EFFECTS OF COLLAR ROT AND RING BARKING ON THE RHIZOSPHERE MICROFLORA AND CERTAIN CHEMICAL CONSTITUENTS OF SWORD BEAN PLANTS

BY

P. N. KANAKAMBARAN, B. Sc. (Agri)

THESIS

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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 bona fide research work carried out by Shri P.N. Kanakambaran, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

(P. KUMARA PILLAI)
Principal.

(J. SAM RAJ)
Professor of Plant Pathology.

Agricultural College &
Research Institute,
Vellayani, Trivandrum,
August 1968.

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P.N. KANAKAMBARAN.

C O N T E N T S

INTRODUCTION

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It is known that changes in the metabolism of plants will usually be reflected in the microbial population of the rhizosphere also. In most normal healthy plants, the microbial population of the rhizosphere increases with age, reaches the peak at the time of flowering and then declines. However, if the metabolism of the plant is altered by any means it can bring about quantitative and qualitative changes in the root exudates which in turn will influence the microbial population of the rhizosphere. Thus it has been found by some of the earlier workers that certain chemicals like 2,4-D, urea and gibberellic acid, when applied on the foliage can bring about changes in the microbial population of rhizosphere as a result of altered host metabolism.

Infection of plants by pathogens may also be able to bring about changes in the host metabolism. Whatever be the magnitude and nature of these changes, their effects may be noticeable in the rhizosphere also. However, our knowledge about this aspect is very limited and it is based on the work of Lekshmikumari (1960), Ranganathan (1965) and Balakrishnan (1967) on virus diseases. It was found by these authors that infection of plants by viruses can bring about a beneficial

effect on the microflora of the rhizosphere in so far as their population is markedly increased. Similar changes in the host metabolism are also possible when plants are infected by fungal and bacterial pathogens eventhough we have practically no information about this aspect. It is in this context that the present study was undertaken.

The collar rot of sword bean plants due to Rhizoctonia was selected for this study mainly because the organism usually girdles the stem, renders the phloem tissue inactive and finally kills the plant. The blockage of the phloem tissue will prevent the downward translocation of nutrients. Further, Rhizoctonia is known to produce toxic metabolic products which may diffuse into the host tissues. These pathological conditions can normally bring about profound changes in the metabolism of infected plants from the time of infection till the death of the plant.

In the present work an attempt has been made to assess the extent of changes that are brought about on the carbohydrate and nitrogen content of the root system and also on the microbial population of the rhizosphere as a result of fungal infection. For comparative purpose the rhizosphere of healthy and also those of ring-barked sword bean plants have been studied.

Ring-barking prevents the downward translocation of nutrients and thus brings about the death of the plants eventhough this death is not strictly comparable with that brought about by Rhizoctonia.

REVIEW OF LITERATURE

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A positive rhizosphere effect evidenced by greater incidence of microorganisms in the rhizosphere of different plants as compared to the soil away from the root systems has been reported by various investigators like Hiltner (1904), Starkey (1929 a, b), Adati (1939), Timonin (1940 a, b), Lochhead (1940), Katznelson and Richardson (1943, 1948), Krassilnikov (1944), Katznelson *et al* (1948), Rangaswami and Vasantharajan (1962) and Sundara Rao and Venkataraman (1963). They found that fungi, bacteria and actinomycetes present in the rhizosphere have been differentially influenced by the rhizosphere of the particular crop. A negative rhizosphere effect was, however, reported by Bhuvaneswari (1958) in the case of *Brassica juncea* and *Allium cepa*.

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.

Clark (1947) pointed out that appreciable error may occur in the use of rhizosphere soil on the basis of which microbial number is expressed. When plants are collected from soils having varying degrees of moisture content the amount of

soil adhering to the root system will be considerably different. Rangaswami and Venkatesan (1963) reported a lesser microbial population in the dry soil than in wet soil. Peterson et al (1965) reported that the population of bacteria in the rhizosphere decreased from 90 per cent to 30 per cent of the total moisture holding capacity.

Adati (1939) found that the influence of cereals on the development of microorganisms was generally more powerful than that of legumes. Roy (1949) reported greatest number of fungi at a depth of 4" in the case of paddy soils.

Timonin (1940 a, b), Agnihothurudu (1953), Firsanova (1956) and Rangaswami and Venkatesan (1963) observed a maximum bacterial population at the stage of flowering and a decline there after in the case of annuals.

Katznelson and Richardson (1943) noted marked differences in the rhizosphere of tomato plants as a result of soil sterilization with steam, chloropicrin and formal-dehyde.

Starkey (1929 a, b, 1931) reported the influence of the stage of plant growth upon the abundance of the microorganisms. Krassilnikov et al (1936) while studying the rhizosphere of wheat, maize, sunflower and soyabeans 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 to be diminishing towards the maturity of plants where as the number of fungi and actinomycetes were increasing. Timonin (1940 a, b) noted the establishment of a rhizosphere microflora within three days of seed germination and it was more noticeable with bacteria than with fungi. He obtained 11 to 20 times more rhizosphere population than elsewhere in the same soil. Rovira (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. Rouatt (1959) obtained 92: 1 R:S ratio for bacteria in the rhizosphere of three days old wheat seedlings. Lugauskas (1961) reported maximum population at the most intensive stage of plant growth. He also reported more fungi in sandy soils than in clayey soils. Agnihothurudu (1953) and Rangaswami and Venkatesan (1963) observed a maximum bacterial population at the stage of flowering in the growth period. Rema Devi (1964) observed an increase in the microbial population in the rhizosphere of tomato plants corresponding to increase in the age of the plants and the maximum population was recorded at the flowering stage. Rangaswami and Vasantharajan (1962) found that in perennials the growing roots harbour a much greater number of bacteria compared to non-growing roots. Rangaswami and Venkatesan (1964)

working on paddy reported that the rhizosphere effect varied with maturity of root, depth of soil, age of plant etc. Remigius (1966) observed maximum microbial population in the rhizosphere of paddy at the stage of flowering and a gradual decline thereafter. Balakrishnan (1967) reported a definite rhizosphere population within 3 days of germination of tobacco seeds. The population steadily increased with the age of plant till flowering, after which there was a decline in all the three groups of microorganisms (viz.) bacteria, actinomycetes and fungi.

Timonin (1941) found that the soil amendments as addition of manures were of lesser importance in determining the abundance of microbial population though it increased soil productivity. Timonin and Lochhead (1948) reported that the microflora was most abundant for the central or crown position of the roots and more particularly in vertical direction from the base of the roots. Becker et al (1952) found that the equilibrium of bacteria and fungal population of the rhizosphere altered when the root debris of phanerogams were incorporated in the soil. Contois (1953) noted that the rhizosphere microflora of plants are influenced by altitude, rainfall and soil pH. Rouatt et al (1963) found that microorganisms in the root zone are directly influenced by the temperature. Rangaswami and

Venkatesan (1963) showed that the top layers of soil harbour more population than the deeper layers. Ranganathan (1965) studying the microflora of various root regions of banana plant came to the conclusion that the middle region of the roots harboured the highest population followed by the tip region and then the base. According to him the differences are due to the nature and extent of actively growing lateral roots found in the different regions and to the surface/volume ratio.

Root exudates and rhizosphere effect

It is now widely accepted that the increased activity in the rhizosphere is due to organic materials exuded from the roots. Amino acids, sugars, tanin, alkaloids and numerous other substances are known to be present in root exudates. Hiltner (1904) explained the increased activity of the microorganisms near the root zone as due to the excretions of the roots. Miller (1938) reported that the growing roots liberate more exudates than the non-growing older roots.

Katznelson et al (1954, 1956), Andal et al (1956), Rovira (1956), Bhuvaneswari and Subbarao (1957), Subbarao and Bailey (1961) found that the root exudates of plants were rich in aminoacids. Burton (1956) studying the effect of root exudates on the rhizosphere populations of pea varieties with different

susceptibilities to three races of Fusarium oxysporum f. pisi found that the variety susceptible to race I had more fungi, bacteria, and actinomycetes near its root surfaces than the one resistant to race I. This showed that varieties resistant to Fusarium oxysporum f. pisi either secrete substances that prevent race I spores from germinating or stimulate the production of a soil microflora exerting this effect. Rovira (1956) reported the presence of aminoacids 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. Bawden (1957) suggested that the reasons for the rich populations and greater activity of microbes near the root system may in part reflect the action of root exudates in stimulating dominant microbes but there may be many other contributing factors also. Bhuvaneswari (1960) found that the root exudates of rice variety GEB 24 resistant to Fusarium moniliforme exhibited fungistatic effect on the pathogen and encouraged the growth of saprophytic fungi, bacteria and actinomycetes. The root exudates of resistant varieties inhibited spore germination, germ tube growth and radial growth of pathogen. The root exudates of resistant variety contain more asparagine and cystine than the susceptible varieties. Subbarao and Bailey (1961) while studying the rhizosphere of tomato plants in relation to the varietal reaction to verticillium wilt found

that the aminoacids form the bulk of the root exudates. Also they found that the resistant varieties of 6 weeks old plants exuded only 3 aminoacids whereas the susceptible variety secreted 5 amino-acids. Buxton (1962) reported that banana variety Gros-Michel, susceptible to Panama wilt exuded more quantity of 18 aminoacids. Of these 13 were found to be common for both, resistant (Lacatan) and susceptible varieties. He found that the root exudates of Lacatan variety inhibited the spore germination of Fusarium oxysporum f. cubense. Buxton also reported that the susceptible variety had 1.5 times more carbohydrates in the exudates of young roots than in the resistant variety.

Sulochana (1962) reported that diploid strains of cotton susceptible to Fusarium wilt was found to exude greater amounts of amino-acids and vitamins than amphidiploid and resistant varieties. Singh (1967) was able to isolate six amino-acids from the root exudates of corn plants.

West (1939) reported that flax seedlings excreted significant amount of thiamine and biotin. Timonin (1943) observed that Bison variety of flax resistant to wilt caused by Fusarium lini excreted hydrocyanic acid through roots. Rangaswami and Balasubramonian (1963) also noticed hydrocyanic acid in the root exudates of cholam varieties CO_4 and K_1.

Differential effect of rhizosphere on soil micro-organisms

1. Bacteria:

Several workers have found that various groups of bacteria have been differentially stimulated in the rhizosphere depending upon the type of the plant. Taylor and Lochhead (1938) classified the organisms into eight groups and reported that the non-sporing short rods comprised nearly 90 per cent of the total rhizosphere bacterial population. Lochhead (1940) found that Gram negative rods were activated more in the rhizosphere of red clover, mangels, oats, tobacco, maize and flax. Spore forming bacteria were lesser in the rhizosphere than in the control soil. Lochhead (1940) and Rovira (1956) found that with increasing age of the seedlings there was a corresponding increase in the Gram negative organisms in the rhizosphere. Lochhead and Chase (1943) found that morphological groups of bacteria have got no direct correlation with their nutritional requirements. They also reported a greater percentage of incidence of aminoacid requiring bacteria in the rhizosphere of mangel. Similar observations were made by Rovira (1956), Subbarao (1961) and Andal et al (1956).

Krassilnikov (1944) reported that 95 per cent of the rhizosphere bacteria constituted Gram negative rods. Among the non spore forming types Gram negative forms were more abundant

than Gram positive ones. Krassilnikov (1946) reported 95.5 per cent of the rhizosphere bacteria as non-sporing types. Contois (1953) while investigating the rhizosphere of pine apple observed a constant association of Gram negative non-sporing rods which occurred abundantly along with cocci. King and Wallace (1956) reported that there occurred a selective stimulation of Gram negative rods in the rhizosphere of oats. Rangaswami and Vasantharajan (1962) studying the microflora of citrus plants reported that Gram negative, non-sporing rods were abundant in the rhizosphere than Gram positive rods and spore formers. Rangaswami and Balasubramonian (1963) also observed that Gram negative bacteria were dominating in the rhizosphere of cholam while Gram positive and spore forming bacteria were abundant in the control soil.

. Various workers have reported a greater incidence of different physiological groups of bacteria in the rhizosphere. Lochhead (1940) reported a greater incidence of gelatin liquifiers than nitrate reducing forms in the rhizosphere of red clover, 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 aminoacids to predominate in the rhizosphere.

ii. Fungi:

Chatak and Roy (1940) isolated 23 species of fungi from the rice fields including several phycomycetes, species of Aspergillus, Penicillium, Fusarium demerum, F. orthoceras, F. oxysporum and F. solani. Katznelson and Richardson (1948) reported qualitative differences between the fungi of the rhizosphere and the non rhizosphere soil. They observed that Cladosporium was abundant in strew berry rhizosphere at 100 days' growth where as Verticillium was predominant at 220 days. Roy (1949) revealed that Aspergillus niger was present in all soil samples examined. Chinnaya 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) reported that pine apple plants grown at lower altitudes harboured Aspergillus and Penicillium species abundantly but in higher altitudes Rhizopus nigricans and Circinella simplex were more. Agnihothurudu (1953) and Agnihothurudu et al (1955) isolated species of Aspergillus, Penicillium, Fusarium, Alternaria, Curvularia, Mucor, Rhizopus, Helminthosporium, Trichoderma, Cunninghamella, Phoma, Diplodia, Chaetomium and Macrophomina phaseoli from the rhizosphere of Pigeon pea and sorghum. Montegut (1957) observed that out of the total fungal population in sandy soils 82.5 per cent were Fungi

imperfecti, 6 per cent Phycomycetes, 11 per cent Ascomycetes and 0.5 per cent Basidiomycetes. Rangaswami and Vasantharajan (1962) reported species of Aspergillus, Penicillium, Fusarium, Helminthosporium, Mucor and Rhizopus from the rhizosphere of citrus plants. Das (1963) while studying the ecology of soil fungi in rice fields reported that the fungal associations with rice root changes with the growth of the crop. During the early stage of the vegetative growth common soil fungi such as Aspergillus and Penicillium were found associated with the roots of older plants. Rangaswami and Venkataraman (1963) found that number of fungi gradually increased in the rhizosphere of plants till the time of harvest. Balakrishnan (1967) found that species of Aspergillus and Penicillium formed the predominant group of fungi in the rhizosphere and control soil of tobacco.

iii. Actinomycetes:

There are only few reports regarding the effect of rhizosphere on the different groups of actinomycetes. Timonin (1940 a, b) obtained 7 to 71 times greater actinomycetes population in the rhizosphere of wheat, oats, lucerne and peas. Rangaswami and Vasantharajan (1962) reported the presence of 4 to 20 times more of actinomycetes in the rhizosphere of citrus plants than in the non-rhizosphere soil. They further noted that actinomycetes

with antagonistic effects were more prominent in the rhizosphere than in the non-rhizosphere soil. Rangaswami and Venkatesan (1963) noted a steady increase in the actinomycetes population in the rhizosphere of rice till harvest. Among the actinomycetes identified the most predominantly occuring group was Streptomyces species. Micromonospora and Nocardia species were also present though not frequently. Venkatesan and Rangaswami (1964) in their studies on the microflora of the rhizosphere of rice found that the number of actinomycetes was more in the rhizosphere than in the non-rhizosphere soil. Agnihothurudu (1955) while studying the fungistatic organisms in the rhizosphere of pigeon-pea found that Streptomyces griseus were abundant. Nocardia and Micromonospora species were also obtained.

Rhizosphere microflora in relation to root infection

Certain crop varieties susceptible to infection by soil borne fungi are known to exert a greater rhizosphere effect than the resistant varieties. Thom and Humfield (1932) studying the tobacco plants susceptible to Thielavia root rot reported higher rhizosphere populations in the susceptible varieties. Timonin (1940) reported higher populations in the Fusarium susceptible varieties of flax. Lochhead et al (1940) found that the rhizosphere of wilt susceptible tobacco plants supported an abundant quantity of

Gram negative short rods and harboured lesser number of coccoid forms, spore formers and Gram positive rods. They also found that there was variation in the different nutritional groups also, as amino nitrogen requiring bacteria were supported more by resistant varieties. Timonin (1943) in the comparative studies on the rhizosphere of Bison and Novelty flax, resistant and susceptible, respectively, to Fusarium lini demonstrated that the incidence of Alternaria tenuis group, Cephalosporium humicola, Fusarium bulbogenum, F. culmorum, F. oxysporum, F. solani, Helminthosporium sativum, Verticillium, Penicillium chrysogenum, P. intricatum, P. terrestre, Trichoderma viride and T. album increased by the rhizosphere effect of resistant as compared to susceptible variety. Harper (1950 a, b) and Rombouts (1953) studying the rhizosphere of banana plants in relation to panama wilt, reported higher populations in the susceptible varieties. Aguilhothuradu (1955) noted actinomycetes antagonistic to the wilt organism Fusarium udum in the rhizosphere of resistant pigeon-pea. Subbarao and Bailey (1961) in their studies on the resistance and susceptibility of tomato plants to Verticillium wilt invariably proved that susceptible varieties harbour much higher population than resistant ones. Lacy and Horner (1962) reported lesser population of Verticillium alboatrum in the rhizosphere of resistant species of Mentha than in susceptible varieties.

Rhizoctonia collar rot

Rhizoctonia is considered to be an omnivorous plant parasite, which attacks a wide variety of hosts. In India the fungus is known to attack economic crop plants like cotton, cow pea, green gram, tomato, potato, brinjal, cucurbits, tobacco, sesamum etc. There is no record of the fungus causing collar rot of sword beans (Canavalia ensiformis DC.) in India. Butler (1918) reported that Rhizoctonia solani infected cowpea seedlings on the stem and at the growing points, but in the field the attack usually begins at the ground level. In describing the nature of attack on cotton by Rhizoctonia species he reported that the fungus attacks the base of the stem causing a rotting of the stem at the ground level and the upper part of the tap root chiefly affecting the bast and external tissues which break down into fibrous shreds. The hyphae of the fungus occur in the deeper layer of the bast and on the surface of the root.

Matsumoto (1923) in his studies on the physiology of Rhizoctonia solani reported the staling phenomena observed in cultures of the fungus. The toxic substances may be in activated by heating and the growth of the fungus is promoted by merely eliminating them. Nobecourt (1928) reported the development of substance antagonistic to the growth of orchid bulbs by the

orchid mycorrhiza (Rhizoctonia sp.). Kerr (1957) using the cellophane bag technique demonstrated that the roots of raddish were destroyed due to the growth products of Rhizoctonia probably due to a toxin. Stakman and Harrar (1957) in describing the antagonistic activities of this organism stated that some races of Rhizoctonia can cause distortions and death of roots of certain crop plants at a distance of at least one centimetre without ever touching the roots.

Ring-barking and its effects on plants

In order to demonstrate the conduction of food materials in plants, Malpighi (1686) removed ring like pieces of bark by making two parallel cuts encircling the stems of plants like Prunus, Quince, Quercus, Salix etc. He observed the accumulation of food coming down from the leaves, at the upper portion of the ring. Many workers have subsequently confirmed the above findings. Nageli (1861) proposed the term phloem for the tissues concerned with the conduction of cell sap. Munch (1930) by his experiments with ringing, grafting and shading of the foliage established bark as the principal pathway of food materials. Extensive experimentation by Cotta and Knight placed the concept of movement of food materials in the bark on a firm basis and the discovery of the sieve tubes by Hartig (1837) revealed the particular part of the bark which is concerned with this movement.

Mason and Maskell (1928 a, b) analysed the bark and wood derived from the stem above and below the ring at different time intervals after ringing and found that the ringing resulted in a marked accumulation of sugars above the ring and their decline below the ring. Nitrogenous compounds were generally affected similarly.

Ringing experiments have been used with much success in studies with viruses. Bennett (1956) and Esau (1956) showed that many viruses are translocated in the phloem during their systemic invasion of the plant. Phloem limited viruses are unable to pass a ringed part of the stem.

Leach (1937, 1939) recommended ring-barking as a control measure against root diseases. Working with Armillaria mellea on tea he demonstrated that this organism develops freely as parasite on the roots of many plant species once the tops have been felled. If the trees were killed by ring-barking some time before felling their roots get invaded by harmless saprophytes such as Rhizoctonia bataticola etc. and not by A. mellea. Wappes (1939) observed that in trees ring-barked 4½ months before felling, the roots had lost 52 per cent of their starch content as 3½ to 4 months and 86 per cent at 6 months after treatment.

Ezekiel (1940) reported that topping or girdling of infected cotton plants at the advancing margins of root rot spots in midsummer may greatly shorten the survival period of Phymatotrichum omnivorum.

MATERIALS AND METHODS

MATERIALS AND METHODS

A local bushy variety of sword bean (Canavalia ensiformis DC.) obtained from the Agricultural College Farm was used. The plants were raised in earthern pots of 12" diameter. The potting mixture consisted of farm yard manure, river sand and garden soil in the ratio 1:1:1 (V/V). Only one plant was grown in each pot.

The culture of Rhizoctonia sp. used in the present studies was isolated from naturally infected sword bean plants and maintained on Potato Dextrose Agar medium.

Plants showing uniform growth and size were selected and they were divided into three lots of 25 plants each. One lot was used for inoculation, the second lot was used for ring-barking and the third lot served as control. Inoculation as well as ring-barking were done 25 days after germination of seeds by which time the seedlings had put forth three to four true leaves. These plants were used for rhizosphere studies. A similar set of plants were used for chemical analysis.

I. Inoculation

Inoculations were done at the region just above the cotyledons (approximately 2 to 5" above the ground level) with

five days old culture of the fungus. The region to be inoculated was injured with three to four pin pricks just before inoculation in order to hasten the infection. Culture bits were applied at this region and it was covered with moist cotton wool (Plate I).

II. Ring-barking

Ring-barking was done at the region just above the cotyledons as in the case of inoculated plants using sterile razor blades. Two parallel cuts encircling the stem were made in the bark and the ring like piece of bark formed by the cut was removed from the stem. The width of the bark removed was equal in all cases and was about 1 cm. in size. Care was taken to see that the cut did not extent to the wood region (Plate I).

The control plants did not receive any treatment.

All the experimental plants including the control were kept under identical conditions with regard to watering and other operations.

Control soil was drawn from a separate pot which was kept along with the experimental pots for the purpose.

III. Collection of samples

Watering of the pots was always done in the morning only. Samples for rhizosphere studies and chemical analysis were drawn in the afternoon. Samples were drawn simultaneously from healthy plants, plants which were inoculated, plants which were ring-barked and control soil.

The root samples required for the assessment of the rhizosphere microflora was collected as per details given below. The plants were uprooted with a block of soil and the soil around the root system was removed carefully by gentle shaking. The root system was then tapped gently with a sterile needle to remove the superfluous soil adhering to it. The roots were cut into pieces of about 3 to 4 cm. aseptically and transferred into 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 1 to 2 gm. of soil (Wallace and Lochhead, 1949).

IV. Quantitative determination of the rhizosphere microflora

The assessment of rhizosphere microflora was done as per method described by Timonin (1940). The first sample was drawn on the day of inoculation (25 days after germination). Subsequent samples were drawn at three days interval.

Flasks containing 100 ml. of sterile distilled water into which the roots 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 1:1,000,000. The dilution was prepared by two successive transfers of one ml. aliquot from the lower concentration 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. One ml. of the final suspension was used for plating in sterile petridishes alongwith 15 ml. of the medium. Different media were used for bacteria, actinomycetes and fungi. The media used were:

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

This medium was used for bacteria.

Composition:

Soil extract	:	1000 ml.
K_2HPO_4	:	0.2 g.
Agar agar	:	15.0 g.

For preparing the soil extract 100.0 gm. of soil was autoclaved in 1000 ml. of water for half an hour at 15 lb. pressure.

Then it was allowed to sediment and the supernatent liquid was decanted and filtered. A small quantity of $CaSO_4$ was added to hasten the sedimentation. It was filtered through No. 41 filter paper. The volume was made upto 1000 ml. One gram of glucose was added to hasten the appearance of colonies. The pH of the medium was adjusted to 6.8 prior to sterilization.

ii. Peptone dextrose agar with rose bengal and streptomycin

(Martin, 1950)

Composition:

Dextrose	:	10.0 g.
Peptone	:	5.0 g.
KH_2PO_4	:	1.0 g.
$MgSO_4$	:	0.5 g.
Agar agar	:	15.0 g.
Distilled water	:	1000 ml.
Rose bengal	:	1 part in 30,000 parts of the medium.
Streptomycin	:	30 µg. per ml.

(The streptomycin was added only at the time of plating)

This medium was used for fungi.

iii. Kon-knight's agar

Composition:

Glucose	:	1.0 g.
K_2HPO_4	:	0.1 g.
$MgSO_4$	:	0.1 g.
$NaNO_3$	:	0.1 g.
Kcl	:	0.1 g.
Agar agar	:	15.0 g.
Distilled water	:	1000 ml.

This medium was used for actinomycetes.

The above 3 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. Before plating the media were melted in a water bath, cooled to 45 to 48°C and the plating was done.

At first the plating was done by using the method suggested by Timonin (1940). One 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 48°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). One 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 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 this method.

The dilutions were plated in triplicate for each group of microorganisms. The plates were incubated at room temperature. Counts of fungal colonies were taken 6 to 7 days after plating as soon as the colonies began to appear. Counts of bacteria and actinomycetes were taken after 14 days. 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 soils used for preparing the dilutions, the roots were removed from the flask after washing down all the adhering soil particles into the same flask. The suspension in the flask was evaporated to dryness by placing it in a water bath. After that the flask was kept in a hot air oven

at 105°C to 110°C for six hours. These were then allowed to cool in the oven itself and were weighed. The dry weight of the soil was determined, making necessary corrections for the aliquots of soil removed during dilutions (Herr et al, 1961).

This technique was followed throughout the rhizosphere studies.

V. Qualitative determination of bacteria in the rhizosphere

The bacterial colonies were picked up 14 days after plating and stabcultures were made for further studies. For this the semi-solid soil extract medium was used. (Taylor and Lochhead, 1938).

Composition of the medium:

Soil extract	:	1000 ml.
K_2HPO_4	:	0.2 g.
Glucose	:	1.0 g.
Yeast extract	:	1.0 g.
Agar agar	:	3.0 g.

pH adjusted to 6.8

For taking the bacterial colonies from a plate, the surface area of the plate was divided into four sections and all the colonies within a single sector were picked up to represent each treatment.

The bacterial isolates were classified according to their morphology and Gram reaction. For Gram reaction 24 hours old cultures were used and for the determination of spore formers 48 hours old cultures were used.

VI. Fungi present in the rhizosphere

The fungal colonies that appeared in the plates were identified to the extent possible. All the unidentified species were grouped under other fungi.

VII. Chemical analysis of the root system of healthy, ring-barked and inoculated sword bean plants

i. Collection of samples:

Sampling was done at 3 stages of growth of the healthy, inoculated and ringed plants as given below:

1st sample: On the day of inoculation, i.e., when plants were 25 days' old.

2nd sample: 10 days after inoculation (36 days' old).

3rd sample: 18 days after inoculation (43 days' old).

Samples were collected from the same set of plants that were used for the rhizosphere studies. Only the root system (below the collar level) was taken for analysis.

The root system for the estimation of total sugars was dried at 105°C for 6 hours in a hot-air oven. The percentage of moisture and dry matter were also determined.

ii. Estimation of sugars:

Two grams of the moisture free plant material was taken in an Erlenmeyer flask to which 180 ml. of water and 20 ml. of concentrated hydrochloric acid were added. The flask was provided with a reflux condenser and boiled for two and a half hours, cooled and neutralized with sodium hydroxide initially and then with sodium carbonate. It was then filtered and the filtrate was made upto 200 ml. and the quantity of total sugars was estimated by titration against Fehling's solution.

10 ml. of Fehling's solution (5 ml. of A and 5 ml. of B) was measured into a conical flask, diluted with 20 ml. of water, heated to boiling and then filtrate was added from a burette. When a faint precipitate was obtained 2 to 5 drops of methylene blue was added and the titration was completed at the stage of discolouration of methylene blue. The percentage of total sugars were calculated as follows:

$$\text{Percentage of total sugar} = \frac{\text{Factor for Fehling's solution} \times 200 \times 100}{\text{Titre value} \times \text{weight in gram}}$$

iii. Estimation of crude fibre:

Two grams of the moisture free plant material was taken in an Erlenmeyer flask. About 200 ml. of 1.25 per cent sulphuric acid was added and the solution was heated to boiling. After about 30 minutes digestion the contents of the flask were filtered through a linen supported in a flutted funnel and washed free from acid with boiling water. The residue on the linen was transferred completely to the flask and 200 ml. of 1.25 per cent boiling sodium hydroxide was added to it through the same linen and the contents of the flask was digested for 30 minutes. Afterwards it was filtered through the same linen and washed free of alkali with boiling water and later washed twice with alcohol. The residue was scraped into a silican crucible, dried free of moisture and weighed after cooling. Then the content of the crucible was ignited to white ash in a muffle at 600°C and was weighed again. The differences between the two weights gave the weight of crude fibre and was expressed as percentage.

iv. Estimation of total carbohydrates:

For the determination of total carbohydrates the sugars and crude fibre estimated separately were added and represented as per cent.

v. Estimation of total nitrogen:

Total nitrogen was estimated according to the method given by Piper (1950).

One gram of moisture free plant material was transferred into a kjeldahl digestion flask, containing 20 ml. of concentrated sulphuric acid to which was added 10 gram of potassium sulphate and one gram of copper sulphate and digested. After the completion of digestion as evidenced by the appearance of a light blue colour the flask was cooled and water extracts of the digested material was transferred into a 800 ml. distillation flask. About 100 ml. of 40 per cent sodium hydroxide was added to it and distillation was carried out. The distillate was collected in an Erlenmeyer flask containing a known quantity of 0.1 normal sulphuric acid. When about 150 ml. of distillate was collected distillation was stopped and the excess acid was back titrated with 0.1 normal sodium hydroxide using methyl red as indicator.

Titre value for calculation is the volume of normal sulphuric acid used for neutralisation of with the alkali formed by distillation. The volume of acid not used during distillation was determined by titration with 0.1 normal sodium hydroxide. The difference in the volume of acid taken to the volume determined

by titration, give the volume of acid used for neutralisation during distillation. This volume become the titre value.

$$\text{Percentage of nitrogen in the plant material} = \frac{\text{Titre value} \times 0.0014 \times 100}{\text{Weight of material in grams}}$$

VIII. Effect of ring-barking and inoculation with Rhizoctonia sp. on root development of sword bean plants

The wet weight and dry weight of the root system of healthy, ring-barked and inoculated plants were determined at 3 stages. Average of 10 plants were taken in each case.

IX. Effect of growing sword bean seedlings in the culture filtrates of Rhizoctonia sp.

The fungus was grown in conical flasks containing sterile Richard's solution. After 12 days growth the cultures were filtered through thick layers of cotton wool. Healthy sword bean seedlings (15 days old) grown in pots were carefully removed and the roots were washed thoroughly under a jet of water. The stem and roots of these plants were dipped in 0.1 per cent mercuric chloride solution for 1 minute and then washed in three changes of sterile water. These were then placed in 250 ml. Erlenmeyer flasks each containing 200 ml. of the culture filtrate with the root system completely immersed in the filtrate. Only one seedling was placed in each

flask and this was kept in position by means of sterile cotton wrapped around the stem of the seedling at the mouth of the flask. Seedlings placed in separate flasks containing sterile Richard's solution and sterile water served as controls. The plants were kept under observation.

RESULTS

RESULTS

I. Symptomatology

i. Effect of inoculation with *Rhizoctonia* sp. on sword bean plants:

First visible signs of infection were noted on the third day after inoculation when the felty growth of the fungus was seen at the inoculated region. By this time the leaves showed a number of oily or water soaked lesions. These lesions were more prominent on the lower fully expanded leaves. The fungal growth encircled the stem at the inoculated region in about 5 to 6 days after inoculation. The bark at this region showed a gradual decay and disintegration. The lesions on the leaves enlarged and some of them coalesced forming larger discoloured areas. The colour of these lesions gradually became dirty yellow. In about 8 days after inoculation the leaves became dull grey in colour and started drooping with a characteristic inward rolling. The rolling and drooping progressed upto 13th day by which time most of the leaves became yellow in colour. Complete wilting and death of the plant with shedding of leaves was noticeable by the 16th day of inoculation (Plate 2 & 3).

ii. Effect of ring-barking on sword bean plants:

The leaves of the ring-barked plants exhibited a general discolouration from the third day onwards without developing any

lesion. The green colour almost completely faded by the 6th day. By the 9th day after ring-barking the lower leaves started rolling inwards and became distinctly yellow in colour. Yellowing of the upper leaves was evident by the 11th day after ring-barking by which time the plants started shedding of leaves. Complete wilting and death of the plants occurred by the 13th day (Plate 2 & 3).

II. Quantitative determination of the microflora in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

A steady increase in the rhizosphere microflora of healthy plant was noted as the plant became older. The total microbial population when the plants were 25 days old was only 73.21 millionsand this increased to 218.51 millions when the plants became 43 days old. The population in the control soil at the above two periods were only 18.53 and 19.83 millions.

Ring-barking as well as inoculation with Rhizoctonia sp. exerted a stimulatory effect on the rhizosphere microflora. This stimulatory effect was noticeable in all the three groups of organisms. There was a greater incidence of bacteria, fungi and actinomycetes in the rhizosphere of plants which received the treatment. This increase in the population was noticeable from

the third day after the treatment. From sixth day onwards the increase was greater in plants which were inoculated with Rhizoctonia sp. than those were ring-barked. Thus on the 18th day after the treatment the total microbial population in the rhizosphere of healthy, ring-barked and inoculated plants were 218.51, 245.25 and 306.17 millions respectively (Table I).

i. Bacteria:

The bacterial population in the rhizosphere of healthy, ring-barked and inoculated plants showed a steady and significant increase throughout the period of observation. There was a sharp increase in the bacterial population in the ring-barked plants three days after the treatment and this increase was greater than that noted in the inoculated plant. Thus the bacterial population in the ring-barked plants rose to 70.19 millions from 41.87 millions while that in the inoculated plants increased only to 64.85 millions (Table II). The subsequent increases in the bacterial population were throughout greater in the inoculated plant than in the ring-barked plant. Six days after the treatment the population in the inoculated plant rose from 64.85 millions to 95.30 millions while in the ring-barked plant it rose only to 75.04 millions from 70.19 millions. At the same time the population in the healthy plant rose from 49.35 millions to 58.75 millions. On the 18th

TABLE I

Total microbial populations in the rhizosphere of healthy, inoculated and ring-barked sword bean plants

Treatment	Age of the plant					
	25 days (day of treatment)	28 days (3 days after treatment)	32 days (6 days after treatment)	36 days (10 days after treatment)	40 days (14 days after treatment)	43 days (18 days after treatment)
Healthy	73.21	83.73	97.09	109.57	175.01	218.51
Ring-barked	73.21	108.31	130.71	149.50	216.07	245.25
Inoculated	73.21	104.30	155.62	196.14	283.25	306.17
Control soil	18.53	20.16	19.25	19.11	20.57	19.83

TABLE II

Total bacterial populations in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

Treatment	Age of the plant											
	25 days (day of treatment		28 days (3 days after treatment)		32 days (6 days after treatment)		36 days (11 days after treatment)		40 days (14 days after treatment)		43 days (18 days after treatment)	
	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio
Healthy	41.87	3.41	49.35	4.13	58.75	5.30	61.84	5.53	102.41	8.31	125.54	10.31
Ring-barked	41.87	3.41	70.19	5.87	75.04	6.77	94.07	8.42	120.47	9.73	141.20	11.60
Inoculated	41.87	3.41	64.85	5.42	95.30	8.60	123.38	11.04	163.79	13.30	177.11	14.55
Control soil	12.26	..	11.95	..	11.07	..	11.17	..	12.31	..	12.17	..

TOTAL BACTERIAL POPULATION IN THE RHIZOSPHERE OF HEALTHY, RING-BARKED AND INOCULATED SWORD BEAN PLANTS

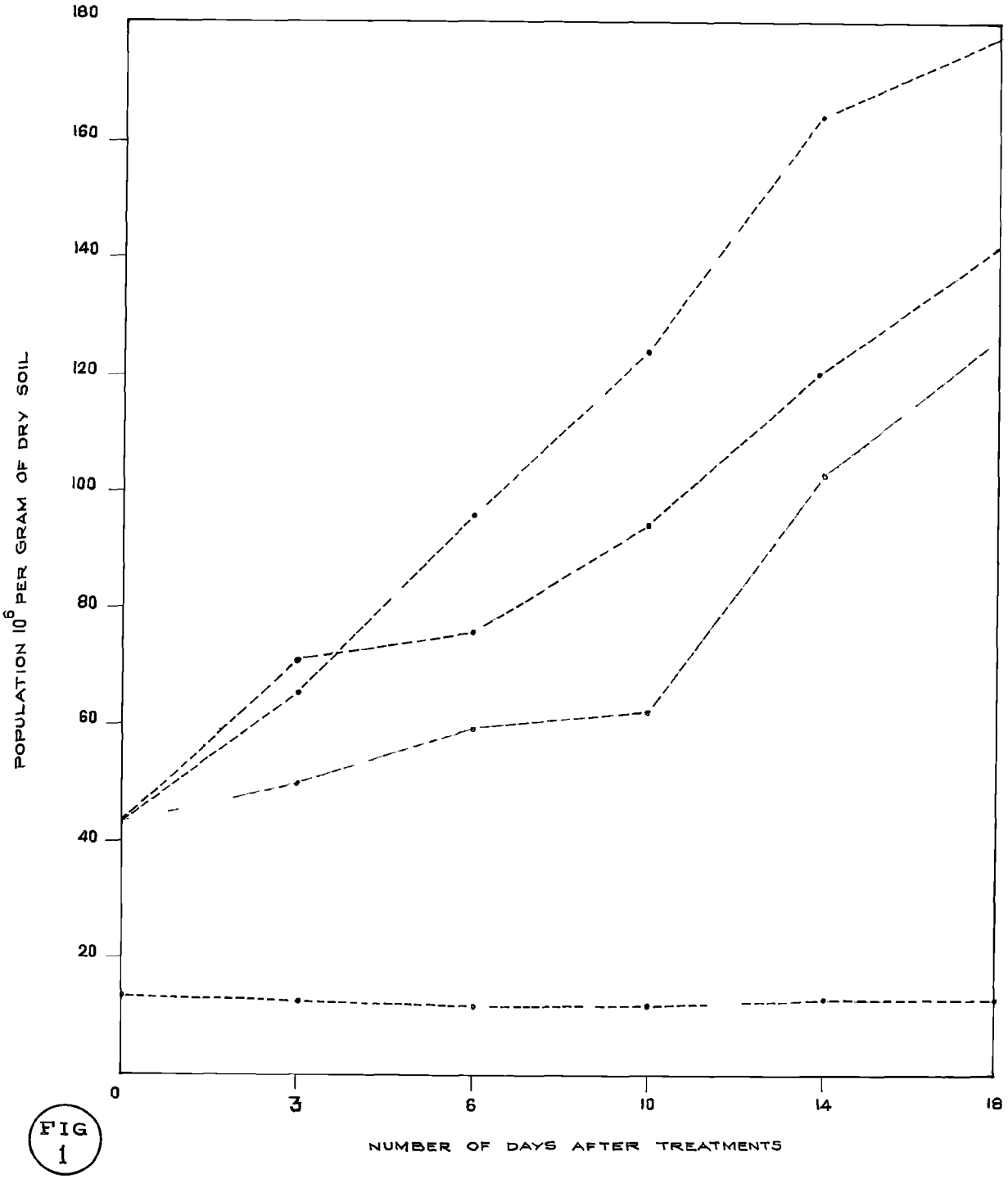

FIG 1

day after the treatment the population in the rhizosphere of healthy, ring-barked and inoculated plant increased to 125.54, 141.20 and 177.11 millions respectively. The corresponding R:S ratios were 10.31, 11.60 and 14.55 (Table II).

ii. Actinomycetes:

The actinomycetes population in the rhizosphere also showed a steady increase as the plant became older. The increase was greater in the rhizosphere of ring-barked and inoculated plant, the increase in the latter being more pronounced. On the third day after the treatment the actinomycetes population in the healthy, ring-barked and inoculated plant were 29.97, 33.70 and 34.81 millions respectively. The corresponding R:S ratios were 4.14, 4.63 and 4.82 respectively. The rhizosphere population of ring-barked and inoculated plant showed a steep increase on the 14th day after the treatment. The population increased from 49.01 millions to 85.48 millions in the ring-barked plant and from 64.64 millions to 103.36 millions in the inoculated plant. On the 18th day the population increased to 80.42, 89.00 and 110.73 millions in the rhizosphere of healthy, ring-barked and inoculated plant. The corresponding R:S ratios were 12.17, 14.07 and 17.01 (Table III).

TABLE III

Total actinomycetes populations in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

Treatment	Age of the plant											
	25 days (day of treatment)		28 days (3 days after treatment)		32 days (6 days after treatment)		36 days (11 days after treatment)		40 days (14 days after treatment)		43 days (18 days after treatment)	
	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio
Healthy	26.91	5.25	29.97	4.14	33.35	4.65	42.43	6.26	65.27	9.26	80.42	12.17
Ring-barked	26.91	5.25	33.70	4.65	49.91	6.96	49.01	7.25	85.48	12.10	89.00	14.07
Inoculated	26.91	5.25	34.81	4.82	53.98	7.52	64.64	9.54	103.36	14.64	110.73	17.01
Control soil	5.12	..	7.24	..	7.17	..	6.77	..	7.06	..	6.33	..

TOTAL ACTINOMYCETES POPULATION IN THE RHIZOSPERE OF HEALTHY, RING-BARKED AND INOCULATED SWORD BEAN PLANTS

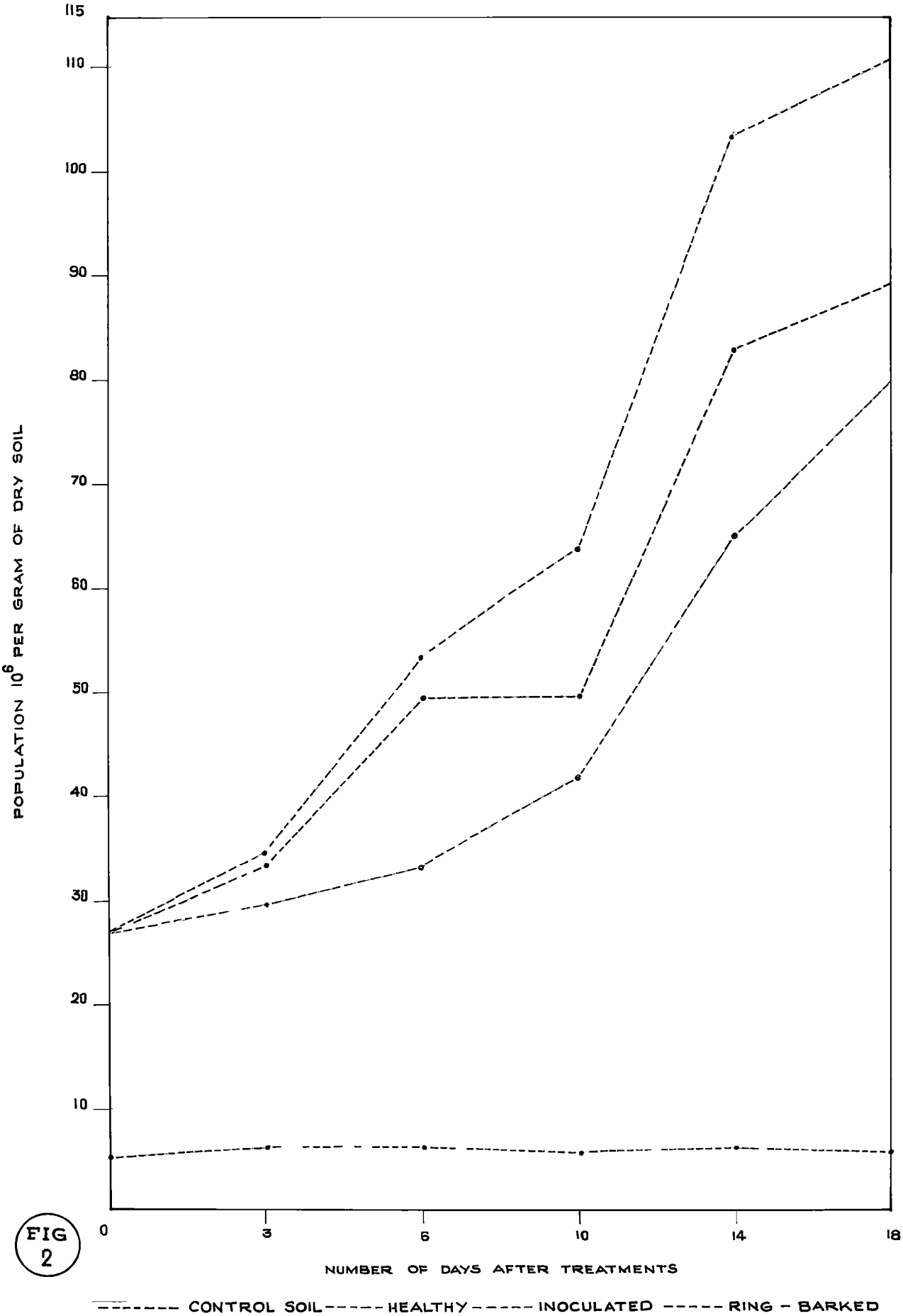

FIG 2

iii. Fungi:

The fungal population in the rhizosphere of healthy, ring-barked and inoculated plant also showed an increase. This increase was more pronounced in the ring-barked and inoculated plant. The increase in the inoculated plant was always greater than in the ring-barked plant. The population on the third day after the treatment on the healthy, ring-barked and inoculated plant were 4.41, 4.42 and 4.64 millions respectively with the corresponding R:S ratios of 4.54, 4.54 and 4.78. There was a steep increase in the fungal population of the rhizosphere of ring-barked and inoculated plant on the 14th day after the treatment. In the ring-barked plant the population of 6.42 millions on the 10th day increased to 10.12 millions and in the inoculated plant the increase was from 8.12 millions to 16.10 millions. The corresponding R:S ratios on this day were 8.35 and 13.12. On the 18th day after the treatment the fungal population in the rhizosphere of healthy, ring-barked and inoculated plant increased to 12.55, 15.05 and 18.33 millions respectively with the corresponding R:S ratios 9.43, 11.31 and 13.77 (Table IV).

III. Qualitative determination of bacteria in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

The treatment did not materially affect the percentage of different morphological groups of bacteria present in the

TABLE IV

Total fungal populations in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

Treatment	Age of the plant											
	25 days (day of treatment)		28 days (3 days after treatment)		32 days (6 days after treatment)		36 days (11 days after treatment)		40 days (14 days after treatment)		43 days (18 days after treatment)	
	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio	Population	R.S. ratio
Healthy	4.42	3.85	4.41	4.54	4.99	4.94	5.30	4.53	7.35	6.10	12.55	9.43
Ring-barked	4.42	3.85	4.42	4.54	5.76	5.70	6.42	5.49	10.12	8.35	15.05	11.31
Inoculated	4.42	3.83	4.64	4.78	6.34	6.27	8.12	6.94	16.10	13.12	18.33	13.77
Control soil	1.15	..	0.97	..	1.01	..	1.17	..	1.20	..	1.33	..

TOTAL FUNGAL POPULATIONS IN THE RHIZOSPERE OF HEALTHY, RING-BARKED AND INOCULATED SWORD BEAN PLANTS

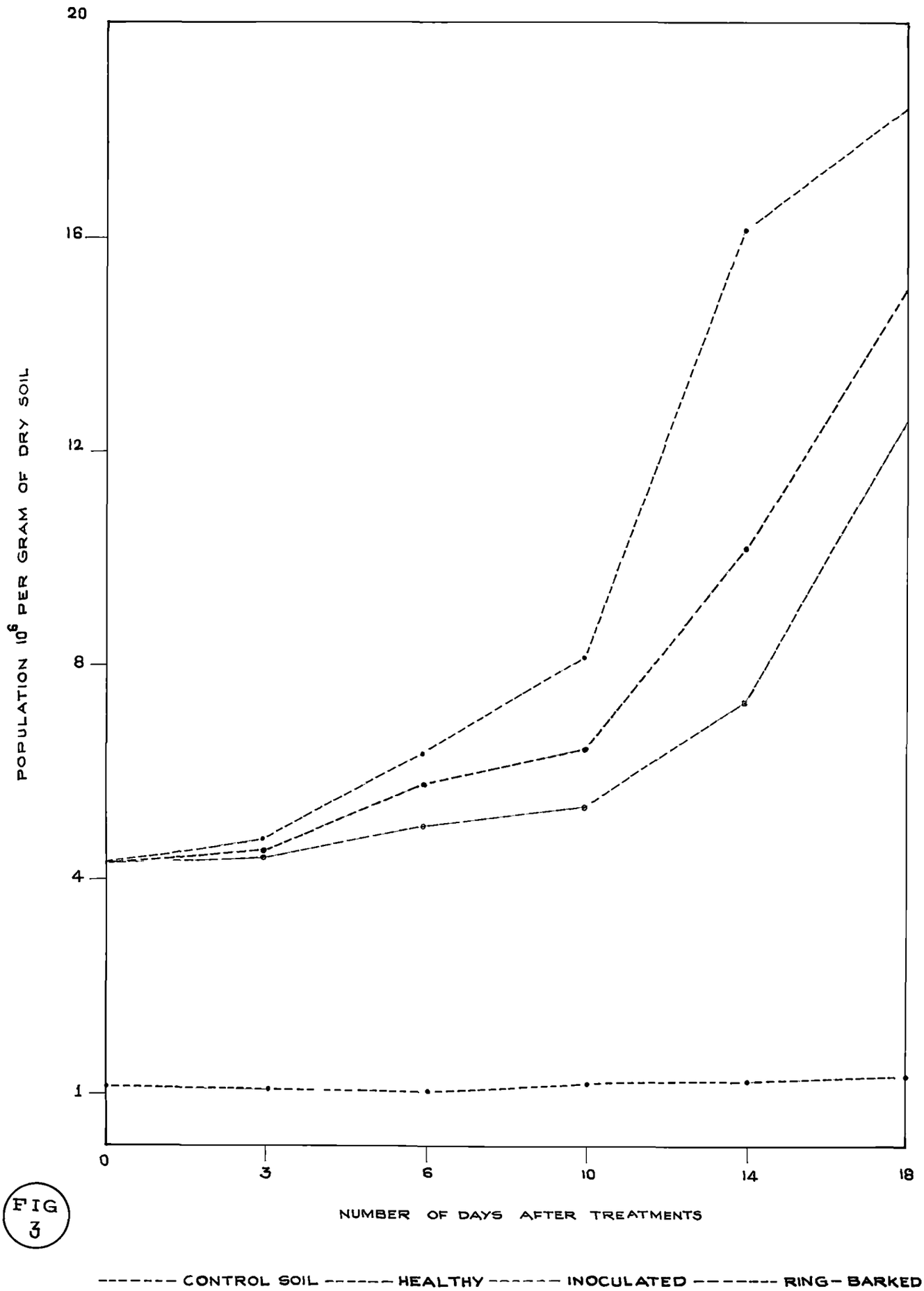

FIG 3

rhizosphere. A higher incidence of Gram negative rods was noticed in the rhizosphere of healthy, ring-barked and inoculated plants while there was a preponderance of Gram positive rods in the soil.

The per cent incidence of spore formers in the control soil was higher than that in the rhizosphere of healthy, ring-barked and inoculated plants. While the percentage of spore formers in the soil ranged between 28.89 and 31.00 that in the healthy, ring-barked and inoculated plants ranged between 10.80 to 12.70, 7.00 to 12.00 and 6.50 to 12.70 (Table VIII).

There was also no appreciable difference in the percentage of the different groups of bacteria present in the rhizosphere at the three stages of plant growth when the samplings were made (Tables V to VIII).

IV. Fungi present in the rhizosphere

Species of Aspergillus and Penicillium were predominant in the control soil and in the rhizosphere of healthy, ring-barked and inoculated plants. In the Aspergillus group of organisms, Aspergillus niger was more abundant and formed more than 50 per cent of the total number occurred. Other important genera of fungi noted were Rhizopus, Trichoderma, Chaetomium, Fusarium and Pestalotia.

TABLE V

Distribution of morphological groups of bacteria in the rhizosphere of

of healthy sword bean plants on the day of treatments

Particulars	Control soil		Healthy plant	
	Percentage	Population	Percentage	Population
Total population	..	12.26	..	41.87
Gram -ve rods	17.84	2.19	42.78	17.91
Gram -ve cocci	14.28	1.76	21.50	9.00
Gram +ve rods	45.21	5.28	20.20	8.46
Gram +ve cocci	24.67	3.03	15.52	6.50
Spore formers	30.00	3.67	12.70	5.06

TABLE VI

Distribution of morphological groups of bacteria in the rhizosphere of healthy, inoculated and ring-barked sword bean plants, 10 days after the treatment

Particulars	Control soil		Healthy plant		Ring-barked plant		Inoculated plant	
	Per-centage	Popu-lation	Per-centage	Popu-lation	Per-centage	Popu-lation	Per-centage	Popu-lation
Total population	..	11.17	..	61.84	..	94.07	..	123.38
Gram -ve rods	22.23	2.49	45.50	28.14	49.25	46.33	49.14	60.63
Gram -ve cocci	16.66	1.86	18.50	11.44	21.50	20.23	22.61	27.90
Gram +ve rods	38.22	4.26	15.25	9.43	16.75	15.75	18.12	22.36
Gram +ve cocci	22.89	2.56	20.75	12.83	12.50	11.76	10.13	12.49
Spore formers	28.89	3.23	10.80	6.68	7.35	6.91	6.50	8.02

TABLE VII

Distribution of morphological groups of bacteria in the rhizosphere of healthy, inoculated and ring-barked sword bean plants, 18 days after the treatments

Particulars	Control soil		Healthy plant		Ring-barked plant		Inoculated plant	
	Per-centage	Popu-lation	Per-centage	Popu-lation	Per-centage	Popu-lation	Per-centage	Popu-lation
Total population	..	12.17	..	125.54	..	141.20	..	177.11
Gram -ve rods	22.20	2.70	46.00	57.74	50.20	70.88	52.00	92.09
Gram -ve cocci	15.00	1.83	22.00	27.62	22.60	31.91	23.25	41.18
Gram +ve rods	40.35	4.91	16.00	20.09	17.00	24.00	18.17	32.18
Gram +ve cocci	22.45	2.73	16.00	20.09	10.20	14.41	6.58	11.66
Spore formers	31.00	3.77	11.00	13.81	7.00	9.88	7.35	13.02

TABLE VIII

Percentage distribution of the different morphological groups of bacteria in the rhizosphere of healthy inoculated and ring-barked sword bean plants at three stages after the treatment

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
Healthy	42.78	45.50	46.00	21.50	18.50	22.00	20.20	15.25	16.00	15.52	20.75	16.00	12.70	10.80	11.00
Ring-barked	42.78	49.25	50.20	21.50	21.50	22.60	20.20	16.75	17.00	15.52	12.50	10.20	12.70	7.35	7.00
Inoculated	42.78	49.14	52.00	21.50	22.61	23.25	20.20	18.12	18.17	15.52	10.13	6.58	12.70	6.50	7.35
Control soil	17.84	22.23	22.20	14.28	16.66	15.00	43.21	38.22	40.35	24.67	22.89	22.45	30.00	28.89	31.00

A = day of inoculation B = 10 days after inoculation C = 18 days after inoculation

The per cent incidence of Mucoraceous fungi was greater in the rhizosphere of healthy and ring-barked plants than in the soil and in the rhizosphere of inoculated plants. While the percentage incidence of the Mucoraceous fungi was only 5.72 per cent in the control soil those in the rhizosphere of healthy, ring-barked and inoculated plants were 12.29, 8.09 and 5.04 per cent respectively (Table IX).

Rhizoctonia sp. was not obtained from any of the samples used for plating.

V. Chemical analysis of the root system of healthy, ring-barked and inoculated sword bean plant

i. Percentage of moisture and dry matter:

A decrease in the percentage of moisture in the root system of healthy, ring-barked and inoculated plant was noticed as the plants became older. The reduction in the moisture content was greater in the inoculated and ring-barked plant. The maximum reduction was noticed in the ring-barked plant. While the moisture content in the root system of healthy plant fell from 82.80 to 70.72 per cent within a period of 18 days that in the ring-barked plant fell to 61.52 per cent during the same period. The moisture content of inoculated plant fell from 82.80 to 63.88 (Table X).

TABLE IX

Percentage incidence of different fungi in the rhizosphere of healthy, ring-barked and inoculated sword bean plants

Treatments	Group of organisms		
	Penicillium and *Aspergillus* spp.	Mucoraceous fungi	Other fungi
Control soil	93.28	5.72	1.00
Healthy	89.00	10.29	0.71
Ring-barked	90.00	8.09	1.91
Inoculated	93.42	5.04	1.54

TABLE X

Percentage of moisture and dry matter in the root system of healthy, ring-barked and inoculated sword bean plants

Treatments	Percentage of moisture			Percentage of dry matter		
	Healthy	Ring-barked	Inoculated	Healthy	Ring-barked	Inoculated
Day of treatment (25 days)	82.80	82.80	82.80	17.20	17.20	17.20
10 days after treatment (36 days)	74.01	64.84	67.52	25.99	35.16	32.48
18 days after treatment (43 days)	70.72	61.52	63.88	29.28	38.48	36.12

ii. Percentage of total sugars:

The root system of healthy, ring-barked and inoculated plant showed a decrease in the total sugars on dry weight basis as the plant became older. This reduction was very highly pronounced in the case of ring-barked plant. While the percentage of total sugars in the healthy and the inoculated plant fell from 9.63 to 6.35 and 5.36 respectively within 18 days that in the ring-barked plants fell from 9.63 to 2.20 during the same period (Table XI).

iii. Percentage of crude fibre:

The root system of healthy plant showed an increase in the percentage of crude fibre as the plant became older. But in the ring-barked and inoculated plant there was a drastic reduction in the percentage of crude fibre. While the percentage of crude fibre which was 21.60 on the day of treatment rose to 32.23 per cent in the healthy plant in 18 days it fell to 4.30 per cent in ring-barked plant and 10.86 per cent in inoculated plant during the same period (Table XII).

iv. Percentage of total carbohydrates:

The percentage of total carbohydrates in the healthy plant was found to increase with age on dry basis. In the ring-barked

TABLE XI

Percentage of total sugars in the root system of healthy, ring-barked and inoculated sword bean plants

Number of days after treatment	Healthy (Dry basis)	Ring-barked (Dry basis)	Inoculated (Dry basis)
Day of treatment (25 days)	9.63	9.63	9.63
10 days after treatment (36 days)	8.55	5.13	6.95
18 days after treatment 43 days)	6.35	2.20	5.36

TABLE XII

Percentage of crude fibre in the root system of healthy, ring-barked and inoculated sword bean plants

Number of days after treatment	Healthy (Dry basis)	Ring-barked (Dry basis)	Inoculated (Dry basis)
Day of treatment (25 days)	21.60	21.60	21.60
10 days after treatment (36 days)	28.25	10.21	17.43
18 days after treatment (43 days)	32.23	4.30	10.86

and inoculated plant on the other hand the percentage of total carbohydrates decreased as a result of the treatment. This reduction was very highly pronounced in the ring-barked plant when the percentage of total carbohydrates increased to 38.58 per cent from 31.23 per cent in 18 days it fell to 16.22 per cent in the inoculated plant and to 6.50 per cent in the ring-barked plant during the same period (Table XIII).

v. Percentage of total nitrogen:

The percentage of total nitrogen decreased with age of the plant and also as a result of ring-barking and inoculation. But this decrease was very pronounced in the ring-barked and inoculated plant. Maximum reduction occurred in the ring-barked plant. While the percentage of nitrogen in the healthy plant fell from 2.56 to 2.06 per cent it fell to 0.43 per cent in the inoculated and to 0.18 per cent in the ring-barked plant (Table XIV).

vi. Carbohydrate/nitrogen ratio:

The carbohydrate/nitrogen ratio in the root system of healthy, ring-barked and inoculated plant widened as the plant became older. But this widening was more pronounced in the ring-barked and inoculated plant. In the healthy plant the initial C/N ratio of 12.20:1 changed to 18.72:1 in 18 days.

TABLE XIII

Percentage of carbohydrates in the root system of healthy, ring-barked and inoculated sword bean plants

Number of days after treatment	Healthy (Dry basis)	Ring-barked (Dry basis)	Inoculated (Dry basis)
Day of treatment (25 days)	31.23	31.23	31.23
10 days after treatment (36 days)	36.80	15.34	24.38
18 days after treatment (43 days)	38.58	6.50	16.22

TABLE XIV

Percentage of total nitrogen in the root system of healthy, ring-barked and inoculated sword bean plants

Number of days after treatment	Healthy (Dry basis)	Ring-barked (Dry basis)	Inoculated (Dry basis)
Day of treatment (25 days)	2.56	2.56	2.56
10 days after treatment (36 days)	2.23	0.25	0.82
18 days after treatment (43 days)	2.06	0.18	0.43

But in ring-barked and inoculated plant it changed to 36.11:1 and 37.72:1 respectively during the same period. No significant difference could therefore be noted in the final C/N ratio of the ring-barked and inoculated plant. An abrupt widening in the C/N ratio of ring-barked plant from 12.20:1 to 61.36:1 was noticed 10 days after the treatment and this fell to 36.11:1 18 days after the treatment. The widening of the C/N ratios in the healthy and inoculated plant was on the other hand gradual (Table XV).

VI. Effect of ring-barking and inoculation with Rhizoctonia sp. on the root development of sword bean plant

The ring-barked and inoculated plant exhibited poor root development after the treatment. There was a progressive decline in the dry weight of their root system after the treatment, the ring-barked plant showing a more pronounced decrease. When the healthy plant showed an increase in weight of root system from 1.07 g. to 3.95 g. within a period of 18 days the ring-barked and inoculated plant showed a decrease to 0.14 g. and 0.28 g. respectively (Table XVI). It was further noticed that nodulations was practically absent on the root system of ring-barked and inoculated plant while the healthy plant had profuse nodulations on their roots.

TABLE XV

Carbohydrate/nitrogen ratio in the root system of healthy, ring-barked and inoculated sword bean plants

Number of days after inoculation	Healthy	Ring-barked	Diseased
Day of inoculation (25 days)	12.20:1	12.20:1	12.20:1
10 days after inoculation (36 days)	16.50:1	61.36:1	29.73:1
18 days after inoculation (43 days)	18.72:1	36.11:1	37.72:1

TABLE XVI

Development of root system in the healthy, ring-barked and inoculated sword bean plants

Number of days after treatment	Healthy (Dry weight) gm.	Ring-barked (Dry weight) gm.	Inoculated (Dry weight) gm.
Day of treatment (25 days)	1.07	1.07	1.07
10 days after treatment (36 days)	2.66	0.36	1.16
18 days after treatment (43 days)	3.95	0.14	0.28

VII. Effect of growing sword bean seedlings in the culture filtrates of Rhizoctonia sp.

The lower leaves of the seedlings which were placed in the culture filtrates of the fungus developed light greyish yellow lesions within twenty four hours. These lesions enlarged involving the entire leaf lamina in 3 days. The tender leaves also turned pale. These symptoms are similar to those noted on plants which were artificially inoculated. The plants wilted completely within five days. The seedlings placed in flasks containing sterile Richard's solution and sterile water did not show any symptom of wilting (Plate 4).

DISCUSSION

DISCUSSION

Ring-barking as well as inoculation with Rhizoctonia sp. brought about marked increases in the rhizosphere microbial population of sword bean plants. This increase was noticeable in the case of all the three groups of organisms, namely, bacteria, fungi and actinomycetes and it became evident on the third day of the treatment. The microbial population in the rhizosphere of healthy sword bean plants also showed a steady increase with age, but the increase noted as a result of treatments was significantly greater. While the total microbial population in the healthy plant rose from 73.21 millions to 218.51 millions in 18 days, it rose to 245.25 millions and 306.17 millions in the ring-barked and inoculated plant respectively, during the same period.

The increase in the microbial population was greater in the ring-barked plant than that in the inoculated plant 3 days after the treatment. The population in the healthy, ring-barked and inoculated plant at this time were 83.73, 108.31 and 104.30 millions respectively. This pattern soon changed and the population in the inoculated plant rose above that of the ring-barked plant 6 days after the treatment. From then onwards the population in the inoculated plant increased at a higher pace

as compared to that in the ring-barked plant.

Ring-barking can usually be expected to result in a sudden stoppage of downward translocation of nutrients, thereby causing a much faster depletion of reserve food materials in the root system. Inoculation with Rhizoctonia, on the other hand, can bring about such a situation only gradually. Complete stoppage of downward translocation of nutrients in this case can occur only when the entire phloem tissue around the stem is rendered inactive. This possibly explains why the initial increase in the microbial population was greater in the ring-barked plants as compared to the inoculated plants.

Ring-barking as well as inoculation can be expected to influence the metabolism of the plant, which in turn can bring about quantitative and qualitative changes in the root exudates. These changes will normally be reflected in the microbial population of the rhizosphere. The trend of increase in the microbial population of the rhizosphere in inoculated plant is indicative that the changes in these plants are not brought about exclusively by the mechanical blocking of the phloem tissue. It is apparent that some other factor also came into operation soon after inoculation as was evidenced by the

sudden rise in the microbial population 3 days after inoculation, before the fungus could completely girdle the stem. It is possible that as the fungus grew on the stem its metabolic products might have diffused into the tissues of the host. This seems to have exerted a greater influence on the rhizosphere microflora than that brought about by the mechanical blockage of the phloem tissue.

That Rhizoctonia is able to produce toxic metabolic products is known from the works of Kerr (1957). Using the cellophane bag technique he demonstrated that the roots of raddish were destroyed by the growth products of Rhizoctonia probably due to a toxin present therein. Stakman and Harrar (1957) have also mentioned about some antagonistic substances of Rhizoctonia which can cause distortions and death of roots of certain plants at a distance of atleast one centimetre without ever touching the roots. In the present studies also the fungus was found to produce some unknown toxic substances in vitro. Sword bean plants which were grown in the culture filtrates wilted, manifesting symptoms more or less similar to those produced due to infection.

Results of chemical analysis showed a marked reduction in the carbohydrate, nitrogen and moisture content in the root

system of ring-barked and inoculated plants. This reduction was greater in the ring-barked plant. Total carbohydrates in the ring-barked plant fell to 6.5 per cent from 31.23 per cent, 18 days after the treatment while it fell only to 16.22 per cent in the inoculated plant. Similarly total nitrogen in ring-barked plant fell from 2.56 per cent to 0.18 per cent while in the inoculated plant fell only to 0.43 per cent. This is clear indication that the reserve food materials in the root system of ring-barked plant were depleted faster than that in the inoculated plant.

Some what similar results have been obtained by Mason and Maskell (1928), Leach (1937) and also by Napper (1938), in other plants. These authors noted a faster depletion of starch and other nutrients from the root system of plant which were ring-barked. Leach (1937) therefore suggested ring-barking of trees in advance of clearing jungles for the establishment of new plantations, so that the roots of such trees will encourage the growth of saprophytic fungi which in turn will eliminate root disease fungi.

It is evident that ring-barking and also infection by Rhizoctonia were able to influence favourably the microbial population in the rhizosphere. Though the nature of changes

that were brought about in the metabolism of plants by ring-barking and inoculation does not appear to be similar, their ultimate effects on the rhizosphere were more or less similar in so far as they helped to increase the microbial activity of this region. In both cases the plant tissues died though the death was quicker in the ring-barked plant as a result of the faster depletion of nutrients. Such roots can be expected to encourage the growth of organisms that decompose dead organic matter especially cellulose. Perhaps a study of the nutritional requirements of the different groups of organisms present in the rhizosphere may be necessary for a better understanding of the changes that are brought about in the microbial population of the rhizosphere as a result of ring-barking and infection by Rhizoctonia.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The collar rot disease could be induced artificially in sword bean plants by inoculation with Rhizoctonia sp. The fungus girdled the stem in 5 to 6 days and finally brought about the death of the plant within 16 days. Ring-barking on the other hand brought about the death of the plant in 13 days.

Inoculation of sword bean plants with Rhizoctonia was found to influence favourably the rhizosphere microflora of sword bean plants. Ring-barking also brought about a more or less similar beneficial influence. The microbial population in the rhizosphere of healthy sword bean plants increased as the plant became older but the increase noted in treated plants was markedly greater than that in healthy plants.

The initial increase in the microbial population was greater in ring-barked plants than that in plants inoculated with Rhizoctonia. This is considered due to the sudden stoppage of downward translocation of nutrients in the ring-barked plants thereby bringing about a much faster depletion of the reserve food materials in the root system.

The population in the rhizosphere of inoculated plants increased more rapidly after the 3rd day and it became greater

than that of the ring-barked plants on the 6th day. This position was maintained till the last sampling.

The increase in the population was noticeable in all the three groups of organisms, namely, bacteria, fungi and actinomycetes.

Gram negative bacteria were predominant in the rhizosphere of healthy and treated plants throughout while Gram positive organisms were predominant in the soil.

Aspergillus and Penicillium group of fungi were abundant in the rhizosphere as well as in the soil. Rhizoctonia could not be obtained from the rhizosphere and also from the soil.

There was a fall in the carbohydrate and nitrogen content of the root system of ring-barked and inoculated plants. This fall was more rapid in the ring-barked plants. The rapid depletion of carbohydrates and nitrogen in the root system is considered to encourage the growth of cellulose decomposing organisms in the rhizosphere.

The fungus was found to produce some unknown toxic substances in vitro. Sword bean plants which were grown in the culture filtrates of the fungus wilted, manifesting symptoms

more or less similar to those produced due to infection. The increase in microbial population noted in the rhizosphere of inoculated plants is therefore considered due to changes in the metabolism of plants brought about by the combined action of mechanical blockage of phloem tissue and the toxic substances produced by the fungus.

The ultimate effects of infection and ring-barking on the rhizosphere were more or less similar in so far these treatments favoured the microbial population.

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PLATES

PLATE I

The plants on the day of treatments (25 days old)

H = Healthy

R = Ring-barked

D = Inoculated

PLATE II

The plants on 10 days after treatments (36 days old)

H - Healthy

R - Ring-barked

B - Inoculated


H
R
D

PLATE III

The plants on 18 days after inoculation (43 days old)

H = Healthy

R = Ring-barked

I = Inoculated

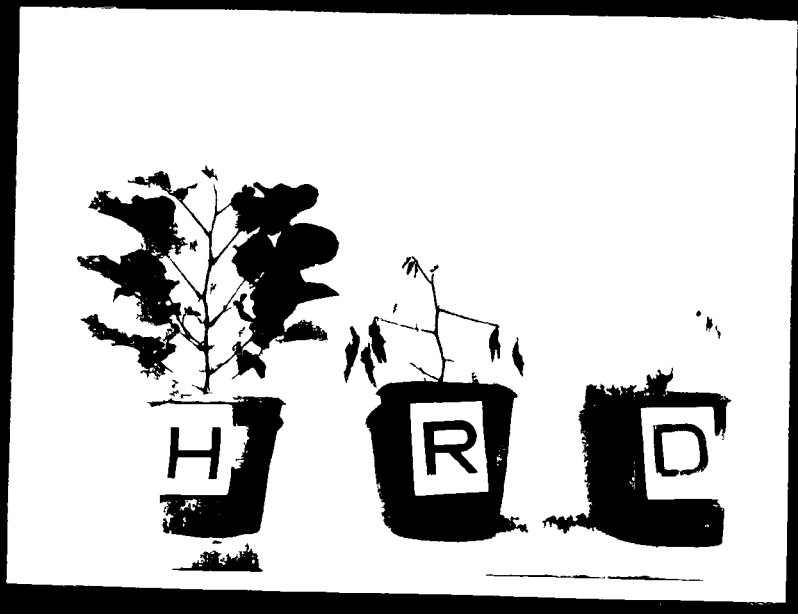
H
R
D

PLATE IV

The growth of the sword bean seedlings in the culture filtrates of fungus and the control plants

1. Culture filtrate
2. Sterile Richard's medium
3. Sterile distilled water


1
2
3