hyphea
Rhizomophs
Soyabean
Protoplasm
B
X 1200
Bacillus Mengaterium

Fig. 24

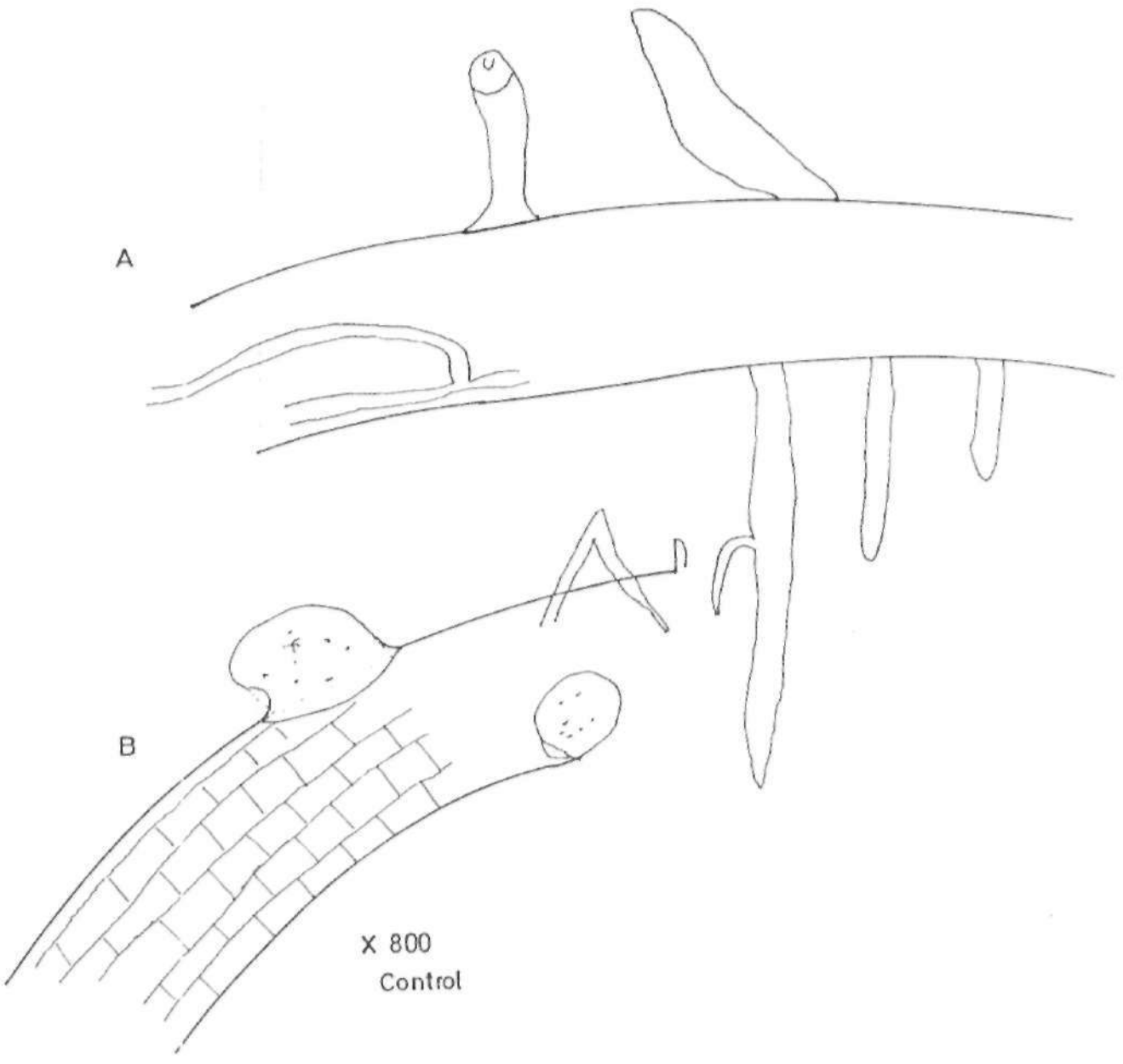


Fig.25

5.0 Summary and Conclusion

Sixty one phosphate solubilizing micro-organisms were isolated from soils taken at five locations in Samaru soils. Their effectiveness at solubilizing insoluble phosphates and subsequent release of P was studied in the laboratory. Twenty of these organisms were observed to solubilize rock phosphate to an appreciable level and were further screened in their pattern of phosphorus release. Ten of the twenty organisms were then finally selected for study in the greenhouse. The amount of P released as a result of the solubilizing activities of these organisms ranged from 31.9 - 51.0μg/g P_2O_5.

The green house study consisted of inoculating the organisms on soil supplied with SSP, Togo rock and Sokoto rock phosphate. Two test crops (soyabean and maize) were cropped twice consecutively. At the first cropping, the agronomic parameters of soyabean significantly increased by SSP while at the second cropping soyabean gave higher agronomic yields to application of rock phosphates relative to SSP. The micro-organisms had significant influence on soyabean growth at both cropping.Inoculated pots gave better yields than uninoculated pots. However the magnitude of response at the second cropping was more than the first cropping.

Soyabean also responded positively to combined effect of P sources and inoculation particularly at the second cropping. SSP was enhanced by inoculation at the first cropping while rock phosphates were enhanced by inoculation at the second cropping.

The maize crop gave significantly higher response to SSP at the first cropping. Whereas its response to rock phosphates at the second cropping were significant higher relative to SSP. Togo rock however, appeared to be more efficient in its agronomic efficiency relative to Sokoto rock. Also at the first cropping inoculation brought about retarded growth of maize crops when compared with uninoculated pots. Whereas, at the second cropping maize responded to inoculation positively and significantly. At the first cropping the organisms rather than releasing P, assimilated P from the soil and so increasing the deficiency of soil P. Whereas, at the second cropping, micro-organism immobilization of P appeared to have been satisfied and so crop responded to inoculation as microbial activities resulted in P release.

Inoculation was also observed to favour all the P sources suplied. Maize responded to inoculation of SSP at first cropping while at second cropping, it responded more to inoculation of rock phosphates.

Five organisms were selected, based on their performance in the greenhouse for field study. These organisms were characterized and identified as *Rhizopus* sp, *Aspergillus niger*, *Penicillium* sp, *Pseudomonas striata* and *Bacillus megaterium*. The field trial also consisted of SSP, Togo rock, Sokoto rock phosphates and zero-P-control. The P source were inoculated with the organisms in a split plot design. Maize and soyabean

were used as test crops.

Togo rock phosphate gave higher yield than SSP when applied to soyabean indicating that legumes could be efficient at utilizing rock phosphates. Inoculation with *Rhizopus* sp and *P. striata* also promoted grain production in soyabean. Response of soyabean to combined effect of P sources and organisms were however selective. Different organisms promoted different P sources. SSP was enhanced in its performance by *Rhizopus* sp, *B. megaterium* and *P. striata*. While Togo rock was enhanced in its performance by *A. niger* and Sokoto rock was enhanced by *P. striata*. The organism inoculated also interracted positively with the legume *Rhizobium*.

SSP gave higher grain and straw yield than the rock phosphates. However, among the rock phosphates, Togo rock was superior to Sokoto rock. Maize did not appear to respond to inoculation in 1993. However, by the second year (1994) the maize showed more response to inoculation. Inoculation did not appear to improve the effectiveness of rock phosphate when applied to maize. SSP and zero-P-plots however seemed to benefit from inoculation. *A. niger* and *B. megaterium* benefitted SSP while zero-P-plots benefitted from inoculation of *A. niger* and *Penicillium* sp.

The treatments were observed to improve the CEC, available P, organic carbon and total nitrogen. The pH of the final soil was slightly reduced.

The study tends to highlight the following:

(1) The presence of micro-organisms capable of solubilizing rock

phosphates in our soils.

(2) Legumes benefit from inoculation than cereals

(3) Legumes are capable of efficient use of rock phosphates than cereals.

(4) The organisms, immediately on inoculation, immobilized P and substantial P released takes place much later

(5) A period of incubation is necessary between inoculation combined with application of P sources and cultivation.

(6) Togo rock phosphate is superior to Sokoto rock phosphates in its agronomic efficiency.

(7) Microbial effectiveness increases with continual annual inoculation.

Incubation studies showed that P-immobilization proceeded for 8 weeks after inoculation prior to substantial P release into the soil by micro-organism. And mycorrhizal study on inoculated soils showed that inoculation influenced root infection by mycorrhizal and the subsequent development of mycorrizal organism depended on the organism inoculated.

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Appendix 1: Biochemical characterization of bacterial isolates

Isolates	Catalase	Amylase activity	Ammonia production	Carbohydrate utilization: Frutose A	Frutose G	Manitol A	Manitol G	Maltose A	Maltose G	Glucose A	Glucose G	Sucrose A	Sucrose G	Lactose A	Lactose G	Metyl red	Nitrate reduction
Pseudomonas sp	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
Bacillus sp	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-

Isolates	Nigrate reduction	voges Proskauer	indole	Citrate	H_2S production	O/F	Gelatin Hydrolysis	Oxygen relationship
Pseudomonas sp	-	-	-	+	+	O	+	AE
Bacillus sp	-	-	-	-	-		-	AN

Profile No.1 **Appendix 2**

General Site Information

Location	:	IAR UI Farm
Taxonomic Classification	:	
Soil Parent Material	:	Thin aeolian deposit over residual deposit from the Basement complex rocks.
Geology	:	Basement Complex Rocks
Geomorphology/Physiography:		Slightly undulating plain
Topography	:	Mid-slope with 0.2%
Vegetation and use	:	Northern Guinea Savanna, cultivated to maize
Erosion	:	None, but slight deposition of aeolian materials.
Soil Moisture Conditions	:	Profile Ustic
Drainage	:	Well drained
Depth to water table	:	Not encountered at 150cm
Dept to Impenetrable Layer	:	None encountered at 150cm
Described by	:	Kparmwang and Raji
Date Sampled	:	18th June, 1993

HORIZONTAL DESCRIPTIONS

Horizons	Depth(cm)	Description
Ap	0-16	Brown (10YR 4/6) light brown (7.5YR 6/4 dry) sandy loam with few fine distinct dark red (10R 3/6) mottles; weak medium to coarse subangular blocky structure; slightly sticky and non plastic (wet) friable (moist) slightly hard (dry); common fine pores; many very fine to fine roots; common fine Fe-Mn concretions; many fine quartz and mica flakes; common termite holes; abrupt wary boundary.
Bt 1	16-43	Strong brown (7.5YR 5/8) reddish yellow (7.5YR 6/8 dry) sandy clay with few medium dsitinct red (10R 5/6) mottles; strong coarse subangular blocky structure; sticky and plastic (wet) firm (moist) very hard (dry); many fine to medium pores; many fine to medium, and few coarse roots; few medium soft Fe nodules; few fine quart and mica flakes; common ant/termite nest; clear smooth boundary.
Bt 2	43-74	Strong brown (7.5YR 5/8) reddish yellow (7.5YR 6/6 dry) clay with many medium distinct red (10R 5/6) mottles; strong medium subangular blocky structure; sticky and plastic (wet) friable (moist) hard (dry); very few thin clay and Iron-oxyhydroxide cutans on ped faces; many medium Fe-Mn concretions; few quartz and mica flakes; common ant/termite nest; gradual smooth boundary.
BT 3	74-116	Strong brown (7.5YR 5/8) reddish yellow 7.5YR 7/6 dry) clay with many medium distinct red (10R 5/6 mottles; moderate coarse subangular blocky structure; sticky and plastic (wet) friable (moist) hard (dry); very few thin clay cutans on ped faces; many medium pores; few medium and common very fine to fine roots; common Fe-Mn concretions and many Iron nodules; few quartz and mica flakes; common ant/termite nest; gradual smooth boundary.

BT 4	116-150	Brownish yellow (10 YR 6/6) very pale brown (10YR 7/4 dry) clay with many medium distict dark red (2.5YR 3/6) mottles; weak medium subangular blocky structure; sticky and plastic (wet) friable (moist) slightly hard (dry); few thin clay cutans on ped faces; many medium pores; few very fine to fine roots; many iron nodules; many medium quartz grains; common ant/termite nest.

Profile fit 2

Horizon	Depth (cm)	Description
Ap	0 - 22	Yellowish red (5YR 5/8) reddish yellow (7.5YR 6/8 dry) sandy loam with common fine faint red (10R 4/8) mottles; moderate medium subangular blocky structure; shghtly sticky and slightly plastic (wet) friable (moist) hard (dry); common very fine to fine pores; few fine and common very fine roots; few Fe-Mn concretions; many fine quartz and mica flakes; few ant/termite holes; clear smooth boundary.
AB	22 - 60	Yellowish red (5YR 5/8) reddish yellow (5YR 6/8 dry) sandy clay with few fine faint red (10R 4/8) mettles; moderate coarse subangular blocky struture; sticky and plastic (wet) firm (moist) hard (dry); common fine porse; few fine to coarse and common very fine roots; few medium Fe-Mn concretions; few quartz and mica flakes; common ant/termite holse; few vertical cracks less than 1 - 2mm thick; diffuse smooth boundary.
Bt	60 -95	Yellowish red (5YR 5/8) reddish yellow (5YR 5/8 dry) clay with common fine faint red (10R 4/8) mottles; moderate redium subangular blocky structure; sticky and plastic (Wt) friable (moish) slightly hard (dry); very few thin clay cutans on ped common fine pores; few fine to concretions; few quartz and mica flakes; few ant/termites holes; few vertical cracks less 2mm wide; clear smoothe boundary.
Bt2	95 - 137	Reddish yellow (5YR 6/8) reddish yellow (7.5YR 6/8 dry) clay with common medium distinct red (10R 4/8) mottles; moderate medium, subangular blocky structure; sticky and plastic (wet) firm (moish) hard (dry); few thin clay and Iron-ixyhydroxide cutans on ped faces; few medium pores; few fine to medium roots; common Fe-Mn concretions; few quartz and mica flakes; common ant/termite holes; few cracks less than 2mm wide; clear smooth boundary.

Bt3	137 - 165	Strong brow (7.5YR 5/6) reddish yellow (7.5YR 6/6 dry) clay with many medium distinct dark red (10R 3/6) mottles; moderate meddium subangular blocky structure; sticky and plastic (Wet) firm (moish) hard (dry); few thin clay cutans on ped faces; common very fine to fine pores; few fine root; common Fe-Mn concretions; few quartz grains; few ant/termite holes.

Appendix 3: Characterization and Classification of Soil (Profile Pit 1) and (Profile Pit 2)

Soil Parameters	Pit 1 HORIZONS 0-16	16-43	43-74	74-116	116-150	Classification	Pit 2 HORIZONS -22	22-60	60-95	95-137	137-165	Classification
												% cLAY
15.6	35.6	34	32	28		Order: Alfisol	14	31	31	30	26	Order: Alfisol
% Silt	28	22	24	24	24	Suborder: Ustalf	32	25	25	26	26	Suborder: Ulstalf
% Sand	56	42	44	48	48	Great group	54	44	44	44	48	Great group: Haplustalf
pH/H$_2$	5.7	5.9	6.0	6.1	6.0	Haplustalf	5.4	5.5	5.5	5.5	5.8	
pH/0.01MCaCl$_2$	4.7	5.1	4.7	4.7	4.6	Class:Typic Haplustalf	4.6	4.7	4.6	4.5	4.6	Class:Typic Haplustalf
Carbon (%)	0.54	0.06	0.03	0.02	0.01	Class:Typic Haplustalf	0.6	0.2	0.1	0.1	0.1	(USDA 1990)
Total N (%)	0.034	0.034	0.017	0.017	0.051	(USDA 1990)	0.07	0.07	0108	0.03	0.03	OR
Exchangeable Bases (Meg/100g soil)						OR						
Ca	3.1	4.1	7.0	6.4	6.6	Haptic Luvisol	3.7	1.9	5.2	5.6	5.9	Haptic Luvisol
Mg	2.4	2.1	3.5	3.2	3.7	(FAO 1988)	0.9	0.6	2.7	2.8	2.4	(FAO 1988)
K	0.3	0.2	0.2	0.4	0.4		0.4	0.3	0.4	0.5	0.5	
CEC	5.1	6.2	25.9	10.0	8.4		5.6	5.2	9.2	10.0	8.2	

Appendix 4: Mean Monthly rainfall, Relative humidity and Temperature from 1993 - 1994.

	Rainfall			Relative Humidity			Soil Temperature		
Month	1993	1994	1995	1993	1994	1995	1993	1994	1995
January	0.0	0.0	0.0	21.4	20.9	26.3	19.0	21.7	28.19
February	0.0	0.0	0.0	21.4	13.2	18.3	23.2	23.8	30.5
March	0.0	0.0	0.0	30.6	22.7	28.05	26.3	28.6	35.8
April	8.3	20.4	90.5	38.3	42.65	36.1	30.0	29.1	36.2
May	113.6	120.1	45.3	54.4	55.95	56.25	29.9	27.8	34.2
June	155.8	231.9	125.4	62.3	65.25	70.6	26.3	25.3	31.4
July	269.0	169.2	193.7	67.6	74.25	86.9	24.9	24.7	29.8
August	300.8	218.6	258.6	72.0	56.7	79.0	24.0	23.8	28.7
September	181.8	82.5	159.1	67.5	78.45	75.5	24.8	24.3	29.9
October	32.4	69.2	32.5	58.6	70.7	64.4	26.4	24.8	32.0
November	0.0	0.0	0.0	NR	35.7	40.8	23.9	21.6	28.4
December	0.0	0.0	0.0	28.4	31.65	NR	21.6	19.1	NR

N= Not recorded