# STUDIES ON THE RHIZOSPHERE MICROFLORA OF TOMATO AND TRANSLOCATION OF STREPTOMYCIN IN THE PLANT


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# 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 Smt. L. Rema Devi under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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# C O N T E N T S

## INTRODUCTION

The term rhizosphere was introduced by Hiltner (1904) who defined it as the region of the soil which is in close and intimate contact with the plant roots and subject to the influence of the root system. It is a widely accepted fact that the plant roots and soil microorganisms form an inseparable relationship. Interactions may occur between the exudates of the plant roots and the products liberated by the activity of the microorganisms.

The occurrence of a greater number of microorganisms in the rhizosphere than in the soil free from the influence of the roots is well established. The width of the zone of the soil influenced by the plant roots varies with the type of the plant, age of the plant, soil conditions, environment and other factors. The microorganisms in the soil exert a profound influence upon the plant which include a multiplicity of reactions, such as, liberation of plant foods by decomposition of organic matter, transformation of non-available substances to readily available forms, synthesis of vitamins and

auxins etc. It is now known that the microbiology of this region concerns not only the growth of the plant, but also its health and well being. The microorganisms in the rhizosphere are also known to play an important role in determining the susceptibility and resistance of the plant to some of the root disease pathogens. It has been established by many workers that varieties susceptible to root infecting pathogens harbour a much higher microbial population than the resistant ones.

In the present studies an attempt was made to determine whether there is any difference in the microbial population in the rhizosphere of three different tomato varieties which showed some differences in susceptibility to bacterial wilt. Counts were taken from the fifth day of sowing the seeds until harvest at twelve intervals.

The influence of soil depth on the population in the rhizosphere and of the soil was also studied.

A comparative assessment of the rhizosphere microflora in freshly wilted and healthy plants was also carried out to determine the extent of change that is brought about by wilt. This work was prompted by the fact that the root system of the plants showing early

symptoms of wilt appeared externally healthy with only a few lateral roots discoloured.

It is a well established fact that streptomycin sulphate is absorbed and translocated in tomato and many other plants. It was therefore considered worthwhile to ascertain whether the antibiotic influenced the metabolism of the plant in such a manner as to alter the rhizosphere population.

Bacterial wilt of tomato being a serious problem, a field experiment was also conducted to determine the comparative susceptibility of some of the available tomato varieties.

## REVIEW OF LITERATURE

Greater percentage incidence of bacteria, fungi and actinomycetes in the rhizosphere and the consequent positive rhizosphere effect have been repeatedly observed by many workers. Timonin (1940), Lochhead (1940), Katznelson and Richardson (1943), Krassinilkov (1944), Katznelson et al (1948), Rangaswami and Vasantharajan (1962) and many others found that the fungi, bacteria and actinomycetes present in the rhizosphere have been differentially influenced by the rhizosphere of the particular crop.

The special character of the microflora of a given rhizosphere is well pronounced and considered to persist throughout the growing period of the plants. Starkey (1929 and 1931), Timonin (1940), Agnihothurudu (1953) and Rangaswami and Venkatesan (1963) observed the maximum bacterial population at the flowering stage of the crop when it was in the grand stage of vegetative growth in the case of annuals. Rangaswami and Vasantharajan (1962) found that in perennials the growing roots harbour a much greater number of bacteria compared to non-growing roots.

Age of the plant has got a remarkable influence on the development of the rhizosphere microflora. Timonin (1940) noted the establishment of a rhizosphere microflora within three days of seed germination and it was more noticeable with the bacteria than with fungi. He obtained 11 to 28 times as great a rhizosphere population as elsewhere in the same soil. Rouatt (1959) also noted a R:S ratio of 2:1 for bacteria in the rhizosphere of wheat three days after planting.

Krassinilkov et al (1936) while studying the rhizosphere of wheat, maize, sunflower and soybeans found a close correlation between the vital activities of the plants and the quantitative composition of the soil microflora. In the case of peanuts he found the number of bacteria diminishing towards the maturity of plants where as the number of fungi and actinomycetes were increasing.

The microorganisms may vary with the soil type. Adati (1939) reported that sandy soils have much less population compared to clayey loams which were rich in organic matter. Rangaswami and Venkatesan (1963) also showed that dry soils are poorer in microbial population than wet soils and top layers of the soil harbour more population than the deeper layers.

Timonin (1940) found that soil amendments as addition of manures were of lesser importance in determining the abundance of microbial population though it increased the soil productivity. Katznelson and Richardson (1943) observed marked difference in the number of fungi and actinomycetes in the rhizosphere of tomatoes due to soil sterilisation with steam, chloropicrin and formaldehyde.

## Rhizosphere effect on bacteria

### Morphological groups in the rhizosphere

Different morphological forms of bacteria such as Gram negative and Gram positive rods, cocci etc. are present in the rhizosphere and soil. It is known that each of the group is differentially stimulated in the rhizosphere. Lochhead (1940) reported that the Gram negative rods were activated more in the rhizosphere of red clover, mangels, oats, tobacco, maize and flax. Spore forming bacteria were less abundant than in control soil. Krassinilkov (1944) had noted that 95 percent of the rhizosphere bacteria constituted the Gram negative rods. Among the non-sporing types Gram negative forms were more abundant than Gram positive ones. King and Wallace (1956)

reported that in the rhizosphere of oats a selective stimulation of Gram negative rods occurred. Rovira (1956) found that with increasing age of the seedlings there was a corresponding increase in the Gram negative organisms in the rhizosphere. Rangaswami and Vasantharajan (1962) also reported from their studies on the microflora of citrus plants that Gram negative non-sporing rods were more abundant in the rhizosphere than Gram positive rods and spore formers.

Rhizosphere effect on fungi

Williams et al (1952) in a comparative study of the steamed and unsteamed soil around tomato plants found that Penicillium spp. were more numerous in the unsteamed soil, where as Mucor spp. were more in the steamed soil. Ebben (1959) also reported that the rhizosphere and root surface population of tomato plants were not very different in the steamed and unsteamed soils.

Agnihothurudu (1953) and Agnihothurudu et al (1955) isolated the following fungi from the rhizosphere of pigeon pea and sorghum. Species of Aspergillus, Penicillium and Fusarium, Macrophoma phaseoli, Neocosmospora vasinfecta, Alternaria, Curvularia, Mucor,

Rhizopus, Helminthosporium, Trichoderma, Cunnighamella, Phoma, Diplodia and Chaetomium.

Chinnayya and Agnihothurudu (1953) found that the number of fungi, bacteria and actinomycetes in the rhizosphere of mesophytic plants were higher than those of aquatic and marsh species. Contois (1953) while studying the rhizosphere of pineapple found that plants in lower altitude harboured predominently species of Aspergillus and Penicillium where as in the higher altitudes Rhizopus nigricans and Circinella simplex were most common. Subba Rao and Bailey (1961) found a species of Fusarium predominent in the rhizosphere of Verticillium wilt susceptible varieties of tomato plants and Trichoderma viride in the resistant varieties.

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

Rangaswami and Venkatesan (1963) found that the number of fungi gradually increase till the time of harvest in the rhizosphere of rice plants.

Rhizosphere effect on actinomycetes

Timonin (1940) noted the presence of 7 to 71 times greater population of actinomycetes and bacteria in the rhizosphere of wheat, oats, lucerne and peas. Rangaswami and Vasantharajan (1962) reported that the actinomycetes were 4 to 20 times more in the rhizosphere of citrus plants compared to the non-rhizosphere soil and actinomycetes with antagonistic effect were found greater in the rhizosphere. Rangaswami and Venkatesan (1963) noted a gradual increase in the number of actinomycetes in the rhizosphere of rice plants till harvest. A moisture level of 20 percent of the total water holding capacity of the soil was found optimum for actinomycetes. Application of potassic and nitrogenous fertilizers increased the actinomycetes population while potash had a significant effect. The predominantly occurring group of actinomycetes in the rhizosphere was reported to be Streptomyces sp. Less frequently Micromonospora and Nocardia species also are seen.

Rhizosphere and root diseases

Comparative studies carried out by many workers like Lochhead (1940), Timonin (1940), Lochhead et al (1940)

Harper (1950), Rombouts (1953), Agnihothurudu (1961) and Subba Rao and Bailey (1961) on the rhizosphere microflora of susceptible and resistant varieties of plants to various root diseases have shown that invariably the susceptible varieties harbour a much higher microbial population than the resistant ones. Lochhead et al (1940), and Agnihothurudu (1961) found that the bacterial and fungal populations were affected much significantly while in the case of actinomycetes the difference was less apparent. In some cases the resistance has been reported to be associated with a higher percentage incidence of organisms antagonistic to the pathogen in question in the rhizosphere. Agnihothurudu (1955) reported the presence of actinomycetes antagonistic to the wilt organism Fusarium udum in the rhizosphere of resistant pigeon pea (Cajanus cajan). Sadasivan and Subramanian (1960) have found that the interactions between the various physical, chemical and biotic factors of the soil in influencing root diseases from their studies on cotton wilt caused by Fusarium vasinfectum.

Root exudates and microbial population

Hiltner (1904) claimed that the increased activity of microorganisms in the rhizosphere was due to root

excretions. Katznelson et al (1954), Andal et al (1956), Bhuvaneswari and Subba Rao (1957), Rovira (1956) and Subba Rao and Bailey (1961) found that the root excretions of plants were rich in amino acids. Rovira (1956) reported the presence of amino acids in the root exudates of tomato, subterranean clover and Phalaris tuberosa. He found that the exudations were greater in the first two weeks of growth. Subba Rao and Bailey (1961) while studying the rhizosphere of tomato plants in relation to varietal resistance or susceptibility to Verticillium wilt found that the amino acids form the bulk of root exudates.

Absorption and translocation of antibiotics by plants

Effective use of antibiotics especially streptomycin against plant pathogenic bacteria has been repeatedly claimed by several authors including Ark (1947), Zaumeyer et al (1953), Mitchell et al (1954), Robison et al (1954) and Rangaswami et al (1959) and (1962). In many cases streptomycin was reported to be absorbed and translocated by the sprayed plants.

Mitchell et al (1954) showed that streptomycin was absorbed by stems of bean. Kramer (1954) reported the systemic penetration of streptomycin in the tissues of

both bean and tomato plants. Napier et al (1956) showed that streptomycin sulphate applied to the primary leaves of dwarf beans exhibited a marked and consistent systemic action and it was removed from the point of application upto the fourth trifoliate leaves. Dowler and Goodman (1958) noted the downward translocation of streptomycin by the Coleus sp. There are, however, a few reports of streptomycin not acting in a systemic manner. Dye (1956) noted that streptomycin entered peach leaves, but it acted only as a penetrant. Crosse and Garrett (1958) also obtained similar results in the case of streptomycin treated cherry leaves.

Dowler and Goodman (1958) concluded from their studies that absorption was greater when the antibiotic was applied to the lower leaf surfaces. Lockwood (1958) reported that more streptomycin was absorbed by leaves incubated in a moist atmosphere than by leaves allowed to dry rapidly and leaf age and portion of the leaf sampled were important sources of variability in leaf disc assays.

Grey (1955 and 1956) studied the extent to which the absorption of streptomycin is influenced by the addition of various chemicals. He found that glycerol

increased the absorption of the antibiotic by the leaves of bean, tomato, pepper and tobacco. Other substances like sorbitol, diethylene glycol and other polyhydroxy alcohols were effective in increasing the absorption of streptomycin by bean leaves, but less so than glycerol. Rangaswamy et al (1959) also found that addition of glycerol increased the absorption of streptomycin by citrus leaves.

Rangaswami and Vasantharajan (1961) assessed the rhizosphere population of streptomycin sprayed plants in order to examine the possible effect of streptomycin causing any physiological change in the plant. They concluded that there was no appreciable change in the number of microorganisms present in the rhizosphere of the sprayed and unsprayed plants. Rangaswami, et al (1962) found that streptomycin had no effect on nodulation and it did not significantly alter the microbial population in the rhizosphere of daincha (Sesbania aculeata) and sunnhemp (Crotalaria juncea)

Varietal resistance of tomato to bacterial wilt

Evaluation of resistance in tomato to Pseudomonas solanacearum by artificial inoculation was attempted by

Winstead (1952) and Winstead and Kelman (1952). They found that the root injury technique was the best in evaluating resistance to the bacteria and that the susceptibility to the wilt decreased in the resistant tomato plants as the age of the plants increased from four to eight weeks.

## MATERIALS AND METHODS

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

Marglobe, Bonnybest and Redcherry were the three varieties of tomato selected for the rhizosphere studies. These varieties were selected because Marglobe showed the highest percentage of wilt while Redcherry showed the lowest with Bonnybest falling between the two.

Seeds of the above tomato varieties were sown in different beds of size 15' x 3'. Soon after germination, the beds were thinned out. Assessment of the rhizosphere microflora was started from the fifth day after sowing, when the cotyledons were fully emerged. The method adopted in the present studies was the soil dilution and plate counts. (Timonin 1940). For the first twenty days root washings were taken every fifth day and thereafter once in ten days. A total of twelve samples were collected during the life of the plant.

Collection of samples

Plants were selected at random from the three varieties for the rhizosphere samples. They were uprooted, care being taken to keep the root system as intact as possible. Simultaneously soil from the same field was collected and used as control. It was drawn from the bed sufficiently away from the influence of the root system and from the same depth from which the roots were taken.

In the laboratory, plants were shaken gently to remove the superfluous soil adhering to the root system. The entire root system was then cut off aseptically and transferred into a previously weighed flask 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 Lochhead, 1949).

The control soil used for the test was sieved through a nine sieve mesh and two samples each of 2 grams were weighed separately. One sample was placed in a previously weighed clean china dish and placed in a hot air-oven at about 105 to 110°C for six hours, so as to

evaporate the entire moisture. Then it was cooled and weighed and the moisture content calculated.

The other sample was transferred into a weighed conical flask containing 100 ml. of sterile distilled water and dilutions were prepared.

To find the weight of the rhizosphere soil used for making the dilutions, the roots within the flasks were removed carefully taking precautions to see that all the adhering soil was well washed off from the root system, the washed water being collected in the original flask itself. The flasks were then placed in a water bath and evaporated to dryness. They were then dried in a hot air-oven at 105 to 110°C for six hours and the weight of the soil was determined making due corrections for the aliquots removed for the dilutions. The counts were expressed per gram weight of the soil on dry weight basis.

This technique was adopted throughout the rhizosphere studies and in all cases non-rhizosphere soils were collected from the same depth from which the roots were taken but away from the influence of the root system.

Determination of the total bacteria, fungi and actinomycetes populations.

Conical flasks containing 100 ml. of sterile distilled water to which the plant root system and non-rhizosphere soils were added were placed in a shaker for half an hour. Appropriate dilutions were then prepared from this suspension. Care was taken to transfer the aliquots under utmost aseptic conditions using sterile pipettes, sterilised previously with one side plugged with cotton wool. In all the further dilutions each flask of the dilution series was shaken for two minutes before transferring an aliquot to the next flask care being taken to remove the suspension while it was in motion.

The final dilution prepared for the counts of the microorganisms was one in million. One millilitre of this final dilution was plated in sterile Petri dishes along with 15 ml. of the medium used. Different media were used for the studies of bacteria, fungi and actinomycetes. The media used were

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

This medium was used for bacteria.

Composition:

| | | |
|---|---|---|
| Soil extract | - | 1000 ml. |
| $K_2HPO_4$ | - | 0.2 gm. |
| Agar agar | - | 15.0 gm. |

For preparing the soil extract 1000 gm. of soil was autoclaved in 1000 ml. of water for half an hour. Then it was allowed to sediment and the supernatent liquid was decanted and filtered through No. 41 filter paper. In order to hasten the sedimentation of the soil particles a small quantity of $CaSO_4$ was added to the suspension before filtering. One gram of glucose was also added to the above medium to hasten the appearance of the colonies. The pH of the medium was adjusted to 6.8.

2. Peptone-dextross agar with rose bengal and streptomycin. (Martin, 1950).

This medium was used for fungi.

Composition:

| | | |
|---|---|---|
| Dextrose | - | 10.0 gm. |
| Peptone | - | 5.0 gm. |
| $KH_2PO_4$ | - | 1.0 gm. |
| $MgSO_4$ | - | 0.5 gm. |
| Agar agar | - | 15.0 gm. |
| Rose bengal | - | 1 part in 30,000 parts of the medium. |
| Distilled water | - | 1000 ml. |
| Streptomycin | - | 30 u per ml. or 0.3 ml. per 100 ml. of the cooled medium. |

(This was added only at the time of plating).

3. Jensen's medium.

This was used for the counts of actinomycetes.

Composition:

| | | |
|---|---|---|
| Glucose | - | 2.0 gm. |
| Caesin | - | 0.2 gm. |
| $K_2HPO_4$ | - | 0.5 gm. |
| $FeCl_3$ | - | trace |
| Agar agar | - | 15.0 gm. |
| Distilled water | - | 1000 ml. |

pH adjusted to 7 to 7.5.

The above three media were prepared and autoclaved at 15 lb. pressure for half an hour in test tubes, each tube containing about 15 ml. of the media. Before plating the media were melted and cooled to 45-48°C and the plating was done using two methods.

Method 1:

One millilitre of the final dilution was transferred to a sterile Petri dish using a sterile 1 ml. pipette. The plate was rotated gently so as to get a uniform spread of the soil dilution in the plate. The cooled medium was then poured over the solution and the Petri dish was again rotated in a swirling movement so as to get an even spread of the soil dilution in the medium. (Timonin, 1940).

Method 2:

In this method one millilitre of the final soil dilution was added directly to the test tube containing the melted agar at about 48°C. The test tube was then rotated well inside the palms and the solution mixed medium was poured directly into the sterile Petri dish. The Petri dish was then gently rotated to get an even spreading of the medium.

Dilution plates were plated in triplicates for each method and for each medium. The plates were then incubated at room temperature ie., at about 30-32°C for about fourteen days. For fungi the counts were taken after six to seven days as soon as the colonies began to appear in the Petri dishes. For bacteria and actinomycetes ten to fourteen days incubation was necessary. For taking the counts of the colonies of bacteria and actinomycetes Spencer's Dark Field Qubec colony counter was used.

Qualitative determination of rhizosphere microflora

Bacteria:

The bacterial colonies that appeared on the dilution plates after fourteen days incubation under the laboratory conditions were picked up and slab cultures were made in the soil extract semi-solid medium for further studies. (Taylor and Lochhead, 1938).

Composition:

| | | |
|---|---|---|
| Soil extract | - | 1000 ml. |
| $K_2HPO_4$ | - | 0.2 gm. |
| Glucose | - | 1.0 gm. |
| Yeast extract | - | 1.0 gm. |
| Agar agar | - | 3.0 gm. |

pH adjusted to 6.8.

For taking the bacterial colonies from a plate, the place was divided into four sectors and twenty five colonies within a single sector were picked up to represent each treatment. From plates where the number of colonies were very low, all the colonies were picked up. The purity of isolates were maintained during the period of study by subculturing them in the soil extract semi-solid medium.

The bacterial isolates were classified according to their morphology into Gram negative or Gram positive cocci and rods and spore formers. Isolates were taken from the rhizosphere of 10, 50 and 90 days old plants of the three varieties ie., at the seedling, flowering and harvesting stages and from the control soils. For Gram reaction twenty four hour old cultures were used and for the determination of spore formation forty eight hour old cultures were used.

2. Fungi:

The fungal colonies that appeared in the plates were broadly grouped under four categories namely Aspergillus spp., Penicillium spp., Mucoraceous fungi and other fungi.

## II. Comparison of the rhizosphere microflora of wilted and healthy tomato plants

Plants showing the early symptoms of wilt were selected for this study. Healthy plants of the same variety and age served as control.

In the early stages of wilt only a few of the branch roots usually showed decay. These may possibly be the roots through which the pathogen have gained entry. The bulk of the root system appeared normal.

Both the healthy and wilted plants were uprooted carefully and brought to the laboratory along with the control soil and soil dilution plates were made as mentioned earlier.

## III. Estimation of rhizosphere microflora at different soil depths in the same plant

The technique already described namely, soil dilution and plate counts was utilised for determining the rhizosphere microflora in the roots of plants collected from different soil levels. Roots were collected from 80 days old Bonnybest plants, from depth of 1"-3", 3"-6" and 6"-10". Control soils were also collected from the same depths.

## IV. Absorption and translocation of streptomycin within tomato plants and its influence on the rhizosphere microflora

For the determination of the absorption and translocation, a series of pots with three plants of about six weeks old in each pot, were sprayed with streptomycin sulphate (Glaxo laboratories 745 µ per mg.) at concentrations of 100 ppm., 1000 ppm. and 10000 ppm. A second set of plants received the same treatment with 1 percent glycerol added to the antibiotic.

For the rhizosphere studies plants sprayed with streptomycin with and without glycerol at concentrations of 1000 ppm. and 10000 ppm. only were used. Of the three plants in every pot, one each was sprayed with the two concentrations of streptomycin and the third served as the control.

In both the cases the antibiotic was sprayed on the aerial portion of the plants by means of an atomizer. Care was taken to prevent the antibiotic from reaching the roots through the soil. For this purpose the soil surface around the plants was covered with a thick layer

of cotton wool before spraying. Unsprayed plants were maintained as control.

Determination of the rhizosphere microflora of both sprayed and unsprayed plants along with control was done five days and ten days after spraying by the technique already mentioned.

Presence of the antibiotic in the leaves, stems and roots of the sprayed plants was assayed after 24, 48, 72 and 96 hours. Persistence of the antibiotic in the leaves was assayed by the leaf disc method. The diameter of the leaf disc used was 8 mm. In the case of stem, discs of 1 mm. thickness were used. Small bits of about 5 mm. long are used in the case of roots. They were planted in nutrient media in which the test organisms were incorporated. The following bacteria were used as the test organisms:-

(i) Pseudomonas solanacearum. (Culture obtained from Annamalai University)

(ii) Bacillus subtilis.

In order to avoid contamination of host tissues, they were washed thoroughly in running tap water first

and then surface sterilised before planting in the test organisms. Whole tissues were used for sterilisation. They were later on cut into bits of suitable sizes.

For surface sterilisation the following methods were tried:-

1. Washing the plant tissues in five series of sterile water.
2. Surface sterilising in 1:1000 mercuric chloride for 15 seconds and then washing in series of sterile water.
3. Surface sterilising in 1:10000 mercuric chloride for 15 seconds and then washing in sterile water.
4. Sterilising by freezing in the ice compartment of the refrigerator overnight. (Slight modification of Gray's method 1955).
5. Sterilising in propylene oxide vapours in partial vaocum. The method adopted was a modification of the one devised by Ark (1947).

Technique of surface sterilisation with propylene oxide vapours:

Propylene oxide at a concentration of 1 ml. per litre of air for ten minutes was used in this method.

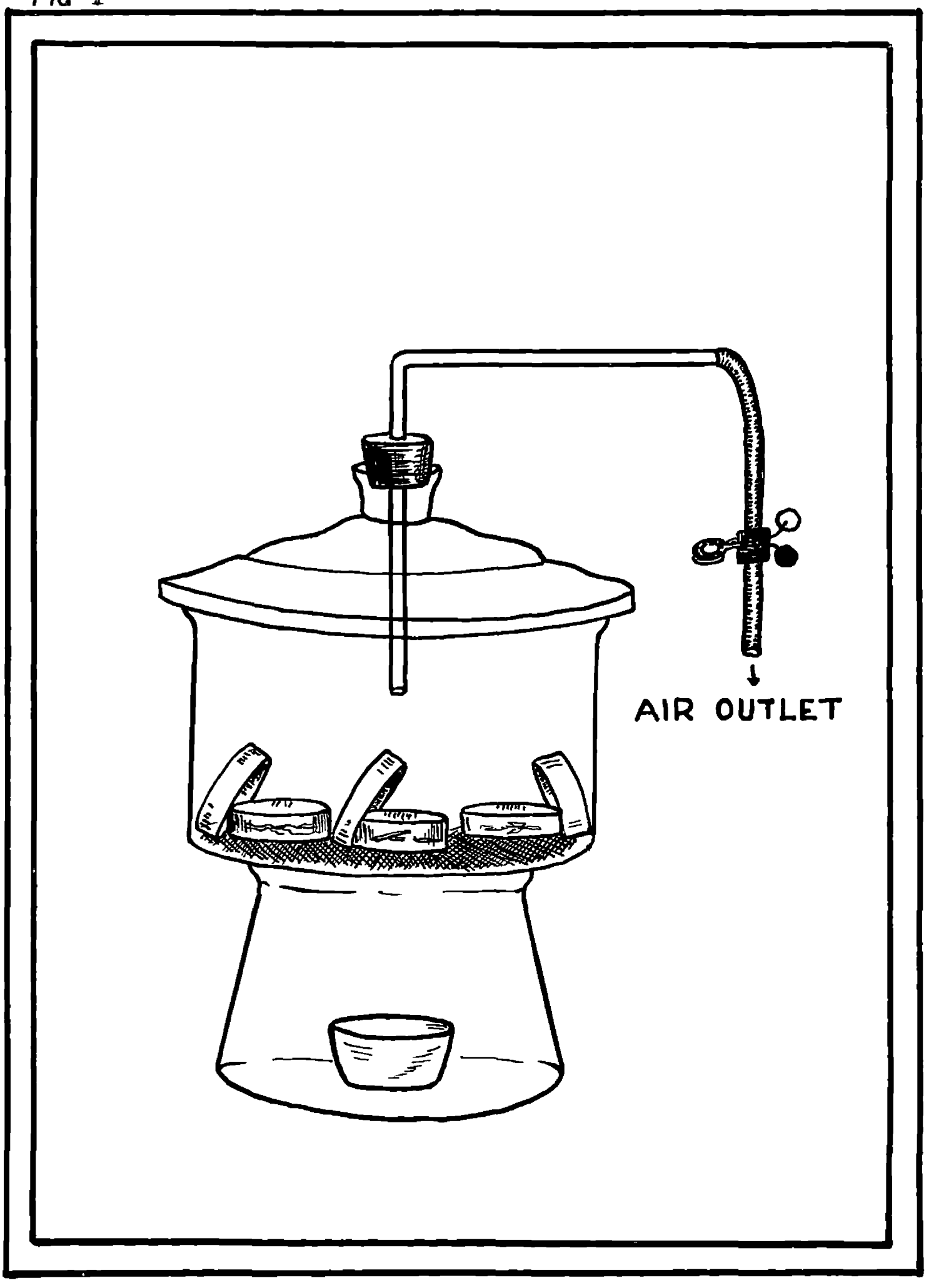
For sterilisation, whole tissues of both sprayed and unsprayed plants were first washed in running tap water and the leaves, stem and roots were separated. They were blotted dry and placed in open Petri dishes. One millilitre of propylene oxide liquid was poured in a clean china dish and placed in the lower compartment of a dessicator of about one litre volume. The Petri dishes containing the plant tissues were then arranged over a wire gauze inside the dessicator and it was closed air tight. (Fig. 1). The air inside the dessicator was drawn out through the out let and the outlet closed. The propylene oxide vapours was allowed to act on the plant tissues for ten minutes in the partial vacuum and then the dishes were closed and removed from the dessicator.

Leaf discs of about 8 mm. diameter were then cut by using a sterile sharp cork borer. Stem and roots were cut to the appropriate size by using a sharp sterilised blade and they were planted in nutrient media containing the test organisms. Before incubation the Petri dishes were placed in the refrigerator for one hour to facilitate easy and rapid exudation of the plant sap.

Observations were recorded after 24 hours, by measuring the width of the inhibition ring measured from

Fig. 1. Apparatus used for the surface sterilisation of the plant tissues for antibiotic assay

FIG I



the edge of the test material. To serve as a comparison sterilised filter paper discs of the same diameter as that of the leaf discs were immersed in the original dilutions of the antibiotic used for the sprayings and planted in the test organisms. The inhibition zones produced in this case was compared with that produced by the plant tissues.

Tissues of the unsprayed plants sterilised by propylene oxide vapours served as control.

V. Studies on the varietal resistance to bacterial wilt.

The experiment was laid out in a randomised block design with eight varieties and four replications. The varieties used for the studies were:-

(1) Bonnybbst (2) Pritchard (3) Yellow plum
(4) Ponderosa (5) Marglobe (6) Redcherry
(7) Sunray (8) Ox-heart

(The seeds of these varieties were received from Pestonjee P. Pocha and Sons, Poona).

Seeds of the above varieties were sown in earthen pots which were filled with soil and compost in the ratio

3:1. The seedlings were transplanted to the main field after 21 days. The bed size used was 15'x3', the spacing given within plants being 9" and between rows 1

In order to get a uniform treatment, known sick soil was spread over the beds in equal quantity. One basket of compost was incorporated to each bed before transplanting. Casuality during transplanting was replaced with excess seedlings remained in the respective pots.

Daily observations were taken from the date of planting till the harvesting stage and the wilt index was calculated for each variety. The method adopted by Winstead and Kelman (1952) was followed with slight modification suitable in rating the plants. The plants showing the symptoms of wilt were rated as follows:-

| Rating | Symptoms |
|---|---|
| 0 | Healthy plant with no symptoms of wilt |
| 1 | First one or two leaves wilted |
| 2 | All leaves wilted |
| 3 | Plant dead. |

Wilt index was calculated as follows:-

Disease index = Number of plants in each symptom category x the corresponding numerical grade and the products were added. The summation was then divided by the maximum numerical grade for the given number of plants expressed as a percentage.

Wilt index was calculated for all the varieties taking 100 days as the average life period of the crop when it was ready for harvest.

VI. Chemical analysis of the soil samples

Since a much higher incidence of actinomycetes was found in the control soils, samples were taken both from the field and from the pots where the experiments were conducted and analysed chemically to determine the nitrogen, phosphorus and potash contents.

The presence of nitrogen, both total and available forms was estimated by the Kjeldahl's method, phosphorus by the volumetric method and potash by the gravimetric method.

# RESULTS

## I. Rhizosphere microflora in relation to age of tomato plants

A definite rhizosphere population was found to be established within five days after sowing in all the varieties. Counts of bacteria, fungi and actinomycetes present in the rhizosphere and also in the control soil were therefore started from the fifth day of sowing when the cotyledons were fully emerged. Both quantitative and as far as possible qualitative studies were made of the bacterial, fungal and actinomycetes population.

### A. Quantitative assessment.

#### (i) Total bacterial population in the rhizosphere and the control soils

There was a marked tendency for the bacterial population in the rhizosphere to increase along with the growth of the plant and the maximum number was found during the flowering stages of the different varieties. Germination started on the third day of sowing and the

Cotyledons were fully emerged on the fifth day. Within two days of germination, a R:S ratio of 7.37 was noticed for the variety Marglobe and 9.91 and 9.03 for the varieties Bonnybest and Redcherry respectively. From then on there was a steady rise in the bacterial population till the flowering stage. In Bonnybest flowering started at about 46 days after sowing and the maximum bacterial population of 159.1 million per gram was noticed during this stage. In Marglobe and Redcherry flowering commenced on the 52nd day after sowing and in this case also the maximum bacterial population of 181.4 million and 167.6 million per gram respectively coincided with their flowering periods. The R:S ratios during this period for the varieties Marglobe, Bonnybest and Redcherry were 13.64, 12.05 and 12.60 respectively. After attaining this peak the intensity of population was found to decrease gradually. This decrease was found to be more or less uniform for all the varieties.

In the case of non-rhizosphere soil which served as control, samples were collected from the same depth from which the roots were taken. When the depth from which the samples were collected was increased there was a noticeable increase in the bacterial population. The

first sample collected on the fifth day after sowing was drawn from soil less than one inch deep. For the next fifteen days soil samples were collected from depths ranging from one inch to three inches. From there on till the 60th day samples were taken from a depth of three to six inches. In the last stages of growth ie., upto 100 days the depth was gradually increased upto ten inches.

In the surface soil of about one inch deep the population was only 3.5 million per gram of soil and the population steadily increased and reached the peak of 13.3 million per gram at a depth of six inches. In the subsequent samples which were collected from still deeper layers though there was a fall in the bacterial population a fairly high count, namely, over nine million per gram of soil was maintained upto the depth of ten inches.

Though the maximum bacterial population in the rhizosphere was noted at the time of flowering, the highest R:S ratio was reached between the 20th and 30th day of growth. The R:S ratios for Marglobe, Bonnybest and Redcherry at this period were 15.24, 14.05 and 14.72 respectively. Inspite of the fact that there was a fall in the bacterial population after flowering, the R:S

ratios were found to be maintained more or less uniform till the harvest of the crop. (Table I).

A graphical representation of the incidence of total bacterial population in the different stages in the rhizosphere and control soil is given in Fig. II.

(ii) Total fungal population in the rhizosphere and the control soils

There was a steedy increase in the fungus population of the rhizosphere upto the 15th day after sowing. From then on there was no appreciable fluctuations in the population till the 70th day. The maximum number of fungi was found at about the time of maturity of the plant growth. The highest figures for Marglobe, Bonnybest and Redcherry were 0.41 million, 0.39 million and 0.48 million per gram of rhizosphere soil respectively.

In the control soil also there was a gradual increase in the number of fungi upto a depth of six inches. The fungal population was higher in the deeper layers of six to ten inches the maximum recorded being 0.26 million.

TABLE I

Total Bacterial Population in the Rhizosphere and Control soil at 12 stages of growth

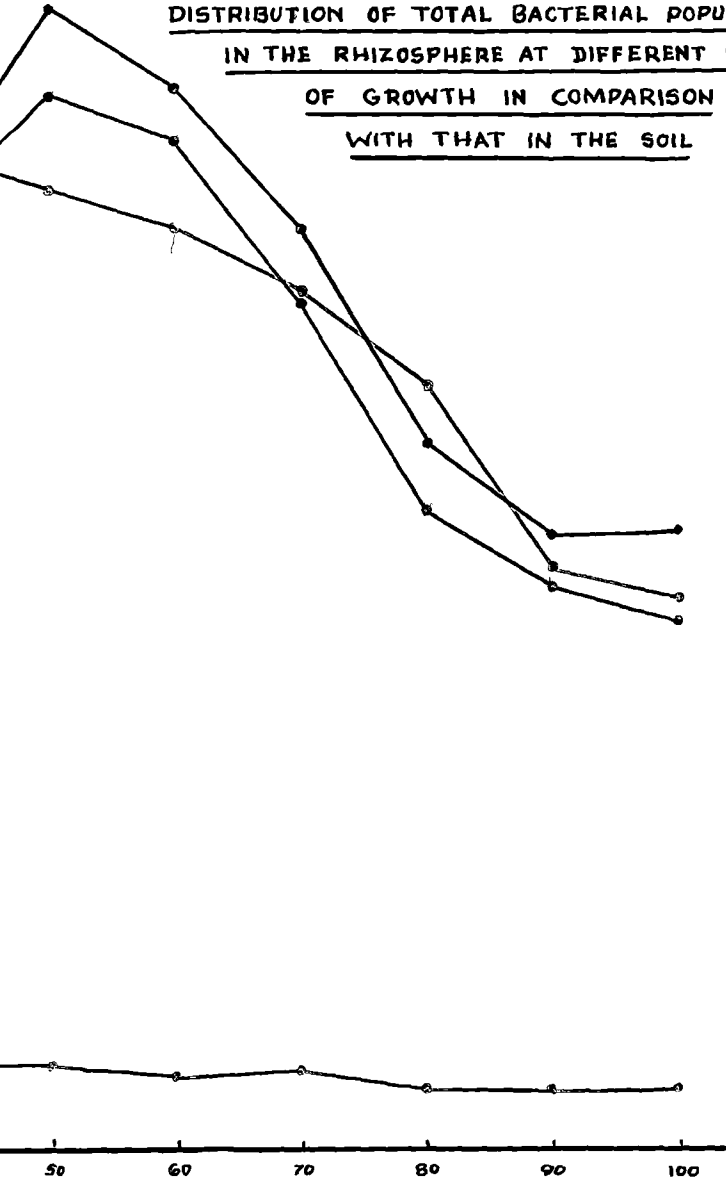
(Population $10^6$ per gram of dry sample)

| Age in days | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control soil | 3.5 | 4.5 | 5.6 | 7.5 | 9.4 | 13.2 | 13.3 | 11.5 | 12.9 | 9.9 | 9.1 | 9.8 |
| Marglobe | 25.8 | 53.4 | 83.1 | 114.3 | 128.8 | 152.0 | 181.4 | 168.1 | 146.5 | 112.5 | 97.9 | 98.9 |
| R:S ratio | 7.37 | 11.86 | 14.84 | 15.24 | 13.70 | 11.52 | 13.64 | 14.61 | 11.35 | 11.36 | 10.75 | 10.09 |
| Bonnybest | 34.7 | 50.0 | 76.6 | 92.3 | 138.4 | 159.1 | 152.7 | 140.6 | 136.4 | 121.0 | 92.2 | 87.9 |
| R:S ratio | 9.91 | 11.11 | 13.68 | 12.3 | 14.72 | 12.05 | 11.48 | 12.74 | 10.57 | 12.22 | 10.13 | 8.97 |
| Redcherry | 31.6 | 45.3 | 68.5 | 105.4 | 123.6 | 147.8 | 167.6 | 160.2 | 135.3 | 101.5 | 89.8 | 84.4 |
| R:S ratio | 9.03 | 10.06 | 12.23 | 14.05 | 13.15 | 11.19 | 12.60 | 13.93 | 10.48 | 10.25 | 9.87 | 8.61 |
| S.E. | 4.24 | 5.95 | 10.57 | 15.39 | 11.43 | 13.92 | 12.05 | 12.23 | 12.85 | 4. 88 | 8.20 | 7.06 |
| C.D. at 5% level | 9.78 | 13.72 | 24.37 | 35.49 | 26.36 | 32.10 | 27.79 | 28.20 | 29.63 | 11.25 | 18.91 | 16.28 |

Fig. II. Graphical representation of the distribution of the bacterial population in the rhizosphere of the three varieties of tomato at different stages of growth, in comparison with that in the soil.

FIG II

DISTRIBUTION OF TOTAL BACTERIAL POPULATION IN THE RHIZOSPHERE AT DIFFERENT STAGES OF GROWTH IN COMPARISON WITH THAT IN THE SOIL



The R:S ratio in relation to fungi was highest 15 days after sowing and then there was a gradual fall. The highest R:S ratio for Marglobe, Bonnybest and Redcherry fell respectively from 2.97 to 1.21, 3.74 to 1.18 and 3.25 to 1.24. (Table II).

(iii) Total actinomycetes population in the rhizosphere and the control soils.

There was a marked increase in the actinomycetes population between 5th and 20th days after sowing. The increase was more prominent between 5th and 10th day. After the 20th day there was some change in the population but the pattern of the change was not uniform for all the varieties. There was practically no increase in the population in Redcherry but there was a slight fall in the population in Marglobe. There was a definite increase in the population in Bonnybest towards the later stages of its growth. The maximum number of actinomycetes colonies recorded for Marglobe was 19.03 million, for Bonnybest 19.50 million and for Redcherry 21.20 million.

In the soil there was a steady increase in the actinomycetes population with increasing depth upto about six inches and from then on there was no appreciable

TABLE II

Total Fungal population in the Rhizosphere and control soil at 12 stages of growth

(Population $10^6$ per gram of dry sample)

| Age in days | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control soil | 0.06 | 0.11 | 0.09 | 0.14 | 0.19 | 0.19 | 0.20 | 0.26 | 0.26 | 0.25 | 0.24 | 0.22 |
| Marglobe | 0.10 | 0.20 | 0.26 | 0.34 | 0.33 | 0.32 | 0.33 | 0.32 | 0.31 | 0.33 | 0.41 | 0.32 |
| R:S ratio | 1.66 | 1.81 | 2.88 | 2.43 | 1.75 | 1.69 | 1.65 | 1.23 | 1.19 | 1.32 | 1.79 | 1.45 |
| Bonnybest | 0.09 | 0.22 | 0.32 | 0.33 | 0.33 | 0.32 | 0.30 | 0.28 | 0.35 | 0.33 | 0.39 | 0.35 |
| R:S ratio | 1.50 | 2.00 | 3.55 | 2.35 | 1.75 | 1.69 | 1.50 | 1.08 | 1.35 | 1.32 | 1.62 | 1.59 |
| Redcherry | 0.14 | 0.15 | 0.28 | 0.29 | 0.34 | 0.33 | 0.30 | 0.31 | 0.48 | 0.43 | 0.36 | 0.33 |
| R:S ratio | 2.33 | 1.36 | 3.33 | 2.08 | 1.79 | 1.75 | 1.50 | 1.19 | 1.85 | 1.72 | 1.50 | 1.50 |
| S.E. | 0.13 | 0.61 | 1.40 | 1.61 | 1.63 | 1.46 | 1.45 | 0.53 | 2.11 | 0.71 | 0.78 | 2.74 |
| C.D. at 5% level | 0.29 | 1.41 | 3.23 | 3.71 | 3.76 | 3.37 | 3.34 | 1.22 | 4.87 | 1.64 | 1.80 | 6.32 |

change in the population. The maximum number found in the control soil was 8.50 million.

The R:S ratios ranged from 2.01 to 3.66 for Marglobe, 1.37 to 3.93 for Bonnybest and 2.43 to 4.04 for Redcherry. (Table III).

In general the population of bacteria both in the rhizosphere and control soil was greater than that of actinomycetes and fungi. In the rhizosphere the percentage incidence of bacteria in relation to the total population ranged from 70.4 to 91.41 percent for Marglobe, 73.06 to 90.2 percent for Bonnybest and 73.04 to 89.6 percent for Redcherry. The percentage incidence of actinomycetes ranged from 8.56 to 29.4 percent, 9.5 to 26.7 percent and 10.21 to 26.2 percent for Marglobe, Bonnybest and Redcherry respectively. The range in the percentage incidence of fungi was found more or less uniform for all the varieties namely between 0.1 to 0.4 percent. The population of bacteria in the rhizosphere was found to be 3 to 10 times as that of actinomycetes and about 200 to 600 times as that of fungi.

In the control soil the range in the percentage incidence of bacteria, fungi and actinomycetes was less pronounced. The bacterial population varied from 51.1

TABLE III

Total Actinomycetes population in the Rhizosphere and Control soil at 12 stages of growth

(Population $10^6$ per gram of dry sample)

| Age in days | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control soil | 3.83 | 4.20 | 4.79 | 5.20 | 6.83 | 7.30 | 7.13 | 6.33 | 8.50 | 8.17 | 8.13 | 7.80 |
| Marglobe | 10.80 | 15.23 | 16.43 | 19.03 | 17.70 | 18.07 | 17.00 | 16.70 | 17.19 | 17.00 | 18.50 | 18.13 |
| R:S ratio | 2.82 | 3.62 | 3.43 | 3.66 | 2.59 | 2.48 | 2.38 | 2.63 | 2.02 | 2.08 | 2.28 | 2.32 |
| Bonnybest | 12.70 | 16.53 | 17.13 | 16.93 | 17.20 | 16.83 | 17.60 | 18.73 | 19.50 | 18.57 | 19.23 | 19.00 |
| R:S ratio | 3.31 | 3.93 | 3.57 | 3.26 | 2.52 | 2.31 | 2.47 | 2.95 | 2.29 | 2.27 | 1.37 | 2.44 |
| Redcherry | 11.30 | 16.57 | 15.50 | 21.00 | 20.60 | 20.37 | 19.10 | 20.53 | 21.20 | 19.83 | 20.47 | 20.30 |
| R:S ratio | 2.95 | 3.94 | 3.23 | 4.04 | 3.02 | 2.79 | 2.68 | 3.24 | 2.49 | 2.43 | 2.52 | 2.60 |
| S.E. | 2.24 | 2.52 | 2.85 | 3.26 | 2.59 | 1.78 | 1.85 | 1.61 | 2.45 | 3.81 | 2.19 | 2.27 |
| C.D. at 5% level | 5.17 | 5.81 | 6.57 | 7.52 | 5.97 | 4.10 | 4.27 | 5.55 | 5.65 | 8.78 | 5.05 | 5.23 |

to 64.4 percent, the actinomycetes population from 34.5 to 47.6 percent and the fungus population from 0.9 to 2.5 percent. It is interesting to note that the total bacterial population in the soil is only 1.07 to nearly 2 times as that of actinomycetes. In relation to fungi, however, the bacterial population was 30 to 60 times as that of the former. (Fig. III).

B. Qualitative determination of the rhizosphere microflora

1. Bacteria: Morphological groups

There was invariably a much higher percentage incidence of Gram negative organisms in the rhizosphere of the three varieties as compared to the control soil. Gram negative rods were found to be about 60 percent and Gram negative cocci (which include coccoid rods also) about 20 percent in the rhizosphere which was about twice as much as in the non-rhizosphere soil. In the control soil on the other hand Gram positive organisms and sporeformers were more prominent. The mean percentage incidence of the different morphological groups in the rhizosphere and control soil in the three different stages of growth is presented in Fig. IV.

Fig. III. Bardiagram showing the percentage incidence of bacteria, actinomycetes and fungi in the rhizosphere and

Fig III

PERCENTAGE INCIDENCE OF BACTERIA ACTINOMYCETES AND FUNGI IN THE RHIZOSPHERE AND CUNTROL SOILS

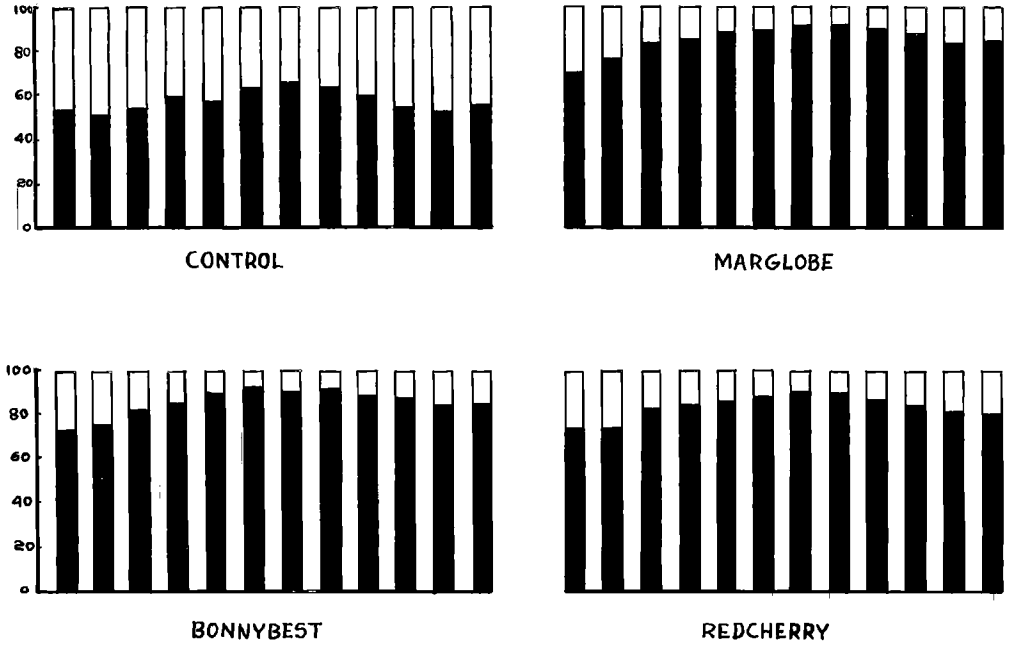
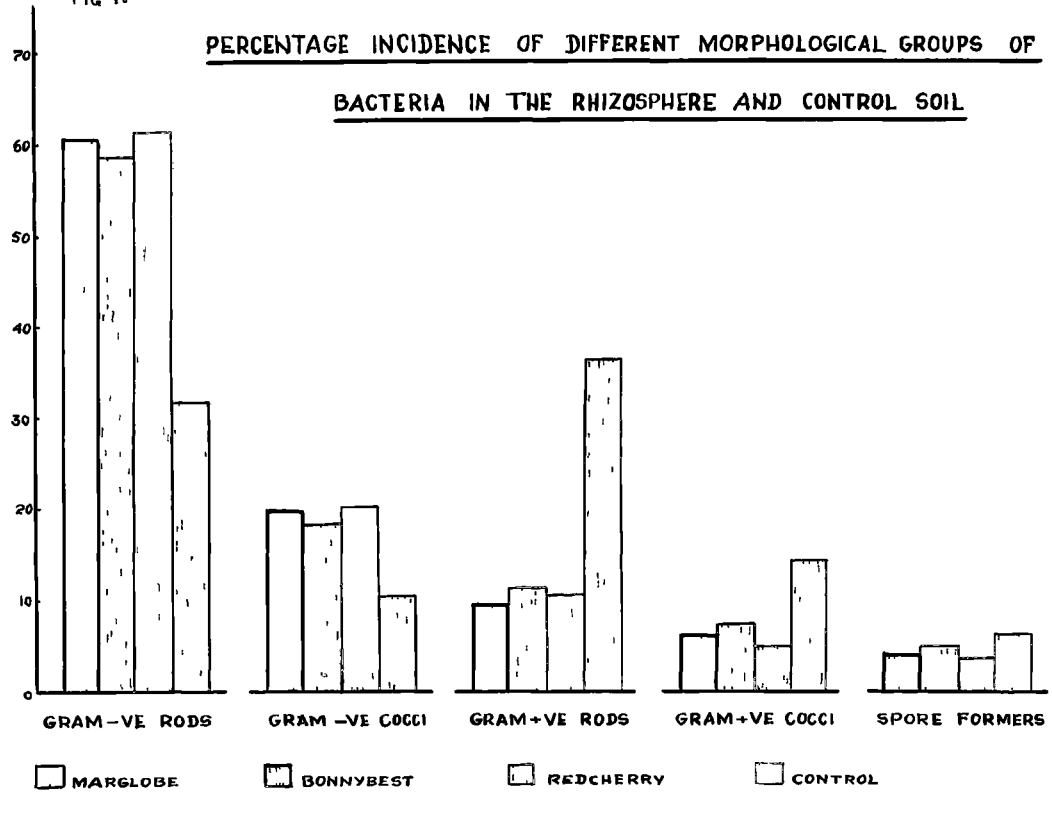


Fig. IV. Bardiagram showing the percentage incidence of different morphological groups of bacteria in the rhizosphere and control soils.

Fig IV


PERCENTAGE INCIDENCE OF DIFFERENT MORPHOLOGICAL GROUPS OF
BACTERIA IN THE RHIZOSPHERE AND CONTROL SOIL
70
60
50
40
30
20
10
0
GRAM-VE RODS
GRAM -VE COCCI
GRAM+VE RODS
GRAM+VE COCCI
SPORE FORMERS
MARGLOBE
BONNYBEST
REDCHERRY
CONTROL

On the total population basis there was a remarkable variation in the number of the different morphological groups of bacteria present in the rhizosphere at the three different stages of growth. The population of the Gram negative rods in the rhizosphere of Marglobe rose from 28.41 million on the 10th day to 120.99 million on the 50th day. Subsequently it fell to 60.35 million on the 90th day. The same trend was true for Bonnybest and Redcherry also whose population of Gram negative rods on the 50th day were 103.70 and 99.05 millions respectively.

A more or less similar pattern was noted in the control soil also where the maximum number of Gram negative rods, namely, 5.21 million were found at a depth of about 6" and the number present upto a depth of 1-3" and 6-10" being 1.39 million and 2.28 million respectively.

Similarly in the case of Gram negative cocci also the population became 2 to 3 times at the age of 50 days and subsequently falling down though the fall was less marked thanthat of the Gram negative rods. The maximum number of Gram negative cocci found in the rhizosphere of Marglobe, Bonnybest and Redcherry were 24.13, 21.80 and 30.51 million respectively. In the case of control the maximum at 6" depth was 1.16 million.

Almost the same pattern of population change was noted in the case of Gram positive organisms and spore-formers with only slight fluctuation in the case of Bonnybest only. (Table IV to VII).

Though the variation in the number of the individual groups of organisms was well marked when the total population was taken into consideration there was not much difference in the percentage of any particular group of organism at the three stages of growth. In other words the rise or fall in the total population was proportionately reflected in the various groups of organisms in the different stages.

2. Fungi:

Both in the soil and in the rhizosphere Aspergillus and Penicillium groups formed the predominent fungus flora. They comprised 86 percent of the total population in the soil. In the rhizosphere they formed 75 to 78 percent of the total population.

Mucoraceous group of fungi were found to be about 3 percent both in the control soil and Marglobe and 7 to 9 percent in Bonnybest and Redcherry.

TABLE IV

Distribution of Morphological groups of Bacteria in the Rhizosphere and in the Control soils - Stage of the crop: 10 days after sowing

(Population $10^6$ per gram of dry sample)

| Particulars | Soil | | Marglobe | | | Bonnybest | | | Redcherry | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Population | % | Population | R:S ratio | % | Population | R:S ratio | % | Population | R:S ratio |
| Total Population | - | 4.41 | | 53.40 | 11.9 | - | 50.04 | 11.3 | - | 45.34 | 10.3 |
| Gram -ve rods | 31.5 | 1.39 | 53.2 | 28.41 | 20.1 | 56.0 | 28.02 | 20.2 | 64.4 | 29.20 | 21.0 |
| Gram -ve cocci | 12.5 | 0.55 | 22.8 | 12.18 | 21.8 | 21.4 | 10.71 | 19.3 | 23.0 | 10.43 | 18.9 |
| Gram +ve rods | 44.2 | 1.95 | 11.4 | 6.09 | 3.1 | 11.2 | 5.61 | 2.9 | 9.2 | 4.17 | 2.14 |
| Gram +ve cocci | 6.3 | 0.28 | 7.6 | 4.06 | 14.5 | 5.6 | 2.80 | 10.0 | 3.4 | 1.54 | 5.5 |
| Spore formers | 5.5 | 0.24 | 5.0 | 2.66 | 10.7 | 5.8 | 2.90 | 12.1 | 0 | 0 | 0 |

TABLE V

Distribution of Morphological groups of Bacteria in the Rhizosphere and in the control - Stage of the crop: 50 days after sowing.

(Population $10^6$ per gram of dry sample)

| Particulars | Soil | | Marglobe | | | Bonnybest | | | Redcherry | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Population | % | Population | R:S ratio | % | Population | R:S ratio | % | Population | R:S ratio |
| Total population | | 13.30 | | 181.4 | 13.6 | | 152.4 | 11.5 | | 167.6 | 12.6 |
| Gram -ve rods | 39.2 | 5.21 | 66.7 | 120.99 | 23.2 | 68.0 | 103.7 | 19.9 | 59.1 | 99.05 | 19.0 |
| Gram -ve cocci | 8.7 | 1.16 | 13.3 | 24.13 | 20.8 | 14.3 | 21.8 | 19.0 | 18.2 | 30.51 | 26.3 |
| Gram +ve rods | 26.1 | 3.47 | 10.0 | 18.14 | 4.5 | 7.1 | 10.8 | 3.1 | 13.6 | 22.79 | 6.5 |
| Gram +ve cocci | 17.3 | 2.30 | 6.7 | 12.15 | 5.3 | 7.1 | 10.8 | 4.7 | 4.5 | 7.54 | 3.2 |
| Spore formers | 8.7 | 1.16 | 3.3 | 5.99 | 5.2 | 3.5 | 5.3 | 4.6 | 4.6 | 7.71 | 6.6 |

TABLE VI

Distribution of Morphological groups of Bacteria in the Rhizosphere and in the control - Stage of the crop: 90 days after sowing

(Population $10^6$ per gram of dry sample)

| Particulars | Soil | | Marglobe | | | Bonnybest | | | Redcherry | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Population | % | Population | R:S ratio | % | Population | R:S ratio | % | Population | R:S ratio |
| Total population | | 9.13 | | 97.96 | 10.7 | | 92.16 | 10.1 | | 89.8 | 9.8 |
| Gram -ve rods | 25.0 | 2.28 | 61.6 | 60.35 | 27.7 | 52.4 | 48.29 | 22.1 | 60.2 | 54.1 | 24.8 |
| Gram -ve cocci | 10.0 | 0.91 | 23.1 | 22.63 | 24.9 | 19.0 | 17.51 | 19.2 | 20.0 | 18.0 | 19.8 |
| Gram +ve rods | 40.0 | 3.65 | 7.7 | 7.54 | 2.1 | 14.3 | 13.18 | 3.6 | 8.6 | 7.7 | 2.1 |
| Gram +ve cocci | 20.0 | 1.83 | 3.8 | 3.72 | 2.0 | 9.5 | 8.76 | 4.8 | 5.6 | 5.0 | 2.7 |
| Spore formers | 5.0 | 0.46 | 3.8 | 3.72 | 8.1 | 4.8 | 4.42 | 9.6 | 5.6 | 5.0 | 10.9 |

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TABLE VII

Percentage incidence of the different morphological groups in the rhizosphere and control soils at three stages of growth

| Treatments | Gram -ve rods | | | Gram -ve cocci | | | Gram +ve rods | | | Gram +ve cocci | | | Spore formers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C |
| Marglobe | 53.2 | 66.7 | 61.6 | 22.3 | 13.3 | 23.1 | 11.4 | 10.0 | 7.7 | 7.6 | 6.7 | 3.8 | 5.0 | 3.3 | 3.8 |
| Bonnybest | 56.0 | 68.0 | 52.4 | 21.4 | 14.3 | 13.0 | 11.2 | 7.1 | 14.3 | 5.6 | 7.1 | 9.5 | 5.8 | 3.5 | 4.8 |
| Rehcherry | 64.4 | 53.1 | 60.2 | 23.0 | 18.2 | 20.0 | 9.2 | 13.6 | 8.8 | 3.4 | 4.5 | 5.6 | 0.0 | 4.6 | 5.6 |
| Soil | 31.5 | 39.2 | 25.0 | 12.6 | 8.7 | 10.0 | 44.1 | 26.1 | 40.0 | 6.3 | 17.3 | 20.0 | 5.5 | 8.7 | 5.0 |

A = 10 days after sowing  B = 30 days after sowing  C = 90 days after sowing

Fungi other than the above mentioned two groups formed about 10 percent in the control soil and 14 to 20 percent in the rhizosphere.

## II. Comparison of the rhizosphere microflora in wilted and healthy tomato plants

It was found that the roots of the wilted plant harboured a much higher microbial population, namely, 202.58 million as compared to 174.65 million in healthy plants.

The difference was more marked in the bacterial population which was 189.1 million for the wilted plant as compared to 158.5 million for the healthy one. Less apparent difference was noticed in the case of fungi and actinomycetes. (Table VIII).

## III. Determination of the rhizosphere microflora of a single plant at different depths

There was variation in the total rhizosphere population in the roots of the same plant collected from different depths, namely, 1"-3", 3"-6" and 6"-10".

TABLE VIII

Population of Bacteria, Actinomycetes and Fungi in the Control soil and rhizospheres of wilted and healthy tomatoes

(Population $10^6$ per grom of dry sample)

| Particulars | Total Population | Bacteria | | Actinomycetes | | Fungi | |
|---|---|---|---|---|---|---|---|
| | | Population | R:S ratio | Population | R:S ratio | Population | R:S ratio |
| Control soil | 20.81 | 13.8 | - | 6.80 | - | 0.21 | - |
| Rhizosphere of wilted tomato | 202.58 | 189.1 | 13.70 | 13.20 | 1.94 | 0.28 | 1.33 |
| Rhizosphere of healthy tomato | 174.65 | 158.5 | 11.49 | 15.82 | 2.30 | 0.33 | 1.57 |

The maximum population namely 152.73 million occurred in the root surfaces collected from a depth of 3"-6". In the control soil also the maximum of 20.07 million was found in this zone. (Table IX).

There was a fall in the population both in the rhizosphere and control soil at lower depths of 6" to 10", but this fall did not appear to be very marked. The total population at this region was 142.72 million and 17.82 million for the rhizosphere and control soils respectively. In the upper layers of 1"-3" depth the population in the rhizosphere and control were 108.88 and 12.84 million respectively.

There was a rise in the bacterial, actinomycetes and fungus population from depth of 3" onwards both in the rhizosphere and control soils. The peak population was noticed at 3"-6". Then there was a fall in the bacterial population below 6", where as no appreciable change was noted in the case of actinomycetes and fungi.

In the upper layers of about 1"-3" depth the bacterial population was 93.9 million and the corresponding numbers obtained at 3"-6" and 6"-10" depths were 134.8 million and 123.5 million respectively.

TABLE IX

Incidence of Bactoria, Actinomycetes and Fungi in different depths in the rhizosphere of a single plant and Control soil

(Population $10^6$ per gram of dry sample)

| Particulars | Total population | | Bacteria | | | Actinomycetes | | | Fungi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Population | | R:S ratio | Population | | R:S ratio | Population | | R:S ratio |
| Depth in inches | C | R | C | R | | R | R | | C | R | |
| 1" - 3" | 12.84 | 108.88 | 7.5 | 93.9 | 12,52 | 5.20 | 14.72 | 2.83 | 0.14 | 0.26 | 1.85 |
| 3" - 6" | 20.07 | 152.73 | 11.5 | 134.8 | 11.72 | 8.30 | 17.61 | 2.11 | 0.24 | 0.32 | 1.33 |
| 6" - 10" | 17.82 | 142.72 | 9.8 | 123.5 | 12.60 | 7.80 | 18.86 | 2.41 | 9.22 | 0.36 | 1.63 |

C = Control R = Rhizosphere

The same tendency was noted in the control also, as the maximum bacterial population recorded being 11.5 million at a depth of about 6". So the R:S ratio for bacteria was slightly lesser at this depth when compared to the other two zones, though the actual count was higher.

## Relative efficiency of the techniques used in plating the soil dilution

In the first method wherein the soil dilution was poured into the dish before the incorporation of the medium, the colonies were found crowding more towards the sides of the Petri dishes.

The second method which consisted of incorporating the soil dilution directly into the medium in the test tube was found more easy in handling and also when plated it gave a more uniform spread of the colonies. In this method since the soil dilution was transferred directly into the medium in the test tube and thorough rotation was done immediately, there was lesser chance of the sudden cooling of the media and hence less difficulty was experienced in pouring the media uniformly in the Petri dishes.

The draw back in this method was that the colonies were found to appear in different layers. Since the colonies were fairly wide spread this offered no hindrence in counting the colonies. The second method is preferable to the first one because it is more convenient and at the same time efficient.

A preliminary comparative study made about the two methods of plating has indicated that there is no appreciable difference in the total counts of the colonies. However, since the first method is the more established one it has been followed in this work.

## IV. (a) Effect of streptomycin spray on the rhizosphere microflora

A slight decrease in the microbial population was noted in the rhizosphere of plants sprayed with streptomycin at a concentration of 1000 ppm. with and without one percent glycerol five days after spraying. This reduction in the population was more apparent in plants which were sprayed with 10,000 ppm. with and without glycerol. There was a slight increase in the population ten days after spraying. (Table X a and b).

TABLE X (a)

Effect of streptomycin spray on the rhizosphere microflora

(Concentration of antibiotic used - 1000 ppm. with and without 1 percent glycerol)

| Particulars | Total population | | Bacteria population | | Bacteria R:S ratio | | Actinomycetes population | | Actinomycetes R:S ratio | | Fungi population | | Fungi R:S ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| Control soil | 21.39 | 21.57 | 12.2 | 13.1 | | | 8.98 | 8.23 | | | 0.21 | 0.24 | | |
| Unsprayed plant | 166.40 | 170.72 | 149.2 | 152.6 | 12.23 | 11.64 | 16.89 | 17.81 | 1.88 | 2.16 | 0.31 | 0.31 | 1.48 | 1.29 |
| Sprayed with streptomycin alone | 149.64 | 158.84 | 132.1 | 139.6 | 10.82 | 10.65 | 17.21 | 18.90 | 1.91 | 2.29 | 0.33 | 0.34 | 1.57 | 1.42 |
| Sprayed with streptomycin + 1 percent glycerol | 130.84 | 128.09 | 116.4 | 110.2 | 9.61 | 8.41 | 14.2 | 17.6 | 1.6 | 2.14 | 0.24 | 0.29 | 1.14 | 1.21 |

A = 5 days after spraying B = 10 days after spraying

TABLE X (b)

Effect of streptomycin spray on the rhizosphere microflora

(Concentration of antibiotic used - 10000 ppm. with and without 1% glycerol)

| Particulars | Total population | | Bacteria population | | Bacteria R:S ratio | | Actinomycetes population | | Actinomycetes R:S ratio | | Fungi population | | Fungi R:S ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| Control soil | 21.33 | 22.67 | 12.6 | 13.4 | | | 8.49 | 9.01 | | | 0.24 | 0.26 | | |
| Unsprayed plant | 169.31 | 176.99 | 151.8 | 158.7 | 12.04 | 11.84 | 17.21 | 17.98 | 2.02 | 1.99 | 6.30 | 0.31 | 1.25 | 1.19 |
| Sprayed with streptomycin alone | 144.66 | 147.83 | 128.2 | 130.5 | 10.17 | 9.73 | 16.18 | 17.03 | 1.90 | 1.89 | 0.28 | 0.30 | 1.17 | 1.15 |
| Sprayed with streptomycin + 1 percent glycerol | 114.92 | 120.86 | 98.6 | 106.3 | 7.8 | 7.93 | 16.1 | 14.3 | 1.8 | 1.58 | 0.22 | 0.26 | 0.91 | 1.00 |

It was the bacterial population that was chiefly reduced in the rhizosphere after the antibiotic spray. There was not much reduction in the population of fungi and actinomycetes. The reduction was more marked in the plants sprayed with higher concentration of the antibiotic.

(b) Presence of streptomycin in the plant tissues

The antibiotic was present in a detectable form in the leaf and stem tissues 24 hours after spraying in all the three concentrations tried, namely, 100 ppm., 1000 ppm. and 10000 ppm. After 72 hours the presence of the antibiotic was noticed in the roots also. When higher concentration of 10000 ppm. was used the inhibitory property was found in the roots within 48 hours.

The inhibition zones were comparatively larger in the case of plants sprayed with higher concentrations of the antibiotic. The inhibitory property was found reduced with time on leaves sprayed with 100 ppm. and 1000 ppm. of streptomycin with and without glycerol and they failed to produce any inhibition after 96 hours of spraying.

In the case of the stem there was an increase in the inhibitory property 48 hours after spraying and it

gradually decreased with time. The addition of glycerol one percent was found to increase the absorption of the antibiotic.

Pseudomonas solanacearum was found sensitive to streptomycin sulphate in vitro. But it was less sensitive when compared to Bacillus subtilis. (Table XI a and b).

### (c) Phytotoxicity of streptomycin sulphate on tomato plants

No toxic symptoms were observed in plants, sprayed with 100 ppm. and 1000 ppm. of streptomycin sulphate with and without glycerol. But chlorotic effect was noticed in plants sprayed with the higher concentration of 10000 ppm. The chlorotic areas were more pronounced in the younger leaves near the base of the leaf-lets. Yellow patches appeared within 24 hours after spraying and the patches were found coalescing within 72 hours. The yellow colour of the chlorotic patches was found to decrease in intensity with age. The newly formed leaves were completely free from the toxic effects. No toxic effect could be noted in any other part of the plant.

TABLE XI (a)

Absorption and Translocation of streptomycin in Tomato plants

(Growth inhibition of Bacillus subtilis)

Width of ring in mm.

| Plant samples collected in .. hr. after spraying | Strep. 100 ppm. Leaf | Stem | Root | Strep. 1000 ppm. Leaf | Stem | Root | Strep. 10000 ppm. Leaf | Stem | Root |
|---|---|---|---|---|---|---|---|---|---|
| 24 | 1.0 | ++ | 0 | 3.5 | 1.0 | 0 | 6.0 | 2.25 | 0 |
| 48 | ++ | ++ | 0 | 3.25 | 1.75 | 0 | 5.0 | 3.00 | ++ |
| 72 | + | 0 | 0 | 1.0 | + | ++ | 3.25 | 1.25 | 1.0 |
| 96 | 0 | 0 | 0 | 0 | 0 | 1.0 | + | + | 1.75 |
| Control | 5.0 | | | | 8.0 | | | 10.75 | |

| | Strep. 100 ppm. + 1% glycerol | | | Strep. 1000 ppm. + 1% glycerol | | | Strep. 10000 ppm. + 1% glycerol | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| 24 | 3.0 | + | 0 | 5.0 | 2.25 | 0 | 9.25 | 4.0 | 0 |
| 48 | 1.25 | ++ | 0 | 4.25 | 2.75 | 0 | 6.75 | 3.75 | + |
| 72 | + | ++ | + | 1.50 | 1.75 | ++ | 2.0 | + | 1.0 |
| 96 | 0 | 0 | 1.75 | 0 | 0 | 1.25 | + | + | 1.75 |
| Control | | 6.0 | | | 9.0 | | | 13.5 | |

++ = inhibition above .5 to 1 mm. + = inhibition above .1 to .5 mm.

0 = No indication ofinhibition

TABLE XI (b)

Absorption and Translocation of Streptomycin in Tomato plants

(Growth inhibition of Pseudomonas solanacearum)

Width of ring in mm.

| Plant samples collected in .. hr. after spraying | Strep. 100 ppm. | | | Strep. 1000 ppm. | | | Strep. 10000 ppm. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| 24 | + | + | 0 | 1.75 | ++ | 0 | 3.0 | ++ | 0 |
| 48 | + | + | 0 | 1.75 | + | 0 | 2.25 | ++ | 0 |
| 72 | 0 | 0 | 0 | ++ | 0 | 0 | 1.75 | ++ | ++ |
| 96 | 0 | 0 | 0 | 0 | 0 | ++ | 0 | 0 | ++ |
| Control | | 1.5 | | | 3.5 | | | 6.0 | |
| | Strep. 100 ppm + 1% glycerol | | | Strep. 1000 ppm. + 1% glycerol | | | Strep. 10000 ppm. + 1% glycerol | | |
| | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| 24 | 1.0 | + | 0 | 3.0 | 1.0 | 0 | 4.25 | 2.0 | 0 |
| 48 | ++ | + | 0 | 1.75 | ++ | 0 | 3.50 | 1.5 | 0 |
| 72 | + | + | 0 | 1.50 | + | ++ | 1.0 | + | ++ |
| 96 | 0 | 0 | ++ | 0 | 0 | ++ | 0 | 0 | ++ |
| Control | | 3.0 | | | 5.0 | | | 5.25 | |

(d) Comparative merits of the different methods of surface sterilisation

Of all the methods tried, sterilisation of the tissues with propylene oxide was found to be the best. Tissues of non-treated plants when sterilised in propylene oxide and planted in the test organisms no inhibitory effect was produced. This showed that the chemical had completely evaporated from the material. Also the plant tissues were completely free from contaminents. Apparantly the chemical did not exert any adverse effect on the antibiotic present in the plant tissue. This technique of sterilisation can therefore be considered a very easy, quick and safe one.

Washing the plant tissues in sterile water alone did not prove successful in disinfecting the tissues. Contaminents began to grow around the plant tissues which interfered with the normal inhibitory effects of the antibiotic.

Sterilisation with mercuric chloride 1:1000 and 1:10000 was not helpful since traces of mercuric chloride were present in the plant tissues even after repeated washings in sterile water and this proved to exert an

inhibitory effect on the test organisms. Tissues of plants which were not sprayed with the antibiotic when treated in the above manner showed a definite inhibitory zone. This method was therefore discarded.

Freezing the tissues in the ice compartment of a refrigerator overnight was not satisfactory because the plant tissues turned flaccid and were difficult to handle. Moreover, this method did not help completely to rid the tissues of the contaminents.

## V. Resistance of different tomato varieties to bacterial wilt

All the eight varieties tested were found susceptible to the wilt. The maximum susceptibility in every case was observed in plants five to eight weeks old. Marglobe and Yellow plum showed symptoms of wilt even in the nursery stage.

The wilt index of the different varieties were statistically analysed and it was found that there was significant difference in the wilting coefficients among the eight varieties. The analysis of variance table is given in Table XII.

TABLE XII

Analysis of variance table.

| Particulars | Sum of Squares | D.F. | Variance | F ratio | Inference |
|---|---|---|---|---|---|
| Total | 4870.49 | 31 | | | |
| Block | 75.81 | 3 | 25.27 | <1 | Not significant |
| Treatment (varieties) | 3402.49 | 7 | 486.07 | 7.33 | Significant at 5% level |
| Error | 1392.19 | 21 | 66.29 | | |

S.E. = 5.75 C.D. = 11.96

| Ranks | Varieties | Wilt index | Mean | Difference |
|---|---|---|---|---|
| 1 | V5 | 389.29 | 97.32 | |
| 2 | V3 & 8 | 341.66 | 85.42 | 11.90 |
| 3 | V4 | 325.00 | 81.25 | 4.17 |
| 4 | V7 | 316.54 | 79.14 | 2.11 |
| 5 | V2 | 289.26 | 72.32 | 6.82 |
| 6 | V1 | 269.03 | 67.26 | 5.06 |
| 7 | V6 | 253.32 | 63.33 | 3.93 |

Eventhough all the varieties were susceptible to wilt, Marglobe showed the maximum susceptibility with a wilting coefficient of 97.32. Yellow plum and Ox-heart ranked second in the order of susceptibility with a wilting coefficient of 85.42. Redcherry with a wilting coefficient of 63.44 showed relatively less susceptibility among the varieties.

On the basis of the daily observation it was seen that over 60 percent of the plants wilted in between the fifth and eighth weeks after sowing and the most susceptible variety Marglobe showed a percentage at this period as high as 91. In the comparatively least susceptible Redcherry only 58 percent wilting was noticed during the period. But since then, more plants of this variety was also lost. (Table XIII).

VI. Fertility status of the soil.

The fertility status of the soil from the field was found to be too low. The percentage of nitrogen, both the total and available forms, was found to be only 0.056 percent and 0.0056 percent respectively and phosphorus 0.105 percent and potash 0.19 percent.

TABLE XIII

Weekly record of the number of plants wilted in each variety after transplanting

| No. of weeks after sowing | 4 (trans-planted) | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bonnybest | 1 | 12 | 23 | 19 | 10 | 18 | 8 | 7 | 2 | 1 | 11 |
| Pritchard | 1 | 4 | 13 | 24 | 22 | 15 | 19 | 6 | 1 | 0 | 7 |
| Yellow plum | 3 | 11 | 9 | 40 | 29 | 8 | 3 | 3 | 2 | 0 | 4 |
| Ponderosa | 4 | 9 | 19 | 40 | 16 | 8 | 4 | 2 | 1 | 0 | 7 |
| Marglobe | 6 | 11 | 42 | 36 | 12 | 1 | 3 | 0 | 0 | 0 | 1 |
| Redcherry | 4 | 14 | 12 | 23 | 19 | 12 | 6 | 7 | 1 | 0 | 14 |
| Sunray | 7 | 21 | 22 | 33 | 8 | 8 | 1 | 3 | 1 | 0 | 8 |
| Ox-heart | 5 | 15 | 32 | 38 | 7 | 6 | 5 | 0 | 0 | 0 | 7 |

Though the samples from the pot yielded as much as twice the quantity of nitrogen as in the field, namely, 0.1 percent total and 0.0171 percent available forms, this also can be considered too low. The phosphorus and potash content were 0.188 percent and 0.175 respectively. The pH of the field soil was 5.1 and that of the pot soil 5.8. Detailed data is presented below:-

| | Field Soil percentage | Pot Soil percentage |
|---|---|---|
| Total nitrogen | 0.056 | 0.1 |
| Available nitrogen | 0.0056 | 0.0171 |
| Phosphorus | 0.105 | 0.188 |
| Potash | 0.19 | 0.175 |
| pH | 5.1 | 5.8 |

## DISCUSSION

The total microbial population in the rhizosphere of three varieties of tomato at all the stages of growth was significantly higher than that of the corresponding control soils in the respective stages. This result is to be naturally expected and is in confirmity with the findings of earlier workers including Timonin (1940), Lochhead et al (1940), Agnihothurudu (1958) and Rangaswami and Vasantharajan (1961) who found an increased microbial population in the rhizosphere. The increased population is attributed to the presence of root exudates and sloughed-off tissues in the rhizosphere which stimulate the growth and multiplication of the microorganisms.

In correlating the population with the age of the plant it was noted that the peak population coincided with the flowering stage of the crop. This was true for all the three varieties. A similar pattern was observed by many workers like Starkey (1929), Timonin (1940), Agnihothurudu (1953) and others. The flowering period is the peak vegetative stage when the maximum production of root exudates and sloughed-off tissues are expected. While studying the nature of the root exudates and sloughed-off tissues many workers have reported the

presence of various amino acids and sugars in the former and protein, cellulose etc. in the later. Rovira (1959) and Subba Rao and Bailey (1961) reported that in tomato generally the bulk of the root exudates consisted of amino acids. In the present studies it was noted that the bacterial population reached its highest during the flowering period. Thereafter, there was a gradual fall. As the plant grows there will be many changes in its metabolism which may affect the nature and amount of root exudates. This naturally will have some bearing on the bacterial population. It is possible that the amino acids and other substances present in the exudates may be more favourable for the multiplication of bacteria than that of other organisms.

In all the three varieties under study there was a demonstratable microflora far higher than that in the soil was present in the rhizosphere of seedlings five days after sowing which means two days after germination. This observation agrees with that of Timonin (1940) and Rouatt (1959) who reported a similar rhizosphere effect in three days old seedlings.

It appears that the depth from which the roots were collected also influenced the soil microflora. Studies on

the effect of depth on the rhizosphere of a single plant showed that the maximum population occurred at a depth of 3"-6". In 80 days old plant the maximum number of actively growing roots and rootlets were found in this zone. In soils above 3" and below 6" the number of actively growing roots was comparatively less. Hence, presence of the actively growing roots may be one of the reasons for the higher incidence of microbial population. The same trend was noted in the control soil also, the maximum population being obtained between 3"-6" as in the case of roots. It is therefore possible that the soil environment itself will be having some influence on the rhizosphere population of the same plant at different depth.

Waksman (1952) believes that the various groups of microorganisms are largely concentrated in the surface layers of the soil. In the cultivated soils he found a gradual change in the number of microorganisms in the different depths. Although the bacteria and actinomycetes diminish with increase in depth the proportion is far greater for bacteria than for actinomycetes.

The low population in the top 1" soil can to some extent be due to the effect of agronomic operations. The

environmental conditions existing in this layer also may not be favourable for the rapid growth of microorganisms compared to the deeper layers. In the surface layers the incidence of fungi and actinomycetes was found less while their population was found to be increasing in the lower layers both in the rhizosphere and control soils.

The differential stimulation of the various groups of bacteria in the rhizosphere of plants has been reported by many workers. Lochhead (1940), Krassimilkov (1944) King and Wallace (1956), Rovira (1956) and Rangaswami and Vasantharajan (1961) noted a higher incidence of the Gram negative rods in the rhizosphere of plants, whereas a higher incidence of Gram positive organisms occurred in the control soil. It is interesting to observe that throughout the life of the tomato plant, the proportion of different morphological groups of bacteria in the rhizosphere was maintained though there were marked change in the total population.

Of the eight varieties of tomato tried none showed appreciable resistance to wilt and the difference between the varieties was only very narrow. Except in the case of Redcherry, the maximum number of plants wilted when

they were four to eight weeks old. However, three of the varieties among them, namely, Marglobe, Bonnybest and Redcherry were selected for the rhizosphere studies based on their comparative susceptibility to wilt.

There was no significant difference in the rhizosphere microflora of the three varieties mentioned above during their entire life period. This can be accounted for the fact that there was only a narrow margin of difference in the susceptibility among these varieties as pointed out earlier. Redcherry, however, appeared more resistant before flowering and during this period this variety showed a slightly lower population which is in keeping with the findings of earlier authors. It is worth repeating this work with varieties which show marked difference in their susceptibility to infection by the wilt organism. The present results can therefore be taken to indicate that in varieties with only a narrow margin of difference in wilt resistance, no appreciable change in the microbial population can be expected.

A higher incidence of actinomycetes both in the field and pot soils was noticed during the course of this study. On the basis of chemical analysis it was found that the soil was very poor in nitrogen. Since the

nutrient status of this soil is low the population of the more effective competitors may be diminishing with the result that actinomycetes might have gained prominence.

The roots of freshly wilted plant harbour a higher population than that of the healthy one. The rise in the population was chiefly noted in the case of bacteria. Most of the roots of the freshly wilted plants appeared normal. There is a likelihood for a marked change in the metabolism of the plant in the initial stages of wilt, before it succumbs. This might have resulted in a change in the quality of the root exudate and possibily also in its quantity. The presence of a higher microbial population at the time of wilt may be due to the above factors. It may be interesting to understand a detailed study of the changes brought about in the root exudate and the microbial population in plants from the time it is infected till it succumbs.

For the detection of streptomycin in treated plants, it is necessary to disinfect the tissues before the assay is made to destroy the microorganisms which are resistant to the action of streptomycin. Otherwise these organisms will interfere with the results. Of the different

techniques tried, sterilisation with propylene oxide was found to be the best and also the easiest. This is considered due to the efficient disinfecting property compiled with the highly volatile nature of the chemical.

Streptomycin sprayed on the leaves was absorbed and translocated into the various parts of the plants including the roots. The addition of one percent glycerol enhanced the absorption. This is in keeping with the observations of earlier workers.

There was a reduction in the total microbial population in the rhizosphere of the streptomycin sprayed plants five days after spray. It is likely that the antibiotic might have exerted some influence on the metabolism of the plant especially in those which received 10000 ppm. as expected from the initial chlorosis. The reduction in number is chiefly reflected in the case of bacteria. To what extent has the antibiotic diffused through the root system is not known. This work is only of a preliminary nature and hence, no definite conclusion can be drawn with the results now available.

## SUMMARY AND CONCLUSIONS

Studies on the microbial population, viz., bacteria, fungi and actinomycetes in the rhizosphere of three varieties of tomato plants, which showed slight differences in their susceptibility to bacterial wilt, revealed that there was no significant difference between the rhizosphere population of the different varieties at twelve stages of plant growth. In all cases control soil definitely showed a significantly lower population.

The rhizosphere soil was found to favour the growth of Gram negative organisms whereas the control soil favoured Gram positive organisms. Throughout the life of the plant the proportion of the different morphological groups of bacteria was maintained steady.

There was positive correlation between the age of the plant and the microbial population up to the flowering stage, at which the maximum population was recorded. Thereafter, there was a gradual reduction in the population.

The soil depth influenced the rhizosphere of the same plant and also the non-rhizosphere microflora, the maximum number in both cases being noted in the middle

zone of 3"-6" depth. The microbial population decreased gradually in deeper zones of above 6".

✓ Rhizosphere of freshly wilted tomato plant contained a higher microbial population compared to the healthy plant.

A decreasing tendency in the population, which was marked with bacteria, was observed in the rhizosphere 5 days after streptomycin spray. The effect was pronounced when streptomycin was applied at a concentration of 10000 ppm.

Absorption and translocation of streptomycin readily took place when it was applied as a foliar spray. Addition of 1 percent glycerol was found to increase the absorption of the antibiotic. Presence of the antibiotic was traced in the roots of the sprayed plants 72 hours after the spray. The antibiotic could not be detected in the leaves 96 hours after spraying.

Streptomycin at a concentration of 10000 ppm. was toxic to the plants and produced chlorotic symptoms.

Treatment of the plant tissues with propylene oxide vapours was found superior to the other disinfection methods tried during the antibiotic assay.

## REFERENCES


ADATI, 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.

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

* AGNIHOTHURUDU, V. (1955). Incidence of fungistatic organisms in the rhizosphere of pigeon pea (Cajanus cajan) in relation to resistance and susceptibility to wilt caused by Fusarium udum Butler.
Naturwissenschaften, 42, 373.

AGNIHOTHURUDU, V., BHUVANESWARI, K. & SURYANARAYANAN, S. (1955). Fungi isolated from rhizosphere. I.
Proc. Indian Acad. Sci., Sect. B., 42, 98-104.

AGNIHOTHURUDU, V., (1961). Rhizosphere microflora of Tea (Camellia sinensis (L). O. Kunze) in relation

to the root rot caused by Ustulina zonata.(Lev) Sacc.
Soil Sci., 91, 133-137.

ANDAL, R., BHUVANESWARI, K. & SUBBA RAO, N.S. (1956). Root exudates of Paddy.
Nature, 178, 1063.

ARK, P.A. (1947). Effect of crystalline streptomycin on phytopathogenic bacteria and fungi.
Phytopathology 37, 842.

ARK, P.A. (1947). Disinfecting power of propylene oxide and propylene chloride in relation to phytopathogenic bacteria and fungi.
Phytopathology 37, 842

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

* CHINNAYYA, E.J. & AGNIHOTHURUDU, V. (1953). Rhizosphere microflora of plants growing in different ecological habitats.
J. Madras Univ. Sect. B., 23, 182-192

CONTOIS, D.E. (1953). Microflora of the rhizosphere of pineapple plant.
Soil Sci., 76, 259-272.

CROSSE, J.E. & GARRETT, C.M.E. (1958). Experiments on the movement of streptomycin in cherry trees. Ann. appl. Biol., 46 (3), 310-320.

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

DYE, M.H. (1956). Intake of streptomycin by peach leaves. Nature, Lond., 178, 551.

EBBEN, M.H. (1959). Brown root rot of tomatoes. II Fungal flora of the rhizosphere. Ann. appl. Biol. 47, 17-27.

* GRAY, R.A. (1955). Increasing the effectiveness of streptomycin against the common blight of beans with glycerol. U. S. Dept. of Agrl. Pl. Dis. Rptr. 39, 567-569.

GRAY, R.A. (1956). Increasing the absorption of streptomycin by leaves and flowers with glycerol. Phytopathology 46, 105-111.

HARPER, J.L. (1950). Studies in the resistance of certain varieties of banana to Panama disease. Plant and soil, 2, 383-394.

* HILTNER, L. (1904). Uber neuere Erfahrungen und Probleme auf dem Gebiet der Dodenbakteriologia und unter besonderer Berucksichtegung der Grundungung und Brache.
Arb. deut. landw. Ges. 98, 59-78.

KATZNELSON, H. & RICHARDSON, L.T. (1943). The microflora of the rhizosphere of tomato plants in relation to soil sterilisation.
Can. J. Research., C., 21, 249-255.

KATZNELSON, H., LOCHHEAD, A.G. & TIMONIN, M.I. (1948). Soil microorganism and the rhizosphere.
Bot. Rev. 14, 543-587.

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

KING, H.de L. & WALLACE, R.H. (1956). Morphological and physiological groups of soil bacteria from the roots of barley and oats.
Can. J. Microbiol., 2, 473-481.

* KRASSINILKOV, N.A., KRISS, A.E. & LITVINOV, M.A. (1936). The effect of root system on the soil microflora. Mikrobiologia. 2, 270-286.

* KRASSINILKOV, N.A. (1944). Microflora of soils as influenced by plants. Mikrobiologia., U.S.S.R., 3, 348-358.

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., TIMONIN, M.I. & WEST, P.M. (1940). The microflora of the rhizosphere in relation to resistance of plants to soil borne pathogens. Sci., Agric., 20, 414-418.

LOCKWOOD, J.L. (1958). A method for studying absorption of streptomycin by using leaf disks of Sedum purpureum. Phytopathology, 48, 150-155.

MITCHELL, J.W., ZAUHEYER, W.J. & PRESTAN, W.H. (1954). Absorption and translocation of streptomycin by bean plants and its effect on halo and common blight organisms. Phytopathology, 44, 25-30.

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, 145-151.

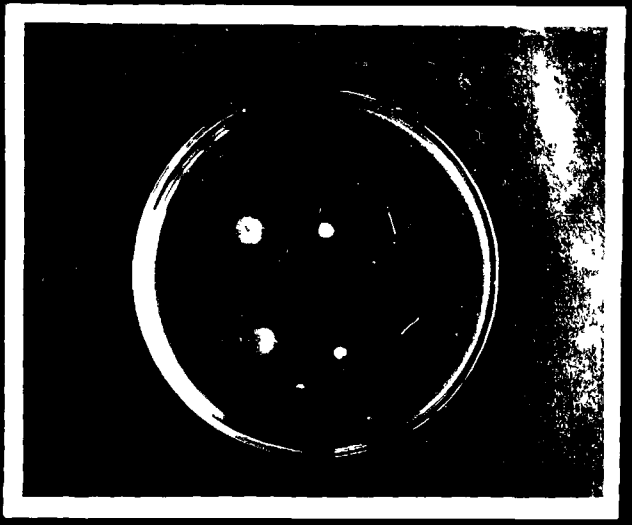
* PRAMER, D. (1954). The movement of chloramphenicol and streptomycin in broad bean and tomato plants. Ann. Bot. Lond., 18, 463.

RANGASWAMI, G., RAO RAMA RAO &LAKSHMANAN, A.R. (1959). Studies on the control of citrus canker with streptomycin. Phytopathol. 49, 224-226.

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

RANGASWAMI, G. & VASANTHARAJAN, V.N. (1962). Studies on the rhizosphere microflora of citrus trees. I. Quantitative incidence of microorganisms in relation to root and shoot growth. Can. J. Microbiol. 8, 473-477.

72 hours after spray



96 hours after spray

Plate IV. Chlorotic symptoms produced on tomato leaves as result of streptomycin spray at a concentration of 10000 ppm.

Normal leaf. Leaf 72 hours after spray

RANGASWAMI, G.& VASANTHARAJAN, V.N. (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 citrus trees. III. Fungal and actinomycetes flora of the rhizosphere. Can. J. Microbiol., 8, 485-489.

RANGASWAMI, G. & VASANTHARAJAN, V.N. & BALASUBRAMONIAN, A. (1962). Studies on the effect of streptomycin spray on the nodulation and rhizosphere microflora of two greem manure plants. Hindustan Antibiotic Bulletin, 4, 30-33.

RANGASWAMI, G. & VENKATESAN, R. (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.

ROBISON, R.S., STARKEY, R.L. & DAVIDSON, O.W. (1954). Control of bacterial wilt of chrysanthemums with streptomycin. Phytopathology 44, 646-650.

* ROUMBOUTS, J.E. (1953). The microorganisms in the rhizosphere of Banana plants in relation to susceptibility or resistance to Panama disease. Plant and Soil, 4, 276-88.

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

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

* ROVIRA, A.B. (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.

SADASIVAN, T.S. & SUBRAMANIAN, C.V. (1960). Interaction of pathogen, soil, other microorganisms in the soil and host. Plant Pathology an advanced treatise. Edited by J.G. Horsfall and A.E. Dimond. Vol. II. The Pathogen Academic Press, New York & London. Chap. 8, 275-315.

SANNE GOWDA, S. & RANGASWAMI, G. (1962). Control of bacterial soft-rot of onion with streptomycin. Hindustan Antibiotic Bulletin, 5, 13-15.

STARKEY, R.L. (1929). Some influence of development of higher plants upon the microorganisms in the soil. I. Historical and introductory. Soil Sci., 27, 319-334.
II. Influence of the stage of the plant growth on the abundance of organisms. Soil Sci., 27, 355-374.
III. Influence of stage of plant growth upon some activities of the organisms. Soil Sci., 27, 433-444.

STARKEY, R.L. (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, 1747-1756.

TIMONIN, M.I. (1940). The interaction of higher plants and soil microorganisms. 1. Microbial population of rhizosphere of seedlings of certain cultivated plants.

II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases.
Can. J. Research, C. 18, 307-317 and 444-456.

WAKSMAN, S.A. (1952). Soil Microbiology. John Wiley & Sons, Inc., New York, Chapman & Hall, Limited, London.

WALLACE, R.H. & LOCHHEAD, A.G. (1949). Qualitative studies of soil microorganisms. VIII. The influence of various crop plants on the nutritional group of soil bacteria.
Soil Sci., 67, 63-69.

* WILLIAMS, P.H. et al (1952). Plant diseases. Chemical investigation. Grower's notes.
Rep. exp. Res. Sta. Cheshnut, 1951, 22-40, 78-83, 85-87.

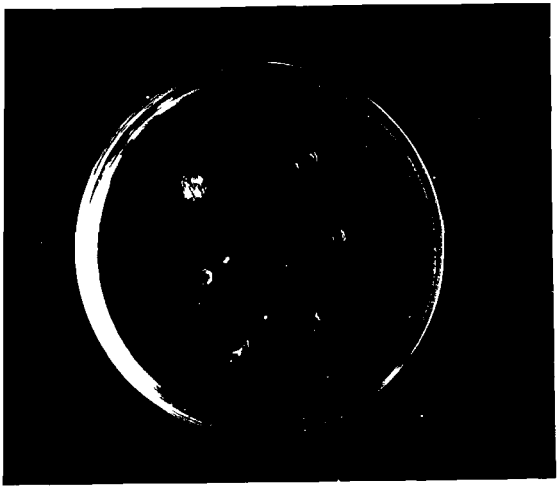
WINSTEAD, N.N. (1952). Evaluation of resistance in tomato to Pseudomonas solanacearum by artificial inoculation methods.
Phytopathology 42, 23.

WINSTEAD, N.N. & KELMAN, A. (1952). Inoculation technique for evaluating resistance to Pseudomonas solanacearum.
Phytopathology 42, 628-634.

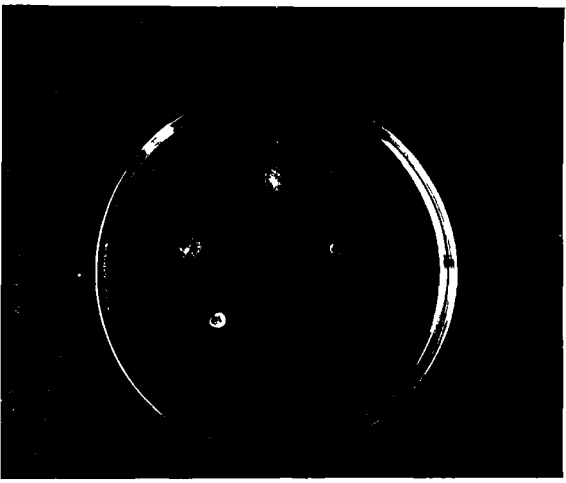
ZAUMEYER, W.J., THOMAS, H.R., MITCHELL, J.W. & FISHER, H.H. (1953). Field control of halo blight of leaves with streptomycin.
Phytopathology 43, 407.

* Originals not seen.

Plate I. Inhibition zones produced by the different plant tissues viz., leaf, stem and root after 24 hours and 48 hours after the antibiotic spray.



24 hours after spray



48 hours after spray

Plate II. Inhibition zones produced by the different plant tissues viz., leaf, stem and root after 72 hours and 96 hours after the antibiotic spray.