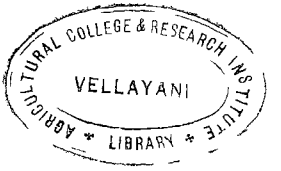
RHIZOSPHERE MICROFLORA OF TOBACCO

INFLUENCE OF TOBACCO MOSAIC VIRUS AND CERTAIN CHEMICALS ON THE MICROBIAL POPULATION



BY

S. BALAKRISHNAN

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY) OF THE UNIVERSITY OF KERALA

DIVISION OF PLANT PATHOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
VELLAYANI, TRIVANDRUM

1967

C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri.S. Balakrishnan, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

(C.K.K.Nair)
Principal & Additional
Director of Agriculture
(Research)

(J. Sam Raj)
Professor of Plant Patholo y.

gricultural College &
Research Institute, Vellayani,
Trivandrum, August, '07.

A C K N O W L E D G E M E N T S

I express my grateful indebtedness to Dr. Sam Raj, Professor of Plant Pathology, for suggesting this problem and for his inspiring guidance and constant help through out the progress of this work.

I am extremely thankful to Dr. C.K.K. Nair, Principal & Additional Director of Agriculture (Research) for kindly providing necessary facilities for this investigation and various helps rendered.

I place on record my grateful thanks to Dr. K.J. Wilson, Senior Lecturer cum Assistant Virologist for his valuable suggestions and helpful criticisms.

My thanks are also extended to Shri. T.J. Thomas, Junior Professor of Agricultural Statistics for necessary helps rendered for the statistical analysis of the data.

Finally I wish to thank all the members of the staff of Plant Pathology Division for their help and timely advice.

S. BALAKRISHNAN.

C O N T E N T S

INTRODUCTION

INTRODUCTION

The region of soil in close proximity to the root system of plants provide a unique ecological habitat, which favours the increased occurrence of microorganisms. This region was termed by Hiltner (1940) as the 'rhizosphere'. After the appearance of Hiltner's report regarding the abundance of microorganisms in the rhizosphere region, this subject has received the attention of a large number of workers who made detailed investigations on different aspects of microbial population in relation to plant growth.

Many factors such as type of plant, age of plant, soil conditions, environmental conditions etc. are known to exert considerable influence on the rhizosphere microflora. Young and growing roots are found to harbour more microorganisms than dead or nongrowing roots. The increased activity is believed to be due to various root secretions such as amino acids, vitamins, sugars etc. and also to the piculiar soil environment.

When a plant becomes diseased as a result of infection by virus or other organisms, the effect of the

disease is usually reflected on the rhizosphere microflora also, on account of the physiological changes brought about by the disease on the host. Lakshmikumari (1960) found an increase in the number of microorganisms in the rhizosphere of Dolichos lablab plants infected by the Dolichos enation mosaic virus. Sadasivan (1963) has also made references to the above phenomenon. A similar effect was noticed by Ranganathan (1965) in the rhizosphere populations of banana plants infected by the bunchy top virus.

In a like manner, the introduction of many substances, especially those having systemic action, into the plants was shown by earlier workers like Halleck and Cochrane (1950), Venkat ram (1960), Lakshmikumari (1961), Sadasivan (1963) and Kandasamy and Rangaswami (1967), to bring about changes in the rhizosphere microflora. Depending on the substance used, these changes were found to be either beneficial or harmful to the microorganisms in the rhizosphere. It is also known as a result of the work of Lakshmikumari (1961) on the D.L.M.V. affected Dolichos lablab plants, that the effect of virus infection on the rhizosphere microflora could be counteracted by the application of certain

chemicals. However, the present state of our knowledge on the above subjects is very limited and it was therefore thought that further work in this direction may be useful.

An attempt was therefore made to determine the effect of T.M.V. and also certain chemicals on the rhizosphere microflora of tobacco plants. The individual effects of the virus and the chemicals on the plant as well as the effect of pretreating the plants with the chemicals before inoculation were determined. Urea, one of the materials used in the work, was known to exert a depressing effect on the rhizosphere microflora, while the effects of terramycin and 2,4-D were not known. Terramycin, being an antibiotic, was expected to exert a depressing effect on the rhizosphere microflora while 2,4-D which induces malformations similar to those brought about by virus infection was expected to exert a stimulatory effect.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hiltner (1904) found that the root-system of plants influenced the type and population of microorganisms in the region of soil which is in close proximity to it. He used the term 'rhizosphere' to denote this region. Since then, intensive investigations on the microbial population in the rhizosphere have been carried out by many workers.

Starkey (1929, 1931) reported greater incidence of microorganisms in the rhizosphere compared to that in the soil away from the influence of root-system. This positive rhizosphere effect has been noted in many plants by various workers like Krassilnikov et al (1936), Lochhead (1940), Timonin (1940), Katznelson and Richardson (1943), Rangaswami and Vasantharajan (1962) and Sundara Rao and Venkataraman (1963). A negative rhizosphere effect was, however, noted in Brassica juncea and Allium cepa by Bhuvaneshwari (1958).

Various factors such as type of plant, age of plant, soil conditions, environmental conditions etc are known to exert considerable influence on the rhizosphere microflora.

Adati (1939) found that the influence of cereals on the development of microorganisms was generally more

powerful than that of legumes. Timonin (1940), Lochhead (1940), Katznelson and Richardson (1945), Krassilnikov (1944), Rangaswami and Vasantharajan (1962) and many others found that the bacteria, actinomycetes and fungi present in the rhizosphere are differentially influenced by the particular crop. Vagyaraj and Rangaswami (1966) reported that the rhizosphere effect varied with crop, soil depth, plant age and type of microorganisms.

Age of the plant exerts a remarkable influence on the rhizosphere microflora. Timonin (1940) noted the establishment of a rhizosphere microflora in wheat plants within three days of seed germination. Rouatt (1959) noted a R:S ratio of 2:1 for bacteria in the rhizosphere of three days old wheat seedlings.

Novira (1956) and many others found that, with an increase in age of plants, there was a corresponding increase in the microbial population in the rhizosphere. Timonin (1940), Agnihotbrudu (1953), Firsanova (1956) and Rangaswami and Venkatesan (1963) reported that in the case of annuals the bacterial population increases till flowering stage, when it reaches the maximum vegetative growth and thereafter declines. Rema Devi (1964) observed an increase in the microbial population in the rhizosphere

of tomato plants corresponding to the increase in the age of plants, and the maximum population was recorded at the flowering stage. While studying the rhizosphere microflora of rice, Remigius (1966) observed maximum microbial population at the flowering stage and a gradual reduction thereafter.

The soil conditions also are known to influence the rhizosphere microflora. Rangaswami and Venkatesan (1963) found a lesser microbial population in the dry soil than in a wet soil and that the top layers of the soil supported more population than deeper layers. Peterson et al (1965) reported that the population of bacteria in the rhizosphere and rhizoplane of wheat increased as soil moisture decreased from 90% to 30% of the total moisture holding capacity. Katznelson and Richardson (1943) noted marked differences in the number of actinomycetes and fungi in the rhizosphere of tomato plants as a result of soil sterilization with steam, chloropicrin and formaldehyde.

Contois (1953) noted that the rhizosphere microfloras of plants are influenced by altitude, rainfall and soil pH. Rouatt et al (1963) found that the microorganisms in the root zone is directly influenced by the temperature.

Root exudates and rhizosphere effect.

The increased activity of microorganisms near the root zone was explained by Hiltner (1904) as due to root excretions. It is now widely accepted that the increased microbial activity in the rhizosphere is due to the organic materials exuded from the roots. Amino acids, vitamins, sugars, tannin, alkaloids and various other substances are known to be present in root exudates.

West (1939) reported that flax seedlings excreted significant amounts of thiamine and biotine. Timonin (1941) observed that 'Bison' variety of flax, resistant to wilt caused by Fusarium lini, excreted hydrocyanic acid through the root system. Hydrocyanic acid in the root exudates has been noted by Rangaswami and Balasubramanian (1963) in cholam varieties CO_4 and K_1. Katznelson et al (1954), Andal et al (1956) Rovira (1956), Bhuvaneshwari and Subba Rao (1957) and Subba Rao and Baily (1961) found that root excretions of plants contained amino acids. Singh (1967) was able to isolate six amino acids from the root exudates of corn plants.

Bhuvaneshwari (1960) found that the root exudates of rice varieties resistant to Fusarium moniliforme

exhibited fungistatic effect on the pathogen and encouraged the growth of saprophytic bacteria actinomycetes and fungi. The root exudates of the resistant variety inhibited spore germination, germ tube growth and radial growth of the pathogen.

Rhizosphere effect on the microorganisms.

Several workers have found that various groups of bacteria have been differentially stimulated in the rhizosphere depending upon the type of the plant. Lochhead (1940) found that the gram negative rods were activated more than the other forms in the rhizospheres of red clover, mangels, oats, tobacco, maize and flax. Spore forming bacteria were lesser in the rhizosphere than in control soil. Krassilnikov (1944) reported that 95% of the rhizosphere bacteria constituted gram negative rods. King and Wallace (1956) reported that there occured a selective stimulation of gram negative rods in the rhizosphere of oats. Rangaswami and Vasantharajan (1962) observed a more abundant occurrence of gram negative non-sporeforming rods than gram positive rods and spore formers, in the rhizosphere of citrus plants.

Lochhead (1940) reported a greater incidence of gelatin liquifiers than nitrate reducing forms in the

rhizosphere of redclover, mangels and oats. Katznelson and Richardson (1943), while studying the rhizosphere of tomato plants found a tendency for the bacteria with simple requirements and those requiring amino acids, to predominate in the rhizosphere.

The actinomycete population also have been found to be greatly stimulated in the rhizosphere of plants.

In the rhizosphere of wheat, oats, lucerne and peas, Timonin (1940) reported a population of actinomycetes 7 to 71 times greater than that in the control soil. Rangaswami and Vasantharajan (1962) reported that actinomycete were 4 to 20 times more in the rhizosphere of citrus plants, that in the non-rhizosphere soil, and those with antagonistic effect were more predominant in the rhizosphere. Rangaswami and Venkatesan (1963) noted a steady increase in the actinomycete population in the rhizosphere of rice till harvest. Venkatesan and Rangaswami (1964) observed actinomycetes which are antagonistic to bacteria and fungi, in the early stages of plant growth than at crop maturity.

The fungal population in the rhizosphere, shows qualitative and quantitative difference from that in the control soil. Katznelson and Richardson (1948) found that

the fungus Cladosporium occurred abundantly in the rhizosphere of strawberry plants at the age of 100 days and Verticillium was predominant at the age of 270 days.

Agnihothrudu (1953) and Agnihothrudu et al (1955) have been able to isolate species of Aspergillus, Penicillium, Fusarium, Alternaria, Curvularia, Mucor, Rhizopus, Helminthosporium, Trichoderma, Cunninghamella, Phoma, Diplodia, Cheatomium and Macrophoma phaseoli from the rhizospheres of pigeon pea and sorghum.

Rangaswami and Vasantharajan (1962) isolated species of Aspergillus, Penicillium, Fusarium, Helminthosporium, Mucor, and Rhizopus from the rhizosphere of citrus plants.

Contois (1953) reported that pineapple plants grown at lower altitudes harboured Aspergillus and Penicillium species abundantly, but in higher altitudes Rhizopus - nigricans and Circinella simplex were more common.

Subba Rao and Bailey (1961) found a species of Fusarium predominant in the rhizosphere of Verticillium wilt susceptible varieties of tomato plants and Trichoderma - viride abundant in the resistant varieties.

Das (1963) reported that the fungal association with rice root changes with the growth of the crop. During the

early stage of vegetative growth, common soil fungi such as Aspergillus and Penicillium were found associated with the roots, while Trichoderma viride and Cephalosporium spp. were associated with the roots of plants belonging to all ages.

Influence of virus infection on the rhizosphere microflora

Virus infection causes many complicated physiological changes within the host plants and it is now known that these changes can be reflected in the rhizosphere of the plants. Lakshmikumari (1960) while studying the rhizosphere of Dolichos lablab plants infected with Dolichos enation mosaic virus, observed that bacteria, actinomycetes and fungi showed an increase in their population as infection advanced. By the 25th day after infection there was a fall in the fungal population and she attributed this to the very high number of bacteria and actinomycetes in the rhizosphere which may have antagonistic action on the fungal population.

Sadasivan (1963) quoting the work of Lakshikumari (1960) reported three phases in the host parasitic interaction at the rhizosphere region of D.E...V infected Dolichos lablab plants. The first phase was when

active virus multiplication took place (5 days), the second production of stunting (10 days) and third the development of various foliar abnormalities (20 days). Observations on the rhizosphere of healthy and inoculated plants in varying environmental conditions revealed considerable variation in the time lag for the appearance of maximum rhizosphere effect on the three major groups of soil organisms. The various physiological and nutritional groups of bacteria also responded readily to change in the infected host. Cellulose decomposing organisms considerably increased in the rhizosphere of infected plants. Ammonifiers and nitrifiers increased on the 5th and 25th days after inoculation. At 10 days there was a notable decrease in almost all groups. A somewhat similar shift characterises the incidence of nutritional groups of bacteria in the rhizosphere of infected plants. Initially there was a stimulation of the aminoacid requiring group. At 10 days the predominant group was the one requiring complex substrates of yeast and soil extracts. Twenty days after infection there was a shift towards more simple groups.

Ranganathan (1965) found that the rhizosphere populations of banana plants infected with bunchy top disease were much higher than those of the non-infected plants of

the same age. On the 10th day after inoculation he observed a significantly higher population in the rhizosphere of the inoculated plants.

Influence of chemicals and antibiotics on the rhizosphere microflora

Halleck and Cochrane (1950) reported that bordeaux mixture, malachite green and dithane Z-78 applied to the leaves of bean plants reduced the relative numbers of bacteria in the rhizosphere, whereas spergon, phygon-XL, Cetyl trimethyl ammonium bromide and proflavine have the opposite effect, in varying degrees, increasing the relative number of rhizosphere bacteria. Remigius (1966) found that bordeaux mixture and ceresan lime dust have no effect on the rhizosphere microflora of rice plants.

Ramachandra Reddy (1959) reported that two to five sprayings of 0.1 M. solution of urea with a detergent on the foliage of a strain of rice susceptible to foot rot disease caused by Fusarium moniliforme showed that the treated plants registered in the rhizosphere a higher fungal count, than untreated control, along with a concomitant decrease in the bacterial and actinomycete population.

Venkatram (1960) while studying the effect of foliar application of nutrients on the rhizosphere microflora of

Camellia sinensis found that foliar spray with certain inorganic or organic nutrients including urea, reduced the bacterial population of the rhizosphere. Horst and Herr (1962) while studying the effect of foliar treatment with urea on the number of actinomycetes antagonistic to Fusarium roseum f. cerealis, in the rhizosphere of corn seedlings, found that at the first sampling urea caused increase in the numbers of actinomycetes and this increase was smaller in second sampling and there was a decrease in the third sampling. Vrany (1963) found that the bacterial numbers in the rhizosphere of wheat, increased markedly as fungi decreased in the first two days after foliar treatment with urea.

Kandaswamy and Ranganswami (1967) found that the qualities of rhizosphere microflora of sorghum plants are greatly altered by the different nutrient sprays and such changes not only depended on the nutrient sprayed but also on the strain and age of plants. They observed that spraying with ammonium sulphate and urea resulted in the reduction of bacteria and actinomycetes in the rhizosphere of sorghum plants. Disodium hydrogen phosphate spray reduced bacteria actinomycetes and fungi.

Fraser (1953) reported that chloromycetin and streptomycin were absorbed by the root system of cucumber

seedlings and translocated to the leaves. Further, he reported the systemic penetration of streptomycin in the tissues of bean and tomato plants. Panier et al (1956) found streptomycin sulphate spray on the primary leaves of dwarf beans exhibited marked systemic action and that it could be detected even in the fourth trifoliate leaf. Bowler and Goodman (1958) detected the downward translocation of streptomycin by Coleus sp. and found that greater absorption of the antibiotic was occurred when applied to the lower leaf surfaces.

Pangavani and Vasantharajan (1961) reported that there was no appreciable change between the number of microorganisms in the rhizosphere of plants sprayed with streptomycin, and unsprayed plants. Rema Devi (1964) found a slight decrease in the microbial population in the rhizosphere of tomato plants sprayed with streptomycin. Remigius (1966) observed that rice plants sprayed with streptomycin, streptocycline and blasticidin-S showed a decrease in their rhizosphere populations.

Sadasivan (1963) reported that a reversal of an abnormal rhizosphere effect to that of normal, was noticed

when pathological stunting of D.L.M.V. infected Dolichos lablab plants was nullified by post-inoculum sprays of gibberellin. A similar approximation to a normal picture was again seen when the active virus multiplication was inhibited by post-inoculum sprays of thiouracil administered daily. This effect seen at 5 days after inoculation, however wore out later.

Mace (1965) noted that concentrations of oxytetracycline greater than 5.6×10^{-4} M. were inhibitory to P.M.V. invivo when applied within 5 hours after inoculation and the virus was not inhibited invivo by streptomycin.

MATERIALS AND METHODS

Jaffna variety of tobacco was used in the present studies. The experiments was conducted on potted plants. The potting mixture used, was made up of farm yard manure, river sand and garden soil in the ratio 1:1:1. The seeds were also sown in pots and they germinated on the 7th days after sowing. Transplanting was done on the 50th day.

I. Determination of the rhizosphere microflora in relation to the age of tobacco plants.

The plants needed for this experiment were raised in 18" pots. The assessment of the rhizosphere microflora was started on the 10th day after sowing. For the first 30 days the samples were drawn at 5 days intervals and thereafter once in 10 days. The method adopted in the present studies was the soil dilution and plate counts (Timonin, 1940).

1. Collection of samples: The plants were uprooted with a block of soil and then the soil around the root system was removed carefully. The plants were shaken and the root system was tapped gently with a sterile needle to remove the superfluous soil adhering to the root system. Then the roots were cut off aseptically and transferred into

previously weighed Erlenmeyer flasks containing 100 ml of sterile distilled water. Sufficient quantity of the roots were added to attain a turbidity equivalent to the addition of 2 to 3 grams of soil. (Wallace and Loch head, 1949).

The soil samples used as control, were drawn from separate pots without any plants which were kept for this purpose. The soil samples were drawn from the same depth from which the roots were taken. Two grams each of the control soil were weighed separately. One sample was taken in a previously weighed clean china dish and placed in a hot air oven kept at 105 to 110 ° C for six hours to evaporate the entire moisture. It was allowed to cool in the oven itself. Then it was weighed and the moisture content was calculated. The other sample was transferred into a weighed Erlenmeyer flask containing 100 ml of sterile distilled water.

ii. Determination of the total bacteria actinomycetes and fungi populations: Flasks containing 100 ml of sterile distilled water, into which plant roots and control soil were added, were shaken for about half an hour on a mechanical shaker. Appropriate dilutions were prepared

from this suspension. The final dilution used for plating was one in million. This dilution was prepared by two successive transfers of 1 ml aliquot from the lower dilution to 99 ml of sterile distilled water. In all the dilutions each flask of the dilution series was shaken for two minutes before transferring the next aliquot, care being taken to pipette the suspension while it was in motion. All the transfers were done under aseptic conditions using pipettes sterilized with one end plugged with cotton wool. 1 ml of the final dilution was plated in sterile petridishes along with 15 ml of the medium. Different media were used for bacteria, actinomycetes and fungi. Soil extract agar, Kenknight's agar and Peptone - dextrose agar with rose bengal were used for bacteria, actinomycetes and fungi respectively.

At first the plating was done by using the method suggested by Timonin (1940). 1 ml of the final dilution was transferred into a sterile petridish using a sterile pipette. The plate was rotated gently to spread the suspension uniformly in the plate. The medium, melted and cooled to 40°C, was then poured over this and the plate was again rotated to get an even spread of the

suspension and the medium. In this method the colonies appeared to get crowded towards the periphery of the petridish. Therefore a modification in the method was made as suggested by Rema Devi (1964). 1 ml of the final dilution was poured directly to the test tube containing melted medium at 48°C. The test tube was then rotated well between the palms and the medium was then poured into the sterile petridish. The petridish was then rotated gently to get an even spread of the medium. The colonies appeared uniformly distributed when the plating was done by using this method.

The dilutions were plated in triplicate for each group of microorganism. The plates were then incubated at room temperature for about fourteen days. Counts for fungal colonies were taken 6 to 7 days after plating as soon as the colonies began to appear. For bacteria and actinomycetes an incubation period of 10 to 14 days was necessary. Spencer's Dark Field Quebec Colony counter was used for counting the colonies of bacteria and actinomycetes. The counts were expressed in millions per gram of the soil on dry weight basis.

To find the dry weight of the rhizosphere soil used for preparing the dilution, the roots were removed from the flask after washing down all the adhering soil particles into the same flask. Then the flask was evaporated to dryness by placing it in a waterbath. After that the flask was kept in a hot air oven at 105 to 110°C for six hours. Then it was allowed to cool in the oven itself and it was weighed. The dry weight of the soil was determine, making necessary corrections for the aliquots of soil removed during dilutions.

Composition of the different media used:

(1) Soil extract agar (Taylor and Lochhead, 1938)

Soil extract	..	1000 ml
K_2HPO_4	..	0.2 gm
Agar agar	..	15.0 gm

Soil extract was prepared by autoclaving 1000 gm of the soil with 1000 ml of tap water for 20 minutes at 15 lb pressure. The soil was allowed to sediment and the supernatent liquid was decanted to another flask. In order to hasten the sedimentation of the soil particles a small quantity of $CaSO_4$ was added to the suspension before

filtering. The extract was then filtered through a No.41 filter paper. The volume of the filtrate was made up to 1000 ml by adding distilled water. One gram of glucose was added to the above medium to hasten the appearance of colonies. The pH of the medium was adjusted to 6.8 prior to sterilization. This medium was used for bacteria.

(2) Ken knight's agar

Glucose	..	1.0 gm
K_2HPO_4	..	0.1 gm
$MgSO_4$	..	0.1 gm
$NaNO_3$	..	0.1 gm
KCl	..	0.1 gm
Agar agar	..	15.0 gm
Distilled water	..	1000 ml

This medium was used for Actinomycetes.

(3) Peptone-dextrose agar with rose bengal and streptomycin (Martin, 1950)

Dextrose	..	10.0 gm
Peptone	..	5.0 gm
KH_2IO_4	..	1.0 gm
$MgSO_4$	..	0.5 gm
Ager agar	..	15.0 gm

Rose bengal	..	1 Part in 30000 parts of the medium
Distilled water	..	1000 ml
Streptomycin	..	30 u g per ml

(Streptomycin was added only at the time of plating). This medium was used for fungi.

The above three media were prepared and autoclaved in test tubes at 15 lb pressure for 20 minutes. 15 ml of the medium was taken in each tube.

II. The influence of T.M.V. on the rhizosphere microflora of tobacco plants

Healthy tobacco plants, 65 days old and grown singly in 5" pots were inoculated with the sap of T.M.V. affected tobacco leaves. Root samples were taken 24 hours, 48 hours, 5 days, 10 days, 15 days and 25 days after inoculation. Healthy plants of the same age served as control plants.

A single plant constituted one replication and there were three replications for each sample. Dilutions were prepared for the root samples of healthy and inoculated plants and for control soil. Plating was done as described earlier.

III. Effect of foliar application of Urea, 2,4-D and Terramycin on the rhizosphere microflora of diseased and healthy plants

Tobacco plants, 65 days old and grown singly in 5" pots were used for this work. A single plant represented one replication and there were three replications for each sample.

The plants were devided into three lots and each lot received a foliar spray of an aqueous solution of one of the following materials.

1. Urea 0.83 M. solution
2. 2,4-D 10 ppm
3. Terramycin 0.00112 M. solution

Half the number of plants under each treatment were inoculated with T.M.V., 24 hours after receiving the spray. The remaining plants served as sprayed control. In addition, unsprayed controls were also kept.

20 ml of the spray material was used for each plant. Spraying was done by using atomizers. The plants were completely covered with a thin film of the liquid. The surface of the soil was covered with cotton wool, to prevent the materials from reaching the root system. The cotton wool was removed as soon as the spray got dried up.

Determination of the rhizosphere microflora was done on the date of spraying on the date of inoculation and 6 days, 16 days and 26 days after spraying.

Samples were taken from the plants which received the following treatments and also from the control soil.

1. Urea + Inoculation
2. 2,4-D + Inoculation
3. Terramycin + Inoculation
4. Urea only
5. 2,4-D only
6. Terramycin only
7. Inoculated, without chemical treatment
8. Uninoculated, without chemical treatment.

RESULTS

RESULTS

I. Influence of age of the plant on the rhizosphere microflora of tobacco plants

There was a marked increase in the population of the rhizosphere microflora with the age of the plants. This increase became pronounced from the 15th day onwards and a sharp increase was noticeable between 40th and 50th day. Then there was a steady increase till flowering, followed by a gradual decline. This was found to be true with all the three groups of organisms, namely, bacteria, actinomycetes and fungi.

(i) Bacteria

The bacterial population was found to increase with the growth of the plants till flowering. On the 10th day after sowing the bacterial population in the rhizosphere was 5.86 millions and the B:S ratio was 1.15. The B:S ratio rose to 1.44 on the 15th day. After that there was a steady increase in the bacterial population and this increase was comparatively more pronounced between the 40th and 50th day after sowing. The population on the 40th day was 24.56 millions, while that on the 50th day was 49.72 millions. The corresponding B:S ratios were 8.62 and 9.26. This increase in the population continued upto the 110th day when the

Table 1

Total bacterial popu ation in the rhizosphere of tobacco plants and i the co trol so ls at different stages of plant growth

(opul tio 10^6 per gram of dry soil)

ge in days	10	15	20	25	30	40	50	60	70	80	90	100	110	120	130	140
Control soil	5.10	83	4 96	5 21	5 16	4 08	4 94	4 59	4 54	4 60	4 56	4 62	4 68	4 59	4 96	4 80
Rhizosphere	5 6	6 94	8 90	12 52	16 35	4 6	45 72	50 64	61 50	79 46	95 58	118 76	127 46	122 24	118 35	110 46
R:S ratio	1 15	1 44	1 79	2 40	3 17	02	9 26	11 03	13 55	17 27	20 96	25 71	27 24	26 63	23 86	23 01

BACTERIAL POPULATION IN THE RHIZOSPHERE OF TOBACCO PLANTS
AS INFLUENCED BY THE AGE OF THE PLANTS

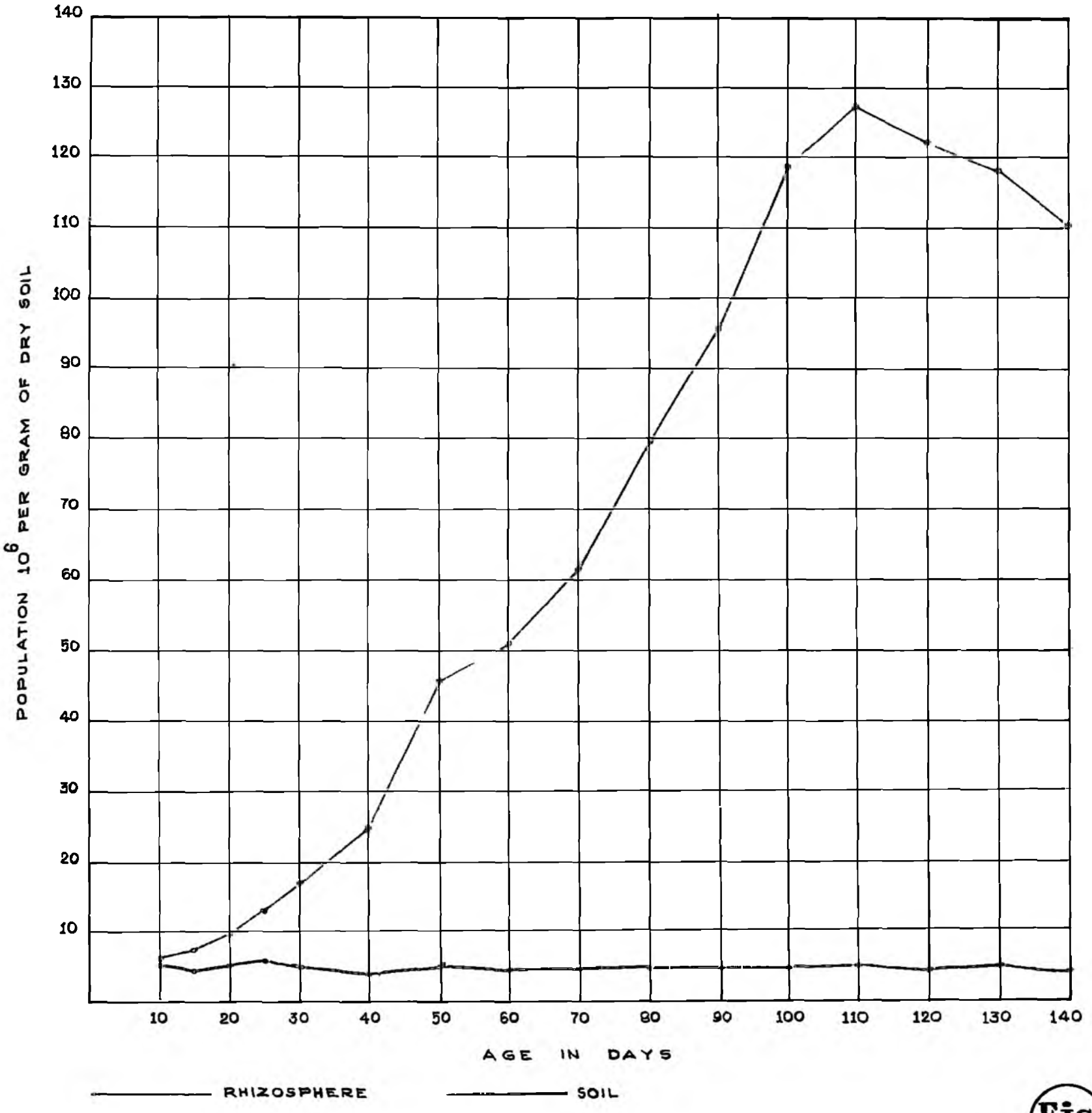


Fig 1

maximum population of 127.46 millions was reached. The plants flowered on the 110th day and subsequently there was a gradual fall in the population and on the 140th day the population was only 110.46 millions (Table 1).

(ii) Actinomycetes

The population of the actinomycetes also showed a marked tendency to increase with the age of the plants till flowering.

In 10 days old plants the population was 2.92 millions and that in the 15 days old plants was 3.13 millions. The corresponding R:S ratios were 1.30 and 1.34. A population of 7.17 millions was reached on the 40th day and this rose sharply to 12.50 millions on the 50th day. The corresponding R:S ratios were 2.78 and 4.22. After this, there was a steady increase in the population and the maximum population of 30.25 millions was recorded on the 110th day corresponding to the flowering stage.

After the 110th day there was a gradual decline in the actinomycete population in the rhizosphere and the population was only 24.68 millions on the 140th day (Table 2).

(iii) Fungi

The fungal population in the rhizosphere, also increased as the plants became old r till flowering, after

able 2

otal actinomycete popul tion in the rhizosphere of tobacco plants and in the co trol soils at different stages of plant growth

(Populatio 10^6 per gram of dry soil)

ge in days	10	15	20	25	30	40	50	60	70	80	90	100	110	120	130	140
Control soil	4	2 26	2 30	2 32	2 28	2 8	2 96	2 87	3 02	2 96	3 10	2 94	2 05	2 86	2 90	2 64
Rhizosphere	2 92	3 13	3 41	4 1	4 7	7 17	12 50	13 46	14 87	18 90	21 46	26 30	30 5	27 45	26 20	24 68
R: ratio	1 30	1 34	1 48	1 77	2 00	2 78	4 22	4 69	4 98	6.39	6 92	8 95	10 25	9 59	9 03	9 34

ACTINOMYCETE POPULATION IN RHIZOSPHERE OF TOBACCO PLANTS AS INFLUENCED BY THE AGE OF THE PLANTS

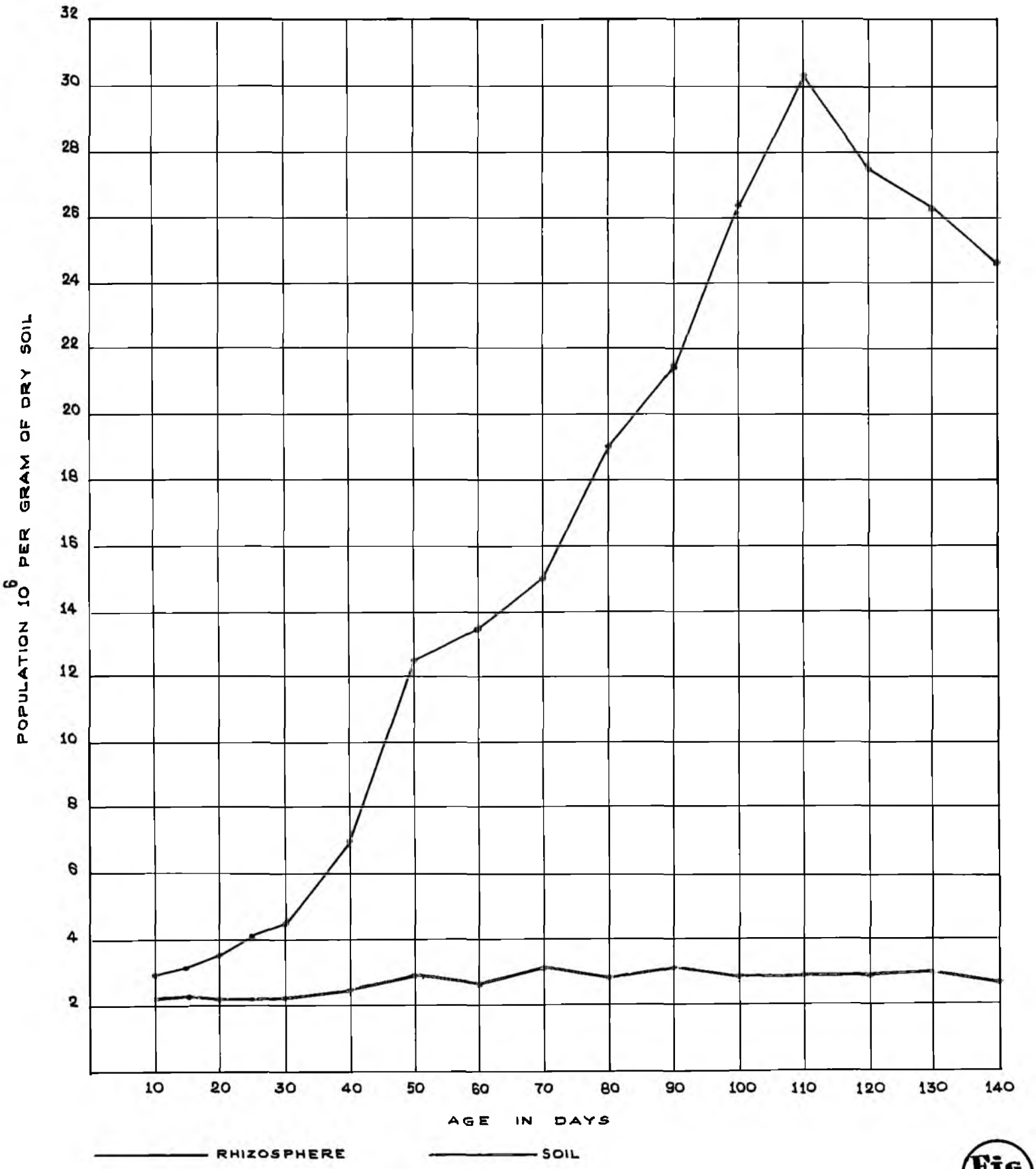


Fig 2

ab e 3

otal f dul pop ation in he r izoe here of t o o l ts
and in he c t ol soilu at d f er t st res o nt rro th
(o u a io O p r gr m d 1)

ge in daye	0	1	20	25	30	40	5	0	0	0	0	00	0	20	30	40
C ntrol soil	0 7	76	0 80	C 79	0 80	4		4				3	4		2	20
zosphere		8	0 93			3	5	4				6	52	52	42	
ratio	12	5	7	4	2 45				0	52	79	86	86	3 03	3 05	

FUNGAL POPULATION IN THE RHIZOSPHERE OF TOBACCO PLANTS AS INFLUENCED BY THE AGE OF THE PLANTS

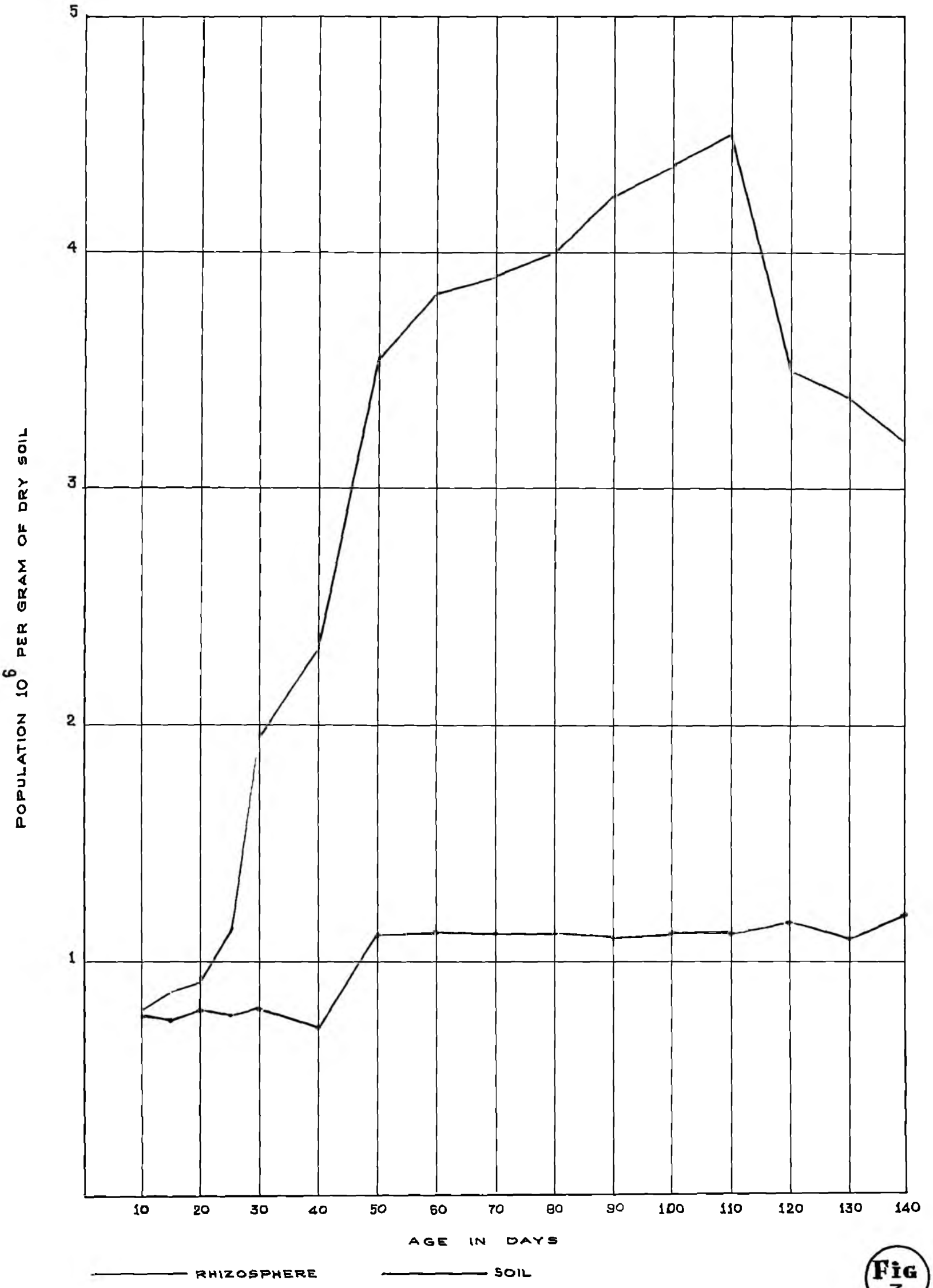


Fig 3

Table 4

Percentage incidence of different fungi in the rhizosphere and in the control soil

	Penicillium and *Aspergillus* spp.	*Fusarium* spp.	Mucoraceous fungi	Other fungi
Rhizosphere	70.29	14.85	8.91	5.94
Control soil	82.95	8.52	4.28	2.25

which there was a gradual fall.

The population of fungi on the 10th and 15th day after sowing were 0.86 million and 0.68 million respectively. The corresponding F:S ratios were 1.12 and 1.15. On the 40th day, the fungal population become 2.33 millions which increased sharply to 3.57 millions on the 50th day. Then there was a gradual increase in the population till the flowering stage and the population on the 110th day was 4.52 millions which was the maximum.

After the flowering stage the fungal population also showed a tendency to decrease. It came down to 3.25 millions on the 140th day and the corresponding F:S ratio was 2.71 (Table 3).

species of Penicillium and Aspergillus formed the predominant group of fungi in the rhizosphere and in the soil, and these constituted 70.29% and 82.95% respectively of the total fungal population. Fusarium spp. came next followed by Mucoraceous fungi and then by the other fungi (Table 4).

B. Influence of V.M.I. infection on the rhizosphere mycoflora of tobacco plants

The microbial population in the rhizosphere of virus infected plants were found to be higher than that in the

healthy plants of the same age. Practically no effect was noted in the rhizosphere of inoculated plants 24 hours after inoculation. But an increase in the microbial population in the rhizosphere was noticeable 48 hours after inoculation. While the bacterial population in the rhizosphere of healthy plants at this time was 55.91 millions, that in the inoculated plants was 58.68 millions. The actinomycete population in the healthy and inoculated plants 48 hours after inoculation were 12.13 millions and 16.22 millions respectively.

The bacterial population in the rhizosphere of inoculated plants increased to 62.81 millions on the 5th day after inoculation as against 57.75 millions in the healthy plants. Similarly the actinomycete population also increased to 18.34 millions in the inoculated plants as against 12.98 millions in the healthy plants.

The plants developed mosaic symptoms on the 7th day after inoculation. On the 10th day the bacterial population in the rhizosphere of inoculated plants was 74.63 millions while that in the healthy plants was only 65.43 millions. The corresponding actinomycete populations were 18.80 millions and 15.43 millions.

This increase in the microbial population in the rhizosphere of diseased plants over that in the healthy

Table 5

Influence of T.M.V infection on the bacterial population in the rhizosphere of 65 days old tobacco plants.

(Population 10^6 gram of dry soil)

Days after inoculation.	1	2	5	10	15	25
Control soil	4.82	4.87	4.65	4.62	4.84	4.87
Healthy plants	54.81	55.91	57.75	65.43	77.02	92.34
Inoculated plants	55.18	58.68	62.81	74.63	85.73	100.01

Table 6

Influence of T.M.V. infection on the actinomycete population in the rhizosphere of 65 days old tobacco plants

(Population 10^6 per gram of dry soil)

Days after inoculation	1	3	5	10	15	25
Control soil	2.67	2.81	3.03	3.14	3.15	3.21
Healthy plants	11.69	12.13	12.98	15.43	16.03	19.01
Inoculated plants	11.98	16.22	18.34	18.80	19.98	22.03

ACTINOMYCETE POPULATION IN THE RHIZOSPHERE OF MOSAIC INFECTED TOBACCO PLANTS AS COMPARED TO THAT OF HEALTHY PLANTS AND IN THE CONTROL OF SOIL

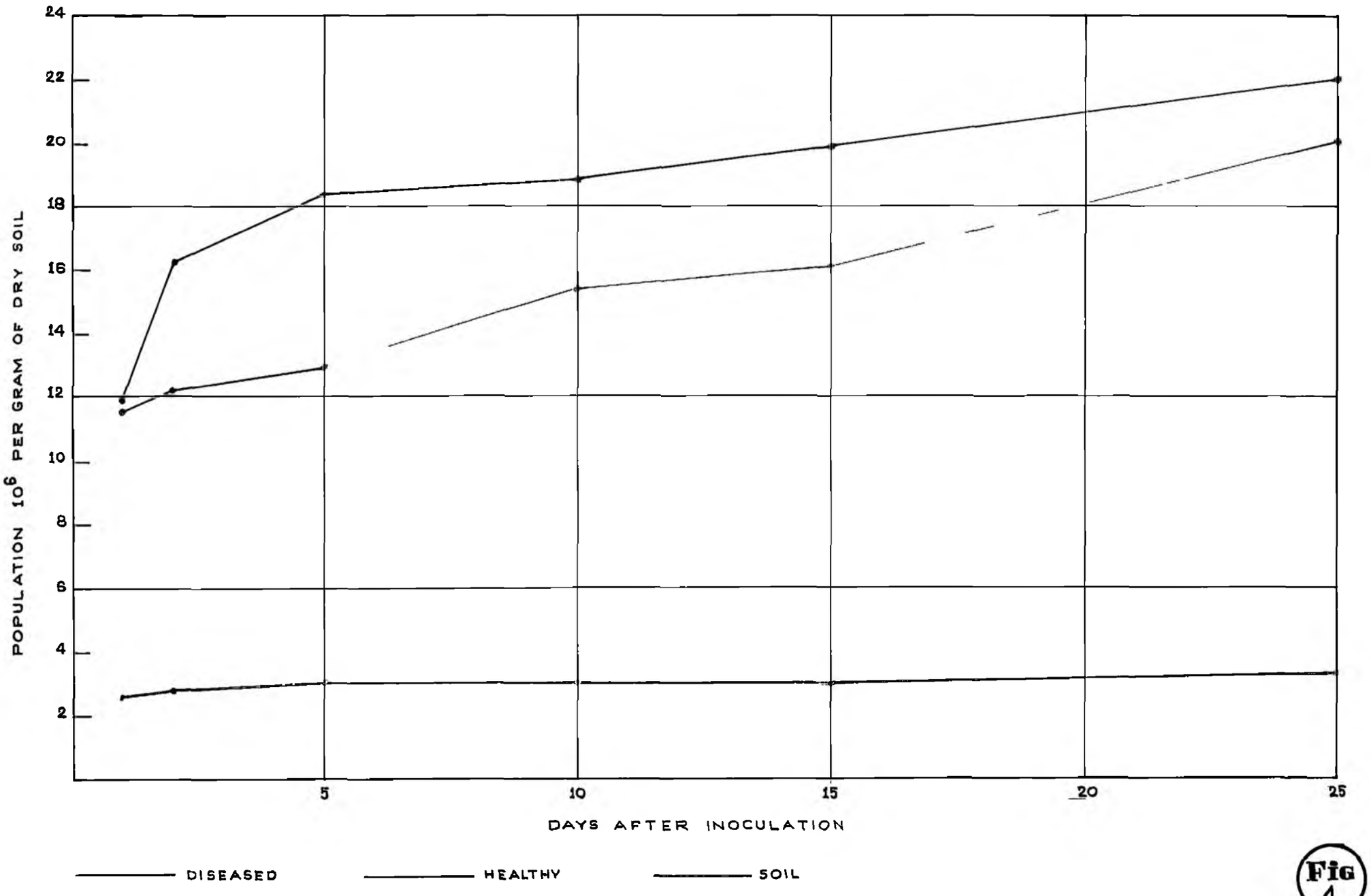


Fig 4

Table 7

Influence of T.M.V. infection on the fungal population in the rhizosphere of 65 days old tobacco plants

(Population 10^6 per gram of dry soil)

Days after inoculation	1	2	5	10	15	25
Control soil	1.16	1.17	1.14	1.16	1.16	1.23
Healthy plants	2.96	3.15	3.23	3.40	3.57	4.30
Inoculated plants	3.05	3.17	3.32	3.49	3.77	4.83

FUNGAL POPULATION IN THE RHIZOSPHERE OF MOSAIC NFECTED TOBACCO PLANTS AS COMPARED TO THAT OF HEALTHY PLANTS AND IN THE CONTROL SO L

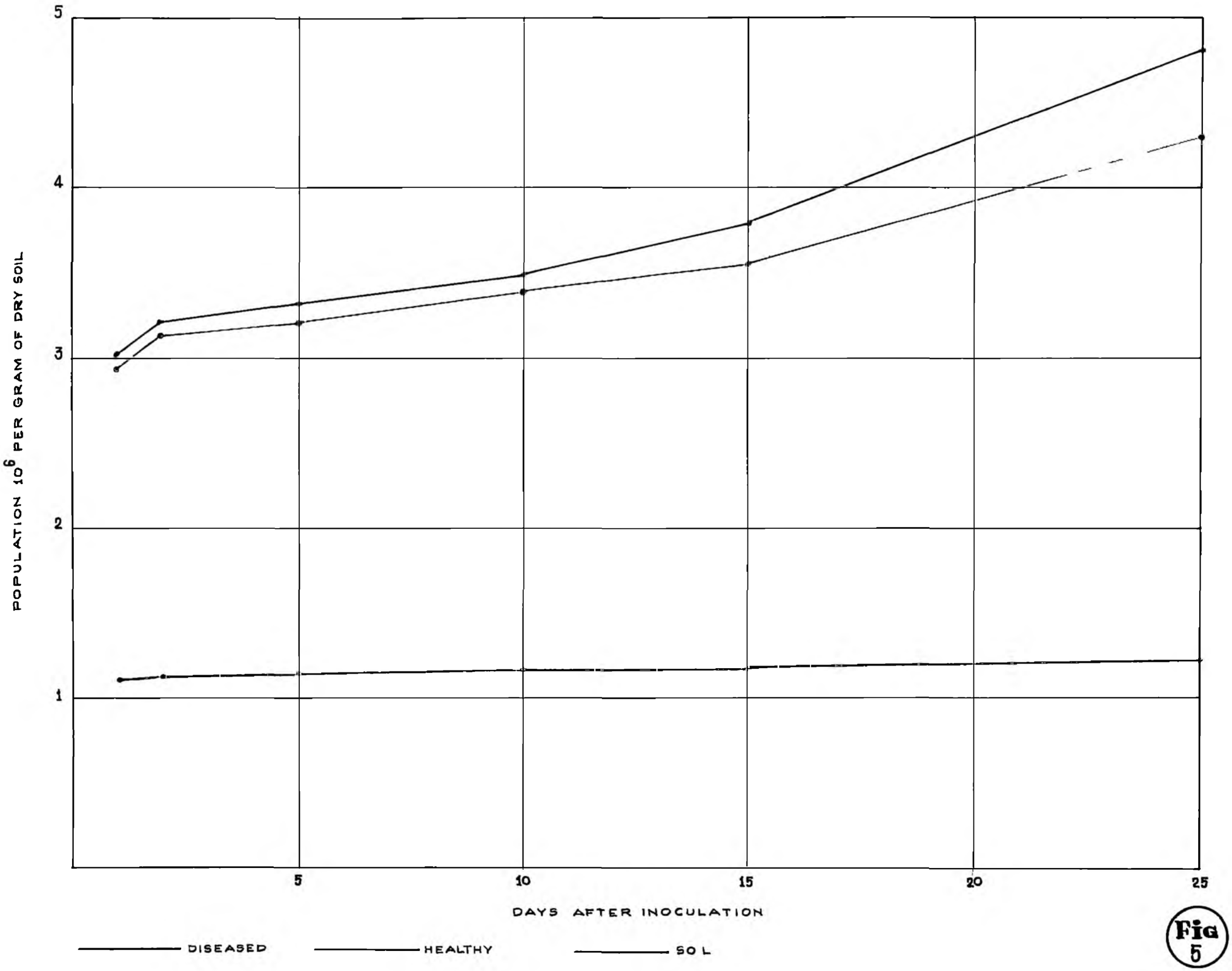


Fig 5

plants was noticeable on the 25th day after inoculation also, when the last sample was taken.

On the 25th day after inoculation the bacterial populations in the inoculated and healthy plants were 100.01 millions and 92.34 millions respectively. The corresponding actinomycete populations were 22.03 millions and 19.01 millions respectively.

A slight increase in the fungal population was also noted in the rhizosphere of the inoculated plants. The population on the 25th day after inoculation in the rhizosphere of diseased and healthy plants were 4.83 millions and 4.30 millions respectively (Tables 5, 6 & 7).

III. Effect of foliar application of Urea, 2,4-D and Terramycin on the healthy and inoculated tobacco plants

(1) Effect of treatment on the plant

Among the three materials applied on the foliage, Urea caused slight scorching on the leaves. The newly emerged leaves were, however, unaffected. 2,4-D caused abnormal elongation of the newly emerging leaves and a reduction in the width of the lamina. This abnormality was more pronounced on the treated plants which were also

inoculated with the virus. Terramycin did not produce any visible effect on the plants.

(2) Effect of treatment on the rhizosphere

Urea, 2,4-D and Terramycin applied on the foliage were all found to exert some influence on the microbial population in the rhizosphere of healthy and inoculated tobacco plants. The effect of 2,4-D was more pronounced.

(i) Urea

A slight depressing effect on the microbial population of the rhizosphere was noticeable in healthy plants which received urea as a foliar spray.

The bacterial population in the rhizosphere on the date of spraying was 53.24 millions. This has increased only 54.81 millions after 6 days in the treated plants, while in the untreated plants the corresponding population was 57.75 millions. This difference narrowed down on the 16th day, the bacterial populations in the treated and untreated plants at this time were 76.02 millions and 77.02 millions respectively. On the 26th day these populations became 91.86 millions and 92.34 millions respectively.

On the other hand urea seemed to exert a stimulatory

Table 3

ffect of fol ar ap l ca n of u oa o he rhizosphere
m c flora of daye old bac o lents
ion 6^5 p E a of dry o l)

	To al popu at on			acter al p o			ot on ce e pop ation		Pungal p pulat on		
			C	A		C		C	A	P	C
Co trol soil	79	9	5 30		4	4 7	03	5	2	4	3
l y l od	7 98	9	6 5 5	75		3		6 03	C	3	5 30
ea pl pr d th ur 0 3			8 4 55	54 8	0	9 86	2 4	06	8 5	4	76 4 3
oc t p u s r yed	47	9	4 6 87	2 8	3	0		9	03	32	77 4 3
l ocul ted p e spr ye w th ure	9 7	6	29 8	7 02	3	0 08	9 6	65	2	5	6 4

A d ft r ying B duye p C daye e p eyi r

effect on the microbial population in the plants which were inoculated with T.M.V. 24 hours after treatment with urea. The populations in these plants were higher than those in the plants which were inoculated with T.M.V. but not pretreated with urea.

The population in the inoculated plants on the 6th day after urea treatment was 67.02 millions, while that in the untreated plants was only 62.91 millions. This effect was present on the 16th day also, the populations in the treated and untreated plants on this day were 90.53 millions and 85.73 millions respectively. This difference is statistically significant. The difference narrowed down and the populations became 102.08 millions and 100.01 millions respectively on the 26th day.

A more or less similar trend, as that of bacteria, was noticed in the actinomycete and fungal populations also as a result of the treatment (Table 8).

(ii) 2,4-D.

Foliar application of 2,4-D was found to exert a marked influence on the microbial population in the rhizosphere of healthy and inoculated tobacco plants.

The bacterial population in the rhizosphere of

healthy treated plants was 67.45 millions on the 6th day after the treatment, while that in the untreated plants was only 57.75 millions. This stimulatory effect was seen on the 16th and 26th days also. On the 16th day after treatment, the population in the treated and untreated plants were 88.36 millions and 77.02 millions respectively and the corresponding populations on the26th day were 100.75 millions and 92.34 millions. These differences were found to be statistically significant.

This stimulatory effect was more pronounced on inoculated plants, which were treated with 2,4-D 24 hours before inoculation. The bacterial population in the rhizosphere of these plants on the 6th day after the treatment was 88.23 millions as against 62.81 millions in the inoculated plants which did not receive the 2,4-D treatment; the difference being statistically significant. On the 16the day after the treatment, the population in the treated plants rose to 98.04 millions while that in the untreated plants was only 85.73 millions. This stimulatory effect was noticeable on the 26th day also, the populations on this day being 115.75 millions and 100.01 millions respectively. These differences also were found statistically significant.

able 9

Effect of f liar applicat on of 2 4 D o the rh zosphere
m c oflor of 65 d ye old tobacco p ante

(opulat n 0^6 per g am of dry soil

	otal populat on			acter al population			ct nomycete populat on		Fungal populat on		
	A	B	C	A	B	C	B	C	A	B	C
Control soil	8 79	9 5	9 30	4 62	4 84	4 87	3 03	3 5	3 2	4	6	23
Healthy plants unsprayed	73 96	96 62	5 65	57 75	7 0	2	2 8	6 03	9 0	23	3 5	4 30
Healthy plants sprayed with 2 4 D 0 ppm	84 80	09 83	25 63	67 48	88 36	0C 5	4 3	7 32	20 2	3 9	4 5	4 67
Inoculated plants unesprayed	84 47	09 48	26 87	62 8	85 73	00 0	8 34	19 98	22 03	3 32	3 77	4 83
Inoculated plants sprayed with 2 4 D 0 ppm	105 77	25 0	44 4	82 23	98 04	5 78	9 63	22 56	23 7	3 9	4 4	5 9

A = 6 days after spr ng B 6 days after spray ng C 26 days after spraying

An increase in the populations of actinomycetes and fungi was also noted in the treated plants, both healthy and inoculated (Table 9).

(iii) Terramycin

The plants sprayed with Terramycin showed a slight decrease in the microbial population of the rhizosphere. This was found to be so in the healthy as well as in the inoculated plants.

The bacterial populations in the rhizospheres of healthy treated and healthy untreated plants, on the 6th day after the treatment were 51.77 millions and 57.75 millions respectively. This difference became narrower on the 16th and 26th days after the treatment. The populations on the 16th and 26th days in the rhizosphere of treated plants were 74.13 millions and 90.70 millions respectively and those in the untreated plants were 77.02 millions and 91.34 millions respectively.

In the inoculated plants the bacterial population in the rhizosphere of treated and untreated plants on the 6th day after the treatment were 59.53 millions and 62.81 millions respectively. On the 16th day after the treatment the population in thetreated plants was only 78.20 millions while that in the untreated plants was 85.75 millions. This difference is statistically significant. The difference

became narrower on the 26th day and the population in the treated plants was 96.43 millions while that in the untreated plants was 100.01 millions.

A reduction in the actinomycete and fungal populations also, was noticed both in the healthy and inoculated plants as a result of terramycin treatment. (Table 10)

nblo 0

Ef ec of fol ar ap l cntio of t r c n o e rhi o ere
icroflor of dnys old to c o plcnts
(o lation 0^6 per -r of d oil

	otel popul tion			terial l ion		c cc o opula ion		n l opulation	
		B	C		C		C	A	C
Control soil	79	5	36	4 62	4 84	7	03	2	6 23
al y plants u sprayed	7 6	96 62		5	0	4	9 03	0	57 4 30
ealthy p nts sprays th terramycin 0 00 2	8	3 9	2 6		4	0	6	82	94 48 4 24
nnoculated l ts unsprayed	84 47	09 48	26 7	8		9 2	03	3 2 3	4 83
noculated la ts s rayed w th terramyc n 00 2	7 59	98 66	2 6	3	20		7 2	0 8	0 4 68

o after s r i r

6 yn e r

C s fter s rs i c

EFECT OF FOLIAR APPLICATION OF UREA, 2,4-D AND TERRAMYCIN ON THE BACTERIAL POPULATION IN THE RHIZOSPHERE OF HEALTHY AND MOSAIC INFECTED TOBACCO PLANTS

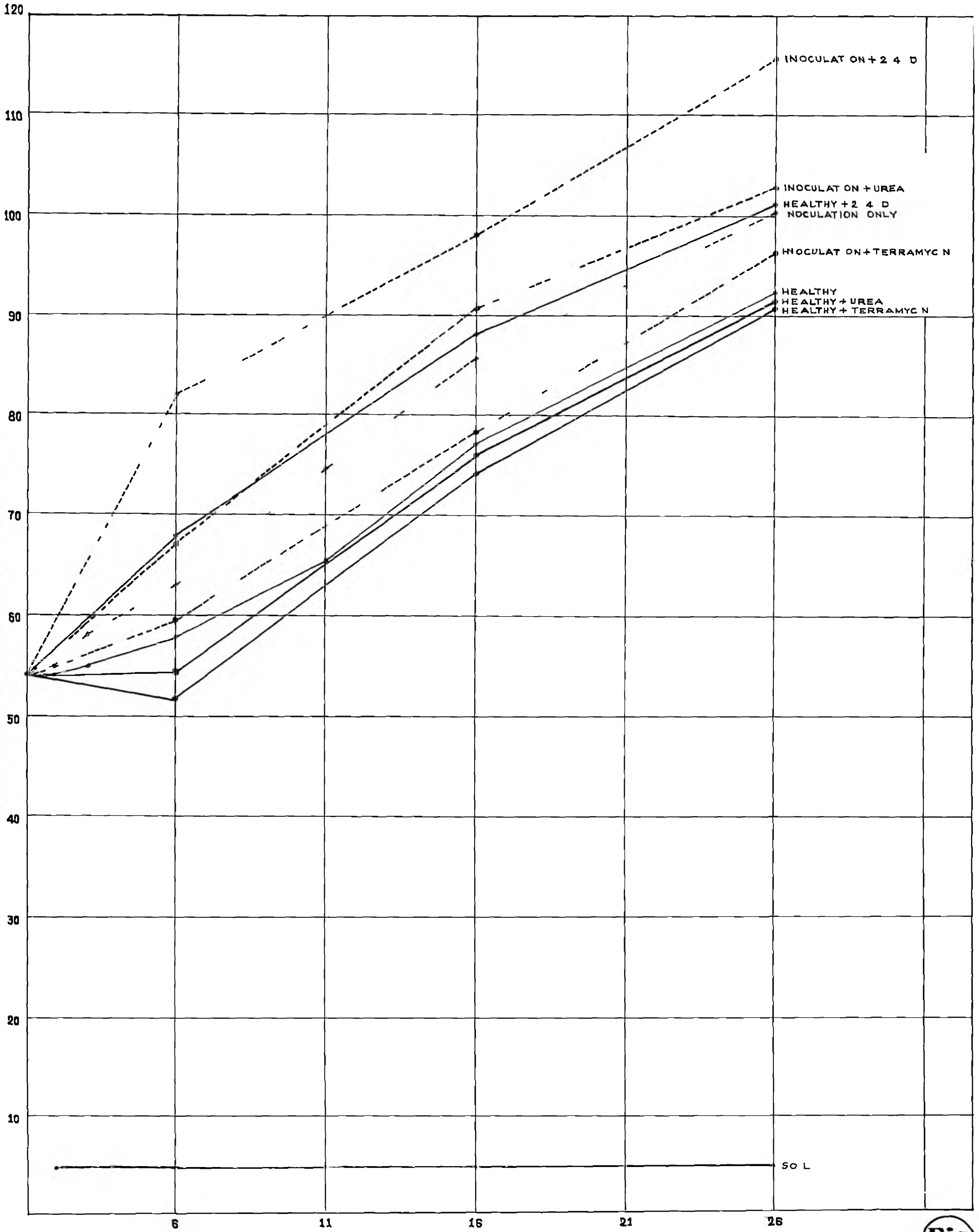


Fig 6

Table 11

Analysis of variance table

(6 Days after spraying

Source	Sum of squares	D.P.	Variance	F. ratio	Critical value of F
Total	11812.64	26	..	..	..
Treatments	10982.70	8	1372.84	$F_{8,18} = 29.76^{**}$	3.71
Between healthy and inoculated plants.	593.51	1	593.51	$F_{1,18} = 12.87^{**}$	8.28
Between chemicals and control plants.	1239.90	3	413.30	$F_{3,18} = 8.96^{**}$	5.09
Interaction	85.29	3	28.43	$F_{3,18} = 0.62^{N.S}$	5.09
Between treatment and control.	9064.94	1	9064.00	$F_{1,18} = 196.57^{**}$	8.28
Error	829.94	18	46.11	..	..

Table 12

Analysis of variance table

(10 days after spraying)

Source	Sum of squares	D.F.	Variance	F. ratio	Critical value of F
Total	18131.33	26	..	..	..
Treatments	18026.45	8	2253.53	$F_{8,18}$ = 386.50**	3.71
Between healthy and inoculated plants	511.79	1	511.79	$F_{1,18}$ = 87.79**	8.28
Between chemicals and control plants	914.56	3	304.85	$F_{3,18}$ = 52.99**	5.09
Interaction	82.54	3	27.51	$F_{3,18}$ = 4.72*	5.09
Between treatments and control	16517.56	1	16517.56	$F_{1,18}$ = 2831.49**	8.28
Error	104.88	18	5.83	..	..

Table 13

Analysis of variance table

(26 days after spraying)

Source	Sum of squares	D.F.	Variance	F. ratio	Critical value of F
Total	25074.92	26	..	..	..
Treatments	24893.36	8	3111.67	$F_{8, 18} = 306.39^{**}$	3.71
Between healthy and inoculated plants.	559.89	1	559.89	$F_{1, 18} = 55.49^{**}$	8.28
Between chemicals and control plants	762.35	3	254.12	$F_{3, 18} = 25.19^{**}$	5.09
Interaction	73.15	3	24.38	$F_{3, 18} = 2.42^{N.S.}$	5.09
Between treatments and control	23497.97	1	23497.97	$F_{1, 18} = 2327.34^{**}$	8.28
Error	181.56	18	10.09	..	..

Table 14

Pooled analysis of variance table

Source	Sum of squares	D.F.	Variance	F.ratio	Critical value of F
Total	67700.52	80	..	..	..
Between duration	13811.84	2	6905.70	$F_{2,\ 54}$ = 314.09**	5.04
Between treatments	52076.99	8	6509.62	$F_{8,\ 54}$ = 314.93**	2.87
Interaction	1812.13	16	113.25	$F_{16,54}$ = 5.48**	2.44
Pooled error	1116.38	54	20.67	..	..

DISCUSSION

A definite rhizosphere population was found to be established in the tobacco plants, within three days of germination of seeds. This increased steadily with the increase in the age of plants till flowering. Thereafter, there was a gradual decline. The microbial population in the rhizosphere was significantly higher than that in the control soils at all stages of plant growth.

The steady increase in the rhizosphere microbial population till flowering, followed by a fall afterwards, may be to a large extent due to the qualitative and quantitative changes in the root exudates. These changes appear to be progressively more beneficial to the microorganisms before flowering, but act in the reverse direction after flowering. Upto flowering, the plant is in an active state of growth, after which the growth gradually ceases. This slackening of the growth of plant can be expected to be reflected on the root exudates, which in turn affect the microflora of the rhizosphere. Miller (1938) found that the growing roots, liberate more root exudates as compared to the nongrowing or older roots. It is therefore probably that the changes in root exudates,

coupled with the presence of a greater number of dead roots, could have influenced the reduction in the rhizosphere population after the flowering stage.

The fall in the microbial population after flowering, was reflected equally in all the three groups of microorganisms, namely, bacteria, actinomycetes and fungi. Rangaswami and Vasuntharajan (1961, 1962), Rema Devi (1964) and Remigius (1966), working with other crops, found that while there was a fall in the bacterial population after flowering, the population of actinomycetes and fungi continued to increase even after flowering. They attribute this to the changes in the root exudates, presence of dead roots and greater amount of sloughed off tissues in older plants after flowering. These factors may be operating in the tobacco plants also, but the resultant effect of their action in this crop is reflected more or less in a similar manner in all the three groups of organisms.

With reference to the influence of virus infection on the rhizosphere microflora of tobacco plants, it was noted that the population of bacteria, actinomycetes and fungi increased as the infection advanced. Similar observations have been made by Lakshmikumari (1960) in Dolichos lablab

plants infected by the Dolichos enation mosaic virus and by Ranganathan (1965) in banana plants infected by the bunchy top virus. These authors found an increase in the microbial population of the rhizosphere with the advance of infection. Virus infection is known to alter the host metabolism and this is likely to be reflected in the root exudates which in turn influence the rhizosphere microflora. We have at present very little information on the nature and type of changes that take place in the root exudates of virus infected plants. Whatever be the nature and type of these changes, their effect is felt favourably by the different groups of microorganisms in the rhizosphere.

Urea, applied on the foliage of tobacco plants, exerted a supressing effect on the microbial population in the rhizosphere. Similar effects, on the microbial population in the rhizosphere as a result of foliar application of urea has been noted by earlier workers also. Venkat ram (1960) while studying the effect of foliar application of nutrients on the rhizosphere microflora of Camellia sinensis, found that certain inorganic and organic nutrients including urea, when applied on the foliage, reduced the microbial population in the rhizosphere. Ramachandra reddy (1959) and Pandasamy and Ramaswami (1967), who worked on rice and sorghum respectively,

have also obtained similar results.

Though urea exerted a depressing effect on the rhizosphere microflora of healthy plants, such plants when inoculated with T.M.V. showed a marked increase in the microflora of the rhizosphere. This increase was greater than that in the inoculated plants which did not receive urea. As could be expected, the metabolism of the plant might have been affected by urea. It is possible that in such a plant, the virus might have multiplied faster. This could have affected the root exudates resulting in the population of the microorganisms increasing at an accelerated pace. It is also possible that the plant, whose metabolism was already affected by urea, reacted in a more vigorous manner when the virus was introduced into its system.

Foliar application of 2,4-D resulted in an increase in the microbial population of the rhizosphere, both in the healthy and inoculated plants. The treatments markedly affected the bacterial and actinomycete populations while the fungal population was not much affected.

The increase in the total microbial population as a result of 2,4-D application was more or less equal

to that obtained as a result of inoculation with the virus. The total population in the inoculated and 2,4-D treated plants 6 days after the treatment were 84.47 and 84.60 millions respectively, while that in the control plants was only 73.96 millions. The subsequent rise in the populations in the plants which received the above two treatments were also more or less equal (Table 9).

With regard to the effect of these treatments on the different groups of organisms, it was noted that 2,4-D exerted a greater influence on the bacterial population, while virus infection exerted almost equal influence on the bacterial and actinomycete populations at least during the early period (Table 9). It is therefore possible that the changes that are brought about in the root exudates as a result of 2,4-D application and virus infection, may not be similar, eventhough in both cases these changes favour the increase of the microbial population.

The increase in the total microbial population in the inoculated plants which also received the 2,4-D spray, was much more pronounced and was greater than the total of the increases obtained by either treatments. This is indicative of very serious metabolic changes inthe system

of these plants even though nothing is known about the combined action of 2,4-D and virus on the plants.

An inhibitory effect on the rhizosphere microflora was noticeable in plants which received the terramycin treatment resulting in a decrease in the population of all the three goups of microorganisms. This effect was noted in the healthy as well as inoculated plants. The decrease was more pronounced in the case of bacteria. Possibly terramycin was absorbed and translocated in the system of the plants. It is now known that certain antibiotics are absorbed and translocated in the system of plants. It is also known from the works of Rema Devi (1964) and Benigius (1966) that application of streptomycin on the foliage of tomato and rice plants, can bring about a reduction in the bacterial population in the rhizosphere. They have suggested that this reduction in the bacterial population may be due to the excretion of the antibiotics through the roots or to the changes in the root exudates as a result of altered host metabolism or to both. The data available on the subject is not sufficient to permit further speculation in the matter.

The reduction in the microbial population of the rhizosphere of inoculated plants which were pretreated with

terramycin was found to be very much lower than that of the healthy plants on the sixth day after the treatment. In the healthy plants which received the treatment, the bacterial population fell to 51.77 millions from 57.75 millions, while in the inoculated plants which also received terramycin the bacterial population fell to 59.53 millions from 62.81 millions. The actinomycetes population fell by 1.31 millions and 2.56 millions respectively. However, on the 16th day after the treatment there was a much greater fall in the bacterial population of the inoculated plants which also received terramycin. The population in these plants fell to 78.20 millions from 85.73 millions, while the population in the healthy plants which received the treatment fell to 74.13 millions from 77.02 millions.

Terramycin has been found, by Mace (1965) to inhibit the multiplication of T.M.V. when applied within five hours after inoculation. In the present experiments terramycin was applied 24 hours before inoculation. The effect of such a treatment on virus multiplication and also on the plant itself are not known. However, as far as the microbial population in the rhizosphere is concerned it is seen that the introduction of the virus after treating the plants with terramycin helped initially to

reduce the adverse effects of infection, though later on it was aggravated. Further work in this direction is necessary.

SUMMARY

SUMMARY

A definite rhizosphere population was found to be established in the tobacco plants, within three days of germination of seeds. The population steadily increased with the age of plants till flowering stage, after which there was a gradual decline in all the three groups of microorganisms namely, bacteria, actinomycetes and fungi.

The microbial population in the rhizosphere was significantly higher than that in the control soils at all stages of plant growth.

Species of Penicillium and Aspergillus formed the predominant group of fungi in the rhizosphere and in control soils. Fusarium spp. came next followed by Mucoraceous fungi and then by the other fungi.

The microbial population in the rhizosphere of virus infected plants was found to be higher than that in the healthy plants of the same age. This effect was noticeable within 48 hours after inoculation.

Foliar application of urea caused slight scorching on the leaves and 2,4-D caused abnormal elongation of the newly emerging leaves while terramycin did not produce any visible effect on the plants.

Urea, 2,4-D and terramycin applied on the foliage, were all found to exert some influence on the microbial population in the rhizosphere of healthy and inoculated tobacco plants.

Urea caused a slight depressing effect on the microbial population in the rhizosphere of healthy plants. But in the case of inoculated plants, it exerted a stimulatory effect on the microbial population.

2,4-D was found to exert a stimulatory effect on the microbial population in the rhizosphere of the healthy and also of the inoculated plants. This increase was much pronounced in the inoculated plants and it was greater than the total of the increases obtained separately by inoculation and by 2,4-D treatment.

An inhibitory effect on the rhizosphere microflora was noticeable in plants which received the terramycin treatment, resulting in a decrease in the population of all the three groups of organisms especially those of bacteria. This decrease as a result of the treatment was more pronounced in the healthy plants than in the inoculated plants. It is suggested that the reduction in the bacterial population may be due to the excretion of the antibiotic, through the roots or to the changes in the root exudates as a result of altered host metabolism or to both.

REFERENCES

REFERENCES

ADACI, M. (1939). Studies on the rhizosphere of plants. Second report. On the influence of various cultivated plants on the incidence of microorganisms in the different Formosan soil types.
J. Soc. trop. Agric. Taiwan, xi., 57-65.

G. THOTTAPUDU, V. (1953). Soil conditions and root diseases VIII. Rhizosphere microflora of some of the important crop plants of South India.
Proc. Indian Acad. Sci., B, 37, 1-13.

[illegible]OTTAPUDU, V., BHUVANESHWARI, K. & SUBBARAYALAN, S.(1955). Fungi isolated from rhizosphere. I.
Proc. Indian Acad. Sci., Sect. B., 42, 98-104.

DAL, R., BHUVANESHWARI, K. & SUBBARAO, N.S. (1956). Root exudates of Paddy.
Nature, 178, 1063.

RAGYAMMJ, J & RANGASWAMI, G. (1966) On the variation in rhizosphere effects of some crop plants.
Curr. Sci. 35, 238-39.

BHUVANESHWARI, K. (1958). Studies on the rhizosphere microflora of crop plants.
Doctoral thesis, University of Madras.

_____ (1960). Rhizosphere and root diseases.
Mem. Indian bot. Soc. 3: 152-155.

BHUVANESHWARI, K. & SUBBA RAO, N.S. (1957). Root exudates in relation to the rhizosphere effect.
Proc. Indian Acad. Sci. B, 45, 299-301.

COOTOIS, D.E. (1953). Microflora of rhizosphere of the pineapple plant. Soil Sci. 76, 259-72.

DAS, A.C. (1963).Ecology of soil fungi of rice fields.
1. Succession of fungi on rice roots.
2. Association of soil fungi with organic matter.
Trans. Brit. Mycol. Soc. 46, 431-43

DOWDER, W.M. & GOODMAN, R.N. (1958). Downward translocation of streptomycin in Coleus sp. Phytopathology, 48, 392.

TIKHANOVA, A.N. (1956). The microflora of the rhizosphere of rice in relation to its stage of development. Zap. Leningrad. S. Kh. Inst., 11, 92-99.

HALLOCK, F.E., & COCHRANE, V.W.(1950). The effect of fungistatic agents on the bacterial flora of the rhizosphere. Phytopathology, 40, 715-18.

HILTNER, L.(1904). Über neuere Erfahrungen und probleme auf dem Gebiet der Boden bakteriologie und unter besonderer Berucksichtigung der Grundung und Brache. Arb. Deut. Landw. Ges. 98, 59-78.

HOLL, K.K. & HERR, L.J.(1962). Effect of foliar urea treatment on numbers of actinomycetes antagonistic to Fusarium roseum f. cerealis in the rhizosphere of corn seedlings. Phytopathology, 52, 423-27.

KANDASAMY, D. & RANGASWAMI, G.(1967).Changes in the rhizosphere microflora of sorghum due to foliar nutrient sprays. Indian J. Agric. Sci. 37, 143-50.

KATZNELSON, H.& RICHARDSON, L.T. (1943). The microflora of the rhizosphere of tomato plants in relation to soil sterilization. Can. J. Research., C., 21, 249-55.

_____ (1948) Rhizosphere studies and associate microbiological phenomena in relation to strawberry root rot. Sci. Agr. 28, 293-308.

KATZNELSON, H., ROUATT, J.W. & PAYNE, T.M.B. (1954) Liberation of amino acids by plant roots in relation to dessication. Nature, 174, 1110-11.

PETERSON, E.A. ROVATT, J.W. & KATZNELSON, H. (1965) Microorganisms in the root zone in relation to soil moisture.
Can. J. Microbiol. 11, 483-489.

RAMLE, D. (1953). The movement of chloramphenicol and streptomycin in broad bean and tomato plants.
Ann. Bot. Lond., 18, 463.

[illegible] CHANDRA REDDY, T.K. (1959). Foliar spray of urea and rhizosphere microflora of Rice (Oryza sativa L.)
Phytopath. Z., 36, 236-89.

RAJAPAR SINGH, (1967). Excretion of Amino acids by plant roots.
Curr. Sci., 36, 236-237.

R. RAMATHAN, K. (1965). Observations on rhizosphere microflora in the different region of banana roots as influenced by the infection of the plant by the Bunchy top virus.
M.Sc. Thesis, University of Kerala.

R[illegible]CAS, A.M, G. S. BALASUBRAMANIAN, A. (1963). Studies on the rhizosphere microflora in realtion to hydrocyanic acid content of roots.
Can. J. Microbiol. 9, 719-25.

R. RAGASWAMI, G & VASANTHARAJAN, V.N. (1961). Studies on the rhizosphere microflora of citrus plants as influenced by streptomycin spray.
Curr. Sci., 30, 25-26.

_____ (1962). Studies on the rhizosphere microflora of citrus trees. I. Quantative incidence of microorganisms in relation to root and shoot growth.
Can. J. Microbiol. 8, 473-477.

_____ (1962). Studies on the rhizosphere microflora of citrus trees. II. Qualitative distribution of bacterial flora.
Can. J. Microbiol., 8, 479-484.

ING, M. de L. & WALLACE, R.H. (1956). Morphological and physiological groups of soil bacteria from the roots of barley and oats.
Can. J. Microbiol., 2, 473-81.

KRASSILNIKOV, N.A., KRISS, A.E. & LITVINOV, M.A(1936). The effect of the root system on the soil microflora.
Microbiol., V, 2, 270-86.

KRASSILNIKOV, N.A. (1944) Microflora of soils as influenced by plants.
Mikrobiologia., U.S.S.R., 3, 348-58.

LAKSHMIKUMARI, M. (1960) Rhizosphere in relation to virus infection.
Proc. Indian Bot. Soc. 3, 160-63.

_____ (1961) Rhizosphere microfloras and Host-parasite relationships.
Doctoral thesis, University of Madras.

LOCHHEAD, A.G. (1940). Qualitative studies on soil microorganisms. III Influence of plant growth on the character of the bacterial flora.
Can. J. Research C.18, 42-53.

LOCHHEAD, A.G. THORN, C.I & WEBB, P.W. (1940). The microflora of the rhizosphere in relation to resistance of plants to soil borne pathogens.
Sci., Agric., 20, 414-18.

NAGAI, F.D.(1965). Effects of Oxytetracycline and streptomycin on the metabolism of tobacco leaf tissue and tobacco mosaic virus replication.
Diss. Abstr., 25, 5520.

MILLAR, E.C.(1938). Plant Physiology (2nd Ed.) McGraw Hill Book Co., New York, U.S.A.

NAPIER, E.J., TURNER, D.I., RHODES, A. & TOOTILL, J.P.R.(1956). The systemic action against Pseudomonas medicaginis var. Phaseolicola of a streptomycin spray applied to dwarf beans.
Ann. appl. Biol. 44, 145-51.

PETERSON, E.A. ROVATT, J.W. & KATZNELSON, H. (1965) Microorganisms in the root zone in relation to soil moisture.
Can. J. Microbiol. 11, 488-489.

PRAKER, D. (1958). The movement of chloramphenicol and streptomycin in broad bean and tomato plants.
Ann. Bot. Lond., 18, 463.

[illegible]CHANDRA REDDY, T.K. (1959). Foliar spray of urea and rhizosphere microflora of Rice (Oryza sativa L.)
Phytopath. Z., 36, 286-89.

RAMAIAH JINGH, (1967). Excretion of Amino acids by plant roots.
Curr. Sci., 36, 236-237.

R. CANATHAN, K. (1965). Observations on rhizosphere microflora in the different region of banana roots as influenced by the infection of the plant by the Bunchy top virus.
M.Sc. Thesis, University of Kerala.

RAMASAMI, G. & BALASUBRAMANIAN, A. (1963). Studies on the rhizosphere microflora in realtion to hydrocyanic acid content of roots.
Can. J. Microbiol. 9, 719-25.

RAMASWAMI, G & VASANTHARAJAN, V.N. (1961). Studies on the rhizosphere microflora of citrus plants as influenced by streptomycin spray.
Curr. Sci., 30, 25-26.

_____ (1962). Studies on the rhizosphere microflora of citrus trees. I. Quantative incidence of microorganisms in relation to root and shoot growth.
Can. J. Microbiol. 8, 473-477.

_____ (1962). Studies on the rhizosphere microflora of citrus trees. II. Qualitative distribution of bacterial flora.
Can. J. Microbiol., 8, 479-484.

RANGASWAMI, G. & VASANTHARAJAN, V.N. (1962). Studies on the rhizosphere microflora of cttrus tr cr. III. Fungal and actinomycetes flora of the rhizosphere.
Can. J. Microbiol., 8, 485-489.

RANGASWAMI, G. & VENKATESAN, D. (1963). Scheme for the investigation of Actinomycete population of the paddy soil. Final Report. June 1, 1959 to March, 31, 1963.
Dept. of Agri. Annamalai Univ.

RAJI DEVI, L. (1964). Studies on the rhizosphere microflora of tomato and translocation of streptomycin in the plant.
M.Sc. Thesis, University of Kerala.

REMIGIUS, J.G.G.(1966). Rhizosphere microflore of Rice - Influence of plant growth and foliar application of antibiotics on the microbial population.
M.Sc. Thesis, University of Kerala.

ROVIRA, J.E. (1959). Initiation of the Rhizosphere effect. Can. J. Microbiol. 5, 67-71.

ROVIRA, J.E., PETERSON, E.A., KATZNELSON, H. & HILDERSON, V.E. (1963). Microorganisms in the root zone in relation to temperature.
Can. J. Microbiol. 9, 227-236.

TOMLIN, A.D. (1956). Plant root excretions in relation to rhizosphere effect. IV. Influence of plant species, age of plant, light temperature and calcium nutrition on exudation.
Plant and Soil 11, 53-64.

_____ (1956). A study on the development of root surface microflora during the initial stages of plant growth.
J. Appl. Bact., 19, 72-79.

VARADARAJAN, T.S. (1963) Physiology of virus infected plants.
J. Indian Bot. Soc. XLII, 339-57.

STARKEY, R.L.(1929). Some influence of development of higher plants upon the microorganisms in the soil. I. Historical and introductory. Soil. Sci., 27, 318-34.

II. Influence of the stage of the plant growth on the abundance of organisms. Soil Sci.s 27, 355-74.

III. Influence of stage of plant growth upon some activities of the organisms. Soil Sci., 27, 435-44.

_____(1931). Some influence of development of higher plants upon the microorganisms in the soil.

IV. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci., 32, 395-404.

SUBBA RAO, N.S. & BAILEY, D.L. (1961). Rhizosphere studies in relation to varietal resistance or susceptibility of tomato to Verticillium wilt. Can. J. Botany. 39, 1747-56.

SUNDARA RAO, M.V.B & VENKATARAMAN, K.V. (1963). Studies on the rhizosphere microorganisms of wheat. Ind. J. of Agri. Science, 33 (3), 163-73.

TIMONIN, M.I.(1940). The interaction of higher plants and soil microorganisms.

I. Microbial population of rhizosphere of seedlings of certain cultivated plants. Can. J. Research C. 18, 307-17.

II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil borne diseases. Can. J. Research C.18, 444-56.

_____ (1941) Microbial actitity as influenced by root excretions. Chron. bot., VI, 19-20, 440.

TIMONIN, M.I. & LOCHHEAD, A.G. (1948). Distribution of microorganisms in the rhizosphere of a root system.
Trans. Roy. Soc. Canad. III 42, 175-81.

VENKATESAN, R. & RANGASWAMI, G. (1964). Quantitative and quality studies of actinomycetes population from non-rhizosphere soil, rhizosphere of rice and stubble root surface.
Ind. J. Microbiol. IV, 4, 111-120.

VRANY, J.(1963). Effect of foliar application of urea on root microflora.
Folia. Microbiol., Prague, 8, 351-55.

VENKAT RAM, C.S. (1960). Foliar application of nutrients and rhizosphere microflora of
Camellia sinensis.
Nature Lond. 187, 621-622.

WEST, P.M. (1939). Excretion of thiamine and biotin by the roots of higher plants.
Nature Lond. 144, 1050-51.