AN EVALUATION OF

FUNGAL BIOASSAY PROCEDURES FOR

ASSESSMENT OF SOIL PHOSPHATE STATUS.

Thesis
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by

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I. INTRODUCTION.

Microbiological assays, especially with A. niger, have been used rather widely to assess phosphate status of soils. The merits as well as shortcomings of these procedures have been discussed in the literature by various investigators. The speed, cheapness, and simplicity with which microbiological assays may be carried out have been used as arguments in favour of their use. It was this type of argument, considered in relation to the fact that good correlations have been reported by a number of workers between results obtained by A. niger and by field tests, which suggested to the writer that microbiological assay might have special merit in those developing countries where a rapid assessment of soil potential is required in the interests of food production but where limited finance is available for full-scale soil investigations.

The work reported here was undertaken to investigate further the value of the A. niger procedure as a means of evaluating soil phosphate status and to examine the possibility that other fungi including some not previously employed for this purpose might be even more suitable.

The present investigation was confined to a range of New Zealand soils. As field response data were not available for these soils a pot experiment incorporating a number of crops was conducted to provide plant growth data with which the results of microbiological assay could be correlated.

Chemical testing of soils has found much wider application than microbiological assay and there is a possibility that such methods might provide superior evaluation of soil phosphate status, which could outweigh the advantages of cheapness and simplicity claimed for the biological techniques. As an extension of the present study it was therefore considered worthwhile to determine whether Truog's procedure (1930) for determining available soil phosphate (the method employed by the New Zealand Department of Agriculture) possessed any marked advantage over the biological assays. It was further considered of interest to determine whether any one form of soil phosphate or combinations of forms determined by selective extracting agents would show better correlation with plant growth than shown by biological assay.

II. REVIEW OF LITERATURE

DETERMINATION OF AVAILABLE SOIL PHOSPHORUS BY ASPERGILLUS NIGER

1. The Aspergillus culture.

A. niger has been employed to estimate available plant nutrients in the soil by a large number of workers in various parts of the world. Investigations were carried out in: Belgium (Maercke, 1950), England (Nicholas, 1949, 1960), France (Manil et al., 1956), Germany (Niklas, 1930, 1932; Schlichting, 1962), Holland (Gerretsen, 1948; Mulder, 1948; Jensen, 1953), Indonesia (Gonggrijp, 1938), Japan (Matzuki, 1937), Russia (Simakov, Boushik, 1932), Spain (Sauchez, Marroquin, Tamayo, 1946), South Africa (Rosselet, 1955), U.S.A. (Smith et al., 1935), Venezuela (Schulz-Schomburgh, 1953), Yugoslavia (Njegovan, 1960), and elsewhere. It is intended here to review individual papers, covering aspects similar to the present investigation. Particular attention is paid to work carried out for the estimation of available phosphate of the soil. Work on other elements will be mentioned only if applicable to this report.

The growth of A. niger (and other organisms), in a nutrient medium is influenced by a number of factors. Various investigators use somewhat related procedures or techniques. Noted differences are in media composition, temperatures and periods of incubation, amounts of soil employed, etc. Niklas and his coworkers (1930)

were the first to develop a method for the determination of phosphorus and potassium in soil, which could be used for routine work. Smith et al., (1935), have shown that Cunninghamella species did not grow appreciably in Niklas medium, whereas Aspergillus species grew normally in it. As a comparison with Niklas medium these workers made up what they called "Dextrose medium A", consisting of 1% dextrose, 0.5% peptone, 0.05% $MgSO_4.7aq$, 0.1% $NaNO_3$ and 0.1% K_2SO_4. A "Dextrose medium B" was also used, which was similar to Dextrose medium A, except that it contained 10% dextrose. $CaH_4(PO_4)_2.2H_2O$ was the source of P. A. niger did not produce mycelium in P-free Niklas medium, whereas a considerable growth of both fungi was observed in both Dextrose medium A and B. Apparently the peptone in these media contained some P available to these fungi. Gerretsen (1948) showed that the Niklas strain of A. niger and medium gave conflicting results; he altered the medium and isolated a more suitable strain of A. niger from currents. Gerretsen's final medium for estimation of P comprised: 10% sucrose, 1% citric acid, 0.4% urea, 0.03% $MgSO_4$ as $MgSO_4.7aq$, 0.02% K_2O as K_2SO_4, 0.0005% Fe as $FeSO_4.7aq$. 0.0002% Zn as $ZnSO_4.7aq$, with the addition 1.25 gram Ca-citrate per 75ml culture solution, 12.5mg Na-humate per 75ml culture solution, 1cc saturated yeast extract per litre of culture solution. The faults of Niklas medium were attributed to the following points: (a) the culture solution was not sufficiently buffered, hence Gerretsen's addition of calcium citrate; (b) the weight of mycelium was markedly affected by Ca

content of soil, giving fluctuation in the Niklas solution which did not contain Ca; (c) the culture solution did not give optimum development of the fungus, allowing some soils to favour the growth of A. niger owing to some substances they contained in advantage of other soils; (d) the Niklas strain required peptone for quick growth and this favoured the occurrence of contaminating micro-organisms. Maercke (1950) used a combination of the two media of Sekera (1940) and Gerretsen (1948), i.e. 10% saccharose, 2% tannin, 0.5% $(NH_4)_2SO_4$, 0.035% K_2SO_4, 2% $CaSO_4$, 0.03% $MgSO_4.7aq$, 0.0005% $CuSO_4.5aq$, 0.0005% $ZnSO_4.4aq$, 0.0005% $FeSO_4.7aq$, 0.0001% $MnSO_4.4aq$. Tannin in Sekera's medium was added to give trace elements approaching the demands of A. niger in the soil. The breakdown of tannin was supposed to give tannose and subsequently sucrose and gallactose as a result of fungal activities. Smith and Simpson (1952) in Edinburgh, in assessing soil fertility for advisory purposes used 30ml of a nutrient solution containing: 10% sucrose, 1% citric acid, 0.1% peptone, 0.02% K_2O as K_2SO_4, 0.36% NH_4NO_3, 0.03% $MgSO_4.7H_2O$, 1.5p.p.m. Cu as $CuSO_4$, 1p.p.m. Fe as $FeSO_4$ and 1p.p.m. of Zn as $ZnSO_4$. Under their experimental conditions the weight of the mycelium varied from about 0.2 to 1.0 gram, and the standard error of an individual determination was about 5%. The same medium was also used for K determination, except 0.02% K_2O was replaced by 0.075% P_2O_5 as $NH_4H_2PO_4$. This medium was used previously by Smith and Dryburgh (1934) for examining the soil P by A. niger. Rosselet (1955), for bioassay of P, used a modified medium of Mehlich (1933), viz: 100 grams cane sugar, 10 grams citric acid, 6.67 grams

$(NH_4)_2SO_4$, 1 gram peptone, 0.61 grams $MgSO_4 \cdot 7aq$, 0.005 grams $CuSO_4 \cdot 5aq$, 0.0044 grams $ZnSO_4, 7aq$, 0.005 grams $FeSO_4 \cdot 7aq$, 0.42 grams $CaCO_3$, 0.178 grams K_2SO_4, in 1,000ml distilled water. Rosselet considered that Mo and Mn essential for the growth of A. niger were present in minute amounts in the medium for P determination, but had to be added to the medium when it was used for testing for Mg. It is, therefore, obvious that various workers used different media, depending on the purpose of investigation and the suitability of substrate for the respective experimental conditions.

Different strains affect results. Gerretsen (1948) isolated a suitable strain of A. niger from currents for the modified Niklas' medium. Smith et al (1935) obtained slightly different growth of the fungus as measured by dry weight of the mycelia, because the spores were taken from different batches, other experimental conditions being the same. (However, they did not check the number of spores, to which the slight differences may be attributed). In a study by Smith and Dryburgh (1934) it was shown that in the estimation of K, strain exerts a specific effect, and that the test for P is less delicate than for K as proved by a significant interaction between soil and strain. Nicholas (1960) compared a Mulder strain with a number of other strains including that of Steinberg, and found that the Mulder strain was the most suitable one for his particular medium.

The most suitable source of nitrogen for the medium has

been investigated by many workers. Smith and Dryburgh (1934) have shown that NH_4NO_3 and $(NH_4)SO_4$ behaved similarly as regards mycelium weight and final pH value. NH_4-citrate yielded greater mycelium weight but a higher initial pH of the suspension, permitting bacterial development. This disadvantage of NH_4-citrate may be obviated by sterilization, but it may give rise to more complications in the technique. It was suggested thence that the initial pH of the solution containing citrate might be lowered sufficiently by increasing the concentration of citric acid. This is permissable, because it has been shown that considerable variation in quantity of citric acid does not affect the mycelium weight or final pH to any extent. Gerretsen (1948) found $(NH_4)_2SO_4$ to decrease the pH considerably during fungal growth, because it leaves behind an acid radicle. Similar effect was shown by the use of NH_4NO_3, because the fungus prefers NH_4^+ to NO_3^-. He found that urea was suitable for his strain isolated from currents, it has a buffering effect and hence the pH is more stable. He also tried asparagine successfully, but the substance was too expensive for mass-analyses. Maercke (1950) also found urea to be better than $(NH_4)_2SO_4$ as N source due to its buffering effect. The increase in pH by urea over $(NH_4)_2SO_4$ was 0.45.

In constructing the standard growth curve for determination of available P, a suitable source of P must be chosen. Smith et al. (1935) have compared the effect of various phosphorus compounds on the growth of A. niger. They found that the difference in the weight

of mycelium was due to the different forms of P employed. The average weight of mycelium was increased slightly in the ammonium, sodium and potassium-phosphates over that obtained with Ca-phosphates. Mono-, di-, and tricalcium-phosphates are all equally good. The weight of mycelium obtained with Fe and Al-phosphates decreased as compared with that obtained with Ca-phosphates. These variations may be due to a difference in stability of the various forms of P or to the effect of different cations on the growth of the fungus. Smith et al. (1935) used $CaH_4(PO_4)_2H_2O$ as a source of P, ammonium phosphate was employed by Gerretsen (1948), Maercke (1950), Smith and Simpson (1952) and Rosselet (1954), and Na_2PO_4 by Njegovan (1960). It appears that ammonium phosphate is preferred as P source for the practical investigation of available phosphorus in soil.

Nicholas (1960) reviewed the importance of trace elements for the growth of A. niger. He quoted Raulin (1869), who showed that small amounts of Fe and Zn were indispensible for the growth of A. niger. At that time, however, Raulin's results were disputed by others who assumed, incorrectly, that metals were toxic substances stimulating abnormal growth in the organism. This controversy was finally resolved in favour of Raulin by the results of careful work by Bertrand and Javillier (1911, 1912), who demonstrated the need for Mn and Zn, and Steinberg (1919) showed a 5,000% increase in yields given by the same fungus by returning Fe and Zn to media previously treated with $CaCO_3$ to adsorb the two metals. Bortels (1927) confirmed these results and also found a beneficial effect of including Cu in

the culture solution. Further evidence came from similar work by Roberg (1928), Metz (1930), Gollwick (1936), and others. Other trace metals claimed to be required by the fungus include Mo (Steinberg, 1937, 1939; Mulder 1939, 1948; Nicholas and Fielding, 1947, 1950), Ga (Steinberg, 1938, not supported by Nicholas, 1953, and Bertrand, 1954), and V (Bertrand, 1941), an element not yet proved absolutely essential and requiring further confirmation. As Ca is required in microgram quantities it is regarded as a trace nutrient (Bertrand, 1954). Nicholas quoted Arnon (1950) regarding the definition of "essential" trace metals, who proposed the following criteria: (a) the organism is unable to complete its life cycle without it; (b) the effect must be specific to the nutrient and cannot be replaced by another; (c) it must be directly involved in its metabolism and not in correcting any unfavourable conditions in the growth medium, e.g. change in pH. This definition may be extended to both macro nutrient and higher plants. Smith (1936) quoting Steinberg (1919) pointed out that about 17 metals (mostly heavy) have been shown to accelerate the Aspergillus development. He stated that although claims are made about the stimulating effect of many metals, Fe, Zn, Cu and Mn are definitely necessary for normal growth and sporulation and these elements cannot replace each other. Smith stated that any normal soil, in the presence of 1% citric acid will most likely supply the small amount of Mn, which is required for maximum development of A. niger. This tends to confirm a statement by

Mehlich et al., (1933), that any specific constituents or stimulants possessed by certain soils are unlikely to influence the result for practical purposes.

Impurities present in the chemicals used for preparation of culture medium may affect the result of the analysis, particularly when dealing with trace elements. Purifications of the chemicals must be carried out before they are incorporated in the medium. It is important to check whether or not the reagents used to purify the media have been thoroughly removed and are not depressing the growth of the fungus. This can be checked by comparing the growth of the fungus under optimum conditions using purified and non-purified media. Phytocidal effects are also readily detected, since the standard series for the method become erratic when toxic materials are left in the culture. The amount left may be too small to affect the fungus directly, but they chelate with the test nutrient or other trace elements, resulting in low assay values for the test element. The purification of nutrients has been described in detail by Nicholas (1949, 1960) and he suggested that use of A.R. or C.P. grade chemicals is quite satisfactory.

The effects of the pH of media on the growth of A. niger has been studied by some workers. According to Nicholas (1960) the initial pH of the culture solution has a profound effect on the bioassay of trace metals in soils. Yet the fungus grows well over a wide range of pH values, viz from 1.8 to 7.5. In general the

micronutrients become less available to the fungus as the culture solution becomes more acid. He found different levels of optimum pH for assay of various trace elements, e.g. about 2.0 for Mo, about 7.5 for Mn, and about 5.5 for Cu, Zn and Co. Smith & Dryburgh (1934) studied the effects of the source of N on pH. They observed that ammonium citrate yielded greater mycelium weight but gave higher initial pH of the suspension, permitting bacterial development. Variations in quantity of citric acid did not affect the mycelium weight or the final pH to any appreciable extent. Discussing the effect of soil reaction, these workers stated that the effect of lime largely depends on the initial degree of saturation of the soil. Using infertile sandy loam with an extremely low degree of saturation, they showed that the addition of lime certainly affected the availability of P as estimated from the development of A. niger, but it had little or no effect on K availability. Experiment with clay loam, however, revealed that the growth of A. niger was not influenced by changing the soil reaction. Therefore, whatever the effect of excess $CaCO_3$ in the soil may have on the development of the fungus, the normal applications of lime to correct soil acidity would not appear to be of any appreciable influence. Gerretsen (1948) in determining the available P in the soil observed that pH decreased as result of fungal growth. The main cause was that the fungus produced citric acid from sugars. The quantity of acid produced depended on fungal growth, which corresponded with the amount of available P in the soil. He assumed that

more P in the soil would lower the pH. The pH was kept constant by incorporating Ca-citrate into the medium which acted as a buffer, and urea which he employed as N source acted similarly. Soils, containing appreciable amount of $CaCO_3$ gave difficulties, because the pH at which these soils were extracted was higher than that of soils with negligible quantities of $CaCO_3$. Maercke (1950) confirmed that the pH of his medium decreased as P concentration increased. He stated that urea neutralized sulphate ion, reducing the decrease of pH, while $CaSO_4$ buffered the pH. At 0.009% P the pH was so low that he did not aim at going to any higher concentration. Final pH was too low at high P in his medium and the values were very variable. Maercke was of the same opinion as Gerretsen that Ca-citrate buffers the pH from 3.25 to 3.5. Using sodium citrate instead of calcium citrate he obtained an increase in final pH, a decreased fluctuation of pH and higher weight of mycelium at higher P concentrations. He stated that a pH of 7 was not wanted for P analysis of soil. Swaby (1962 priv. com). is in favour of pH7 for reason that the organisms grow better at this pH, but the soil and media must be sterilized to prevent contamination by other organisms.

Container sizes have some bearing on results. Smith et al. (1935) showed that different volumes of medium used were associated with a variation in the weights of individual pads, but the average weights of mycelia increased with the volumes of medium from 30cc to 90cc. At greater volumes the weights were reduced again. Smith & Dryburgh (1934) tried different quantities of soils and culture solutions and they found that the area of growth was a much more important factor

than the actual amount of suspension. This may be associated with better aeration. This last point has been confirmed by the work of Njegovan (1960) that a logarithmic line of growth can only be obtained with a partially suppressed aeration using cotton wool plugs. With airtight condition using rubber plugs the growth was very irregular.

Regarding size of inoculum, Smith & Dryburgh (1934) showed that the differences in mycelium weights obtained over a millionfold range of concentrations of inoculum were negligible. Smith et al. (1935) studied the influence of size of inoculum on the weight of mycelium using 0.1., 0.5, 1.0, 2.0, and 5.0cc spore suspensions for inoculation. A sharp rise in the weight of mycelium was recorded with an increase in the size of inoculum up to 1.0cc, whilst above 1.0cc gave only small increase in the weight of mycelium. These results explain variations in weight of mycelium in replicate cultures. Variations may also be brought about by the differences in spore numbers within each volume of inoculum.

It was felt that lack of agreement between results of pot tests and A. niger experiments might be due to the fact that the soil is examined on a volume basis in the pot method, whilst, on account of the small quantities required, the soil is weighed in the A. niger method. Smith (1936) carried out a series of experiments with varying weights of soil and it was shown that the Aspergillus method was not as sensitive to the amount of soil taken as might be expected. A large number of samples giving mycelium yields varying

from about 0.2 to 1.0 gram was specially examined. For P estimation 4, 5 and 6 grams of soil were taken and the average figures were 0.362, 0.398 and 0.452 respectively. Thus a difference of 20% in the weight of soil taken led to a difference of only 10% in the mycelium weight. Similar investigations have been made by other workers, and it seems that minor variations in the apparent densities of mineral soil samples are not likely to lead to serious discrepancies in the results obtained in routine examination. However, soils rich in organic matter necessarily receive special consideration, because greater portions of total P are not readily available to the organism. It has been suggested that better results can be obtained by using greater quantities of soil for major elements than those used for minor element estimations. Roschach (1961) using samples up to 100mg in the estimation of Zn, Cu and Mg in different soils by the A. niger method gave a highly significant positive linear correlation between mycelium yield and sample weight. This result indicates that under these conditions the mycelium yield was directly proportional to the minor element content of the sample. Since this linear relationship did not hold true with samples of greater weight, the use of small samples is tentatively suggested for comparative microbiological determinations of trace elements in soils.

Several ways of interpreting the amount of the test nutrient absorbed by the fungus appear in the literature. The most common one is the comparison of dry weights of mycelium. Before weighing, the mycelium is washed and dried in an oven and then cooled in a dessicator. This method has been adapted by many investigators (Gerretsen, 1948;

Mulder, 1949; Nicholas, 1949, 1960; Rosselet, 1954; Niklas, 1930; Smith & Dryburgh, 1934; Smith et al., 1935; Smith & Simpson, 1952; etc.) Various levels of temperature and periods of drying have been adopted by different workers, e.g. at 105°C for 8 hours (Gerretsen, 1948); 50 to 60°C overnight and finally at 100°C for an hour (Smith & Simpson, 1952). The weights obtained from the test materials such as soils, plant tissues, ash, etc., are compared with the weights obtained from standard series which have been previously prepared by additions of known amounts of the nutrient under test and results plotted in a standard growth curve. By plotting the weights of mycelium obtained from the test material together with that obtained from the standard series, the amount of the test nutrient taken up by A. niger can be estimated. Some workers have argued that the total weight of mycelium can be misleading, because the composition of mycelia are not always constant (Smith & Dryburgh, 1934; Smith & Simpson, 1952.) In the case of K for instance, the rate of increase of mycelial weight is smaller than the rate of K uptake by the fungus. This can be overcome by determining the K content chemically in the ashed mycelium, and then comparing the content with that of the standard series similarly prepared. This procedure, however, involves chemical determination, which renders the biological method more complicated. Njegovan (1960) has employed an indirect estimation of the tested nutrient. He claims that an accurate estimation of available phosphorus assimilated by A. niger from test material can be carried out by determining the amount of assimilated nitrogen by the same organism from the nutrient medium. The assimilated N is

determined by "Formol" titration. By this procedure, he obtained a straight line relationship between the levels of N and P assimilated by the fungus. He later discovered that this line actually corresponded with the logarithmic line derived from Mitcherlich's equation. Only at the beginning was the line approximately straight, followed by a part of a curve. For measurement of fungal growth after incubation Gerretsen (1948), Mulder (1949), Nicholas (1960), and others used a preliminary visual examination of the thickness of the mycelial pad or of the abundance and/or colour of the spores. As this method was not accurate and required a great deal of experience, other means of measurement had to be investigated to obtain more precise result. Gerretsen (1948) used a photronic cell to measure fungal growth in his determination of magnesium. He considered this method more accurate and objective for determination of this element without resorting to weighing. The procedure involved the measurement of the intensity of light reflected by the mycelium when it is strongly illuminated. A tube carrying the photronic cell was placed on the top of the Erlenmeyer incubation flask. The resulting photocurrent was measured with a sensitive galvanometer. The electric current through the illuminating lamp was kept constant. Where Mg contents rose to 100p.p.m., the mycelium became denser, whiter and reflected more light. Above 100p.p.m. there was a sudden increase in sporulation, which was indicated by a decreased reflection of light. Thus, the curve obtained by plotting Mg concentrations against galvanometer readings indicated that one galvanometer reading may give 2 different Mg contents. According to Gerretsen (1948) this does not create difficulties, for

it can easily be established whether the reading is made on the ascending or descending slope of the curve, as indicated by the absence or presence of spores. However, this method can only be used satisfactorily up to a certain level of Mg in the soil. It is not suitable for Zn and other elements, because with Zn there is no region in which the spores fail to grow, which makes visual and photoelectric measurement difficult. In such cases he resorted to weighing the mycelium, which is the most commonly used method.

2. Results achieved by the application of the procedure:

Smith & Dryburgh (1934) in their determination of available P and K compared results obtained from the A. niger method with those obtained from the Mitscherlich's method by constructing regression lines. They found, that in the estimation of P, r = 0.77 which was highly significant for 40 observations, and in the case of available K, r = 0.40 was also significant. These results differ from thos of Niklas et al. (1930), who found good correlations between the Aspergillus method and pot test for K, but in the case of P the Aspergillus method agreed better with chemical extraction methods. This is to be expected, since the growth of the fungus depends on the quantities of P or K absorbed from the soil, and these in turn are largely determined by the citric acid in the nutrient solution. Smith (1936) in estimating responses to K fertilizer, observed that all soils giving values below 0.3 gram of mycelium weight can be regarded as seriously deficient in available K. Addition of K from 0.8 to 2 cwt. per acre are responsible for significant increase in mycelium weight, the recovery of K is practically quantitative.

Addition of P to soil corresponding to common dressing of 3 and 7 cwt superphosphate per acre, do not give any considerable increase in yield of mycelium. It is obvious that a small addition of P is fixed by those soils in such a manner that it does not become available completely to the fungus during the period of incubation. Niklas (1941) was able to determine the amount of P fixation by means of the A. niger technique. He also established absolute and relative fertilizer effects, and a method of evaluation of forms and amounts of fertilizers applied by the use of a microbiological fertilizer experiment. Schlots et al. (1931) compared the results of the A. niger method with those secured by the Truog method, and they found that A. niger indicated changes in the availability of P in a soil. They expressed the opinion that this method may be calibrated for use on various soil types. Gonggrijp (1938) working with Indonesian soils found a parallel result between total analysis and physiological analysis using A. niger. A. niger was grown in the presence of known amounts of P_2O_5 and $P_2O_5 + K_2O$ to determine fertilizer requirements of tropical soils. In comparison with European soils, he found that the sufficiency levels of P for tropical soils were lower than those for European soils. These findings were confirmed by those of Nicholas (1960) who stated that the levels of nutrient availability are lower for tropical soils than for soils of the temperate regions. Matzuki (1937) found the A. niger method suitable for P determination but not for K, because the amount of mycelium is influenced more by the amount of P than that of K. Mooers (1938) has published a tentative standard for determination of the fertilizer needs of a soil. According to his data for P_2O_5,

1 - 50lbs per acre is regarded as very low level and the soil as extremely deficient in P_2O_5. At 51 - 90lbs per acre the level is low and the soil is deficient in P_2O_5 for all crops. A medium level is 91 - 130lbs per acre in which the response to P_2O_5 may be moderate to none. The level of 131 - 170lbs per acre is regarded as a high level and indicates no immediate need of fertilizer, and above 170lbs per acre is considered a very high level, which would allow more than a year's cropping without response to P_2O_5 dressings. Schulz-Schomburgh (1953) determined available P, K and Mg in tropical soils and his findings confirmed those of Smith (1936), pointing at considerable P fixation in some P deficient soils of heavy texture. Jensen (1953) determined the P content of 82 soil samples varying widely in their P contents, pH and humus contents. The amount of assimilable P showed a very close correlation ($r = + 0.98$) with P soluble in 0.2 N H_2SO_4 and amounted to approximately 96% of a latter. In 70% of the soils, assimilable P and H_2SO_4 soluble P agreed within $\pm$ 25%. Low assimilability (less than 50%) could be seen in certain alkaline soils, especially those rich in Fe. High assimilability (greater than 50%) occurred in a few organic soils of low P content. P added to strongly P-fixing soils and not extractable by H_2SO_4 or HNO_3 or exchangeable against zeoliths is unavailable to A. niger as well. On the other hand Aspergillus utilizes P in rock phosphate and in this respect it agrees with the H_2SO_4 extraction, but contrasts with the zeolith method. He found the A. niger method as reliable as chemical methods in indicating

phosphate deficiency in soils and a well defined fraction of the total soil phosphorus. Smith & Simpson (1952) have employed A. niger to estimate the available P and K of soils in Edinburgh for 20 years for advisory purposes. They found that when the mycelial weights were below 300mg the soils were almost invariably deficient in P and gave a substantial response in crop yield on application of phosphate fertilizers. The upper limit, above which P-fertilizers were not needed, was more difficult to define, because (a) most of field experiments have been carried out on P-deficient soils, and (b) the response in the field to phosphate was greatly influenced by seasonal conditions. They concluded that the percentage recovery in the crop of fertilizer phosphorus was the best measure of the fertilizer treatment. There was also a strong correlation between P recovery in the crop and exchangeable Ca in the soil. This may indicate that the form of phosphate in the soil was mono calcium phosphate instead of tri-calcium phosphate which is of very low availability. They also found a good negative correlation between phosphate response and mycelium weight. In the case of K, the mycelium weights of 300 to 450 milligrams were regarded as a range of values over which fertilizer may or may not be required. A potato crop responded to fertilizer when soil yielded less than 300 milligrams of mycelium. When the weight of mycelium exceeded 400 or 450 milligrams the soil was able to provide an amount of readily available K for healthy growth of potato crop. These findings confirmed those of Smith (1936). Rosselet (1954) used the A. niger method to determine phosphate levels of citrus fertilizer plots in South African

soils. He found that the P content of virgin soil was very low, i.e: 111 lb per acre, and the application of P fertilizer was recommended. This method also showed that phosphatic fertilizers were more effective than manure in increasing the P content of soils. In the case of manure, he suggested that part of P did not originate so much from the manure itself as from increased microbial activity, which in turn releases phosphate from P combinations of reduced availability. This case has been pointed out by Gerretsen (1949) in his paper dealing with "the effects of microbial activity on the availability of various phosphatic compounds." The sufficiency level of available P has been found by Rosselet to be about 400lb per acre, giving a yield of 730lb per tree. In his experiment the phosphate was added in graded quantities, calculated on the basis of lbs per acre over a 0 - 6 inch depth, assuming such a layer of soil to weigh two million pounds. Where convenient, the number of lb per acre was converted to parts per million. Manil et al. (1956) found, that for sandy and clayey soils the P values obtained from the A. niger method were 10 - 30% higher than corresponding values obtained from the Egner chemical method, but were comparable with those from the König and Ferrari methods. Although they obtained little agreement between the results of these analyses and observations on the vegetation, the A. niger method was recommended for P analysis, due to its good repeatability and reproducibility. It was not recommended for K, because the values they obtained varied widely. The A. niger technique has also been used by various workers for estimating the content of available Mg and trace elements in soils. Figures for deficiency and

sufficiency levels have been published for these elements. (Gerretsen, 1948; Mulder, 1949; Nicholas, 1960; Schlichting, 1962).

3. Advantages and shortcomings of the A. niger method:

Various workers have recognized the merits of the A. niger method for estimating available plant nutrients, but they also discussed its shortcomings. Smith & Simpson (1952) have stated that the peculiar merit of the A. niger method lies in the fact that it does not require an accurate chemical determination. There is no justification for precise analytical results obtained in many methods, when due consideration is given to errors of soil samplings and uncertainty of results. The simplicity of the method and the fact that it gives results closely parallel to those obtained with acid extractions have been instrumental in their decision to adopt the method for routine advisory purposes. It has been employed for 20 years in Edinburgh, exclusively for K and frequently for P. Even now the method is still used in some European countries, and was especially so during the last war (Gerretsen, 1948). Smith & Simpson noted that the A. niger method was closely related to a chemical method inasmuch as the soil is in contact with a 1% citric acid solution for 6 days. A good agreement was also obtained between mycelium weights and the amounts of P or K dissolved from a series of soils by a dilute acid. After comparing the results with field experimental results, they concluded that the A. niger method was more sensitive for K than for P. The response of the fungus to small addition of P was small, whereas the response to addition of K

was almost quantitative. This may be brought about by a reaction between soil and added P, where P may be fixed in insoluble form by the soil and rendered unavailable to the fungus. Mulder (1949) claimed that microbial tests (A. niger) have the advantage over chemical analyses in that the estimation of a certain element is possible without separating it from other compounds. Thus it saves time. As the requirement of A. niger for trace elements is very low, a very small quantity of these elements can be estimated, e.g. in cases of Cu and Mo, amounts in the range of 10^{-4}, 10^{-3} and 10^{-6}, 10^{-5} milligram respectively may be determined. By chemical methods it is often impossible to detect such small amounts. The Aspergillus method applied to soil problems has shown Mg deficiency in the presence of K deficiency, a point which may not be shown by chemical analysis of plant tissues. It can also detect Zn deficiency in horticultural soil where fruit trees showed "little-leaf" effects, and Cu deficiency in alkaline fen soil in East Anglia and other centres. (Nicholas, 1949; Rosselet, 1954). The cheapness and speed of the method are claimed to be of advantage, but it is suggested that its reliability must be checked by means of examining, with it, a much greater variety of soils from suitable field experiments. This opinion is shared by several workers, (Smith & Dryburgh, 1934; Gerretsen, 1948; Mulder, 1949; Smith & Simpson, 1952; Njegovan, 1960). Gerretsen pointed out, that for P determination the A. niger method is the best method compared with three other methods (Egner, citric acid extraction and Neubauer seedling methods), whereas the reproducibility is of the same order of magnitude as that of chemical

methods. For K, the accuracy is even greater than that of chemical methods. The correlation with results of fertilizer experiments compares favourably with the other methods. He also claimed that the same personnel was able to make two to three times as many determinations (both in duplicate) as with chemical methods.

On the other hand, the A. niger method, like any other method, also has its limitations and shortcomings. The greatest limitation of the procedure is that it can not be used to determine the exact quantity of fertilizers required to remedy nutrient deficiencies of the soil. Various workers have emphasized the point that the results obtained by this method must be calibrated against the yields of a large number of fertilizer field experiments on different crops, (Gerretsen, 1948; Smith & Dryburgh, 1934; Mulder, 1949; Rosselet, 1954; Manil et al., 1956; and Schlots et al., 1931). Work carried out by Rosselet (1954) indicates that it is not possible to show differences in available Fe in soil and fruit trees by means of A. niger. He attributed this to the fact that Fe-deficient leaves usually contain adequate total Fe, but that the metal is immobilized in plant tissues. According to Mulder (1949) the result of the A. niger method is less accurate than chemical methods for the estimation of total Mg in plant tissue, although the former has the advantage that a very small sample may be investigated and that it is much quicker. Many investigators have reported that the A. niger method is not nearly as sensitive as other common methods such as those of Mitscherlich and Neubauer (Smith & Dryburgh, 1934). This may be due to the fact that the mycelium is not a direct measure of the nutrient uptake, for the

composition of the mycelia varies. The nutrient absorbed by the fungus does, indeed, agree more closely than mycelium weight with the results of other methods, but the need to analyse the mycelium chemically would make the method impracticable. Although the technique certainly enables us to place soils into two or three large groups according to their fertilizer requirement, within which small variation in the mycelium weight and its composition are of little consequence, there remain the difficulties of assessing the border lines between the groups. This, however, is common to all methods of estimation of soil fertility.

OTHER FUNGOUS SPECIES USED FOR SOIL PHOSPHORUS DETERMINATION:

Very limited evidence concerning the use of other fungous species for the estimation of available plant nutrient in the soil is found in the literature on this subject. Swaby (1958) has used Curvularia geniculata for determining the availability of phosphatic minerals. He used a medium employed by Donald et al. (1952), consisting of 50 gr sucrose, 5 gr K NO_3, 0.75 gr Mg $SO_4.7H_2O$, 320 microgram Fe as $FeSO_4.7H_2O$, 250 microgram Zn as Zn $SO_4.7H_2O$, 80 microgram Cu as Cu $SO_4.5H_2O$, 8 microgram Mn as $MnSO_4.4H_2O$, and 3.2 microgram Mo as $(NH_4)_2MoO_4$, made up to 1 litre with distilled water. The pH was adjusted to 7.5. According to his observation the fungus maintained a constant pH of 7.5 throughout the incubation period of 7 days at 25°C. In comparison with A. niger, Swaby found that A. niger has greater ability to extract phosphate than C. geniculata. This may be due to

the ability of A. niger to produce a suite of acids, citric, gluconic, oxalic, whereas C. geniculata does not produce noticeable acid. According to Swaby, Curvularia will not grow in the presence of acid. Curvularia has also been used for assaying Mg, K, S and trace elements. The main thought directing the use of this fungus in preference to A. niger is that it is not producing acids and thus more closely approximates higher plants.

No reference was found in the literature to Penicillium lilliacum and Fusarium species, used here for estimating the available phosphate in the soil. Donald et al. (1952) tried to estimate the availability of trace elements with Penicillium roqueforti, but they were unable to obtain satisfactory results, although the culture was incubated for 17 days at 22°C.

III. MATERIALS.

Description of Soils:

The soils used are described in Table: 1. Each soil type, with the exception of Tokomaru silt loam and Arapohue clay loam, is represented by a phosphated and a non-phosphated sample. Tokomaru silt loam is represented by three samples, one with phosphate only, one with phosphate and lime, and one without phosphate addition. Arapohue clay loam is represented by one sample only, the phosphate fertilizer history of which is unknown. The descriptions of the soils are derived from the published data of Fieldes & Taylor (1961), the Soil Bureau bulletin No. 5 (1954), and from information received through private communications with the Farm Advisory Officers in the respective districts.

Table 1.

Description of Soils.

Serial No.	Soil Type	Parent Material	Reference No.	Genetic No.	Genetic Classification	Predominant clay minerals	Phosphate fertilizer history.	Location of soils
1	Tokomaru silt loam	Alluvium from greywacke	13	2	Yellow-grey earth, moderately leached, forest melanized.	Illite, vermiculite, montmorillonite, hydrous mica intermediates, and amorphous hydrous iron and aluminium oxide.	superphosphate topdressing at 4 cwt. annually	Nutrition block A_2, Sheepfarm of Massey College, Palmerston North.
2	Tokomaru silt loam	Same as No. 1 above	13	2	Same as No. 1 above.	Same as No. 1 above	4 cwt. superphosphate topdressing plus lime annually	Nutrition block B_4, Massey College Sheep farm, Palmerston Nth
3	Tokomaru silt loam	Same as No. 2 above.	13	2	Same as No. 2 above.	Same as No. 2 above	None	Clifton Terrace Palmerston North.
4	Carnarvon black sandy loam	Wind-blown quartz-felspatic sand.	23b	17	Sandy gley soil, moderately leached.	Illite with partially expanded micas and vermiculite	topdressed with 4 cwt. superphosphate annually	Himatangi farm
5	Carnarvon black sandy loam.	Same as No. 4 above.	23b	17	Same as No. 4 above.	Same as No. 4 above	None	Roadside, Himatangi
6	Arapohue clay loam	Argillaceaus lime stone.	7	11	Rendzina.	Montmorillonite, hydrous mica	Unknown	Waikato area
7	Galatea sand	Kaharoa ash on pumice gravel	14b	13a	Yellow-Brown pumice soil.	Allophane, and amorphous hydrous silica	topdressed with 4 cwt. superphosphate annually	Farm, Rotorua district
8	Galatea sand	Same as No. 7 above.	14b	13a	Same as No. 7 above.	Same as No. 7 above	None	Roadside, Rotorua district.
9	Koro Koro silt loam	Greywacke	35bH	5b	Yellow-Brown earth, moderately leached, moderately weathered.	Clay-vermiculite metahalloysite	2 cwt. superphosphate topdressed annually	Farm, Kahuteraua Valley
10	Koro Koro silt loam	Same as No. 9 above.	35bH	5b	Same as No. 9 above.	Same as No. 9 above	None	Roadside, Kahuteraua Valley

Table: 1. (Cont'd)

Description of Soils.

Serial No.	Soil Type	Parent Material.	Reference No.	Genetic No.	Genetic Classification.	Predominant clay minerals	Phosphate fertilizer history	Location of the soils
11	Kairanga silt loam	Alluvium, mainly from greywacke and tertiary sediment.	2	17	Gley soil, weakly leached.	clay-vermiculite, hydrous micas, quartz	2 cwt. superphosphate annually	Kairanga, near Longburn, Mr. Rowland's farm.
12	Kairanga silt loam	Same as No. 11 above	2	17	Same as No. 11 above.	Same as No. 11 above	None	Mr. Rowland's property, Kairanga, near Longburn.
13	Taupo sandy silt	Taupo rhyolitic pumiceous ash	18	13a	Yellow-Brown rhyolitic pumice soil.	Allophane, also hydrous silica	3 cwt. superphosphate topdressing annually since 1952	Mr. F.McKenzie's property, Ngakuru, Rotorua.
14	Taupo sandy silt	Same as No. 13 above	18	13a	Same as No. 13 above	Same as No. 13 above	None	Roadside, Ngakuru, Rotorua.
15	Ramiha silt loam	Pleistocene silts, mainly from grey-wacke with some volcanic ash.	77	14 or 6	Strongly leached, Yellow-Brown earth, in Yellow-Brown loam intergrade.	Not known, but related to "Dannevirke" soil. - vermiculite, ethrite, chlorite, hydrous mica.	2 cwt. superphosphate annual topdressing	Farm, Greenroad.
16	Ramiha silt loam	Same as No. 15 above	77	14 or 6	Same as No. 15 above	Same as No. 15 above.	None	Roadside, Pahiatua track.
17	Taupo light sandy loam	Shallow rhyolitic pumice of Taupo over andesitic ash of Tongariro.	18g	13	Primary podzolic rhyolitic pumice soil, moderately to strongly leached	Allophane and amorphous hydrous silica	3 cwt. superphosphate topdressing annually	From a farm, Hihitahi, Wanganui.
18	Taupo light sandy loam	Same as No. 17 above	18g	13	Same as No. 17 above	Same as No. 17 above	None	Roadside, Hihitahi, Wanganui.
19	Stratford sandy loam	Andesitic Egmont ash.	66b	14	Yellow-Brown loam moderately leached, forest melanized, volcanic ash im-mature.	Allophane and hydrous feldspar	5 cwt. superphosphate annually since 1955	Stratford, farm, Taranaki.
20	Stratford sandy loam	Same as No. 19 above.	66b	14	Same as No. 19 above.	Same as No. 19 above	None	Roadside, Stratford, Taranaki.

IV. METHODS.

1. Soil sampling and preparation of samples:

The localities from which the samples were collected are shown in table: 1. About 6 cwt. of each soil was collected from the 0 - 3" depth by random sampling using a spade. All the fertilized soils were obtained from topdressed paddocks, whereas most of the unfertilized samples were obtained from roadside sites. The exceptions in this latter respect were samples 3 and 12, which were obtained from untop-dressed paddocks.

The samples were spread out on trays in the glasshouse, and when their moisture had been reduced sufficiently by natural evaporation, they were shredded with a mechanical shredder (Plate I). The shredded soils were placed back on the trays and left to dry for about 3 days with periodic turning over to hasten the drying; they were then sieved by hand to pass through $\frac{1}{4}$" holes. The soils in this state of subdivision were reserved for the pot experimental work. Sub-samples passing a 2m.m. sieve were prepared from these bulk samples for use in the fungal bioassays and chemical determinations.

2. Microbiological Assays:

(a) Species of fungus investigated:

The following four species of fungus isolated from the rhizo-sphere of plants were investigated for use in the microbiological assays.

(i) *Aspergillus niger*, a black spored fungus.

(ii) *Penicillium lilliacum*, a pinkish-white spored fungus.

(iii) *Curvularia geniculata*, a greenish-black spored fungus.

(iv) *Fusarium* species, a creamy-white spored fungus.

Plate I.
Mechanical Shredder and
hand sieve for soil preparation.

Plate II.
Part of the lab where
the bioassays were
carried out. Some of the
equipment used are shown.

(b) Production of spores:

For spore production, the fungi were grown on a modified Czapek Dox Agar medium. This was prepared by dissolving 51.4g. of the agar in 1 litre of warm distilled water, followed by sterilization by autoclaving for 20 minutes at 10 p.s.i. pressure.

Spore production for Penicillium, Curvularia and Fusarium, was carried out in petri dishes. The sterilized, solidified agar medium was re-liquified by immersing the flask containing the agar in boiling water for a few minutes. The liquid agar was then poured into sterilized petri dishes inside an isolation cabinet to prevent contamination by undesired organisms. When the medium was cool, it was inoculated with spores of the stock cultures.

For A. niger the production of spores was carried out in slope cultures or test tube. Approximately 10ml. of the liquid agar was poured into each tube and then sterilized and cooled as above, followed by inoculation with the stock culture.

The temperaturesand periods of incubation varied with each species, viz:

A. niger - 35^{0}C for 4 days (96 hours)
P. lilliacum- 28^{0}C " 6 " (144 hours)
C.geniculata- 28^{0}C " 5 " (120 hours)
Fusarium sp.- 28^{0}C " 14 " (336 hours)

These optimum conditions for spore production for the different species had been determined by preliminary experiment.

(c) Preparation of P-deficient medium:

The fungi were grown in liquid media containing all the essential

nutrients, except phosphate. The medium employed was one originally used by Gerretsen (1948), but it was necessary to modify it slightly to suit the strains of fungi employed and the purpose of the investigation. The final composition of the medium used was as follows:

Sucrose	10%
Citric acid	1%
Urea	0.4%
$MgSO_4$	0.03% as $MgSO_4.7H_2O$
K_2O	0.02% as K_2SO_4
Fe	0.0005% as $FeSO_4.7H_2O$
Zn	0.0002% as $ZnSO_4.7H_2O$
Mn	0.0001% as $MnSO_4.4H_2O$
Cu	0.00005% as $CuSO_4.5H_2O$.

The chemicals used in the preparation of the medium were of A.R. grade, except the urea which was the common field fertilizer. All the constituents were shown to be phosphate-free by application of the molybdenum blue test.

The major constituents of the medium were weighed out into an R.F.B. flask of required capacity, and the micro-element additions were made by pipetting from stock solutions of the appropriate salts. (Details of the preparation of micro-nutrient stock solutions are given in the appendices). The contents of the flask were made up to the required volume with water, and sterilized by autoclaving for 20 minutes at 10 p.s.i.

For the bioassays 50ml. of the above solution was placed in each test flask to which was then added 1g. sterile calcium citrate and

1 ml. of sterile solution containing 10.3 mg. of dissolved sodium humate. The test flasks used were 250 ml. wide-neck (1½" diameter) Erlenmeyer. (Plate II).

(d) Setting up of test flasks for standard growth curves and bioassays of soils.

In preparing the standard growth curves for the different fungi the media were supplemented by the addition of phosphate in the form of $NH_4H_2PO_4$ solution to provide a range of phosphate concentrations in the test flasks plus the required volume of sterile water to bring the total addition to 10 ml. The completed growth media were then immediately inoculated with 1 ml. of thick spore suspension.

The spore suspension of A. niger was prepared by shaking the spore culture with sterile water, followed by further dilution to give sufficient volume for the number of inoculations needed. The spores of the other 3 species, grown on petri dishes, were too difficult to detach by shaking. They were therefore scraped off with a sterilized glass rod and then diluted as above.

After inoculation, the flasks were plugged with cotton wool bungs and incubated at the appropriate temperatures and for the appropriate periods previously determined optimal for spore production.

The final volume of the culture was 62 ml. per flask, which derived from:

50 ml. of phosphate-free nutrient solution.

10 ml. of phosphate solution.

1 ml. of sodium humate solution.

1 ml. of thick spore suspension.

The procedure for the bioassay of available soil phosphate was essentially that used in the preparation of the standard growth curves, except that 1 g. of soil and 10 ml. of sterile water were added to each flask in view of the standard phosphate solution.

The soils were sterilized before bioassaying as suggested by Swaby (1962, priv. com.). Sterilization was carried out by adding a few drops of propylene oxide into each flask containing 1 g. of soil and left overnight in an air-tight condition at room temperature. The fumigant was expelled from the flasks by placing them uncovered in an incubator at 40 - 50°C for 2 hours. The nutrient culture solution was added at this stage.

(e) Harvesting the mycelia:

At the end of the incubation period the mycelia were quantitatively transferred with a spatula on to a filter (NO. 41 Whatman paper), and washed with distilled water. The filtrate was discarded. When completely drained, the mycelia together with the filter paper were placed on corrugated carton sheet and dried in the oven at 90°C overnight. After drying, the mycelia and paper were cooled in a desiccator for about 15 minutes. The mycelia and paper were weighed and the total dry weight was recorded. The dry weight of the mycelia was obtained by subtracting the weight of the filter paper from the total dry weight.

The weights of the filter papers used were determined individually, instead of taking the average weight of several papers. This technique was employed in view of the considerable variation found between the weights of individual filter papers, which could seriously affect the mycelial

weight calculated by difference. Before weighing, the filter papers were placed in desiccators overnight as suggested by Fife (1963, priv. com.) to remove the hygroscopic moisture. All weighing was done on Mettler Model B6.

The pH of the media, before and after incubation, were measured using a Radiometer pH meter to determine the change of pH brought about by fungal activity.

3. Pot experiment:

(a) Species of plants employed:

The plants were grown in the glass house during the summer months. The plant species used were chosen to suit these conditions and were as follows:

(i) Japanese millet representing a cereal crop,

(ii) Yellow Globe turnip representing a root crop,

(iii) Lucerne representing a leguminous crop.

These crops were known to be phosphate responsive.

(b) The glass house and type of pots:

The glass house used during the period of experiment (January to March, 1963) is shown in Plate III. It has 2 compartments of unequal size. Before the plants were sown, the roof was sprayed with oil distemper white paint to reduce the excessively high summer temperatures.

The type of metal pot used is shown in Plate IV. The pot dimensions were:

top diameter..... $7\frac{1}{4}$ inches.

bottom diameter..... $6\frac{1}{4}$ inches.

height ..... 8 inches.

Plate III

The glasshouse used
during the period of pot experiment.

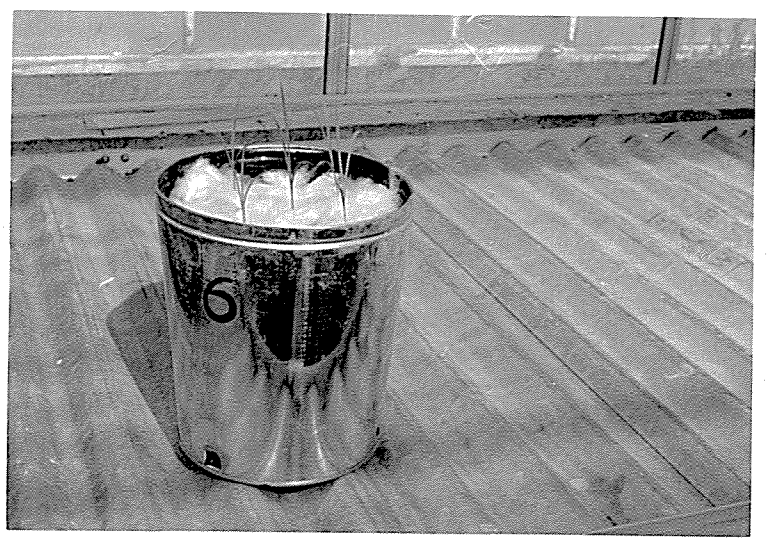


Plate IV

Type of metal pot used.
After thinning of seedlings
the surface soil is covered
with glass-wool.

Each pot was provided with 3 side holes drilled close to the base to provide drainage and adequate aeration. The inside of the pots was painted with black bituminous paint to prevent any harmful contamination which might originate from the metal. A total of 245 pots were required for the experiment.

(c) Determination of water holding capacity (W.H.C.) of soils.

W.H.C. of soils was determined by Keen-Razkowski method. (Details of the method are given in the appendices). The W.H.C., both before and after diluting the soils with vermiculite (50% by volume), were determined to examine the effect of vermiculite on absorbtion of water. The results are shown in Table: 2.)

(d) Determination of soil pH:

The Radiometer pH meter was employed to determine the pH of the soils (Table: 2).

(e) Procedure for filling the pots:

The soil was diluted with an equal volume of vermiculite, aimed to improve the physical condition of the potted soils. The weight of vermiculite added in each instance is shown in Table: 3.

The filling of the pots and the packing of the potted soils was carried out according to techniques used by Fergus and Stirk (1961), Schuffelen et al. (1952), and Stewart (1932). Prior to potting, the diluted soil was mixed with fertilizer, containing all the essential nutrients, except phosphate. Mixing was carried out by hand, followed by sieving through a $\frac{1}{4}$" holed screen. The fertilizer was applied in solution form, the volume of liquid added being that required to bring the soil to a moisture content equivalent to 40% W.H.C. of the undiluted

soil. This W.H.C. was selected as providing a satisfactory moisture regime and mechanical condition in the soils. Preliminary trial showed that 40% W.H.C. of the diluted soil rendered the soil too wet and sticky for handling. Stewart (1932) quoted Mitcherlich, who found that it was difficult to effect complete transfer of wet soil into pots.

Before adding the soil a $\frac{1}{2}$" layer of gravel was placed on the bottom of each pot to assist drainage. The soil was then added in successive $1\frac{1}{2}$" layers. Each layer was carefully pressed down and tapped gently several times by dropping the pot vertically from about 3" high on to the concrete floor. Each layer was superficially loosened with a fork before being covered with the following layer. The pots were filled up to approximately 1 inch from the top. After filling, the total amount of water was increased up to 60% W.H.C. of the undiluted soil. (The actual amounts of water added in each instance are shown in Table: 4). This level of water in pot experiment was recommended by Piper (1942) and considered by Fergus and Stirk (1961) as providing a moisture tension in the vicinity of pF2.

To avoid any possibility of germination injury, the upper layer of the soil (about 1" thick) did not include fertilizer.

Table: 2.

PH and Water Holding Capacities of soils.

Serial No.	Phosphate content.	pH	W.H.C. of undiluted soils (% O.D.W.)*	W.H.C. of diluted soils. (% O.D.W.)*	Increase in W.H.C. (% O.D.W.)*
1	4 cwt. super	5.25	76.6	115.3	38.7
2	4 cwt. super + lime	6.50	73.5	110.4	36.9
3	no P	5.30	84.7	137.3	42.6
4	with P	5.65	77.0	128.9	51.9
5	no P	5.75	89.4	122.3	32.9
6	?	7.70	81.4	141.3	59.9
7	with P	5.50	65.8	145.7	79.9
8	no P	6.00	62.9	119.6	55.7
9	with P	5.05	89.1	133.6	44.5
10	no P	5.30	91.7	146.2	54.5
11	with P	5.80	74.8	128.7	53.9
12	no P	5.65	73.2	131.6	58.4
13	with P	5.45	134.2	190.4	56.2
14	no P	5.60	124.5	170.3	45.8
15	with P	6.10	90.5	148.9	58.4
16	no P	5.40	94.5	157.7	63.2
17	with P	5.55	105.2	169.7	64.5
18	no P	5.90	107.9	168.1	60.2
19	with P	5.90	108.2	165.4	57.2
20	no P	6.40	79.8	126.3	46.5

* O.D.W. = OVEN DRY WEIGHT.

Table: 3.

Weights of soils and diluents per pot.

Serial Number of soils	Weight of soil per pot. (kg.)	Weight of vermiculite per pot. (kg.)	Total weight of soil+diluent per pot. (kg.)
1	2.0	0.246	2.246
2	2.0	0.263	2.263
3	2.0	0.2725	2.2725
4	2.0	0.255	2.255
5	2.0	0.2665	2.2665
6	2.0	0.253	2.253
7	2.0	0.2555	2.2555
8	2.0	0.207	2.207
9	2.0	0.208	2.208
10	2.0	0.227	2.227
11	2.0	0.260	2.260
12	2.0	0.298	2.298
13	2.0	0.261	2.261
14	2.0	0.253	2.253
15	2.0	0.280	2.280
16	2.0	0.336	2.336
17	2.0	0.369	2.369
18	2.0	0.378	2.378
19	2.0	.370	2.370
20	2.0	0.223	2.223

Table: 4.

Amount of water retained by undiluted soils in each pot.

Serial No. of soils.	W.H.C. of soils as % of oven dry weight.	Weight of soils per pot. (kg.)	Total H_2O retained by 2kg. ofsoils. (ml.)	Amount of water at 60% of W.H.C. (ml.)	Amount of water at 40% of W.H.C. (ml.)	Differences of the amount of water at (60%–40%) of W.H.C. (ml.)
1	76.6	2.0	1532	919	612.8	306
2	73.5	2.0	1470	882	588	294
3	84.7	2.0	1694	916	677	239
4	77.0	2.0	1540	924	616	308
5	89.4	2.0	1788	1073	715	358
6	81.4	2.0	1628	977	651	316
7	65.8	2.0	1316	790	526	264
8	62.9	2.0	1258	755	503	252
9	89.1	2.0	1782	1069	713	356
10	91.7	2.0	1834	1100	734	366
11	74.8	2.0	1496	898	598	300
12	73.2	2.0	1464	878	585	293
13	134.2	2.0	2684	1610	1074	536
14	124.5	2.0	2490	1494	996	698
15	90.5	2.0	1810	1086	724	362
16	94.5	2.0	1890	1134	756	378
17	105.2	2.0	2104	1262	842	420
18	107.9	2.0	2158	1295	863	432
19	108.2	2.0	2164	1298	866	432
20	79.2	2.0	1584	950	634	316

(f) Composition and preparation of fertilizer used in pot experiment:

Gerretsen's formula for pot experiment (1948 - 1949), which was designed for determination of available soil phosphate, was used. The amounts of nutrient salts required were modified in accordance with the weight of soil used. Thus each pot, containing 2 kg. soil, was given the following constituents:

A. Macronutrients:

KNO_3	0.655 g.
$Ca(NO_3)_2$	0.435 g.
$CaCO_3$	0.035 g.
$CaSO_4$	0.035 g.
K_2SO_4	0.073 g.
$Mg(NO_3)_2$	0.182 g.

B. Micro-nutrients:

$MnSO_4$	18.18 mg.
$CuSO_4$	0.73 mg.
H_3BO_4	0.73 mg.
$ZnSO_4$	0.73 mg.
KI	0.18 mg.
$(NH_4)_2MoO_4$	0.035 mg.
Ferric citrate	34.545 mg.

As already indicated the nutrients were supplied in liquid form. For this purpose stock solutions of macro- and micro-nutrients, corresponding to the above formula, were prepared in separate bottles and mixed and diluted as required.

Plate V

The seedlings before
being thinned out.

Plate VI

Placement of pots.
A tensiometer is
installed among turnip plants.

(g) Establishment of plants and glasshouse management:

(i) Sowing seeds and selecting seedlings:

Seeds of all species were sown on 3rd January, 1963. The depths of sowing were as follows:

Millet	-	$\frac{1}{2}$ inch.
Turnip	-	$\frac{1}{2}$ inch.
Lucerne	-	$\frac{3}{4}$ inch.

About 30 seeds were sown in each pot at 5 positions, with the object of obtaining enough plants, from which 5 good plants could be selected in each pot.

The seedlings were thinned out to 15 plants per pot when the first leaf started to appear, and then to 5 plants per pot at the appearance of second leaf. At the two-leaf stage, the plants were relatively strong. Weeds were pulled out by hand as soon as they appeared above ground level. The surface of the soil was then covered with glass wool approximately $\frac{1}{2}$" thick to prevent too much disturbance of the soil at watering times and also to prevent excessive heating and evaporation (Plates: IV and V).

(ii) Placing of pots:

The pots were placed on trays, which were in turn placed on benches in the glasshouse (Plate: VI). To avoid mixing of drainage water from the different soils, the pots containing the same soil were placed in the same tray, regardless of plant species. It was assumed that root exudates of one species had no harmful effect on other species.

This practice was adopted due to the shortage of trays. At young stages of growth, all plants were kept in the smaller compartment of the glass-house, but as they matured, they were distributed over both compartments to avoid overcrowding. The pots were shifted around twice a week to mihimize the effect of varying light intensity on plant growth.

(iii) Watering:

As previously indicated (section "e" above), the water contents of the potted soils were adjusted to 60% W.H.C. of the undiluted soils, before the seeds were sown. The next watering was not carried out until germination had started. This took place approximately four days after sowing. Thereafter the plants were watered as often as required. At the young stage of development, especially before the soil surface was protected with a layer of glass wool, extra precaution was taken during watering to avoid disturbance of the soil surface, with consequent damage of the young seedlings. This was carried out by holding a clean metal tin with perforated bottom approximately 2" above the soil surface, into which water was poured from a measuring cylinder. At later stages of plant growth the use of the perforated tin was abandoned, but application of water at high pressure was avoided. When watering, the whole surface of the soil was wetted to achieve more even distribution of water throughout the potted soil. Any drainage water from the pots into the trays was equally redistributed among the pots concerned.

Apart from bulk watering of the soil, the leaves of the plants were sprayed with water at least once a day in the afternoon, or twice a day if the temperature was excessively high, i.e: just before mid-day and

again in the afternoon. This practice kept the plants in fresh condition.

Tap water was used for watering throughout the experiment. It gave a negative phosphate test.

(iv) Determination of water requirement:

Water requirement was determined by installing tensiometers in the pots among the plants (Plates: VI & VII). Five tensiometers of Gallenkamp type were available for use, although one for each soil would have been ideal. At the beginning the diluted soils were grouped into 5 categories according to their water holding capacities. The soil with the lowest water holding capacity in each group was chosen for installation of the tensiometer. The assumption here was that the soil with the lowest water holding capacity would require water before the others and excessive drying out of any member of the group would thereby be obviated. Table: 5 shows the grouping of the soils based on their water holding capacities. The tensiometer pot was buried at 1" below the soil surface. The lower end of the tensiometer pot did not reach the gravel underneath the potted soil.

Table: 5

Grouping of soils according to water holding capacity (W.H.C.) of the diluted soils.

	Group I W.H.C. 110 - 120%	Group II W.H.C. 120 - 130%	Group III W.H.C. 130 - 140%	Group IV W.H.C. 140 - 150 %	Group V W.H.C. over 150%
Serial No. of Soils	1 2* 8	4 5* 11 20	3 9 12*	6* 7 10 15	13 14 16* 17 18 19

* Indicates siting of tensiometers.

Plate VII

Installation of tensiometers among mature turnip plants.

The tensiometers were operated between the readings of 0 and 15 cm. Hg. Each group of soil was brought back to a moisture tension of 0 to 3 cm. Hg. as soon as the tensiometer gauge of that group showed a reading of 15 cm. Hg. Approximately 250 ml. of water per pot was required at each watering.

The frequency of watering at younger stages of plant growth varied inversely with the W.H.C. of the soil. However, as the plants grew bigger (approximately 1½ months after germination), the water requirement depended primarily on the size of plant, regardless of species or W.H.C. New regrouping of soils, therefore, was considered necessary, which was based on plant sizes and not on W.H.C. of soils. The following arrangement was the result of such regrouping:

One tensiometer was now installed in each of the groups,

Millet Pot nos. 1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 19;

Turnip Pot nos. 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, 19, 20;

Lucerne Pot nos. 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 19;

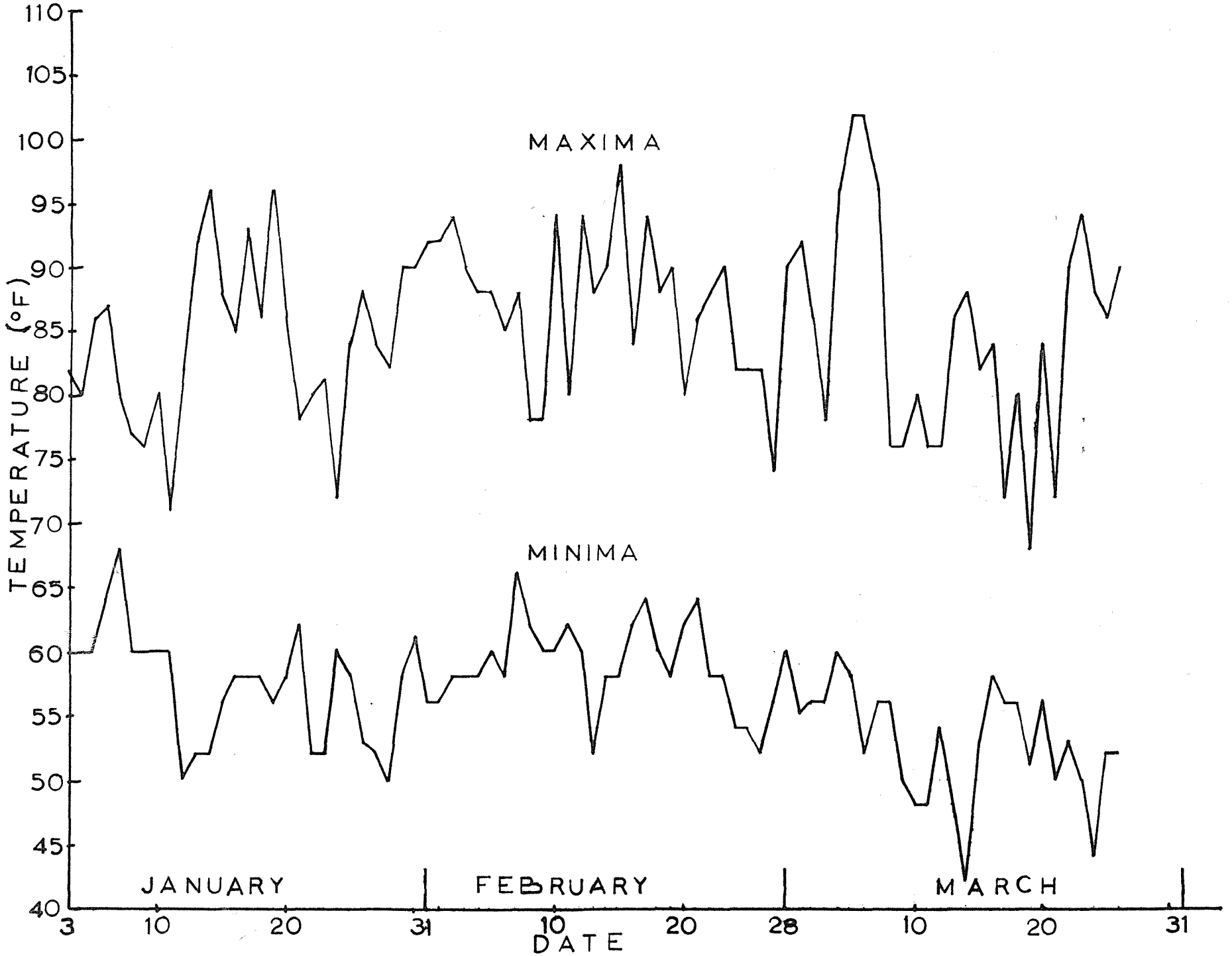
while the other two tensiometers were employed for the remaining soils. This system of grouping was satisfactory throughout the rest of the experimental period.

(v) Climatic conditions in glasshouse:

The glasshouse was not equipped for the control of climate. However, some attempt was made to offset adverse effects arising from high temperatures and low humidity. During hot days, the doors and windows were all widely opened to afford maximum ventilation. The floor was kept constantly moist by spraying water as many times as

Figure 1.

MAXIMUM AND MINIMUM DAILY TEMPERATURES



necessary. This helped reduce temperature and also kept the humidity constantly high. It also reduced excessive evaporation from the soil and from the plant leaves. The maximum and minimum temperatures of each 24-hour day in the glasshouse were recorded; these data are shown in Figure: 1.

(vi) Pests and diseases and means of control:

Damping off: This disease caused by Pithium species, and it affected mainly the turnips and lucerne as soon as germination started. The millet was not infected. This disease was eradicated by watering the plants with a mercuric oxide preparation.

Thrips and sucking insects: These pests attacked the millet and lucerne. Symptoms appeared approximately 6 weeks following germination. The plants were immediately treated with metasystox spray at the rate of 1 ml. per pint of water. The spraying was carried out with a hand spray-gun.

White butterfly caterpillar: This pest attacked the turnips mostly, but also the lucerne slightly. The symptoms were noticed approximately 7 weeks after germination. The grubs were eliminated by spraying with lethaline at 1 ml. per pint of water. The spray-gun mentioned above was employed for this operation.

Rust: This infected the lucerne only and was noted about ten weeks after germination. The plants were immediately sprayed with Zineb at 1 ml. per pint of water. This did not cure the disease, but prevented its spread.

General: The glasshouse was kept free from weeds and organic

debris which could have become the source of inoculum of pests and diseases.

(h) Harvesting of crops and determination of yields:

The millet was harvested on 25th February when the bottom leaves started to become yellow and die off. The turnips were harvested on 2nd March when symptoms of phosphate deficiency had become very marked and the bottom leaves had died off. The lucerne was harvested on 26th March when approximately 50% of the plants had flowered.

The turnips were pulled out carefully, washed under the tap and the total fresh weights recorded. The millet and the lucerne were cut above ground level and the fresh weight of top growth was recorded. The roots of these two species were harvested separately from the tops. The soil was removed from the roots by loosening and shaking, and finally by washing on a sieve.

The relative abundance and size of nodule produced by the lucerne roots growing in the different soils was estimated by counting the number of nodules per 4 sq. inch. of feeding root. The counting was carried out with the root uniformly draped over the palm of the hand. The following groupings were made on this basis.

(i) No. of nodules:

0	(no nodules) ≡	0
1 - 10 nodules	≡	1
10 - 20 nodules	≡	2
20 - 40 nodules	≡	4
40 - 80 nodules	≡	8
more than 80 nodules	≡	10

(2) Size of nodules: (continued overleaf)

(2) Size of nodules:

large : more than 50% are in clusters and had more than 2 mm. in diameter.

medium : smaller than 2 m.m. in diameter but bigger than pin head.

small : mostly of pin head size.

To obtain dry-matter yield values, the harvested crops (tops separately from roots) were dried at 160°F (71°C) for 24 hours. The bigger and thicker stems or roots were split or cut up into small pieces to expedite the drying.

4. Chemical Analyses for Soil Phosphate:

(a) Preparation of soil samples:

The soil samples employed had previously been air-dried and ground to pass a 2 mm. screen. For chemical analyses sub-samples were ground to pass an 80-mesh sieve.

(b) Determination of Aluminium-bound Soil Phosphate:

The simplified procedure of Fife (1962) was employed.

(i) Reagents:

0.5 M.NH_4F: dissolve 18.5 g. of NH_4F crystals in 1 litre of distilled water, and adjust to pH8.5 by adding strong NH_4OH.

5% boric acid: dissolve approximately 50 g. boric acid in 1 litre of warm distilled water.

Ammonium molybdate solution: dissolve 15 g. of $(NH_4)_2MoO_4$ in about 350 ml. distilled water in a 1 litre measuring flask. Add 350 ml. of 10 N.HCl,

rotating the flask during the acid addition. Make up to the mark with distilled H_2O and mix well. Make fresh every week.

Stannous Chloride stock solution: dissolve 10 g. $SnCl_2$ in 25 ml. concentrated HCl and store in a brown bottle. Dilute $SnCl_2$: dilute 0.6 ml. of the above to 100 ml. Make up fresh each time.

(ii) Procedure:

Weigh 0.05 g. of soil into a 50 ml. centrifuge tube. Add 25 ml. of 0.5 M.NH_4F. (pH 8.5). Stopper and shake for 24 hours on an end-over-end shaking machine (40 r.p.m.). Centrifuge for 3 - 4 minutes. Pipette 20 ml. of the supernatan solution into a 50 ml. flask. Add 15 ml. of 5% boric acid solution, followed by 10 ml. $(NH_4)_2MoO_4$ solution and 5 ml. diluted $SnCl_2$ solution. Measure the colour intensity on the Beckman spectrophotometer at 815 mu.

(c) Determination of Aluminium-bound and Iron-bound Soil Phosphates:

The following procedure recommended by Fife (prîv. com.) was adopted.

(i) Reagents:

0.5 M.NaCl.

1.0 N.NaOH.

Acetone.

I.0 N.HCl.

(ii) Procedure:

Weigh out 0.25 g. of soil into a centrifuge tube. Add 20 ml. of 0.5 N.NaCl, shake gently by hand and centrifuge. Pour

off and discard the supernatan liquid. Repeat 5 times.

To the soil residue add 10 ml. acetone, shake gently and centrifuge. Pour off the clear liquid and leave the soil residue to dry overnight. Add exactly 25 ml. of 1 N.NaOH solution and shake for 40 hours. Centrifuge for 3 - 4 minutes. Take a 10 ml. aliquot of the supernatan liquid and add 10 ml. of 1 N.HCl, the acid to be slightly stronger than the NaOH. Shake vigorously by hand and centrifuge to separate the precipitated organic matter. Take a 10 ml. aliquot of the clear liquid (equivalent to a 5 ml. aliquot of the original extract). Add 25 ml. water, 10 ml. $(NH_4)_2MoO_4$ and 5 ml. dilute $SnCl_2$. Read the colour intensity on the Beckman spectrophotometer at 815 mu.

(d) Determination of Iron-bound Soil Phosphate:

This was found by difference between the values obtained by methods 5 (b) and 5 (c) above.

(e) Determination of Calcium-bound Soil Phosphate:

The procedure devised by Fife (priv. com.) was employed.

(i) Reagents:

0.01 N.HCl.

All the reagents used in method 5 (c) above.

(ii) Procedure:

Shake 0.25 g. of soil with 25 ml. 0.01 N.HCl for a minimum of 6 hours in a 50 ml. straight sided centrifuge tube. Centrifuge and pipette a 10 ml. aliquot of the clear extract into a 50 ml. flask. Make up to 35 ml. by addition of water (pipetted) and develop colour in the usual way.

Pour away the remaining supernatan liquid and wash the soil residue in the

tube twice by centrifugation with 20 ml. portions of approximately 0.5 N.NaCl. Pour away the supernatan liquid after each centrifugation. Add about 20 ml. acetone and recentrifuge. Pour off the excess of acetone and leave the soil residue to dry at room temperature.

Add 25 ml. of 1 N.NaOH and shake as in the determination of Iron-bound and Aluminium-bound Phosphates and proceed to the colour development as before.

To obtain the value for Calcium-bound phosphate, subtract the result obtained in method 5 (c) above from the sum of the results obtained in method 5 (c).

(f) Determination of available Soil Phosphate using the Truog method: (see Truog: Jour. Amer. Soc. Ag. 22. No. 10. 1930).

(i) Reagent:

Extracting solution: To a 2 litre glass container add 6 g. of $(NH_4)_2SO_4$, 40 ml. of 0.1 N.H_2SO_4 and dilute with distilled water to 2 litres.

Stannous Chloride Solution: Weigh out 2.5 g. of $SnCl_2$, and dissolve in 10 ml. of concentrated HCl. Add 90 ml. distilled water. Keep in a brown bottle in the dark to prevent oxidation from the air, (or use a $\frac{1}{2}$" layer of paraffin oil on top of the container.)

Ammonium molybdate-sulphuric acid solution: Dissolve 25 g. of $(NH_4)_2MoO_4$ in 200 ml. of water heated to 60°C. and filter. Dilute 280 ml. of concentrated H_2SO_4 to 800 ml. After both solutions have

cooled, add the ammonium molybdate solution slowly with shaking to the sulphuric acid solution. After the combined solution has cooled to room temperature, dilute with water to 1 litre.

(ii) Procedure:

Transfer 0.15 g. of soil to 50 ml. centrifuge tube and add 30 ml. of extracting solution (soil extractant ratio 1 : 200) and shake for 30 minutes. Centrifuge for 3 - 4 minutes, and then pipette 20 ml. of the clear solution into a small flask. Add 0.8 ml. of the ammonium molybdate-sulphuric acid solution and swirl to mix. Add 1 drop of $SnCl_2$ solution and again swirl to mix. Allow 15 minutes for the colour to develop and read the colour intensity on the Beckman spectrophotometer at 815 mu.

V. RESULTS

1. Microbiological Assays:

(a) The growth curves for the fungi:

The growth curves for *A. niger*, *P. lilliacum* and *C. geniculata* are shown in Figures: 2, 3 and 4. The data from which these curves are derived, are given in the appendices. Application of the "least mean square" method has shown that the data of *A. niger* and *P. lilliacum* are straight lines, whereas those of *C. geniculata* form a part of a parabolic curve. The linear regression equation calculated from the data of *A. niger* and *P. lilliacum* are $P = 18.36284\ W$ and $P = 16.84533\ W$ respectively, where P is the amount of phosphate added to each flask expressed as µg. of P, and W is the corresponding dry weight of fungal mycelium expressed as cg. The quadratic regression equation calculated from the data of *C. geniculata* is $P = 5.194065\ W + 0.120460\ W^2$.

Unfortunately, the growth curve for the *Fusarium* species could not be obtained due to the inability of the fungus to grow on the standard phosphate medium. The reason for this is unknown, except that the fungus actually grew satisfactorily in the same medium after the addition of soil as a source of phosphate.

To compare the responses of the fungi to the increased amounts of phosphate added to the standard growth series, the ordinary correlation coefficients for the added phosphate against the resulting

mycelial dry weight were calculated. The results are as follows:

$r = +0.998$ for *A. niger*

$r = +0.881$ for *P. lilliacum*

$r = +0.971$ for *C. geniculata*.

The details of the calculation are given in the appendices. Plate VIII illustrates growth differences found for *A. niger* for a range of phosphate concentrations lying between zero and 725.8 µg. of P per flask. The pH of the *A. niger* medium, before and after incubation, were measured in the preliminary experiment and the results are presented in the appendices.

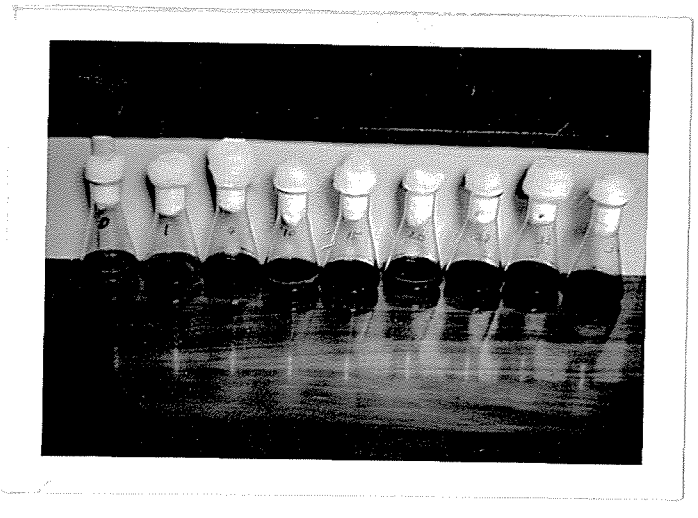


Plate VIII.

Figure 2.

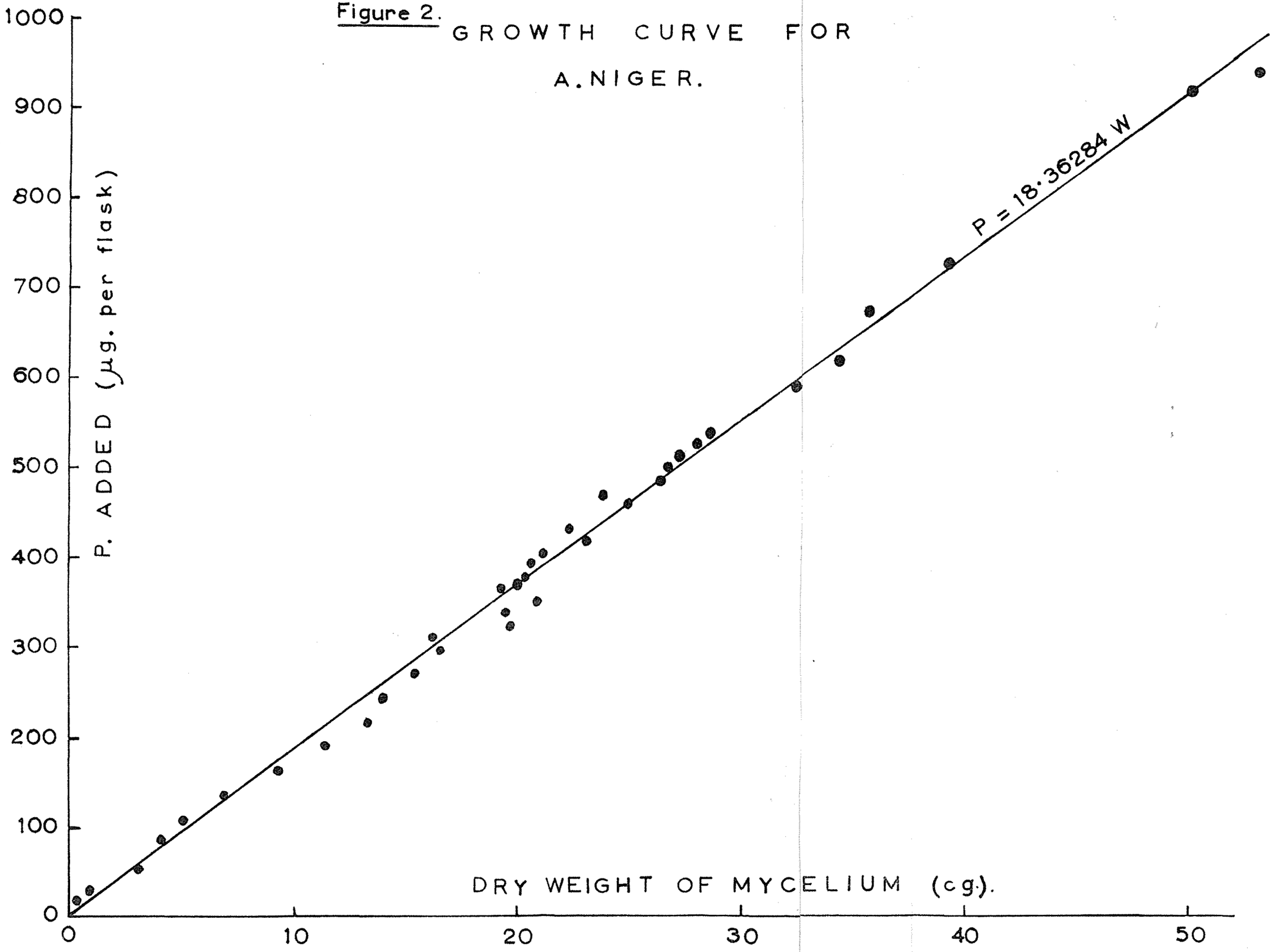

GROWTH CURVE FOR A. NIGER.
P = 18·36284 W
P. ADDED (µg. per flask)
DRY WEIGHT OF MYCELIUM (c.g.).
0
100
200
300
400
500
600
700
800
900
1000
0
10
20
30
40
50

Figure 3.

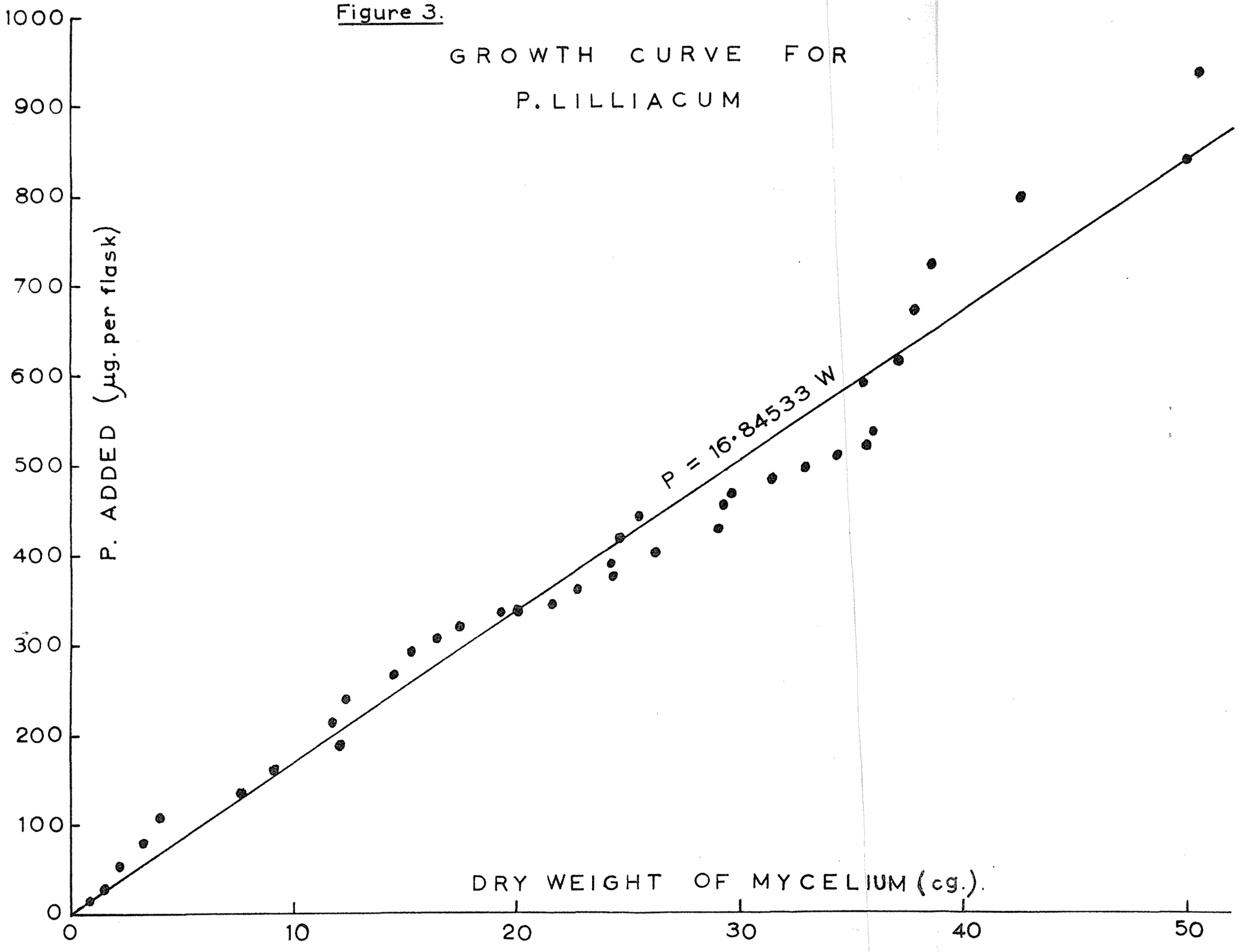
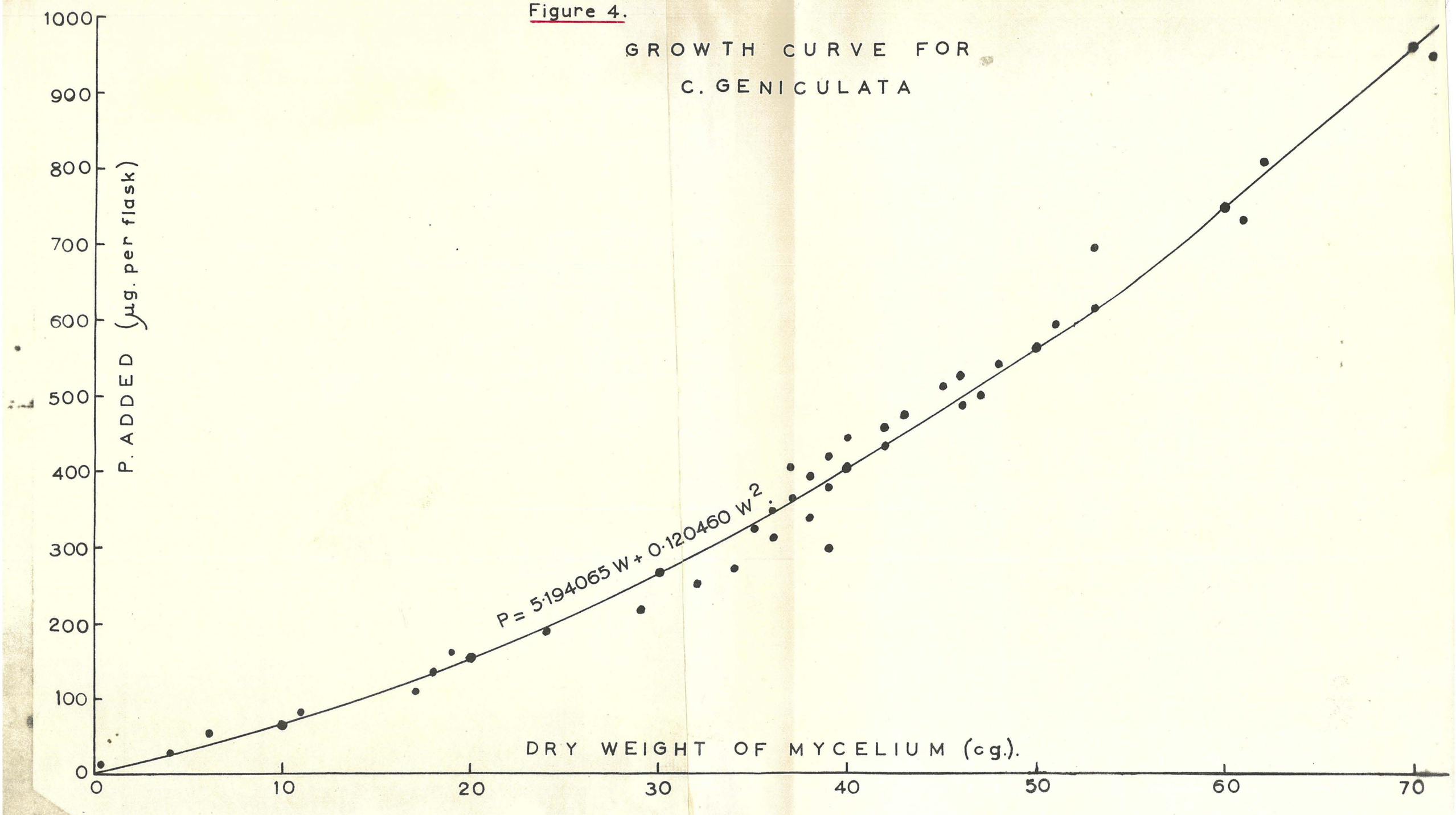

GROWTH CURVE FOR
P. LILLIACUM
P = 16.84533 W
P. ADDED (µg. per flask)
DRY WEIGHT OF MYCELIUM (cg.).
0
100
200
300
400
500
600
700
800
900
1000
0
10
20
30
40
50

Figure 4.


GROWTH CURVE FOR
C. GENICULATA
P. ADDED (µg. per flask)
1000
900
800
700
600
500
400
300
200
100
0
P = 5·194065 W + 0·120460 W²
DRY WEIGHT OF MYCELIUM (cg.).
0
10
20
30
40
50
60
70

(b) Assays of Soil Phosphate:

The dry weights of fungal mycelia obtained from 1 g. of soil added to the media to provide the source of phosphate are given in Tables: 6,7,8 and 9.

Analyses of variances, which are included with each table, were calculated according to the following criteria: If mean and variance are obviously related, - i.e. if the range of variance drops as the mean drops - , the data must be split into groups according to the mean level, and an analysis of variance is done for each group. If inspection shows no obvious differences in ranges of replicates between highest and lowest means, all data can be combined in one analysis. In some cases only one or two treatments may have to be omitted;- if only one, no analysis of the omitted data is required. In those cases where it is impossible to separate data for analyses, a common Standard Error (S.E.) is used for comparing all treatments. This will be too high for comparing low means and too low for comparing high means. Thus real differences between low means can be missed, and false claims can be made for non-existent differences between high means.

Table: 10. shows the ranking of the soils by the different fungi according to the mean dry weights of mycelia, and Table: 11. shows the amounts of phosphate corresponding to mean mycelial dry weights. The standard errors of phosphate are taken into account in these values.

Table: 6

Mycelial dry weights of A. niger
with soil as the source of phosphate.
(Results expressed as cg.)

Serial No. of soils.	1st replicate (cg.)	2nd replicate (cg.)	3rd replicate (cg.)	4th replicate (cg.)	Totals of replicates (cg.)	Means ± S.E. (cg.)
1	35.02	35.39	36.04	35.27	141.72	35.43 ± 0.73
2	32.60	32.65	36.80	34.80	136.85	34.21 ± "
3	12.00	11.55	12.90	12.84	49.29	12.32 ± "
4	26.29	24.04	22.55	23.34	96.22	24.07 ± "
5	13.14	13.53	13.35	13.44	53.46	13.36 ± "
6	20.15	21.45	20.84	19.45	81.89	20.47 ± "
7	25.14	24.14	28.64	24.14	102.06	25.51 ± "
8	15.14	13.09	12.45	10.40	51.08	12.77 ± "
9	20.19	23.29	18.84	20.94	83.26	20.81 ± "
10	13.75	13.10	12.50	13.55	52.90	13.22 ± "
11	12.70	11.75	12.45	14.05	50.95	12.74 ± "
12	10.00	10.35	11.70	11.45	43.50	10.87 ± "
13	33.80	32.00	33.95	37.95	137.70	34.42 ± "
14	9.20	13.15	12.05	11.25	45.65	11.41 ± "
15	12.70	13.30	15.60	15.05	56.65	14.16 ± "
16	12.70	12.70	11.25	11.35	48.00	12.00 ± "
17	19.85	18.15	18.80	21.50	78.30	19.57 ± "
18	15.25	15.15	17.95	17.60	65.95	16.49 ± "
19	37.40	37.20	37.00	38.25	149.85	35.43 ± "
20	27.80	23.55	27.95	26.60	105.90	26.47 ± "
Sums	404.82	399.53	413.61	413.22	1631.18 (Grand total)	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	6244.89	19	328.68	155.77	1.78 (2.24)	**
Error	126.85	60	2.11			
Total	6371.74	79				

(Continued.....)

S.E. = Standard Error.

S.S. = Sum of Squares (corrected.)

df. = degree of freedom.

M.S. = Mean of Squares.

F. ratio = ratio of mean squares.

** = significant at 1% level.

* = significant at 5% level.

Standard Error (S.E.) of the means = $V\frac{\text{Error mean square}}{4}$ =

$V\frac{2.11}{4}$ = V 0.53 = 0.73.

Detectable differences:

$$\underset{(o.o1)}{do.o5} = \underset{(o.o1)}{to.o5}\ (df60)\ V\left(\frac{2 \times 2.11}{4}\right) = \underset{(2.66)}{2}\ V1.057 = \underset{(2.66)}{2 \times 1.028} = \underset{(2.74)}{2.06}.$$

Any two means, which differ by 2.06 or above and/or by 2.74 or above, are significantly different at 5% level (*) and/or 1% level (**).

Table: 7.

Mycelial dry weights of P. lilliacum with soil as the source of phosphate:
(Results expressed as cg.)

Serial No. of soils.	1st replicate (cg.)	2nd replicate (cg.)	3rd replicate (cg.)	4th replicate (cg.)	Totals of replicates (cg.)	Means ± S.E. (cg.)
1	28.00	30.30	27.50	29.75	115.55	28.89 ± 1.31
2	29.95	30.35	32.50	32.45	125.25	31.31 ± "
3	16.10	15.15	16.95	22.40	70.60	17.65 ± "
4	26.25	19.60	34.20	22.65	102.70	25.68 ± "
5	13.45	9.60	14.75	14.65	52.45	13.11 ± "
6	19.25	20.20	23.10	26.60	89.15	22.29 ± "
7	12.90	14.95	14.40	14.15	56.40	14.10 ± "
8	10.85	9.80	14.55	9.35	44.55	11.14 ± "
9	16.50	11.45	20.00	11.75	59.70	14.92 ± "
10	12.25	15.60	17.35	12.80	58.00	14.50 ± "
11	17.45	15.25	16.20	16.15	65.05	16.26 ± "
12	11.30	9.85	11.60	91.50	41.90	10.48 ± "
13	17.50	23.50	19.55	23.10	83.65	20.91 ± "
14	7.25	6.30	7.60	5.80	26.95	6.74 ± "
15	7.00	7.85	12.50	8.00	35.35	8.84 ± "
16	7.60	7.30	8.70	9.40	33.00	8.25 ± "
17	14.45	12.85	12.35	13.55	53.20	13.30 ± "
18	10.80	12.20	11.35	11.55	45.90	11.48 ± "
19	33.10	30.50	28.85	36.70	129.15	32.29 ± "
20	18.00	21.50	20.50	23.65	83.65	20.91 ± "
Sums	329.95	324.10	364.50	353.60	1372.15 (Grand total).	

Analysis of variance:

Source of variance	S.S.	df	M.S.	F. ratio	F. ratio 5% (1%)	Results.
Soils	4471.79	19	235.36	34.56	1.78 (2.24)	**
Error	408.74	60	6.81			
Total	4880.53	79				

$$\text{S.E. of the means} = \sqrt{\frac{6.81}{4}} = \sqrt{1.7025} = 1.31$$

Detectable differences:

$$d_{o.o5\,(o.o1)} = t_{o.o5\,(o.o1)}\,(df60)\,\sqrt{\frac{2 \times 6.81}{4}} = 2 \times \sqrt{3.405}_{(2.66)} = 2 \times 1.845_{(2.66)} = 3.69_{(4.91)}.$$

Any two means, which differ by 3.69 or above and/or by 4.91 or above, are significantly different at 5% level and/or at 1% level.

Table: 8:

Mycelial dry weights of C. geniculata with soil the source of phosphate.

(Results expressed as cg.)

Serial No. of soils.	1st replicate (cg.)	2nd replicate (cg.)	3rd replicate (cg.)	4th replicate (cg.)	Totals of replicates (cg.)	Means ± S.E. (cg.)
1	59.70	55.60	52.70	58.90	226.90	56.73 ± 1.02
2	57.60	57.95	56.25	57.85	229.65	57.41 ± "
3	30.20	36.90	33.50	35.70	136.30	34.08 ± "
4	50.40	58.45	59.10	57.00	224.95	56.24 ± "
5	41.35	45.85	46.05	48.80	182.05	45.51 ± "
6	45.80	44.55	43.90	44.55	178.80	44.70 ± "
7	38.55	39.65	40.05	37.90	156.15	39.04 ± "
8	22.90	24.75	26.50	23.20	97.35	24.34 ± "
9	45.60	45.10	42.50	41.05	174.25	43.56 ± "
10	34.70	35.75	31.60	34.40	136.45	34.11 ± "
11	34.55	34.45	34.55	32.00	135.55	33.89 ± "
12	31.15	31.50	32.05	33.10	127.80	31.95 ± "
13	69.50	72.20	68.80	70.15	280.65	70.14 ± "
14	22.25	23.90	26.46	25.80	98.35	24.59 ± "
15	28.40	31.80	29.50	30.90	120.60	30.15 ± "
16	32.85	30.30	31.90	30.20	125.25	31.31 ± "
17	48.60	49.60	44.46	46.46	189.00	47.50 ± "
18	36.20	36.65	40.00	37.20	150.05	37.51 ± "
19	68.85	66.75	68.50	65.50	269.70	67.43 ± "
20	39.70	39.60	37.25	41.64	158.19	39.55 ± "
Sums	838.85	861.30	845.50	852.34	3397.99 (Grand total)	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	13353.29	19	702.81	169.35	1.78 (2.24)	**
Error	248.98	60	4.15			
Total	13602.28	79				

(Continued.....)

$$\text{S.E. of the means} = \sqrt{\frac{4.15}{4}} = \sqrt{1.04} = 1.02$$

Detectable differences:

$$\underset{(0.01)}{d_{0.05}} = \underset{(0.01)}{t_{0.05}}\,(df 60)\ \sqrt{\frac{2 \times 4.15}{4}} = \underset{(2.66)}{2 \times 1.44} = \underset{(3.83)}{2.88}$$

Any two means, which differ by 2.88 or above and/or by 3.83 or above, are significantly different at 5% level and/or at 1% level.

Table: 9

Mycelial dry weights of Fusarium species with soil as the source of phosphate.

(Results expressed as cg.)

Serial No. of soils.	1st replicate (cg.)	2nd replicate (cg.)	Totals of replicates (cg.)	Means ± S.E. (cg.)
1	26.80	28.40	55.20	27.60 ± 0.92
2	26.05	22.35	48.40	24.20 ± "
3	15.35	15.25	30.60	15.30 ± "
4	29.80	26.50	56.30	28.20 ± "
5	23.70	21.60	45.30	22.70 ± "
6	14.45	11.50	25.95	13.00 ± "
7	16.10	16.70	32.80	16.40 ± "
8	10.40	14.00	24.40	12.20 ± "
9	8.95	12.15	21.10	10.60 ± "
10	8.00	11.05	19.05	9.50 ± "
11	9.30	10.65	19.95	10.00 ± "
12	8.30	9.15	17.45	8.70 ± "
13	53.55	53.65	107.20	53.60 ± "
14	14.85	8.30	23.15	11.60 ± "
15	8.60	7.80	16.40	8.20 ± "
16	23.00	24.90	47.90	24.00 ± "
17	30.80	29.05	59.85	29.90 ± "
18	22.50	23.10	45.60	22.80 ± "
19	51.85	53.75	105.60	52.80 ± "
20	29.20	31.35	60.55	30.30 ± "
Sums	431.55	431.20	862.75 (Grand total)	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	6628.13	19	348.85	103.8	1.96 (2.64)	**
Error	67.24	20	3.36			
Total	6695.37	39				

(Continued ...)

$$\text{S.E. of the means} = \sqrt{\frac{3.36}{4}} = \sqrt{0.84} = 0.92.$$

Detectable differences:

$$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df20) \times \sqrt{\frac{2 \times 3.36}{4}} = \underset{(2.845)}{2.086} \times \sqrt{1.68} = \underset{(2.845)}{2.086} \times 1.296 = 2.$$
$$(3.$$

Any two means, which differ by 2.70 or above and/or by 3.70 or above, are significantly different at 5% level and/or at 1% level.

Table: 10.

Ranking of soils by the different fungi according to the phosphate status of the soils.

Serial No. of soils.	Ranks of the soils by the mean dry weights of mycelia			
	According to A. niger.	According to P. lilliacum.	According to C. geniculata.	According to Fusarium species.
1	2	3	4	6
2	4	2	3	7
3	17	8	14	12
4	7	4	5	5
5	13	14	7	10
6	9	5	8	13
7	6	12	11	11
8	15	16	20	14
9	8	10	9	16
10	14	11	13	18
11	16	9	15	17
12	20	17	16	19
13	3	6	1	1
14	19	20	19	15
15	12	18	18	20
16	18	19	17	8
17	10	13	6	4
18	11	15	12	9
19	1	1	2	2
20	5	7	10	3

Example: Soil No.1 is ranked 2nd by A.niger,
3rd by P.lilliacum,
4th by C.geniculata,and
6th by Fusarium species,
etc., etc.

The data in this table are used to obtain results presented in Table: 26.

Table: 11.

The amounts of "available" soil phosphate as estimated by A.niger, P. lilliacum,& C.geniculata.
(Results expressed as µg. of P per g. of soil)

Serial No. of soils	A.niger (µg.of P per g. of soil).	P.lilliacum (µg. of P per g. of soil).	C.geniculata (µg. of P per g. of soil).
1	650.60± 13.40	486.66 ± 21.98	682.34 ± 19.48
2	628.19 ± "	527.43 ± "	695.34 ± 19.53
3	226.23 ± "	297.32 ± "	316.92 ± 13.80
4	441.99 ± "	432.59 ± "	673.12 ± 18.25
5	245.33 ± "	220.84 ± "	485.87 ± 16.61
6	375.89 ± "	375.48 ± "	472.87 ± 16.18
7	468.44 ± "	237.52 ± "	386.37 ± 15.02
8	234.49 ± "	187.66 ± "	197.79 ± 11.40
9	382.13 ± "	251.33 ± "	454.82 ± 16.13
10	242.76 ± "	244.26 ± "	317.32 ± 14.41
11	233.94 ± "	273.91 ± "	314.38 ± 13.45
12	199.60 ± "	176.54 ± "	288.92 ± 13.27
13	632.05 ± "	352.24 ± "	956.93 ± 23.07
14	209.35 ± "	113.54 ± "	200.56 ± 11.47
15	260.02 ± "	148.91 ± "	266.10 ± 12.83
16	220.35 ± "	138.97 ± "	280.72 ± 13.11
17	359.36 ± "	224.04 ± "	518.51 ± 17.09
18	302.80 ± "	193.38 ± "	364.32 ± 14.64
19	687.87 ± "	543.94 ± "	897.95 ± 21.99
20	486.06 ± "	352.24 ± "	393.85 ± 15.14

The S.E. of phosphate estimations for A.niger and P.lilliacum methods are constant because the growth curves for both fungi are straight lines, whereas the S.E. of phosphate for C.geniculata method increases with the means of phosphate due to the parabolic characteristic of the growth curve.

The above values also reflect the amounts of phosphate in lbs./acre, assuming that 1,000,000 lbs. as the weight of soil per acre at 0"- 3" depth.

2. Pot experiment:

The fresh weights of the harvested plants are given in the appendices. Tables: 12, 13 and 14. show the dry weights of millet top, root, and total yields; Table: 16. shows the dry weights of turnip total yields, and Tables: 18, 19 and 20. show the dry weights of lucerne top, root, and total yields. Table: 19. also includes root nodule data. Each table is followed by an analysis of variance, which was done according to the criteria previously presented.

The ranking of soils in terms of dry matter yields are shown in Tables: 15, 17 and 21.

Plates IX to XVIII illustrate some of the differences in colour, size and general appearance of the various crops arising from the different levels of "available" phosphate in the pots, and Plate XIX shows ramification of roots throughout the potted soils.

Table: 12.

Dry weights of Millet top growth:

(The soils are rearranged according to descending order of mean weights.)

(Results expressed as grams.)

Serial No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
Group I:						
13	71.73	90.87	64.29	107.49	334.38	83.60 ± 4.11
9	67.75	83.80	71.80	92.60	315.95	78.99 ± "
2	58.50	68.60	65.80	78.25	271.15	67.79 ± "
10	50.38	73.10	45.43	55.34	224.25	55.86 ± "
19	66.53	41.60	58.93	54.75	221.81	55.45 ± "
1	47.42	56.00	58.30	46.30	208.02	52.00 ± "
7	49.65	45.82	48.43	47.18	191.08	47.77 ± "
11	52.33	32.88	34.00	53.40	172.61	43.15 ± "
3	40.10	38.80	43.58	50.12	172.60	43.15 ± "
12	31.52	44.60	43.25	38.10	157.47	39.37 ± "
6	26.25	24.00	22.87	22.75	95.87	23.97 ± "
20	20.38	20.80	20.13	23.88	85.19	21.29 ± "
4	18.19	14.80	23.00	22.20	78.19	19.55 ± "
8	13.40	17.62	15.74	13.20	59.96	14.99 ± "
17	15.66	14.86	13.06	15.33	58.91	14.73 ± "
15	11.13	13.84	14.60	12.80	52.37	13.09 ± "
Sums	640.92	681.99	643.21	733.69	2699.81 (Grand Total.)	
Group II:						
14	8.02	9.06	8.72	9.30	35.10	8.78 ± 0.27
18	4.40	5.04	5.01	6.40	20.85	5.21 ± "
5	4.25	4.10	4.10	4.00	16.45	4.11 ± "
16	2.93	2.19	2.19	2.37	9.68	2.42 ± "
Sums	19.60	20.39	20.02	22.07	82.08 (Grand Total.)	

(Analysis Overleaf.....)

Analysis of variance:

Group I:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soil Type	31404.88	15	2093.66	30.92	1.9 (2.4)	**
Error	3250.23	48	67.71			
Total	34655.11	63				

S.E. of the means = $\sqrt{\frac{67.71}{4}} = \sqrt{16.93} = 4.11$

Detectable differences:

$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df48)\ \sqrt{\frac{2 \times 67.71}{4}} = \underset{(2.69)}{2.02} \times \sqrt{33.86} = \underset{(2.69)}{2.02} \times 5.82 = \underset{(15.66}{11.76}$

Group II:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	86.69	3	28.89	99.60	3.5 (6.0)	**
Error	3.47	12	0.29			
Total.	90.16	15				

S.E. of the means = $\sqrt{\frac{0.29}{4}} = \sqrt{0.0725} = 0.27$

Detectable differences:

$d_{0.05} = t_{0.05\ (df12)}\ \sqrt{\frac{2 \times 0.29}{4}} = \underset{(3.06)}{2.18} \times \sqrt{0.145} = \underset{(3.06)}{2.18} \times 0.38 = \underset{(1.16)}{0.83}$

Comparison between Group I and Group II:

S.E. of the difference = $\sqrt{\left\{ (S.E._{I})^2 + (S.E._{II})^2 \right\}} = \sqrt{\left\{ (4.11)^2 + (0.27) \right.}$

$= \sqrt{16.965} = 4.12$

(Continued.....)

Detectable differences:

$$d.05 = t.05\ (60df)\ (4.12) = 2 \times 4.12 = 8.24$$
$$(.01)\quad (.01)\qquad\qquad (2.66)\qquad\quad (10.96)$$

Any mean in Group I differs by 8.24 or above and/or by 10.96 or above from any mean in Group II, the two means concerned are significantly different at 5% and/or 1% levels.

Table: 13:

Statistical Analysis of the Dry Weights of Millet root.

(The soils are rearranged according to descending order of mean weights.)

(Results expressed in grams.)

No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
13	10.73	9.86	9.04	11.69	41.32	10.33 ± 0.46
1	8.18	9.18	14.20	7.10	38.66	9.66 ± "
19	10.04	7.96	9.70	8.91	36.61	9.15 ± "
9	7.40	8.52	7.67	8.62	32.21	8.05 ± "
2	7.40	8.17	7.42	8.10	31.09	7.79 ± "
10	7.20	7.60	6.80	7.40	29.00	7.25 ± "
11	6.50	5.43	5.80	6.75	24.48	6.12 ± "
7	6.90	5.30	6.40	5.73	24.33	6.08 ± "
3	5.85	5.30	6.00	6.53	23.68	5.92 ± "
12	4.83	5.43	5.20	4.90	20.36	5.09 ± "
20	5.60	4.70	4.40	4.78	19.48	4.87 ± "
17	4.80	3.73	3.48	4.00	16.01	4.00 ± "
4	3.20	2.86	4.90	4.80	15.76	3.94 ± "
6	3.90	3.85	3.28	3.10	14.13	3.53 ± "
15	2.74	3.53	4.00	2.97	13.24	3.31 ± "
8	3.18	3.90	3.20	2.56	12.84	3.21 ± "
14	2.26	2.69	2.57	2.93	10.45	2.61 ± "
5	2.30	1.50	1.33	1.30	6.43	1.36 ± "
18	1.04	1.46	1.18	1.51	5.19	1.30 ± "
16	1.20	1.11	0.71	1.13	4.15	1.04 ± "
Sums	105.25	102.08	107.28	104.81	419.42 (Grand total).	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	594.21	19	31.27	37.22	1.78 (2.24)	**
Error	50.36	60	0.84			
Total	644.57	79				

(Continued.....)

S.E. of the means = $V\frac{0.84}{4} = V0.21 = 0.46$.

Detectable differences:

$d\ 0.05 = t0.05\ (df60)V(\frac{2x0.84}{4}) = 2 \times V0.42 = 2 \times 0.648 = 1.29$

$d\ 0.01 = t0.01\ (df60)V(\frac{2x0.84}{4}) = 2.66 \times V0.42 = 2.66 \times 0.648 = 1.72$

Any two means, which differ by 1.29 or above and/or by 1.72 or above, are significantly different at 5% level and/or at 1% level.

Table: 14.

Dry weights of Millet total yields:

(The soils are rearranged according to descending order of mean weights.)

(Results expressed as grams.)

Serial No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
Group I.						
13	82.46	100.73	73.33	119.18	375.70	93.93 ± 5.51
9	75.15	95.32	79.47	101.22	351.16	87.79 ± "
2	65.90	75.77	73.22	86.35	301.24	75.31 ± "
19	76.57	49.56	68.63	63.66	258.42	64.61 ± "
10	57.58	80.70	52.23	62.74	253.25	63.31 ± "
1	55.60	65.18	72.50	53.40	246.68	61.67 ± "
7	56.55	51.12	54.83	52.91	215.41	53.86 ± "
11	58.83	38.31	39.80	60.15	197.09	49.27 ± "
3	45.95	44.10	49.58	56.65	196.28	49.07 ± "
12	36.35	50.09	48.45	43.00	177.89	44.47 ± "
Sums	610.94	650.88	612.04	699.26	2573.12 (Grand Total.)	
Group II:						
6	30.15	27.85	26.15	25.85	110.00	27.66 ± 1.27
20	25.98	25.50	24.53	28.66	104.67	27.17 ± "
4	21.39	17.66	27.90	27.00	93.95	23.69 ± "
17	29.46	18.59	16.54	19.33	74.92	18.73 ± "
8	16.58	21.52	18.94	15.76	72.80	18.20 ± "
15	13.87	17.37	19.00	15.77	66.01	16.50 ± "
14	10.28	11.75	11.29	12.23	45.55	11.39 ± "
Sums	138.71	140.24	144.35	144.60	567.90 (Grand Total)	
Group III:						
18	5.44	6.50	6.19	7.91	26.04	6.51 ± 0.37
5	6.55	5.60	5.43	5.30	22.88	5.72 ± "
16	4.13	3.30	2.90	3.50	13.83	4.66 ± "
Sums	16.12	15.40	14.52	16.71	62.75 (Grand Total)	

(Analysis Overleaf.....)

Analyses of variance:

Group I:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	10074.71	9	1119.41	9.21	2.2 (3.0)	**
Error	3644.41	30	121.48			
Total	13719.12	39				

S.E. of the means = $\sqrt{\frac{121.48}{4}}$ = $\sqrt{30.37}$ = 5.51

Detectable differences:

$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df30)\ \sqrt{\frac{2 \times 121.48}{4}} = 2.04\ (2.75)\ \sqrt{60.74} = 2.04 \times 7.79\ (2.75) = 15.89\ (21.42)$

Group II:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	788.62	6	131.44	20.28	2.6 (3.9)	**
Error	136.08	21	6.48			
Total	924.70	27				

S.E. of the means = $\sqrt{\frac{6.48}{4}}$ = $\sqrt{1.62}$ = 1.27

Detectable differences:

$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df21)\ \sqrt{\frac{2 \times 6.48}{4}} = 2.08\ (2.83) \times \sqrt{3.24} = 2.08 \times 1.8\ (2.83) = 3.74\ (5.09)$

Comparison between Group I and Group II:

S.E. of the difference = $\sqrt{\left((S.E._{I})^2 + (S.E._{II})^2\right)} = \sqrt{\left((1.27)^2 + (5.51)^2\right)}$ =

$\sqrt{31.97}$ = 5.65

Detectable differences:

$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df50)\ (5.65) = 2.008\ (2.678)\ (5.65) = 11.3\ (15.1)$

Any mean in Group I differs by 11.3 or above and/or by 15.1 or above from any mean in Group II, the two means concerned are significantly different at 5% and/or 1% levels.

(Continued.....)

Analyses of variance(Continued).

Group III:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Seils	20.08	2	10.04	18.25	4.3 (8.0)	**
Error	4.96	9	0.55			
Total	25.04	11				

$$\text{S.E. of the means} = \sqrt{\frac{0.55}{4}} = \sqrt{0.14} = 0.37$$

Detectable differences:

$$d_{0.05\,(0.01)} = t_{0.05\,(0.01)}\,(df9)\,\sqrt{\frac{2 \times 0.55}{4}} = 2.26\,(3.26) \times \sqrt{0.28} = 2.26\,(3.26) \times 0.53 = 1.2\,(1.7)$$

Comparison between Group II and Group III:

$$\text{S.E. of the difference} = \sqrt{(S.E._{II})^2 + (S.E._{III})^2} = \sqrt{(1.27)^2 + (0.37)^2} = \sqrt{1.75} = 1.32$$

Detectable differences:

$$d_{0.05\,(0.01)} = t_{0.05\,(0.01)}\,(df30)\,(1.32) = 2.042\,(2.750) \times 1.32 = 2.70\,(3.63)$$

Any mean in Group II differs by 2.70 or above and/or by 3.63 or above from any mean in Group III, the two means concerned are significantly different at 5% and/or 1% levels.

Ranking of soils by Millet top, root, and total yields according to the phosphate status of the soils.

Serial No. of soils	Ranks of the soils by the mean dry weights of plant yields		
	According to Millet top	According to Millet root	According to Millet total
1	6	2	6
2	3	5	3
3	9	9	9
4	13	13	13
5	19	18	19
6	11	14	11
7	7	8	7
8	14	16	15
9	2	4	2
10	4	6	5
11	8	7	8
12	10	10	10
13	1	1	1
14	17	17	17
15	16	15	16
16	20	20	20
17	15	12	14
18	18	19	18
19	5	3	5
20	12	11	12

The data in this table are used to obtain results presented in Table: 26.

Table: 16.

Dry weights of Turnip total yields:

(The soils are rearranged according to descending order of mean weights.)

(Results expressed in g.)

Serial No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
Group I:						
19	57.60	63.33	52.80	58.33	232.06	58.02 ± 1.97
13	51.73	56.46	65.06	50.66	223.91	55.98 ± "
11	33.00	32.10	36.95	38.12	140.17	35.04 ± "
2	30.50	34.12	35.20	30.10	129.92	32.04 ± "
7	32.00	30.80	30.25	33.30	126.35	31.59 ± "
1	34.09	28.10	32.00	31.90	126.09	31.52 ± "
9	30.30	32.15	33.10	28.62	124.17	31.05 ± "
10	32.80	24.80	39.21	25.12	121.93	30.48 ± "
3	28.80	31.50	29.70	29.80	119.80	29.95 ± "
12	34.00	24.20	21.65	33.10	112.95	28.24 ± "
6	27.00	26.10	29.60	29.80	112.50	28.12 ± "
15	26.32	21.30	21.23	22.00	90.85	22.71 ± "
Sums	418.14	404.96	426.75	410.85	1660.70 (Grand total)	
Group II:						
4	19.42	15.03	20.20	13.30	67.95	16.99 ± 1.29
8	14.10	16.00	14.52	15.35	59.97	14.99 ± "
20	14.80	15.11	10.50	13.10	53.51	13.38 ± "
17	10.93	11.17	19.43	11.30	52.83	13.21 ± "
18	8.53	11.67	11.60	13.24	45.04	11.26 ± "
14	12.66	9.33	10.57	9.49	42.05	10.51 ± "
Sums	80.44	78.31	86.82	75.78	321.35 (Grand total)	
Group III:						
5	6.64	6.00	5.75	5.15	23.54	5.89 ± 0.47
16	5.40	3.06	2.93	4.40	15.79	3.95 ± "
Sums	12.04	9.06	8.68	9.55	39.33 (Grand total)	

(Analyses Overleaf.....)

Analyses of variance:

Group I:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	5213.79	11	473.98	30.60	2.15 (2.6)	**
Error	557.48	36	15.49			
Total	5771.27	47				

S.E. of the means $= \sqrt{\frac{15.49}{4}} = \sqrt{3.87} = 1.97$

Detectable differences:

$d0.05\ (0.01) = t0.05\ (0.01)\ (df36) \sqrt{\frac{2 \times 15.49}{4}} = 2.03\ (2.73) \times \sqrt{7.74} = 2.03\ (2.73) \times 2.78 = 5.64\ (7.59)$

Group II:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	113.44	5	22.69	3.41	2.8 (4.3)	*
Error	119.63	18	6.65			
Total	233.07	23				

S.E. of the means $= \sqrt{\frac{6.65}{4}} = \sqrt{1.66} = 1.29$

Detectable differences:

$d0.05\ (0.01) = t0.05\ (0.01)\ (df18) \sqrt{\frac{2 \times 6.65}{4}} = 2.10\ (2.878) \times \sqrt{3.32} = 2.101\ (2.878) \times 1.822 = 3.83\ (5.24)$

Comparison between Group I and Group II:

S.E. of the difference $= \sqrt{(S.E._{I})^2 + (S.E._{II})^2} = \sqrt{(1.97)^2 + (1.29)^2} =$

$\sqrt{5.545} = 2.35$

Detectable differences:

$d0.05\ (0.01) = t0.05\ (0.01)\ (df54)\ (2.35) = 2.006\ (2.683) \times 2.35 = 4.714\ (6.305)$

Any mean in Group I differs by 4.714 or above and/or by 6.305 or above from any mean in Group II, the two means concerned are significantly different at 5% and/or 1% levels.

(Continued........)

Analyses of variance (Continued.)

Group III:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	6.84	1	6.84	7.77	6.0 (13.7)	*
Error	5.28	6	0.88			
Total	12.12	7				

S.E. of the means $= \sqrt{\frac{0.88}{4}} = \sqrt{0.22} = 0.47$

Detectable differences:

$$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df6)\ \sqrt{\frac{2 \times 0.88}{4}} = \underset{(3.707)}{2.447} \times \sqrt{0.44} = \underset{(3.707)}{2.447 \times 0.6633} = \underset{(2.46)}{1.62}$$

Comparison between Group II and Group III:

$$\text{S.E. of the difference} = \sqrt{\left((\text{S.E.}_{\text{II}})^2 + (\text{S.E.}_{\text{III}})^2\right)} = \sqrt{\left((1.29)^2 + (0.47)^2\right)}$$

$$= \sqrt{1.885} = 1.37$$

Detectable differences:

$$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df24)\ (1.37) = \underset{(2.797)}{2.064 \times 1.37} = \underset{(3.83)}{2.83}$$

Any mean in Group II differs by 2.83 or above and/or by 3.83 or above from any mean in Group III, the two means concerned are significantly different at 5% and/or 1% levels.

Table: 17.

Ranking of soils by Turnip total yields according to the phosphate status of the soils.

Serial No. of soils	Rank
1	6
2	4
3	9
4	13
5	19
6	11
7	5
8	14
9	7
10	8
11	3
12	10
13	2
14	18
15	12
16	20
17	16
18	17
19	1
20	15

The data in this table are used to obtain results presented in Table: 26.

Table: 18.

Dry weights of Lucerne top growth:

(The soils are rearranged according to descending order of mean weights.)

(Results expressed in g.)

Serial No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
19	28.13	25.17	27.86	27.95	109.11	26.78 ± 0.93
2	24.10	28.00	20.60	24.90	97.60	24.40 ± "
13	20.46	16.49	25.49	18.26	80.70	20.17 ± "
1	20.40	16.40	22.58	19.32	78.70	19.67 ± "
9	16.43	17.70	16.10	17.10	67.33	16.83 ± "
11	14.38	20.60	17.48	14.42	66.88	16.72 ± "
6	15.30	15.90	16.40	17.65	65.25	16.31 ± "
12	16.60	12.80	17.20	18.48	65.08	16.27 ± "
10	15.82	14.58	16.30	14.10	60.80	15.20 ± "
17	12.57	14.26	14.26	12.63	53.72	13.43 ± "
3	12.90	10.75	10.40	15.53	49.58	12.39 ± "
20	9.75	13.00	11.20	12.45	46.40	11.60 ± "
15	10.88	10.40	10.70	12.78	44.76	11.19 ± "
14	8.26	10.72	9.86	10.66	39.50	9.87 ± "
18	7.86	10.53	9.80	11.09	39.28	9.82 ± "
7	8.00	11.05	8.75	9.38	37.18	9.29 ± "
4	8.34	7.93	8.70	7.40	32.37	8.09 ± "
8	5.70	9.10	6.70	7.70	29.20	7.30 ± "
16	4.66	6.40	5.33	7.20	23.59	5.88 ± "
5	5.58	6.40	4.50	4.89	21.37	5.34 ± "
Sums	266.12	278.18	280.21	283.89	1108.40 (Grand total)	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	2701.22	19	142.17	40.97	1.78 (2.24)	**
Error	208.47	60	3.47			
Total	2909.69	79				

(Continued Overleaf....)

$$\text{S.E. of the means} = \sqrt{\frac{3.47}{4}} = \sqrt{0.8675} = 0.9315 = 0.93$$

Detectable differences:

$$d_{0.05} = t_{0.05}\,(df 60)\,\sqrt{\frac{2 \times 3.47}{4}} = 2 \times \sqrt{1.735} = 2 \times 1.32 = 2.64$$

$$d_{0.01} = t_{0.01}\,(df 60)\,\sqrt{\frac{2 \times 3.47}{4}} = 2.66 \times 1.32 = 3.51$$

Table: 19.

Dry weights of Lucerne root:

(The soils are rearranged according to descending order of mean weights.)

(Results expressed in g.)

Serial No. of soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)	Nodule gradings [1]
19	25.60	29.20	35.20	29.46	119.46	29.86 ± 1.09	10 L
2	25.40	24.90	27.20	25.30	102.80	25.70 ± "	10 L
1	21.20	20.10	22.60	21.30	85.20	21.30 ± "	10 L
13	17.73	11.46	22.26	11.86	63.31	15.83 ± "	1 M
11	13.20	16.70	19.20	10.80	59.90	14.97 ± "	8 M
9	12.75	14.50	15.70	15.70	58.65	14.66 ± "	10 L/M
6	14.30	11.50	14.73	14.90	55.43	13.86 ± "	8 L
17	14.80	11.73	14.73	14.06	55.32	13.83 ± "	2 S
12	14.50	7.80	12.15	13.60	48.05	12.01 ± "	8/4/2 M/S
10	10.22	13.30	9.90	14.30	47.72	11.93 ± "	8 L
20	11.00	9.10	13.60	11.00	44.70	11.17 ± "	8 L
3	11.90	10.30	8.30	13.20	43.70	10.92 ± "	8 L/M
15	10.10	10.00	10.80	12.60	43.50	10.87 ± "	8/4 S
7	9.84	9.70	8.00	7.95	35.49	8.87 ± "	8/4 L/M/S
18	7.86	8.96	7.86	6.60	31.28	7.82 ± "	4 S
14	6.50	8.87	7.49	7.84	30.70	7.67 ± "	1 S
8	5.80	9.38	7.00	7.80	29.98	7.49 ± "	8 L
4	9.00	6.95	7.12	6.55	29.62	7.40 ± "	2/1 M
16	3.73	5.46	4.40	4.40	17.99	4.49 ± "	1 S
5	3.80	3.60	2.70	2.90	13.00	4.25 ± "	1 M/S
Sums	249.23	243.51	270.94	252.12	1015.80 (Grand total)		

[1] Soil 9: 10 L/M means the relative number is 10, and the size ranges from large to medium.

Soil 12: 8/4/2 M/S means the nelative number ranges from 8 to 4 to 2, and the size ranges from medium to small, etc., etc.

(Analysis Overleaf.....)

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	3377.29	19	177.75	37.42	1.78 (2.24)	**
Error	285.03	60	4.75			
Total	3662.32	79				

$$\text{S.E. of the means} = \sqrt{\frac{4.75}{4}} = \sqrt{1.19} = 1.09$$

Detectable differences:

$$d_{0.05\ (0.01)} = t_{0.05\ (0.01)\ (df60)} \times \sqrt{\frac{2 \times 4.75}{4}} = 2 \times \sqrt{2.38} = 2 \times 1.543 = \underset{(4.10)}{3.09}$$

Any two means, which differ by 3.09 or above and/or by 4.10 or above, are significantly different at 5% level and/or at 1% level.

Table: 20.

Dry weights of Lucerne total yields.

(The soils are rearranged according to descending order of mean weights.)

(Results expressed in g.)

Serial No. of Soils	1st replicate (g.)	2nd replicate (g.)	3rd replicate (g.)	4th replicate (g.)	Totals of replicates (g.)	Means ± S.E. (g.)
19	53.73	54.37	63.06	57.41	228.57	55.89 ± 1.74
2	49.50	52.90	47.80	50.20	200.40	50.10 ± "
1	41.60	36.50	45.18	40.62	163.90	40.97 ± "
13	38.19	27.95	47.75	30.12	144.01	36.00 ± "
11	27.58	37.30	36.68	26.22	127.78	31.94 ± "
9	29.18	32.20	31.80	32.80	125.98	31.49 ± "
6	29.60	27.40	31.13	32.55	120.68	30.17 ± "
12	31.10	20.60	29.35	32.08	113.13	28.28 ± "
17	27.37	25.93	28.99	26.69	108.98	27.24 ± "
10	26.04	27.88	26.20	28.40	108.52	27.13 ± "
3	24.80	21.05	18.70	28.73	93.28	23.32 ± "
20	20.75	22.10	24.80	22.45	90.10	22.53 ± "
15	20.98	20.40	21.50	25.38	88.26	22.06 ± "
7	17.84	20.75	16.75	17.33	72.67	18.17 ± "
18	15.72	19.49	17.66	17.69	70.56	17.64 ± "
14	14.76	19.59	17.35	18.50	70.20	17.55 ± "
4	17.34	14.88	15.82	13.95	61.99	15.49 ± "
8	11.50	18.48	13.70	15.50	59.18	14.79 ± "
16	8.39	11.86	9.73	11.60	41.58	10.39 ± "
5	9.38	10.00	7.20	7.79	34.37	8.59 ± "
Sums	515.35	521.63	551.15	536.01	2124.14 (Grand total)	

Analysis of variance:

Source of variance	S.S.	df.	M.S.	F. ratio	F. ratio 5% (1%)	Results
Soils	1918.46	19	100.97	8.86	1.78 (2.24)	**
Error	722.26	60	12.04			
Total	2640.72	79				

(Continued.....)

$$\text{S.E. of the means} = \frac{\sqrt{12.04}}{4} = \sqrt{3.01} = 1.735 = 1.74$$

Detectable differences:

$$d_{0.05\ (0.01)} = t_{0.05\ (0.01)}\ (df 60)\ \sqrt{\frac{2 \times 12.04}{4}} = 2 \times \sqrt{6.02}_{(2.66)} = 2 \times 2.46_{(2.66)} = 4.90_{(6.52)}$$

Any two means, which differ by 4.90 or above and/or by 6.52 or above, are significantly different at 5% level and/or at 1% level.

Table: 21.

Ranking of soils by Lucerne top, root, and total yields according to the phosphate status of the soils.

Serial No. of soils	Ranks of the soils by the mean dry weights of plant yields		
	According to Lucerne top	According to Lucerne root	According to Lucerne total
1	4	3	3
2	2	2	2
3	11	12	11
4	17	18	17
5	20	20	20
6	7	7	7
7	16	14	14
8	18	17	18
9	5	6	6
10	9	10	10
11	6	5	5
12	8	9	8
13	3	4	4
14	14	16	16
15	13	13	18
16	19	19	19
17	10	8	9
18	15	15	15
19	1	1	1
20	12	11	12

The data in this table are used to obtain results presented in Table: 26.

3. Chemical Analyses of Soil Phosphates:

Table: 22. shows the forms of soil phosphate, accompanied by the ranking of soils by each form. Table: 23. shows combinations of forms of soil phosphates and ranking of soils by each combination. The Truog values for "available" phosphate and the ranking of soils by this method are shown in Table: 24. The detailed data from which the above mentioned tables are derived, are presented in the appendices.

Table: 22.

Forms of soil phosphates and ranking of soils.

(The amount of phosphate is expressed as µg. of P/g. of soil.)

Serial No. of soils	Form of soil phosphates [1].			Ranking of soils. [2]		
	Al-bound P. (µg.P/g.soil)	Fe-bound P. (µg.P/g.soil)	Ca-bound P. (µg.P/g.soil)	Al-bound P.	Fe-bound P.	Ca-bound P.
1	462	45	177	4	11	5
2	382	81	208	5	9	2
3	52	55	47	19	10	17
4	96	37	67	10	15	13
5	52	43	43	20	12	19
6	122	-60	85	6	20	10
7	116	29	128	7	16	8
8	68	29	65	16	17	14
9	104	113	93	9	5	9
10	90	116	80	13	4	11
11	90	118	174	12	3	6
12	52	111	141	18	6	7
13	508	15	249	3	18	1
14	78	39	45	14	14	18
15	96	106	72	11	7	12
16	54	89	34	17	8	20
17	108	39	61	8	13	15
18	78	12	56	15	19	16
19	1050	189	188	1	1	4
20	634	121	206	2	2	3

1) The data are used to obtain results presented in Table: 25.

2) " " " " " " " " " Table: 26.

Table: 23.

Combinations of forms of soil phosphate and ranking of soils.
(The amount of phosphate is expressed as µg. of P/g. of soil.)

Serial No. of soils	Combinations of soil phosphate forms.[1]			Ranking of soils.[2]		
	Al + Fe bound P. (µg.P/g.soil)	Al + Ca bound P. (µg.P/g.soil)	Al+Fe+Ca bound P. (µg.P/g.soil)	Al + Fe bound P.	Al + Ca bound P.	Al+Fe+Ca bound P
1	507	639	684	4	4	4
2	463	590	671	5	5	5
3	107	99	154	16	18	19
4	133	163	200	14	14	13
5	95	95	138	18	19	20
6	62	207	147	20	8	17
7	145	343	273	12	6	11
8	97	133	162	17	16	16
9	217	197	310	6	9	7
10	206	170	286	8	11	9
11	208	264	382	7	7	6
12	163	193	304	10	10	8
13	523	757	772	3	3	3
14	117	123	162	15	17	15
15	202	168	274	9	13	10
16	143	88	177	13	20	14
17	147	169	208	11	12	12
18	90	134	146	19	15	18
19	1239	1238	1427	1	1	1
20	755	840	961	2	2	2

1) The data are used to obtain results presented in Table: 25.

2) " " " " " " " " " Table: 26.

Table: 24.

Truog values of available Phosphate and ranking of soils.
(The amount of phosphate is expressed as µg. of P/g. of soil).

Serial No. of soils	Truog's available [1] phosphate (µg.P/g.soil)	Ranks [2] of soils
1	540	2
2	705	1
3	60	14
4	130	8
5	25	17
6	110	12
7	180	6
8	60	15
9	115	10
10	125	9
11	200	5
12	111	11
13	357	3
14	20	18
15	70	13
16	20	19
17	37	16
18	15	20
19	337	4
20	131	7

1) The data are used to obtain results presented in Table: 25.

2) The data are used to obtain results presented in Table: 26.

4. Relationships and comparisons between methods:

The relationships between the results obtained in pot culture and those obtained by fungal assays and chemical extractions are presented in Table: 25. These relationships were obtained by calculating the ordinary correlation coefficients for the average dry weight of plant yields against mean mycelial weights (for the fungal assays) or amounts of phosphate extracted (for the chemical extraction procedures).

Rank correlation coefficients for plant yields and the other methods employed were also calculated, using Spearman's formula*). The results are shown in Table: 26. Complete examples of these calculations are given in the appendices.

Using the pot experiment as the basis for comparison, the overall correlation coefficients for microbioassays were compared with those for chemical extraction of soil phosphate. This was carried out by calculating the overall mean correlation coefficients for fungal bioassays and chemical methods, from which the significant differences were then examined. Table: 27. shows the overall mean correlation coefficients, followed by analyses for significant differences. The data used to obtain these results are derived from Table: 25.

*) Kendall, M.G. Advanced Theory of Statistics Vol. I. Section 16.4, 1948.

Table: 25.

1) Ordinary Correlation Coefficients between plant yields and other methods employed for determination of soil phosphate status.

Other methods employed \ Plant methods	Millet dry weights			Turnip dry weight.	Lucerne dry weights.			Overall	Overall
	Top	Root	Total	Total	Top	Root	Total	Totals	averages
A. niger	+0.572 **	+0.712 **	+0.589 **	+0.610 **	+0.678 **	+0.739 **	+0 722 **	+4.622	+0.660 **
P. lilliacum	+0.529 **	+0.680 **	+0.543 *	+0.583 **	+0.709 **	+0.763 **	+0.761 **	+4.568	+0.653 **
C. geniculata	+0.540 *	+0.624 **	+0.552 *	+0.601 **	+0.630 **	+0.641 **	+0.647 **	+4.235	+0.605 **
Fusarium sp.	+0.270 N.S.	+0.396 N.S.	+0.287 N.S.	+0.445 *	+0.418 N.S.	+0.453 *	+0.435 N.S.	+2.704	+0.386 N.S.
Al-bound P.	+0.412 N.S.	+0.602 **	+0.435 N.S.	+0.603 **	+0.688 **	+0.745 **	+0.718 **	+4.203	+0.600 **
Fe-bound P.	+0.266 N.S.	+0.326 N.S.	+0.277 N.S.	+0.276 N.S.	+0.368 N.S.	+0.389 N.S.	+0.373 N.S.	+2.275	+0.325 N.S.
Ca-bound P.	+0.679 **	+0.761 **	+0.690 **	+0.706 **	+0.744 **	+0.693 **	+0.728 **	+5.00	+0.714 **
Al-bound + Fe-bound P.	+0.423 N.S.	+0.600 **	+0.446 *	+0.623 **	+0.692 **	+0.747 **	+0.695 **	+4.226	+0.604 **
Al-bound + Ca-bound P.	+0.497 *	+0.670 **	+0.516 *	+0.662 **	+0.721 **	+0.760 **	+0.753 **	+4.579	+0.654 **
Total (Al+Fe+Ca-bound) P.	+0.493 *	+0.659 **	+0.514 *	+0.646 **	+0.736 **	+0.771 **	+0.758 **	+4.577	+0.654 **
Truog "available" phosphate	+0.665 **	+0.755 **	+0.673 **	+0.596 **	+0.764 **	+0.800 **	+0.796 **	+5.049	+0.721 **

** Significant at 1% level;
* Significant at 5% level;
N.S. Non-significant.

1) The data, from which these results are obtained, are derived from Tables: 6,7,8,9,12,13,14,16,18, 19,20,22,23, and 24.

Table: 26.

[1] Rank Correlation Coefficients (Spearman's) for Plant yields against other methods employed for assessing "available" soil phosphate.

Other methods employed \ Plant methods	Millet dry weights			Turnip dry weight.	Lucerne dry weights.		
	Top	Root	Total	Total	Top	Root	Total
A. niger	+0.505 *	+0.559 *	+0.551 *	+0.517 *	+0.492 *	+0.547 *	+0.500 *
P. lilliacum	+0.671 **	+0.699 **	+0.685 **	+0.665 **	+0.654 **	+0.669 **	+0.698 **
C. geniculata	+0.504 *	+0.577 *	+0.538 *	+0.454 *	+0.535 *	+0.555 *	+0.575 **
Fusarium sp.	+0.098 N.S.	+0.217 N.S.	+0.133 N.S.	+0.074 N.S.	+0.155 N.S.	+0.201 N.S.	+0.213 N.S.
Al-bound P.	+0.565 **	+0.624 **	+0.590 **	+0.555 *	+0.633 **	+0.687 **	+0.622 **
Fe-bound P.	+0.233 N.S.	+0.347 N.S.	+0.268 N.S.	+0.268 N.S.	+0.337 N.S.	+0.329 N.S.	+0.329 N.S.
Ca-bound P.	+0.665 **	+0.807 **	+0.796 **	+0.740 **	+0.773 **	+0.803 **	+0.774 **
Al-bound + Fe-bound P.	+0.635 **	+0.779 **	+0.679 **	+0.641 **	+0.692 **	+0.708 **	+0.655 **
Al-bound + Ca-bound P.	+0.737 **	+0.785 **	+0.753 **	+0.758 **	+0.765 **	+0.816 **	+0.780 **
Total (Al+Fe+Ca-bound) P.	+0.678 **	+0.771 **	+0.691 **	+0.675 **	+0.722 **	+0.744 **	+0.688 **
Truog "available" phosphate	+0.833 **	+0.872 **	+0.838 **	+0.862 **	+0.711 **	+0.738 **	+0.727 **

1) The data from which these results are obtained are derived from Tables: 10,15,17,21,22,23, and 24.

Table: 27.

Overall total and average "ordinary" correlation coefficients for plant yield method against fungal assays and chemical extraction methods. (The means are arranged in descending order).

Methods	Overall totals of Correl. coeff.	Overall averages of correl. coeff.
Truog	5.049	0.721
Ca-bound P.	5.001	0.714
A. niger	4.622	0.660
Al + Ca-bound P.	4.579	0.655
Al + Ca + Fe-bound P.	4.577	0.654
P. lilliacum	4.568	0.653
C. geniculata	4.235	0.605
Al + Fe-bound P.	4.226	0.604
Al-bound P.	4.203	0.600
		**
Fusarium sp.	2.704	0.386
Fe-bound P.	2.275	0.325

Comparisons of overall average correlation coefficients:

(Ref: Snedecor, G.W., 1956. Statistical methods, section 7.6).

(1). Truog and Fe-bound P.

$r_1 = 0.721 \quad r_2 = 0.325$

$z_1 = 0.909 \quad z_2 = 0.337$

$$t = \frac{z_1 - z_2}{\sqrt{\left(\frac{2}{n \times df}\right)}} = \frac{z_1 - z_2}{\sqrt{\left(\frac{2}{7 \times 18}\right)}} = \frac{0.572}{\sqrt{\frac{2}{126}}} = \frac{0.572}{0.126} = 4.540.$$

$\therefore p < 0.001$ (***)

(Continued Overleaf....)

(2) Truog and Fusarium.

$r_1 = 0.721 \quad r_2 = 0.386$

$z_1 = 0.909 \quad r_2 = 0.408$

$$t = \frac{z_1 - z_2}{0.126} = \frac{0.501}{0.126} = 3.976$$

$\therefore P < 0.001$ (***)

(3) Truog and Al-bound P.

$r_1 = 0.721 \quad r_2 = 0.600$

$z_1 = 0.909 \quad z_2 = 0.693$

$$t = \frac{z_1 - z_2}{0.126} = \frac{0.206}{0.126} = 1.635$$

$\therefore p > 0.10$ (N.S.)

(4) Al-bound P and Fusarium Sp.

$z_1 = 0.693 \quad z_2 = 0.408$

$$t = \frac{z_1 - z_2}{0.126} = \frac{0.285}{0.126} = 2.262$$

$0.02 < p < 0.03$ (Significant at 3% level).

The downward arrow indicates that the best mean within the length of the arrow is significantly better than those below the arrow, while there is no significant difference between any two means within the length of the arrow.

The overall average correlation coefficient for Al-bound P is significantly different from that for Fusarium sp. at 3% level.

Note: *** Significant at 0.1%

** " " 1%

* " " 5%

N.S. Non-significant.

Plate IX.

From left to right:

lucerne, turnip and millet

all at 3 weeks after germination

Top row: soil 2. well supplied with phosphate.

Bottom row: soil 16. poorly supplied with phosphate.

Plate X.

From left to right:

turnip, lucerne, millet

at 6 weeks after germination.

Top row: Soil 1. well supplied with phosphate.

Bottom row: Soil 16. poorly supplied with phosphate.

Plate XI.

Turnips, lucerne, and millet at 6 weeks after germination. Pot No. 19 contains Stratford Sandy loam with high phosphate topdressing, while Pot No. 20 contains the same soil type which never received phosphate topdressing.

Plate XII.

Different levels of available soil phosphate as shown by growth response of millet plants at $6\frac{1}{2}$ weeks after germination. Pot Nos. indicate Serial No. of soils.

Plate XIII.

Different levels of available soil phosphate as shown by growth response of turnip plants at 7 weeks after germination. Pot Nos. (Soil Nos.) are similar to those shown in Plate XII.

Plate XIV.

Different levels of available soil phosphate as shown by growth response of lucerne plants at $6\frac{1}{2}$ weeks after germination. Pot Nos. (Soil Nos.) are similar to those shown in Plates XII & XIII.

Plate XV.

A range of growth shown by millet plants at harvest. On Soil 1. the millets have flowered. (the site of flower is indicated by a piece of brown paper). Soil 16 shows marked symptom of phosphate deficiency.

Plate XVI.

Contrasting conditions shown by the turnip plants at harvest. Left: turnips grown on a soil with adequate phosphate supply. Right: the plants show a marked symptom of P. deficiency in ~~one~~ another soil.

Plate XVII.

Development of turnip "root". Top row: grown on Soil 13 adequately supplied with phosphate. Bottom row: grown on Soil 16 where phosphate is deficient.

Plate XVIII.

State of growth of lucerne at harvest. Nos. 16 & 5 show low available phosphate in the soils. Nos. 1 & 19 show high available phosphate in the soils.

Plate XIX.

Ramification of root in a variety of soils.
From left to right: Soil 1 (Yellow -Grey Earth), Soil 5 (Sandy gley soil), Soil 13 (Rhyolitic - pumice soil), Sil 20 (Yellow - Brown Loam), and Soil 6 (Rendzina).

VI. DISCUSSION OF RESULTS.

1. Fungal bioassay.

From the results obtained it is evident that each fungus produces different mycelial dry weight, in spite of the same conditions for its growth. This indicates that a particular fungus has a specific ability to utilize available phosphate for its growth, provided that other essential nutrients are present in optimum amounts. This statement applied to the standard phosphate series as well as to soil assay with A. niger, P. lilliacum, and C. geniculata, the data for which (Tables: 6, 7, and 8, and Appendix 3) show that in general the best phosphate utilizing organism is C. geniculata, followed by A. niger, and P. lilliacum, this judgement being made on the weights of mycelia produced. The mycelial dry weights of A. niger and P. lilliacum increase proportionally with the phosphate concentrations as indicated by the linear growth curves, while the rate of increase in mycelial weight of C. geniculata is greater at higher phosphate levels leading to the formation of a parabolic-type growth curve.

Fusarium sp. was decidedly the most inefficient phosphate utilizer as shown by the overall total of mean mycelial dry weights obtained (Table: 9), although for some soils such as the sandy soils and pumice soils (parts of which float on the surface of the medium), the fungus produced greater mycelial dry weights than both A. niger and P. lilliacum. This odd result can be attributed to the growth habit of the fungus, which is jelly-like, cord-like, loose mycelia, instead of a firm pad. This makes the light soil particles cling to

the mycelia with a consequent difficulty to obtain an absolutely pure mycelium weight, which in turn leads to an error in the interpretation of results.

In the case of further work this situation might be overcome by wrapping the assayed soil in a very thin filter paper or a sterile cloth and placing them in the medium.

The inability of the Fusarium sp. to grow in the standard phosphate series without soil is probably caused by an absence of specific growth factors such as vitamins or trace elements, which may be supplied by the soils thus promoting the satisfactory growth of the fungus found in the soil assays. This line of thought gets support from a statement by Smith (1936), that A. niger grown on medium without soil did not produce a felt-like mycelia but a cord-like formation, and the yield was always much less than that in presence of even 0.5g. of infertile soil in the same culture solution. It was found later, however, that the "catalytic" effect of the soil was due to the presence of Mn, which was excluded from the soil-free medium.

In the present work it has not been investigated what was the specific growth factor in the soil which brought about the growth of the Fusarium sp. Further studies are required to clarify this problem.

The growth of any one fungus in the phosphate series did not change the pH of the medium regardless of the amounts of phosphate added which ranged from zero to 26.93 mg. of P per flask (Appendix 5), producing mycelial dry weights ranging from 0 to 2.185 g. The highest

mycelial dry weight obtained from the 1g. of soil added was much lower than 2.185 g., and it was therefore assumed that neither the soil pH and/or the amount of soil P nor the acid produced during fungal activity could change the pH of the culture medium. This may be due to the strong buffering capacity of calcium citrate in the medium. This finding agrees with that of Gerretsen (1948) and of Maercke (1950).

The acidity of the culture employed in the current investigation (pH 3.45) did not appear to have any adverse effect on the growth of all fungi employed. Studies by Nicholas (1960) on the effect of pH of medium on the growth of A. niger give support to this finding, whereas the work carried out by Swaby and Sherber (1958) on C. geniculata is contradictory. They found that C. geniculata required pH 7 for its optimal growth, while it grew poorly in more acid conditions. The present evidence suggests that it is not necessary to reduce the acidity of the medium to pH 7.

2. Pot experiment.

The results obtained by this experiment have provided further evidence that different kinds of plant have specific abilities to extract and utilize available nutrients from the soil (Bray, 1963), and they do not necessarily show deficiency symptoms at the same level of nutrient in the soil (Rosselet, 1954). This reasoning has led the writer to treat each plant species individually as a basis of evaluation of the bioassay and chemical extraction methods. The millet was the first crop to show visual symptoms of P deficiency (2 weeks after germination) followed

by the turnip and lucerne. The common symptom was the stunting of growth (Wallace, 1951), plus purpling of the stems and leaves of the millet and turnips, whereas there was no change in the colour of leaf of lucerne. The purplish-red colouration of the millet leaves disappeared after the third leaf stage, while in the turnips the colour became more intense as the plants aged. The differences in growth and colour of plant leaves arising from the different levels of available soil phosphate were clearly revealed in this experiment (Plates IX to XVIII).

The appearance of nutrient deficiency symptoms other than those due to lack of phosphate appeared first in the millets which was indicated by yellowing followed by dying off of the older leaves. This may have been due to shortage of nitrogen which is required by cereal crop at relatively high amount and utilized at a high rate for top growth and which can not be obtained outside the soil in which the millet is growing. Later nutrient deficiency occurred with the turnips which require relatively higher amount of P than the millet does for root production, particularly at later stages of growth. This may explain the difference in colour development of the leaf between the two species growing on phosphate deficient soils. Apparently the millet did not require as much phosphate after the third leaf stage as the turnips did in relation to their nitrogen uptake. On the other hand, the lucerne did not show noticeable change in leaf colouration resulting from phosphate deficiency and it was not affected by nitrogen shortage since it could fix nitrogen from the air in its root nodules by means of symbiotic association with rhizobium species, which was introduced into the soil by inoculation of the lucerne seed before sowing. It was this difference

in their characteristics in showing nutrient deficiencies which led to the different harvesting dates employed for the three crops.

The nodule grading system was considered unreliable for indicating soil phosphate status due to the inconsistency of the results, although there was a slight indication that the more abundant top growth was associated with greater number of nodules.

The dry weight of root, on the other hand, appeared to be most favourable for soil phosphate estimation (discussed further in the oncoming section), although its practical application may be limited by the tediousness in preparation of the yield data. Black (1963) also found it exceedingly difficult to get a satisfactory separation of root from the organic matter in the compost, and the root weights were too variable and inconsistent to be of value.
The present evidence does not agree with the second part of his finding, since as will be shown later the dry weights of roots give better correlations than those of top growth.

There was an even ramification of root throughout the potted soils (Plate XIX) which must be attributed to the favourable mechanical conditions in the soils. The potted soil maintained its firm and crumbly structure throughout the period of experiment, which was the main pre-requisite for adequate aeration and even moisture distribution.

3. Chemical analyses of soil phosphate.

For the majority of the soils Fe-bound P is present in lower amount than either Al-bound P or Ca-bound P. The exceptions to this are

noted in Koro-Koro silt loam and Ramiha silt loam (both are genetically yellow-brown earth, the clay mineralogy of which consists mainly of vermiculite), where there are high amounts of Fe-bound P. This may be due to their high contents of free iron-oxide, resulting from the rapid chemical weathering of the parent materials. Work carried out by Goh (1962) showed that there was good correlation between free iron-oxide and iron-bound phosphate contents of some New Zealand soils. Secondly, the weakly leached gley soils (Kairanga silt loams) used in the current work contain higher amount of Fe-bound P than Al-bound P, but less than Ca-bound P. High amounts of Fe-bound P also occur in Stratford sandy loam (moderately leached yellow-brown loam), but in this instance the amount is higher than Ca-bound P and lower than Al-bound P.
The rendzina soil studied has a negative value for Fe-bound P. This may be due to precipitation of calcium phosphate during the NaOH extraction leading to a low value for Fe-bound + Al-bound phosphates.

Aluminium-bound phosphate appears to be the predominant form of fixed phosphate in the majority of the soils. Calcium-bound phosphate values in general tend to follow the trend of Al-bound P, with the exceptions of Kairanga silt loam (gley soil) and the phosphate-topdressed Stratford sandy loam, in which Ca-bound P tends to follow Fe-bound P in magnitude.

The trend of Truog values for available phosphate strongly follows that of Ca-bound P values. This is not surprising, for in both cases acid extractants of similar properties are used. The absolute values of Truog available phosphate, however, are much higher than those of Ca-bound phosphate, because Truog reagent dissolve all forms of soil

available phosphate, whereas in the case of Ca-bound P the reagents used have a selective property.

VII. GENERAL DISCUSSION.

From the correlation coefficients between the estimates of available phosphate determined by each kind of plant weight measurement and the amounts of phosphate estimated by bioassay and chemical methods, it can be seen (Table: 25) that, in general, the plant methods are highly correlated both with the bioassay and with the chemical methods. Exceptions to this are shown by Fusarium (for the bioassay) and Fe-bound phosphate (for the chemical methods). The reasons for low correlation between the Fusarium and plant methods may be due to the unsatisfactory growth of the fungus as discussed previously. The low correlation coefficients between plant methods and Fe-bound phosphate estimation suggest that iron phosphate is not the main source of plant available phosphate in the soil; this is contradictory to the results obtained on soils of Taiwan as reported by Chang and Juo (1963), who employed the method of Chang and Jackson for the fractionation of inorganic soil phosphorus, and found that the iron phosphate was the main supplier of available phosphate to rice plants as indicated by the high correlation coefficient obtained between the response of rice to phosphatic fertilizer and the amount of iron phosphate in the soil. The high correlation coefficient obtained by Chang and Juo may be due to the exaggerated values for iron phosphate likely to be obtained by the procedure of Chang and Jackson, which has been criticized by Fife (1963, priv. comm.).

The findings of the present investigation are quite strongly in favour of the opinion that aluminium-bound phosphate and calcium-bound phosphate or the combination of both forms are the more important sources of available soil phosphate. The relatively high correlation coefficients obtained between plant yields and the combination of aluminium-bound and iron-bound phosphates are due to the fact that aluminium-bound phosphate is overshadowing the iron-bound phosphate. In case of total (Fe + Al + Ca-bound) phosphate the relatively high correlation coefficients are due to a similar effect produced by aluminium-bound and calcium-bound phosphate together.

Although there was a general agreement in the data obtained by plant methods and the other methods employed (with the exceptions of Fusarium and Fe-bound P) as indicated by the high correlation coefficients, there was frequent and wide disagreement in individual soils. This may be attributed to an inherent source of error as put forth by Mooers (1938) who stated that "every analytical method carries certain inherent error" and "there is no method which is absolutely accurate". Obviously, methods as different as those under consideration may differ in the extent or seriousness of this error. Therefore, different soils or methods may require materially different standards or bases of evaluation. Consequently, using the various plant yield data as a basis of evaluation, it can be seen (Table: 25) that the highest correlations are shown between the following pairs of methods, viz: millet top and Ca-bound P; millet root and Ca-bound P; millet total yield and Ca-bound P; turnip total yield and Ca-bound P; lucerne top and Truog's value; lucerne root and Truog's value; and lucerne total yield and Truog's value. This situation may, however, have no general application.

As far as the plant yield methods are concerned, the lucerne deserves favourable comment, since, in general, lucerne yields give higher correlation both with the bioassay and chemical methods. Where distinctions are made between top, root and total dry weights, such as in lucerne and millet, it appears that the roots, particularly that of lucerne, give the highest correlation.

Rank correlation (Spearman's correlation) coefficients have also been calculated for the various methods (Table: 26) and a similar conclusion was reached. These correlations are merely concerned with the placing of soils according to their relative amounts of phosphate contents.

Considering the overall merits of the bioassay and chemical methods studied, however, it is shown (Table: 27) that the bioassay procedures (except the Fusarium) and the chemical extractions (except the Fe-bound P estimation) are equally reliable and relatively accurate for assessing plant available phosphate for the range of soils examined, and there is no significant difference between the methods used.

From the foregoing discussion it can be deduced that the bioassay procedures are as reliable as the chemical methods for assessing the status of plant available phosphate of the soil, and the use of any method, except the Fusarium and Fe-bound phosphate estimation, is equally recommended for the purpose. The findings of this investigation have practical significance for reasons similar to those discussed by Chang and Juo (1963), and even more so in this case where plants are used as the basis of evaluation. It must be realized, however, that any of these methods will only reveal the qualitative status of soil phosphate, and can not be directly used for routine recommendation on fertilizer usage, unless further work

has been carried out to study the rate of application and the form of phosphate in the fertilizer used, which will give positive results in a particular soil type with a certain plant species.

SUMMARY

A study was made of the merits of microbiological assay for the determination of available soil phosphate using four fungal species, A. niger, P. lilliacum, C. geniculata and Fusarium species. Twenty New Zealand soils representing ten soil types were used in the experimental work. A pot experiment incorporating these soils and utilizing several crops was carried out to provide plant yield data as a basis for evaluation of the microbiological procedures. A variety of chemical extractions for phosphate were also carried out on the soils to provide further comparative data; the methods used were those of Truog (1930) for "available phosphate" and those of Fife (1962 and priv. comm.) for the selective determination of Ca-bound, Al-bound and Fe-bound phosphate.

All the fungi employed showed normal growth response to phosphate addition to the culture medium in the absence of soil, except Fusarium; this fungus however showed better growth in the presence of soil and it was concluded that the soil might be supplying some growth factor.

High correlation coefficients were obtained both between plant weight measurement and the bioassays and between plant weight measurement and the chemical extraction procedures, and it was concluded that the microbiological procedures used in the investigation were as reliable and as accurate as the chemical extraction; the exceptions to this were clearly indicated.

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LIST OF APPENDICES.

APPENDIX 1.

Preparation of micro-nutrient stock solution.

For each element, say Mn, 100 ml. stock solution was prepared containing the calculated amount (x mg.) of the appropriate salt (in this case it is Mn $SO_4.4H_2O$). Of this stock solution 10 ml. aliquot was pipetted into the mixing flask. The value of x is calculated as follows:

Mn required is 0.0001% = 0.0001 g. per 100 ml.

= 1 mg. Mn per litre.

$$1 \text{ mg. Mn} \equiv \frac{\text{M.W. of Mn } SO_4.4H_2O}{\text{A.W. of Mn}} \times 1 \text{ mg. Mn } SO_4.4H_2O \quad \ldots\ldots(1)$$

100 ml. stock solution must contain x mg. Mn $SO_4.4H_2O$.

10 ml. " " " " $\frac{1}{10}$ x mg. $MnSO_4.4H_2O$.

Thus $\frac{1}{10}$ x mg. $MnSO_4.4H_2O$ is required per litre culture solution.

Thus $\frac{1}{10} x = 1 \text{ mg. Mn} = \frac{223}{54.94 \times 0.1}$ ..... from equation (1)

Thus $x = \frac{2230}{54.94} = \underline{\underline{40.6}}$

Thus 40.6 mg. Mn $SO_4.4H_2O$ is required in 100 ml. of the stock solution, of which 10 ml. is used in 1 litre of culture solution, etc., etc.

APPENDIX 2.

Determination of water holding capacity according to Keen - Razkowski method. (Refce: Piper, C.S., 1942. "Soil and Plant Analysis".,pp.82-84.)

Crush the air dry soil in a porcelain mortar and sieve through a sieve having round holes, 2 m.m. in diameter. Continue crushing the coarse residue, so as to disintegrate clay aggregates yet avoiding the actual grinding of any sand particles. When crushing is as complete as possible return the coarser particles remaining on the sieve to the finer fraction and incorporate them by thorough mixing. Crush sufficient soil to enable two determinations to be made for each soil.

Place a thin filter paper (Whatman No. 1 or No. 44) on the bottom of a brass box (Plate XX). Weigh the box and filter paper and record it as Weight of box unfilled (a.gram.)

Transfer the soil from different parts of the heap of the crushed soil to the box until the latter is about ½-full. Pack the soil by tapping gently several times on a wooden bench top or on a sheet of cork. Having properly packed the soil in the box, place it in a basin and add water to the basin until the water level is as high as or higher than the soil surface in the box, and leave to soak overnight. When equilibrium has been reached, remove the box containing the saturated soil, carefully wipe it dry on the outside, and weigh it, recording the weight as weight of box + saturated soil (b. gram).

After weighing, place it in the oven at 105°C for 24 hours. Then cool it in a desiccator and weigh again, recording the weight as weight of box + oven dry soil (c. gram).

Determine the amount of water absorbed by the filter paper by weighing 5 filter papers together, then saturate them with water; place them on a flat glass plate and squeeze gently by rolling with a glass rod. Weigh again to determine the amount of water retained. From this calculate the average amount retained by one paper and expressed as d. gram. (The hygroscopic moisture of the air-dry filter paper can be neglected.)

Calculate the water holding capacity (W.H.C.) as % of oven-dry soil from the expression:

$$\frac{b - c - d}{c - a} \times 100,$$

where a, b, c, d, having the values noted above.

Plate XX.

Brass boxes used for determination of water holding capacity of soil.

APPENDIX 3.

a). STANDARD SERIES FOR ASPERGILLUS NIGER.

Serial No. of flasks	P (ug.)	Mean dry weight of mycel.(c.g).	Serial No. of flasks	P (ug.)	Mean dry weight of mycel.(c.g.)
0	0.00	0.00	19	389.8	20.77
1	13.4	0.30	20	403.2	21.20
2	26.9	0.80	21	416.6	23.22
3	53.8	2.95	22	430.1	22.25
4	80.6	4.17	23	443.5	23.00
5	107.5	5.10	24	456.9	24.87
6	134.4	6.87	25	470.4	23.75
7	161.3	9.35	26	483.8	26.15
8	188.2	11.40	27	497.3	26.82
9	215.0	13.35	28	510.7	27.22
10	241.9	13.95	29	524.2	27.95
11	268.8	15.50	30	537.6	28.55
12	295.7	16.57	31	591.4	32.37
13	309.1	16.30	32	618.3	34.25
14	322.6	19.65	33	672.0	35.57
15	336.0	19.45	34	725.8	39.15
16	349.4	20.85	35	806.4	43.22
17	362.9	19.27	36	940.8	52.90
18	376.3	20.35			
			Sums	13762.6	749.39

(Calculation of "least mean square" overleaf.....)

Linear Regression: test for A. niger Standard series using "Least sum of squares" method:

The Linear Regression equation if $P = a + bW$.

where P = phosphorus level (in ug.) and W = weight of dry mycelium (in cg.)

Since the line passes the point of origin the equation becomes $P = bW$.,

where $b = \frac{\Sigma (PW)}{(\Sigma W^2)}$.

Calculations:

$$\Sigma W^2 = 20,507.56$$

$$\Sigma P^2 = 6,925,240.66$$

$$\Sigma (PW) = 376,577.14$$

Regression line $P = b W.$

$$b = \frac{\Sigma (PW)}{(\Sigma W^2)} = \frac{376,577.14}{20,507.56} = 18.36284.$$

Equation is $P = 18.36284 W$

Thus for

W = 0	20.00	50.00
P = 0	367.26	918.14

For a soil (No. 19.) with W = (374.6 ± 7.3) mg.

= 37.46 ± 0.73 cg.

or rewritten:

W = 36.73	37.46	38.19
P = 674.47	687.87	701.27

then $P = (687.87 \pm 13.4)$ µg.

thus S.E. of P = ± 13.4

Part of (ΣP^2) accounted for by Regression = $\frac{(\Sigma PW)^2}{\Sigma W^2}$

$$= \frac{141810236929}{20508} = 6,914,874,04 \ (= 6,914,874.04).$$

Error S.S. $= 6,925,240.66 - 6,914,874.04$

$= 10,366.62$ (µg.)

APPENDIX 3.

b). STANDARD SERIES FOR P. LILLIACUM.

Serial No. of flasks	P (ug.)	Mean dry weight of mycel. (c.g.)	Serial No. of flasks	P (ug.)	Mean dry weight of mycel. (c.g.)
0	0.00	0.00	19	389.8	24.20
1	13.4	0.95	20	403.2	26.17
2	26.9	1.45	21	416.6	24.72
3	53.8	2.20	22	430.1	28.05
4	80.6	3.32	23	443.5	25.52
5	107.5	4.00	24	456.9	28.32
6	134.4	7.62	25	470.8	28.62
7	161.3	9.10	26	483.8	31.40
8	188.2	12.07	27	497.3	32.87
9	215.0	11.85	28	510.7	34.20
10	241.9	12.37	29	524.2	35.57
11	268.8	14.50	30	537.6	35.85
12	295.7	15.37	31	591.4	35.45
13	309.1	16.45	32	618.3	36.90
14	322.6	17.45	33	672.0	37.72
15	336.0	19.32	34	725.8	38.50
16	349.4	21.67	35	806.4	42.50
17	362.9	22.67	36	940.8	50.65
18	376.3	24.30			
			Sums	13762.6	813.87

(Calculation of "least mean square" overleaf.....)

Here the line also passes through the point of origin, thus the Linear Regression equation is

$$P = bW$$

$$b = \frac{\Sigma\ (PW)}{\Sigma\ W^2}$$

Calculations:

$$\Sigma\ W^2 = 24{,}206.0329$$

$$\Sigma\ P^2 = 6{,}925{,}240.66$$

$$\Sigma\ PW = 407{,}758.607$$

thus $b = \frac{\Sigma\ PW}{\Sigma\ W^2} = \frac{407{,}758.607}{24{,}206.0329} = 16.84533$

Equation is $P = 16.84533\ W$

thus for

W = 0	20	50
P = 0	336.91	842.27.

For a soil (i.e. No: 19.) with W = (322.9 ± 13.05) mg.

= (32.29 ± 1.305) cg.

or rewritten:

W =	30.985	32.29	33.595
P =	521.96	543.94	565.92

then P = (543.94 ± 21.98) ug.

Part of $\Sigma\ P^2$ accounted for by Regression $= \frac{(\Sigma\ PW)^2}{\Sigma\ W^2}$

$$= \frac{166266586564}{24{,}206} = 6{,}868{,}817.09$$

Error S.S. = $6{,}925{,}240.66 - \frac{6{,}868{,}817.09}{56{,}423.57}$ (µg.)

APPENDIX 3.

c). STANDARD SERIES FOR CURVULARIA GENICULATA.

Serial No. of flasks	P (ug.)	Mean dry *weight of mycel. (cg.)	Serial No. of flasks	P (ug.)	Mean dry weight of mycel. (cg.)
0	0.00	0.00	19	389.8	38.0
1	13.4	0.3 (0.0)	20	403.2	37.0
2	26.9	4.0	21	416.6	39.0
3	53.8	6.0	22	430.1	42.0
4	80.6	11.0	23	443.5	40.0
5	107.5	17.0	24	456.9	42.0
6	134.4	18.0	25	470.4	43.0
7	161.3	19.0	26	483.8	46.0
8	188.2	24.0	27	497.3	47.0
9	215.0	29.0	28	510.7	45.0
10	241.9	32.0	29	524.2	46.0
11	268.8	34.0	30	537.6	48.0
12	295.7	39.0	31	591.4	51.0
13	309.1	36.0	32	618.3	53.0
14	322.6	35.0	33	672.0	53.0
15	336.0	38.0	34	725.8	61.0
16	349.4	36.0	35	806.4	62.0
17	362.9	37.0	36	940.8	71.0
18	376.3	39.0			
			Sums	13762.6	1318.0

(Calculation of "least mean square" overleaf.....)

If the data fit well a straight line, it will have a Linear Regression equation and if they fit better a curve, the equation will be of a Quadratic Regression.

a). If it was a "Linear Regression" the equation is

$P = b\,W$ - for the line passing through point of origin.

$$b = \frac{\Sigma\,(PW)}{\Sigma\,W^2}$$

$\Sigma\,W^2 = 57,322.00$

$\Sigma\,P^2 = 6,925,240.66$

$\Sigma\,(PW) = 623,002.00$

$$b = \frac{623,002.0}{57,322.0} = 10.868462$$

Thus $P = 10.868462\,W$

For $W = 50$ c.gram, $P = 543.42$ ug.

Part $\Sigma\,P^2$ accounted for Linear Regression is

$$\frac{(\Sigma PW)^2}{\Sigma\,W^2} = \frac{388,131,492,004}{57,322} = 6,771,073.79$$

Error S.S. $= 6,925,240.66 - 6,771,073.79 = 154,166.87\ (\mu g.)^2$

b). If the data fit better a Quadratic Regression, then the calculation is as follows:

$\Sigma\,W^2 = 57,322.00$

$\Sigma\,W^3 = 2,700,214.00$

$\Sigma\,W^4 = 135,503,602.00$

$\Sigma\,(PW) = 623,002.00$

$\Sigma\,(PW^2) = 30,347,886.00$

$\Sigma\,P^2 = 6,925,240.66$

Quadratic Regression equation (Continued....)

Quadration Equation is $P = b\,W + c\,W^2$, if line passes point of origin.

$$(\Sigma\, W^2)\, b + (\Sigma\, W^3)\, c = \Sigma\, PW \ldots\ldots\ldots \quad (1)$$

$$(\Sigma\, W^3)\, b + (\Sigma\, W^4)\, c = \Sigma\, PW^2 \ldots\ldots\ldots \quad (2)$$

$$57{,}322\ b + 2{,}700{,}214\ c = 623{,}002 \ldots\ldots\ldots \quad (1)$$

$$2{,}700{,}214\ b + 135{,}503{,}602\ c = 30{,}347{,}886 \ldots\ldots\ldots \quad (2)$$

Equation (1) multiplied by $\frac{2{,}700{,}214}{57{,}322} = 47.10606$ to nihilate b.

(1) ..... $127{,}196{,}280.684\ c = 29{,}347{,}132.212.$

(2) ..... $135{,}503{,}602.000\ c = 30{,}347{,}886.000$ –

$8{,}307{,}321.316\ c = 1{,}000{,}753.788$

$c = 0.12046$

(1) ..... $57{,}322\ b + 325{,}267.8 = 623{,}002.0$

$57{,}322\ b = 297{,}734.2$

$b = 5.194065$

Quadratic Equation is Thus $P = 5.194065\ W + 0.120460\ W^2$

Part accounted for by Quadratic Regression is = $b\,(\Sigma PW) + c\,(\Sigma PW^2)$ =

$5.194065 \times 623{,}002$; $0.120460 \times 30{,}347{,}886 = 6{,}891{,}619.23$

Error S.S. = $6{,}925{,}240.66 - 6{,}891{,}619.23 = 33{,}621.43.$

Analysis of comparison between the two Regressions:

Source of Var.	S.S.	df.	M.S.	F. ratio	F.ratio 5% (1%)	Resul
Linear Reg.	6,771,073.79	1				
Quad. Reg.	6,891,619.23	2				
Increment from putting Quad. Reg.	120,545.44	1	120,545.44	10.756	10.1 (34.1)	*
Error	33,621.43	3	11,207.14			
Total	6,925,240.66	5				

(Continued .....)

The analysis shows a significant result at 5%.

Thus the data is better fitted to a curve rather than a straight line.

To draw the curve:

Quadratic equation is $P = 5.194065\ W + 0.120460\ W^2$.

W	0	10	20	30	40	50	60	70	72
P	0	63.987	152.065	264.236	400.499	560.853	745.300	953.839	998.4

APPENDIX 4.

Correlation Coeff. between P levels and fungal weights using phosphate series data to find out the best fungus response to "available" Phosphate.

a) Between P level and weight of A. niger.

Correlation formula:

$$r_{x.y} = \frac{\Sigma(x.y) - \frac{\Sigma x.\Sigma y}{n}}{\sqrt{\left(\Sigma(x^2) - \frac{(\Sigma x)^2}{n}\right)\left(\Sigma(y^2) - \frac{(\Sigma y)^2}{n}\right)}}$$

where: x = P level and y = mycelial weight.

From previous calculations (Linear Regression):

$\Sigma(xy)$	= 376,577.14	Σy^2	= 20,507.56
Σx^2	= 6,925,240.66	Σy	= 749.00
Σx	= 13,763.00		

$$r_{x.y} = \frac{376,577 - \frac{10,307,738}{36}}{\sqrt{\left(6,925,241 - \frac{189,420,169}{36}\right)\left(20,508 - \frac{561,001}{36}\right)}} = \frac{9025}{90420} = \underline{+0.998}$$

b) Between P level and weight of P. lilliacum.

$$r_{x.y} = \frac{407,759 - \frac{11,203,082}{36}}{\sqrt{\left(6,925,241 - \frac{189,420,169}{36}\right)\left(24,206 - \frac{662,596}{36}\right)}} = \frac{86,562}{\sqrt{(9,650,369,570)}}$$

$$= \frac{86,562}{98240} = \underline{+0.881}$$

c) Between P level and weight of C. geniculata.

$$r_{x.y} = \frac{623,002 - \frac{18,139,634}{36}}{\sqrt{\left(6,925,241 - \frac{189,420,169}{36}\right)\left(57,322 - \frac{1,737,124}{36}\right)}} = \frac{119,123}{\sqrt{(15,086,916,330)}}$$

$$= \frac{119123}{122800} = \underline{+0.970}$$

The best fungus to respond to increasing P is the A. niger, followed by C. geniculata and P. lilliacum.

APPENDIX 5.

PH of A. niger medium before and after incubation using $NH_4H_2PO_4$ as the source of P.

The pH of the medium before incubation was 3.45, and after incubation they were as follows:

mg. P/flask	PH after incubation			Mean dry weigh of mycelia (g
	I	II	Average	
0.0000	3.40	3.30	3.35	0.0000
0.0004	3.35	3.35	3.35	0.0590
0.0009	3.45	3.40	3.42	0.0085
0.0018	3.05	3.10	3.07	0.0957
0.0035	3.35	3.35	3.35	0.0885
0.0070	3.35	3.40	3.37	0.0810
0.0140	3.10	3.35	3.22	0.0402
0.0270	3.35	3.10	3.22	0.0472
0.0540	3.40	3.35	3.37	0.0835
0.1070	3.32	3.40	3.36	0.1142
0.2130	3.35	3.30	3.32	0.1815
0.4250	3.40	3.32	3.36	0.3157
0.8500	3.50	3.40	3.45	0.4997
1.6900	3.35	3.33	3.34	0.8098
3.3700	3.00	3.40	3.20	1.8142
6.7400	3.15	3.00	3.07	2.3402
13.4700	3.31	3.00	3.15	2.0420
26.9300	3.40	3.30	3.35	2.1852

APPENDIX 6.

Fresh weights (g.) of Millet yields.

(a, b, c, and d are replicates.)

Serial No. of soils	Top	Root	Total
1 a	250.5	26.40	276.90
b	247.7	36.20	283.90
c	265.0	46.00	311.00
d	246.5	36.3	282.80
2 a	275.5	12.68	287.73
b	295.2	19.6	314.80
c	308.3	11.2	319.50
d	302.5	13.10	315.60
3 a	231.2	12.50	243.70
b	188.7	9.70	198.40
c	229.0	8.90	237.90
d	226.8	13.63	240.43
4 a	143.2	6.00	149.20
b	116.7	11.2	127.90
c	148.5	9.37	157.87
d	155.1	11.2	166.30
5 a	38.5	7.0	45.50
b	39.7	3.24	42.94
c	35.6	2.50	38.10
d	34.9	3.10	38.00
6 a	196.2	6.5	202.70
b	168.5	9.8	178.30
c	178.9	10.55	189.45
d	170.7	5.42	176.12
7 a	267.5	23.8	291.30
b	263.8	16.90	280.70
c	281.3	21.12	302.42
d	290.0	15.40	305.40
8 a	106.5	4.85	111.35
b	127.5	5.85	133.35
c	123.5	4.40	127.90
d	101.5	3.32	104.82

(Continued)

Serial No. of soils	Top	Root	Total
9 a	317.20	9.98	327.18
b	349.0	23.70	372.70
c	301.5	11.30	312.80
d	371.0	14.05	385.05
10 a	277.8	21.80	299.60
b	325.5	21.50	347.00
c	258.0	15.20	273.20
d	332.7	11.0	343.70
11 a	225.9	11.90	237.80
b	255.5	15.37	270.87
c	243.4	16.00	259.40
d	227.2	16.3	243.50
12 a	252.2	18.40	270.60
b	223.1	13.07	236.17
c	227.2	13.50	240.70
d	275.2	29.70	304.90
13 a	315.5	11.84	327.34
b	312.5	12.10	324.60
c	269.0	11.90	280.90
d	323.9	26.20	350.10
14 a	51.0	3.58	54.58
b	54.4	4.84	59.24
c	56.9	4.0	60.90
d	62.1	6.50	68.60
15 a	86.5	4.45	90.50
b	99.5	7.35	106.85
c	107.3	6.60	113.90
d	100.5	5.82	106.32
16 a	19.5	2.80	22.30
b	14.9	2.9	17.80
c	14.9	0.92	15.82
d	17.3	1.60	18.90
17 a	86.5	10.0	96.50
b	91.4	8.60	100.00
c	77.5	11.33	88.83
d	84.4	8.90	93.30

(Continued.....)

Serial No. of soils	Top	Root	Total
18 a	30.2	1.60	31.80
b	35.7	1.80	37.50
c	35.4	1.50	36.90
d	43.7	2.0	45.70
19 a	289.2	9.70	298.90
b	212.5	8.10	220.60
c	239.4	*21.80	261.20
d	256.7	24.90	281.60
20 a	165.5	14.90	180.40
b	142.6	9.15	151.75
c	148.6	7.70	156.30
d	150.7	15.0	165.70

Fresh weights (g.) of Turnip total yields.

Serial No. of soils	Replicate I	Replicate II	Replicate III	Replicate IV
1	321.7	278.10	312.2	311.14
2	298.0	326.9	328.0	284.45
3	251.23	293.0	276.88	220.18
4	163.3	106.0	163.74	95.7
5	52.98	52.0	49.98	46.40
6	221.40	246.5	244.85	320.80
7	327.10	277.80	293.12	322.8
8	117.68	136.90	124.0	134.9
9	328.32	328.08	376.5	278.33
10	315.08	300.22	309.3	264.28
11	305.45	324.24	353.65	384.42
12	335.44	209.75	216.18	309.40
13	388.23	434.10	473.10	488.72
14	79.8	65.9	72.15	61.10
15	242.64	207.5	192.10	222.3
16	30.0	17.15	17.50	24.3
17	68.18	73.50	230.2	73.32
18	53.5	78.6	71.0	80.15
19	426.88	442.55	400.40	510.30
20	110.5	119.43	76.40	98.93

Fresh weights (g.) of Lucerne yields.

(a, b, c and d are replicates).

Serial No. of soils	Tops	Roots	Nodule grades	Total
1 a	91.00	86.60	10 L	177.60
b	74.40	71.80	10 L	146.20
c	100.70	99.50	10 L	200.20
d	84.00	90.50	10 L	174.50
2 a	99.50	70.50	10 L	170.00
b	118.20	86.30	10 L	204.50
c	74.20	93.40	10 L	167.60
d	109.70	85.50	10 L	195.20
3 a	48.30	55.10	8 L	103.40
b	40.60	54.40	8 M	95.00
c	41.10	33.90	8 L	75.00
d	58.5	61.20	8 L	119.70
4 a	31.40	28.70	2 M	60.10
b	28.50	17.80	1 M	46.30
c	33.70	20.90	2 M	54.60
d	26.50	15.80	2 M	42.30
5 a	23.10	13.50	1 S	36.60
b	25.10	10.92	1 M	36.02
c	18.90	12.0	1 M	30.90
d	22.12	11.40	1 S	33.52
6 a	55.80	36.10	8 L	91.90
b	62.20	31.75	8 L	93.95
c	58.30	37.70	8 L	96.00
d	65.70	46.80	8 L	112.50
7 a	36.45	29.40	4 M	45.85
b	49.10	41.00	4 S	90.10
c	37.00	32.10	8 L	69.10
d	38.7	27.30	4 M	66.00
8 a	24.40	16.10	8 L	40.50
b	32.90	29.70	8 L	62.60
c	28.00	22.25	8 L	50.25
d	31.70	26.70	8 L	58.40

(Continued.....) Fresh weights (g.) of Lucerne yields.
(a, b, c and d are replicates.)

Serial No. of soils	Tops	Roots	Nodule grades	Total
9 a	63.20	57.0	10 L	120.20
b	70.40	75.40	10 L	145.80
c	55.5	59.80	10 M	115.30
d	66.30	84.30	10 L	150.60
10 a	65.20	54.2	8 L	119.40
b	51.10	31.40	8 L	82.50
c	61.20	49.50	8 L	110.70
d	51.40	36.00	8 L	87.40
11 a	50.40	39.70	8 M	90.10
b	84.90	59.10	8 M	144.00
c	63.70	48.50	8 M	112.20
d	61.40	34.90	8 M	96.30
12 a	60.30	34.60	4 M	94.90
b	53.20	18.40	2 S	71.60
c	71.50	32.20	8 M	103.70
d	77.60	38.20	8 M	115.80
13 a	60.75	54.40	1 M	115.15
b	59.40	37.80	1 M	97.20
c	69.20	50.50	1 M	119.70
d	66.30	27.30	1 M	93.60
14 a	25.60	16.0	1 S	41.60
b	31.80	21.80	1 S	53.60
c	32.30	15.20	1 S	47.50
d	32.00	18.00	1 S	50.00
15 a	40.9	27.90	8 S	68.80
b	45.5	40.50	4 S	86.00
c	44.9	26.70	4 S	71.60
d	49.70	46.50	4 S	96.20
16 a	17.20	16.20	1 S	33.40
b	21.80	10.20	1 S	32.00
c	18.90	18.10	1 S	37.00
d	25.00	16.5	1 S	41.50

(Continued.....) Fresh weights (g.) of Lucerne yields.
(a, b, c and d are replicates.)

Serial No. of soils	Tops	Roots	Nodule grades	Total
17 a	39.00	45.90	2 S	84.90
b	46.90	38.50	2 S	85.40
c	43.30	46.55	2 S	89.85
d	38.30	45.0	2 S	83.30
18 a	26.00	23.60	4 S	49.60
b	31.70	30.5	4 S	62.20
c	31.40	25.60	4 S	57.00
d	35.90	25.0	4 S	60.90
19 a	86.20	58.30	10 L	144.50
b	77.50	96.0	10 L	173.50
c	73.30	83.30	10 L	156.60
d	86.70	75.90	10 L	162.60
20 a	44.20	56.10	8 L	100.30
b	53.60	33.10	8 L	86.70
c	53.30	44.90	8 L	98.20
d	53.70	51.20	8 L	104.90

Nodule grading:

L = large, larger than 2 mm. dia., clusters more than 50% are large.

M = medium, smaller than 2 mm. in dia., but bigger than pin head.

S = small, mostly pin head size.

Scores of No. of nodules:

0 ≡ 0
1 - 10 ≡ 1
10 - 20 ≡ 2
20 - 40 ≡ 4
40 - 80 ≡ 8
> 80 ≡ 10

8/4 = 8 to 4, ranges from 8 to 4,

M/S = ranges from medium to small, etc.

APPENDIX 7.

Data of Al-bound P determination.

Serial No. of soils	Means of corrected Beckman's reading	p.p.m. of P (x) *)
1	0.222	0.370
2	0.183	0.305
3	0.025	0.042
4	0.046	0.077
5	0.025	0.042
6	0.059	0.098
7	0.056	0.093
8	0.033	0.055
9	0.050	0.083
10	0.043	0.072
11	0.043	0.072
12	0.025	0.042
13	0.244	0.407
14	0.037	0.062
15	0.046	0.077
16	0.026	0.043
17	0.052	0.087
18	0.037	0.062
19	0.504	0.840
20	0.304	0.507

*) The amount of P extracted from 1 g. of soil can be obtained by the expression:

$$1250 \times (x) = \mu g.\ P/g.\ \text{of soil.}$$

Data of Alkali soluble (Fe + Al-bound) Phosphate:

Serial No. of soils	Means of corrected Beckman's reading	p.p.m. of P (x) *)
1	0.304	0.507
2	0.278	0.463
3	0.064	0.107
4	0.080	0.133
5	0.057	0.095
6	0.037	0.062
7	0.087	0.145
8	0.058	0.097
9	0.130	0.217
10	0.122	0.206
11	0.125	0.208
12	0.098	0.163
13	0.314	0.523
14	0.070	0.117
15	0.121	0.202
16	0.086	0.143
17	0.088	0.147
18	0.054	0.090
19	0.745	1.239
20	0.454	0.755

*) The amount of P extracted from 1 g. of soil can be obtained by the expression:

1000 x = μg. P/g. of soil.

Data for Ca-bound phosphate values:

(a) HCl extraction:

Serial No. of soils	Means of corrected Beckman's reading	p.p.m. of P (x) *)
1	0.347	0.578
2	0.412	0.687
3	0.031	0.052
4	0.084	0.140
5	0.015	0.025
6	0.062	0.103
7	0.156	0.260
8	0.036	0.060
9	0.050	0.083
10	0.040	0.067
11	0.110	0.183
12	0.049	0.082
13	0.300	0.500
14	0.006	0.010
15	0.017	0.028
16	0.012	0.020
17	0.013	0.022
18	0.001	0.002
19	0.119	0.198
20	0.041	0.068

*) The amount of P extracted from 1 g. of soil expressed as:

μg. P/g. soil = 500 x.

Data for Ca-bound phosphate values (cont'd).

(b) NaOH extraction:

Serial No. of soils	Means of corrected Beckman's reading	p.p.m. of P (x) *)
1	0.231	0.385
2	0.196	0.327
3	0.077	0.128
4	0.108	0.180
5	0.075	0.125
6	0.057	0.095
7	0.086	0.143
8	0.079	0.132
9	0.161	0.268
10	0.151	0.252
11	0.174	0.290
12	0.158	0.263
13	0.313	0.522
14	0.094	0.157
15	0.156	0.260
16	0.106	0.177
17	0.118	0.197
18	0.087	0.145
19	0.797	1.328
20	0.556	0.927

*) The amount of P extracted from 1 g. of soil can be obtained from the expression:

$$\mu g.P/g.\ soil = 1000x.$$

Data for Truog values of available P.

Serial No. of soils	Means of corrected Beckman's reading	p.p.m. of (x) *)
1	0.501	1.08
2	0.659	1.41
3	0.056	0.12
4	0.118	0.26
5	0.023	0.05
6	0.100	0.22
7	0.166	0.36
8	0.056	0.12
9	0.107	0.23
10	0.113	0.25
11	0.186	0.40
12	0.105	0.225
13	0.330	0.715
14	0.016	0.04
15	0.064	0.14
16	0.016	0.04
17	0.033	0.075
18	0.014	0.03
19	0.312	0.675
20	0.121	0.262

*) The amount of available P extracted from 1g. of soil can be calculated from expression:

μg. P/g. of soil = 500 x.

APPENDIX 8.

Ordinary correlation coeff. and Rank correlation.

(For example: Correlations between A. niger method and Turnip total yields).

Serial No. of soils	Dry weight of A. niger (x cg.)	Dry weight of Turnips (y g.)	Ranking of soils		Differences of ranks = d.
			According to A. niger	According to Turnips	
1	35	32	2	6	4
2	34	32	4	4	0
3	12	30	17	9	8
4	24	17	7	13	6
5	13	6	13	19	6
6	20	28	9	11	2
7	26	32	6	5	1
8	13	15	15	14	1
9	21	31	8	7	1
10	13	30	14	8	6
11	13	35	16	3	13
12	11	28	20	10	10
13	34	56	3	2	1
14	11	11	19	18	1
15	14	23	12	12	0
16	12	4	18	20	2
17	20	13	10	16	6
18	17	11	11	17	6
19	38	58	1	1	0
20	27	13	5	15	10
Sums	408	505			$\Sigma d^2 = 642$

a) Ordinary Correlation Coeff.

$$r_{x.y} = \frac{\Sigma(x.y) - \frac{\Sigma x.\Sigma y}{n}}{\sqrt{\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right)\left(\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right)}} = \frac{11,842 - \frac{206,040}{20}}{\sqrt{(1571)(16,801 - \frac{206040}{20})}} =$$

$$\frac{1540}{\sqrt{(1571)(4050)}} = \frac{1540}{2523} = +0.610..... (**)$$

(Rank Correlation Overleaf

b) Rank Correlation Coeff.

$$r_{x.y} = 1 - \frac{6\,\Sigma d^2}{n(n^2-1)} = 1 - \frac{6\,\Sigma d^2}{7980} = 1 - \frac{6 \times 642}{7980}$$

$$= 1 - 0.483 = +0.517..... (*)$$

ooOoo