STUDIES ON THE RHIZOSPHERE MYCOFLORA OF CELOSIA ARGENTEA (L) AND HIBISCUS ESCULENTUS (L)

A thesis submitted for the degree of Doctor of Philosophy (Botany) of the University of Lagos.

by

Esther Obiageli Igbosuah (Mrs)

B.Sc (Ife), M.Sc (Lagos)

Department of Biological Sciences,

University of Lagos,

Lagos,

NIGERIA.

JANUARY, 1983.

C E R T I F I C A T I O N

This is to certify that the works recorded in this thesis were carried out by the Student, Mrs. E. O. Igbosuah, under the supervision of Dr. (Mrs.) A. E. Uduebo, at the Department of Biological Sciences, University of Lagos, Lagos.

.....E.O.Igbosuah.....
IGBOSUAH E. O. (MRS.)

.....A.E.Uduebo.....
DR. (MRS.) A. E. UDUEBO

.....M.A.Balogun.....
HEAD OF DEPARTMENT OF BIOLOGICAL SCIENCES

CERTIFICATION

We certify that the thesis titled "Studies on the Rhizosphere Mycoflora of Celosia argentea (L) and Hibiscus esculentus (L)" submitted for the degree of Doctor of Philosophy by Mrs E. O. Igbosuah in Botany Unit of the Department of Biological Sciences has been examined by us and recommended for the award of Doctor of Philosophy degree of the University of Lagos.

(M.O. FAWOLE
External Examiner

8th March, 1984.
Date

A Enwueto
Internal Examiner

8TH MARCH, 1984
Date

Internal Examiner

8th March 1984
Date

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

I am very grateful to my Supervisor, Dr. (Mrs.) A. E. Uduebo, who suggested the problem and whose guidance and helpful criticisms have been of great assistance. I wish to express my gratitude to Dr. Olu Odeyemi, of the Department of Microbiology, University of Ife, Ile-Ife for the enthusiastic support he has given this project and for supplying the fungicides.

My thanks also go to the entire members of the staff of the Department of Biological Sciences especially Dr. A. B. Ogunkanmi, for useful advice on various biochemical problems, Dr. (Mrs) T.V.I. Akpata and Prof. T. O. Orebamjo for many assistance and encouragement. I am also very grateful to my husband, Mr. J. U. Igbosuah for his patience and understanding.

I thank the University of Lagos for a two-year bursary, awarded to me for the course. I gratefully acknowledge the skill and patience of Mr. M. A. Nofiu in typing the thesis.

D E D I C A T I O N

This work is dedicated to my daughter, Anwuli and to the memory of my dad - Mr. C.M. Ugoji, who died during the preparation of this thesis.

ABSTRACT

Rhizosphere studies of two local green vegetables, Celosia argentea Linn and Hibiscus esculentus Linn (new name Abelmoschus esculentus) were studied from vegetative stage to senescence.

The viable seeds of both vegetables were collected and planted on garden beds made from sandy loam soil. Samples were collected at two-week intervals at 9.00 hrs in the morning. Morphologically, A. esculentus (okra) had 3 leaves with more dense rooting system at 2 weeks than C. argentea (soko) with 6 leaves. Both plants appeared to reach maturity at the 14th week after planting.

Both plants encouraged the growth of fungi and bacteria in the vicinity of their roots due to their secretions into the soil. In the 14th and 16th weeks of growth however, when the plants fruits were fully developed, fungal and bacterial populations were repressed.

Twenty species of fungi belonging to 9 genera were isolated from non-rhizosphere soil, 14 and 10 species respectively from the rhizosphere and rhizoplane of okra while 12 and 9 were obtained from the rhizosphere and rhizoplane of soko. Aspergillus was the dorminant genus isolated. Other genera include Penicillium, Trichoderma, Fusarium, Curvularia, Rhizoctonia, Gliocladium, Myrothecium Scolecobasidium, Botryodiplodia and sterilia mycelia.

Biochemical studies revealed that no sugars were detected chromatographically from any of the root exudates. Okra exudate was however found to contain alanine and glycine while 'soko' exudate contained aspartic acid, glutamine and an unidentified amino acid.

Most of the isolated fungi were found to grow together in petri dishes without any inhibitions. Trichoderma harzianum Rifai however was found to be antagonistic to Rhizoctonia spp.

Studies on the activities of some fungicides showed that thiram (arasan) which was most effective in reducing microbial population in the soil persisted in the garden soil for 35 days after application.

A few of the isolated fungi were found in nutritional experiments to utilize carboxy methyl cellulose (CMC) as the sole carbon source.

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

TABLE OF CONTENTS

INTRODUCTION

In 1904, Hiltner introduced the term "rhizosphere" to designate that portion of the soil which is subject to the influence of the plant root system, and noted that this soil supported greater microbial activity than soil more distant from the roots.

The term rhizosphere, in general describes that portion of the soil environment which is adjacent to the root system of a plant and is influenced by it. The most satisfactory definition of rhizosphere appears to be that given by Clark (1949). He used the term "rhizosphere" to denote the external surfaces of plant roots, together with any closely adhering particles of soil or debris. The root surface is referred to as the rhizoplane.

One of the most important considerations in the study of the micro-organisms of the rhizosphere is obviously that concerned with the extent of the zone of root influence. It is expected that the effects of root growth and physiology will diminish with distance from this organ.

That bacteria, actinomycetes and fungi of the soil find the root zone a more congenial environment for development than soil away from the root is now generally accepted. As may be expected, numbers of organisms in the root zone are influenced by many factors: nature and age (or stage of growth) of plant; type, moisture content, reaction and treatment of the soil. Frequently, it is impossible, however, to determine which of these phenomena is responsible for the observed effects. Soil treatment, for example may stimulate plant growth which in turn, may cause an increase in numbers of organisms on the root surface (Starkey, 1932).

In so far as the major groups of soil microorganisms are concerned, different plants exert considerably different effects which are also influenced by different stages of growth (Starkey, 1932). Adati (1939) made an extensive study of numbers of bacteria, actinomycetes and fungi in the rhizospheres of different crops growing in sand, loam, clay and humus soils. Counts were given for control soil, for rhizosphere soil directly surrounding the roots and for soil at unspecified distances from them. Much higher numbers of all organisms were found in the rhizosphere directly adjacent to the root, decreasing with distance.

Most of the known 100,000 fungal species are strictly saprophytic. About 50 species can cause diseases in man, and about as many cause diseases in animals while about 8,000 species can cause diseases in plants (Agrois, 1969). Available information on the fungi isolated from Nigerian soil was obtained from the study of pathogenic root-infecting fungi on crops like cowpea (Ezedinma, 1960); tomato (Adeniyi, 1966); cocoa (Westeijn, 1967); groundnut and wheat (Giha, 1976). Groundnut fruit infection has been studied at various stages of growth (McDonald, 1968; 1969; 1970 a and b).

There has been sustained interest in the rhizosphere studies because the interaction between the plant and micro-organisms has a considerable significance for crop production and soil fertility. The plant roots create a rich habitat for the microorganisms. The plant in turn, is markedly affected by the populations it has stimulated since the root is the site for the absorption of inorganic nutrients and through which many pathogens penetrate (Lundergardh, 1945).

Odunfa and Oso (1979) found a high proportion of Rhizopus, Mucor, Pythium and a few other species of phycomycetes on the rhizoplane of plant roots. They concluded that these fungi were frequently associated with the utilization of sugars which are known to be exuded more abundantly at the seedling stage of plant growth.

Root exudation is a phenomenon common to all higher plants, for, a plant which did not exude substances would have no rhizosphere except possibly for that resulting from the stimulatory effect due to thigmotropism.

Exudates may inhibit plant pathogen by supporting an antagonistic microflora. It is suspected that there may be in the rhizospheres of many plants an element of the microflora which is antagonistic to any given pathogen. Its significance in inhibiting disease development would depend to a large extent on whether or not the chemical and biological environment of the rhizosphere was favoured for its growth and multiplication. If development of antagonists are favoured, their activities might result in the repression of disease.

Newman and Watson (1977) used the theory of the involvement of numerous factors to produce a computer model of the rhizosphere. The rate of change of substrate ($\delta s/\delta t$) within this region was assumed to be composed of factors governed by diffusion, microbial growth and maintenance of the indigenous supply of substrate from the soil. They arrived at the following equation.

$$\frac{\delta s}{\delta t} = \frac{D\delta}{r\delta r}\left\{ r\frac{\delta s}{\delta t} - \frac{\mu max}{y}\right\} - \left\{\frac{Xs}{s+K_s\theta}\right\} + mx_o$$

where D is the diffusion coefficient of the substrate in soil ($cm^2\ sec^{-1}$), r is the radial distance from the root axis (cm), max, the maximum specific growth rate (h^{-1}), y, the growth

yield, x (x_0= initially) the microbial concentration in soil, ($\mu g\ cm^{-3}$), K_s, the Michaelis constant ($\mu g\ cm^{-3}$), θ the water content of the soil ($cm^3\ cm^{-3}$) and m, the maintenance coefficient (g substrate g^{-1}dry wt.h^{-1}). With the model, the effect of variations in the assumed values of the characteristics could be assessed.

In the rhizosphere and rhizoplane, several relationships between macro and micro-organisms can be recognized. Pathogenic relationships are not uncommon because roots are attacked by a number of fungi, bacteria and nematodes.

In the last 10 - 20 years, soil microbiologists have been concerned not only with estimating population and identifying isolates of the microflora of the soil but also with the biological basis of production. Estermann and McLaren (1961) consider that the rhizosphere microflora may favour plant development by degrading organic phosphorus substrates and making phosphorus available to the plant. They also think that the plant may benefit from nitrogen transformations in the rhizosphere. Other ways by which micro-organisms can benefit the plant include deammination of nitrogenous compounds and liberation of ammonia which could be taken up by the plant. If a mineral that is available in limited supply is removed from the soil by the plant during active growth, some fungi may suffer as a result.

It is known that several bacteria, actinomycetes and fungi produce in culture at least considerable amounts of growth substances that have an effect on plant development. The products excreted by roots of plants growing under aseptic conditions have been widely studied and the compounds found include amino acids, auxins, carbohydrates, enzymes, flavones, growth factors, nucleic acid derivatives and organic acids (Schroth and Snyder, 1961; Buxton, 1962; Sulochana, 1962). Increase in plant

growth due to production of auxin by soil organisms have been shown by McManus (1960). These auxins, beyond a certain threshold become inhibitory and stop root growth (Katznelson and Sirois, 1961).

Biological control of soil-borne plant pathogens by the addition of antagonistic micro-organisms to the soil is now recognised as a potential non-chemical means for plant disease control. The species of Trichoderma capable of hyper-parasitizing pathogenic fungi are highly efficient antagonists (Barnett and Binder, 1973). Weindling and Fawcett (1936), showed that when Trichoderma lignorum Tode was added to sterilized soil simultaneously with the pathogen Rhizoctonia solani Kuhn, no damping off of citrus seedlings occured. Harder, Chet and Henis (1979) have shown that T. harzianum directly attacked R. solani mycelium. They attributed the control to a thermolabile, alkali - unstable toxic substance produced by the antagonists. Numerous other instances of this type of biological control of plant disease have been reported by Allen and Haensler (1935) and Anwar (1949).

In recent years, bacteria and active substances from bacterial cultures have been used successfully to control several crop pathogens most notably Fusarium (Michael and Nelson, 1972). There is evidence that antibiotics are sythesized as a result of some interactions between micro-organisms in soil (Gottlieb and Siminoff, 1952). Gottlieb, Siminoff and Martin (1952) reported that actidione (a neutral compound) is an antifungal agent and clavacin, an acidic material, which inhibits both bacteria and fungi.

Micro-organisms can often be as effective as, or even better than chemicals in preventing infection of plants (Corke and Rishbeth, 1980). They change the ecological balance so that some other species can take advantage of the lack of competition and become dominant or pathogenic (Hislop, 1976).

Presently, fungicides play an essential role in the production of agricultural crops, in industrial production, and in prolonging the utility of manufactured products. It has been estimated that fungicides are employed in the growth of one-half of the world's crops (Ordish and Mitchell, 1967). Loss of food fiber and ornamental crops through the action of plant pathogens are held to a minimum of 3.3 billion dollars annually in the United States through the use of fungicides (Le Clerg, 1964).

The increase in food production for the enlarging world population will have to come from intensified agricultural practices because most tillable land is being farmed already. Farming in the temperate zones is intensive, with chemical control methods widely employed in the production of food staples. This is not so in the equatorial and tropical regions.

The ever - increasing use of pesticides in agricultural economy and in public health necessitates an investigation into the biological effects of these pesticides on soil micro-flora. This is because a compound that enhances or suppresses carbon mineralization would make its presence known by its faster or slower pace of destruction of native soil, organic matter or plant residues. A compound inhibitory to the nitrifying bacteria causes a decrease in the rate of nitrate formation. Each pesticide to be used therefore must be carefully examined to determine whether it is harmful to soil micro-organisms which are responsible for soil fertility, increased crop yields, development of pest-free plants and other vital processes in the soil that are possible because of the presence of micro-organisms.

It is worthwhile to focus effort on major classes of widely disseminated chemicals in Nigeria especially those where evidence of toxigenicity and bioaccumulation exists as in the case of arasan (Odeyemi, 1979). Since 1927, when some workers patented the use of dithiocarbamates as fungicides and insecticides, this group of organic compounds has played a very important role in the fight against harmful fungi in industry. The compounds are characterized by their high toxicity towards fungi even in minute concentrations while most of them are comparatively harmless to plants (Goldsworthy, Green and Smith, 1943). The possibility of improving agricultural production of A. esculentus and C. argentea would be by studying the effectiveness of some commonly used soil fungicides in controlling soil-borne pathogens in the neighbourhood of their roots.

Pesticide residues in soil may pose several problems to agriculture. Conceivable effects of pesticide residues in soil are injurious to crops grown in later years, production of illegal residues in crops that absorb them or harmful effects on living organisms in the soil. The largest amounts of residues usually result from contamination after crops are directly sprayed or from applying pesticides directly to the soil.

There is a tremendous variation in the rate of decomposition of fungicide by soil microbes. The fungicide may remain active in soil only for one week or as long as a year or even more. The time of effectiveness depends upon the chemical, the rate and method of application and the specific condition of the environment (Schreven, Lindenberg and Koridon, 1970).

Generally, in living plant tissues, cellulolytic enzymes secreted by pathogens play a role in the softening or disintegration of cell wall material and microfibrils. They facilitate the penetration and spread of the pathogen in the host and cause the collapse and disintegration of the cellular structure,

thereby aiding the pathogen in the production of disease. Cellulolytic enzymes may further participate indirectly in disease development by releasing from cellulose chains soluble cellosacharides which serve as food for the pathogen and in the vascular diseases by liberating into the transpiration stream, large molecules of cellulose which interfere with the normal movement of water.

Two widely grown Nigerian vegetables Abelmoschus esculentus (okra) and Celosia argentea (soko) provide the materials for this investigation. Vegetables are generally of high nutritional value. They contain water 88%, carbohydrates 9%, proteins 2% fat 0.3%, ash 0.8%, minerals and vitamins (Jay, 1978).

Vegetables have been described as edible plant products that may be eaten in raw state or in cooked form. The soft edible parts of vegetables include the leaves, petioles, stems, roots, rhizomes, bulbs, tubers, inflorescence, seeds and fruits (Okigbo, 1975). The importance of vegetables lie in their nutritional value as well as their source of energy. The mineral content of vegetables is understandingly high when compared to those of other foods that are not of plant origin (Oke, 1966).

Most perishable vegetables contain sufficient nutrients to support the growth of microorganisms. Therefore, the presence of micro-organisms may lead to reduction in the nutritive values of these vegetables (Rhodes, 1978).

The amino acids in plants may occur in the free form in solution or be chemically combined with protein molecule. The protein-bound amino acids are of nutritive importance for the determination of the quantity and quality of the protein.

Maturation of a plant is followed by senescence, a series of deteriorative events which preceed the death of a mature cell and is triggered by flowering. Many tropical leafy vegetables

have a high content of crude fibre or carbohydrates which tend to increase with age of the plants.

The two vegetable plants A. esculentus (Malvaceae) and C. argentea (Amaranthaceae) were chosen because of their nutritional value, common occurrence and domestic uses. A. esculentrus is grown as a vegetable on almost all West African farms because of its considerable food value. The leaves are used as spinach, leaves and stems as fodder for sheep and goats. The stem contains a fibre of considerable strength which may be used for domestic purposes such as fish lines, traps and hammocks. A preparation of okra can be used as a blood plasma replacement.

Celosia argentea occurs in warm countries of the tropics and sub-tropics. It is an erect, branched annual herb with green leaves. The leaves are linear to lanceolate and sometimes variegated. It is cultivated not only as an ornamental but also as a vegetable (Thonner, 1962). In Nigeria, it is used as a soup vegetable, commonly referred to as 'Soko' by the Yorubas and 'Elimonu' by the Ibos. It has perianth segments which are 6-10 mm long, style 3-7mm long with simple spikes which are continuous with very dense flowers.

Experiments are designed to reveal:-

i. the influence of the two plants on the biology and biochemistry of their rhizospheres,

ii. Associations between plant and the micro-organism communities of each rhizosphere;

iii. Associations between plant and the organic matter components of each rhizosphere;

iv. possible influences of the exudates of the plants on specific micro-organisms;

v. the biological control of micro-organisms compared to that of selected fungicides.

Finally an attempt will be made to study the cellulolytic activities of some of the fungal isolates.

MATERIALS AND METHODS

a. SOIL SAMPLE:

The soil used in this study was obtained from a plot in the Botanical Garden of the University of Lagos, Lagos, which has been continuously used for the past few years in raising vegetables. The soil samples (depth 0-15cm) were carefully collected, avoiding agitation as much as possible. This is because agitation caused marked microbial alterations (Alexander, 1977). The soil was then stored at - 4°C prior to chemical analysis.

b. EXPERIMENTAL PLANTS:

The two plants used were Abelmoschus esculentus (okra) and celosia argentea (Soko).

c. PLANTING AND SAMPLING:

Seeds of the test plants were obtained from the Lagos State Ministry of Agriculture and Natural Resources, Agege, Lagos. Before planting, they were surface sterilized for 10 seconds in 95% ethanol and for 1 minute in a 0.1% mercuric chloride solution. The seeds were then rinsed in three changes of sterile distilled water and dried for 30 minutes at 27°C. In order to estimate mycoflora present on seeds, they were treated with some fungicides. Thiram was applied to slightly moistened seeds at the rate of 5.0 mg g^{-1} of seed. Seeds not treated with fungicides served as controls. The fungicide- treated seeds were left at room temperature for about 3 h to allow the seeds to dry before planting. Five seeds of okra and 20 of 'Soko' were planted in sterile and non-sterile garden soil in plastic cups. Each treatment for the two seeds were replicated 5 times. The soil was watered with sterile

distilled water daily for 9 days. The cups were placed in disinfected polythene bags and placed on a laboratory bench. Mycoflora present on ungerminated seeds were then estimated by placing the seeds on solid agar medium in Petri dishes.

To confirm that seeds were free of contamination they were plated in Petri dishes. The alcohol-sterilized seeds were planted at the rate of 4 seeds per hole 30 cm. apart on garden beds except otherwise specified. A randomized block design was used throughout. The soil was watered with tap water daily. Sampling was carried out fortnightly.

In order to assess the effect of root exudates on the microbial population at various distances from the primary root surface of the plants, ten-day old seedlings were used. Fungal and bacterial populations in 1g of soil sample obtained (Fig.1) were estimated by the Dilution Plate Counting Method (Warcup, 1950)

d. STERILIZING TECHNIQUES:

Conical flasks, McCartney bottles, measuring cylinders, filter paper and sand were sterilized by autoclaving for 15 minutes at 1.06kg/cm^2 steam pressure at 121^{o}C except otherwise stated. Cotton wool plugs were temporarily covered with aluminium foil to prevent wetting by the condensed water during autoclaving. Petri dishes and pipettes were sterilized at 160^{o}C for 5h in the oven. Forceps, inoculating needles and loops were dipped in 95% ethanol, flamed to red-hot and air-cooled before use. The plastic cups with an upper and lower diameter of 8 and 6 cm respectively and of length 9.5cm, used for incubating fungicide - treated soil were, surface - sterilized by wiping them with 95% ethanol. The ultra-violet room where most of the work was carried out was exposed to UV light (300nm wave-length) for 2h before use. Sterilized media were poured thinly into sterile Petri dishes.

FIG.1. SKETCH SHOWING HOW SOIL WAS OBTAINED FOR RHIZOSPHERE STUDIES.

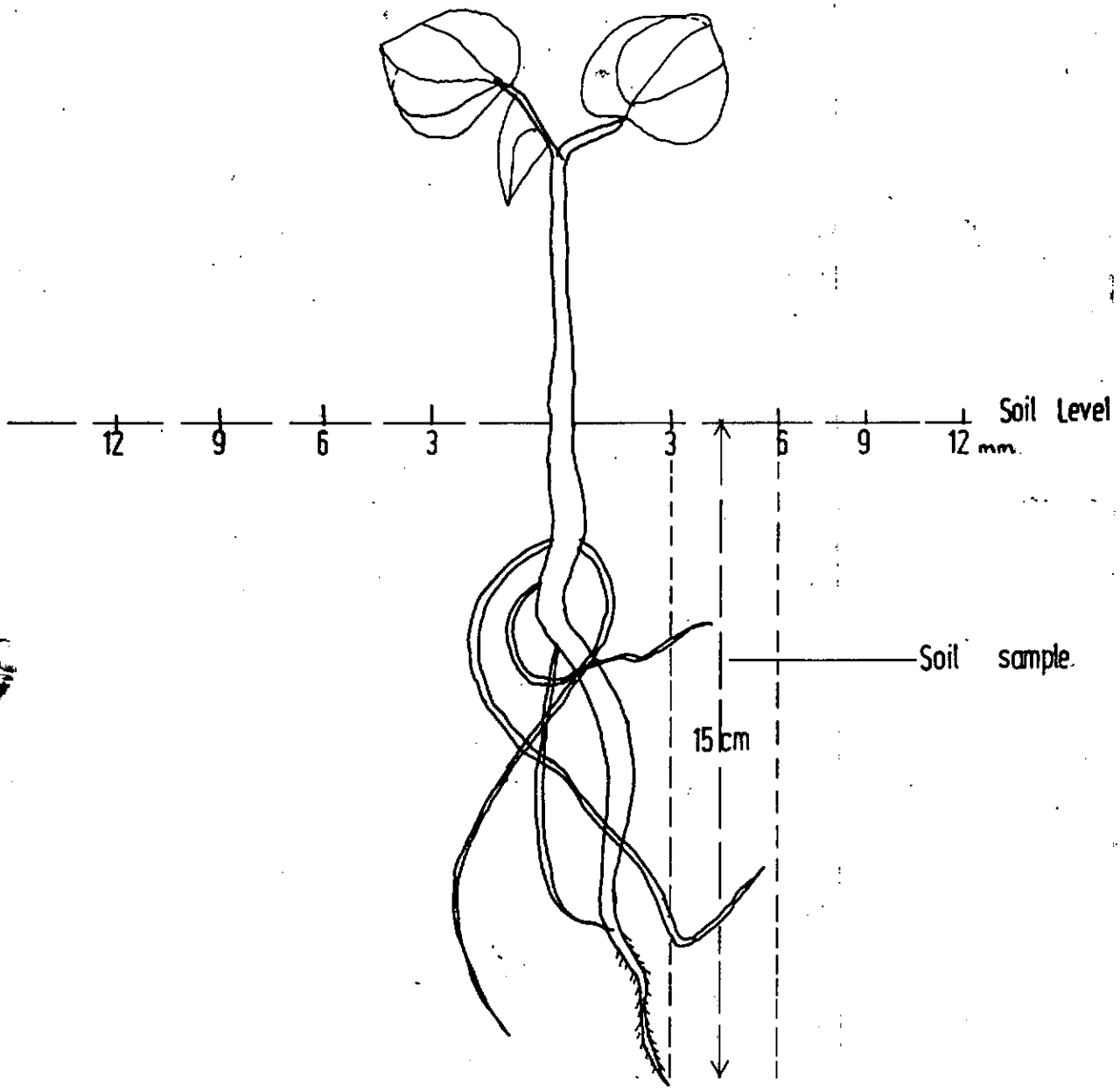


FIG 1

e. CULTURE MEDIA:

Nutrient media particularly suitable for the development of fungi and bacteria were selected. Nutrient agar (NA) and Czapek dox agar (CDA) were found to encourage growth of bacteria and fungi from the soil. It was later found necessary to make the media for fungi as unsuitable as possible for bacteria so that bacterial colonies do not interfere with the development and counting of fungal colonies. This was achieved by the addition aseptically of 2 drops of 'Analar' lactic acid (88%) to 100cm^3 of the sterilized medium with a sterile 1 ml pipette to give a pH of 4 - 4.5.

The following media available in powder form from Oxoid Ltd.— Corn Meal Agar, Czapek Dox Agar, Malt Extract broth, Water Agar, Carboxy Methyl Cellulose Agar (from E. Merck) were used for fungi while Nutrient Agar was used for bacteria. In each case 500 cm^3 was prepared. Other media (with their composition (wt/vol.) shown were prepared and used for fungal cultures also.

Potato Dextrose Agar:

Potato 100g

Dextrose 10g

Agar 10g

Distilled water 500cm^3

The potato was cut into small pieces, covered with water and boiled gently for half an hour. It was cooled and allowed to settle. The supernatant liquid was poured off and made up to 500cm^3. The dextrose and agar were added, heating and stirring until dissolved. It was then autoclaved.

SOIL AGAR:

Garden Soil	200 g
Tap Water	500 cm^3
Agar	7.5 g

The soil was boiled gently in water for 1h. It was filtered with filter paper and made up to 500 cm^3. The agar was added while stirring on a magnetic heater. It was then autoclaved.

CZAPEK DOX AGAR CONSTITUENTS:

Sodium nitrate	2.0g
Potassium chloride	0.5g
Magnesium sulphate	0.5g
Potassium phosphate	0.35g
Ferrous sulphate	0.01g
Sucrose	30.0g
Agar	12.0g
PH 6.8	

FILTER PAPER MEDIUM (PARK, 1976)

Ammonium Sulphate	2.0g
Magnesium sulphate	0.15g
Dipotassium hydrogen phosphate	0.15g
Potassium chloride	0.16g
Filter paper strips	2.0g
Distilled water	1000 cm^3

Fifty millilitre aliquots of the filter paper medium were dispensed into 250 cm^3 Erhlenmeyer flasks. Filter paper strips 0.1g dry weight were immersed into the flasks before autoclaving.

All media were sterilized by autoclaving for 15 minutes at 1.06kg/cm^2 steam pressure at 121^{o}C.

f. ISOLATION OF MICROBES FROM RHIZOSPHERES OF THE TWO PLANTS:

One gram of the non-rhizosphere and rhizosphere soil samples were each shaken up in 10cm^3 distilled water in a flask shaker for 30 minutes at an excursion of 2cm. Three subsequent dilutions were made for bacteria while two were made for fungal counts. Dilutions were done progressively with the sterile shake medium and aliquots removed for analysis. An agar plate was made by pouring sterile molten medium at 45^{o}C into sterile Petri dish.

Counts of soil microbes were made by spreading 0.1cm^3 portions of the final dilutions on the surfaces of hardened agar plates. Plates for counts of fungi were incubated for 5 days at 27^{o}C and for total bacterial counts for 36h.

To obtain microbes growing on the root surfaces, root pieces were shaken vigorously in flasks containing sterile distilled water for 5 minutes. They were again immersed in flasks containing 100 cm^3 sterile distilled water. The flasks were shaken for 20 minutes at an excursion of 2 cm. The root pieces were then subjected to a series of three washings. After washing excess surface moisture was removed from the roots by blotting with sterile filter paper. The roots were then cut aseptically into segments, 5mm long and placed on agar media in Petri dishes at 27^{o}C for 5 days.

A set of fungal isolates maintained on Czapek Dox Agar (CDA) slants in McCartney bottles was stored at -4^{o}C while duplicates were sent to Commonwealth Mycological Institute, Kew, Surrey, England for identification.

g. MEASUREMENT OF RELATIVE HUMIDITY (RH).

The relative humidity in the field was recorded daily at noon throughout the period of study, with a psychrometer after allowing 5 minutes for equilibration.

h. FUNGICIDES:

i. Source and quantity used.

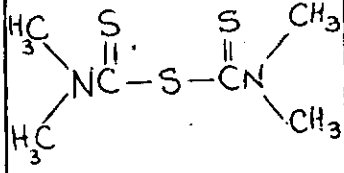
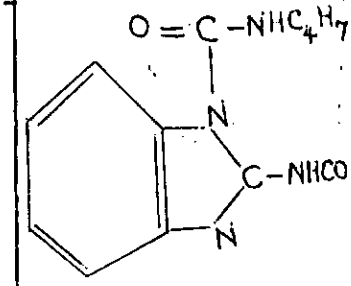
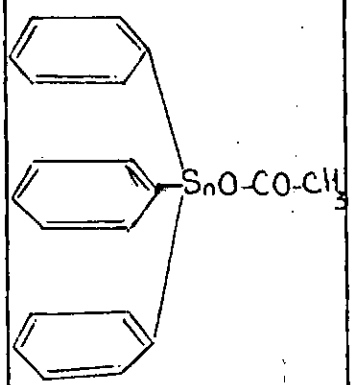
The four fungicides used were kindly supplied by Dr. Olu Odeyemi, Department of Microbiology, University of Ife, Ile-Ife, Nigeria. Table 1 shows the trade names, chemical names - chemical structures and uses of the fungicides investigated.

Known weights of soil brought from locations were, mixed with fungicides as in Horst and Williamson (1974). Benlate of weight 0.00045g was added to $1cm^3$ of water and since $1g = 10^6 \mu g$, $450\mu g$ of benlate was applied per gram of soil. Brestan with application rate of 0.16g per 200 cm^3 of water was applied as $800\mu g$ per gram of soil. Arasan (thiram) was applied at the rate of $100\mu g/g$ of soil. The flasks were then incubated at $27^{\circ}C \pm 2^{\circ}$. Rate of field application of arasan is 0.1g powder/kg of soil which is equivalent to 100 $\mu g/g$ (McKeen, 1950).

h(ii) CHEMICAL ASSAY OF THIRAM:

Four flasks (2 sterilized and 2 unsterilized samples) were analysed for thiram content immediately for the zero day determination. Subsequently, sampling was made at weekly intervals. Thiram content of each flask was extracted 3 times with a total of $100cm^3$ volume of chloroform by means of a $500cm^3$ separating funnel. Excess cuprous iodide was added to the extract to produce a yellowish brown colour.

TABLE 1: CHEMICAL STRUCTURES AND SOME USES OF THE FUNGICIDES USED (ANON, 1976)

COMMON NAMES	CHEMICAL NAMES	STRUCTURAL FORMULA	U S E S
Thiram, Arasan, Tersan, Pomarsol, Normersan	Tetramethyl thiram disulphide		Fungicide suitable for foliage application and for seed treatment, effective against "damping off" when applied to soil
Benlate Benomyl	Methyl-1-(butyl-carbomyl) benzimidazole -2-ylcarbamate.		Effective against a wide range of fungi affecting fruits, nuts, vegetables, field crops, turf and ornamentals. It is effective against mites primarily as an ovicide. It is also used as pre-and post harvest sprays or dips for the control of storage rots of fruits or vegetables
Brestan, Batason, SUZU, TPTA, Phentinoacetate.	Triphenyltin acetate		Non-systemic fungicides recommended for the control of potato blight, leaf spot of sugar beet and celery. It is also used as algicide, molluscide It controls early and late blight of potatoes, Cercospora species on sugar beets, coffee and groundnut; Phytophthora on cocoa etc.

This colorimetric technique was based on the formation of a brown coloured chloroform-soluble copper salt of dialkyldithiocarbamate when an organic solvent solution of thiram is treated with cuprous iodide (Keppel, 1956). Optical density was measured at a wavelength of 420nm, with a spectrophotometer, model Spectronic 20. By means of a standard curve, the residual thiram in the sample was determine. The standard curve was obtained as follows:- a 0.025% stock solution of the thiram was prepared by dissolving 0.025g of thiram in 100cm^3 of chloroform in a volumetric flask. This concentration was equivalent to 250ug of thiram/cm^3 of chloroform. Using 10cm^3 and 1ml pipettes, the following quantities were carefully transferred into 25cm^3 volumetric flasks from the stock solution and were made to mark with chloroform; 0.1cm^3, 0.3cm^3, 0.5cm^3, 0.8cm^3, 2cm^3, 6cm^3, 8cm^3 and 10cm^3. Excess cuprous iodide was then added to the flasks and left to stand for about ten minutes. A colorimetric method was used for chemical determination of thiram. When the optical density of the solution became too high and could not be read on the instrument, a 10:1 dilution as made with chloroform. The standard curve of optical density (OD) against concentration was made (Fig.2).

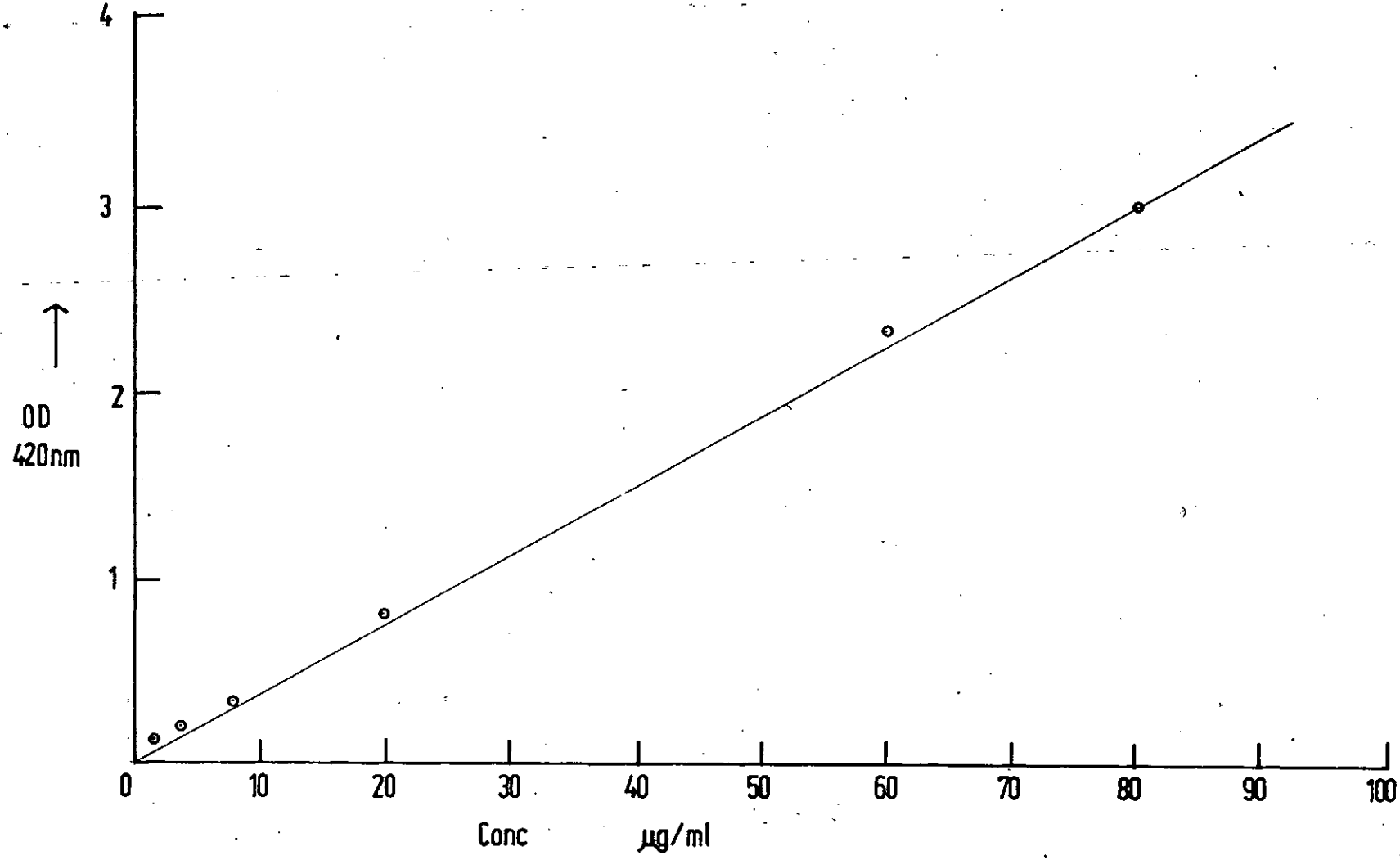


FIG 2 THIRAM STANDARD CURVE

-20-

h(iii) DETERMINATION OF SOIL MICROBIAL POPULATIONS:

The recommended application rates of the fungicides (Horst and Williamson, 1974) were carefully weighed and transferred quantitatively to a known amount of soil sample. The mixture in the plastic cup was mixed throughly to ensure homogeneity. The soil sample was moistened with sterile distilled water before adding fungicides to enhance microbial activity. The mixture was then incubated for 48h. Fungicide - free soil samples moistened with sterile distilled water served as the control. The microbial populations in 1g of each treated soil sample was determined by the Dilution plate counting method (Warcup, 1950).

i. ESTIMATION OF MICROBIAL POPULATIONS IN RHIZOSPHERE AND NON-RHIZOSPHERE SOIL SAMPLES

At sampling time, non-rhizosphere soil was collected by removing 6cm core of soil with a sterile cork borer No.10 from root-free zone. To obtain rhizosphere soil, 5 plants were uprooted and their shoots were cut off by means of a sterilized scalpel. The root systems were then transferred to disinfected polythene bags and taken to the UV room in the laboratory. The roots were gently shaken to remove superfluous soil, while the closely adhering soil which served as the rhizosphere soil sample was shaken onto sterile Petri dishes. Samples collected were then bulked together throughly mixed, air-dried and sieved through 2mm sieve. Non-rhizosphere soil sample or control soil was obtained on an adjacent bed in the garden with no vegetables sown.

Dilution plate counting method of Warcup (1950) was used as in 'f' to estimate microbial population.

j. COLLECTION AND CONCENTRATION OF ROOT EXUDATES:

This was achieved by the sterile sand culture technique. Artificial rhizosphere was prepared by sowing the seedling in coarse sand in the laboratory. Prior to sowing, the sand was washed in running tap water for 2h.; concentrated H_2SO_4 was added to it and the sand was again washed until traces of the acid were eliminated. It was finally washed with distilled water. The pH of the sand was measured by means of a Pusl L pH meter. Four hundred gram portions of the sand were put in separate beakers and sterilized in the oven.

Meanwhile healthy and surface sterilized seeds were grown on Whatman No.1 filter paper for 3 days. 10 sterile germinating seedlings were selected and transferred aseptically to each of the beakers containing sterile sand wet with 60cm^3 sterile distilled water. Sterile water (5cm^3) was added at 2-day interval. At 5-day intervals for 20 days, roots were harvested, washed in 20cm^3 sterile distilled water, bulked with soil washings and filtered asceptically through filter paper. There were 6 replicates for each plant. One hundred and fifty cm^3 of the supernatant was used for sugar, while the remaining 150cm^3 was used for amino acid bioassays.

k. BIO-ASSAY OF SUGAR CONSTITUENT

The pH of 150cm^3 of the supernatant was adjusted to 5.0 with IN HCL. It was then evaporated in a rotary evaporator *in vacuo* at 40°C. The remaining fraction in the flask was dissolved in 80% ethanol and passed through 10cm columns of ion-exchange resins.: Amberlite IR-120 (H^+cycle) and Amberlite IRA 400 (OH^- cycle) for cation and anion removal respectively (White and Hess, 1956). This was again evaporated in vacuo and redissolved in a small quantity of ether. The residue

was taken up in a small quantity of ether, spotted along with other reference sugars at 2.5cm intervals onto Whatman No.1 filter paper and developed in n-butanol-acetic acid-water (5:1:2) $^{v}/_{v}$ for 18h. The chromatograms were developed by drawing the papers through alkaline silver nitrate solution (Trevelyan, Procter and Harrison, 1950).

l. BIO-ASSAY OF AMINO ACID CONSTITUENTS:

The remaining 150cm^3 of the root exudate were passed through Amberlite IR 120 (H^+ cycle) and the column was washed with water. The amino acids were eluted with 3 N NH_4OH using the ninhydrin test to indicate complete elution (Redfield, 1953). Excess ammonia was removed by concentration in vacuo and the residue dissolved in 10cm^{3}water and passed through a column of Amberlite IRA 400 (OH^-cycle). The amino acids were then eluted with concentrated NH_4OH (Clarkson and Kench, 1956). Five hundred milligrams of the reference amino acids were dissolved in 100 cm^3 water. For those that did not dissolve easily, a drop of sodium hydroxide (IN) was added. The developing medium was n-butanol - glacial acetic acid - water (4:1:5) $^{v}/_{v}$ for 7h. Ninhydrin spray (0.1% in n-butanol) was used, as the locating reagent. Identification of amino acids was done by comparing the Rf values of known amino acids run along with those of exudates.

m. THE EFFECT OF ROOT EXUDATES ON FUNGAL GROWTH:

The effect of root exudates on the linear growth of some isolated fungi was studied. The filtrate was divided into 2 equal parts thereafter designated as A and B and sterilized by the following methods.

Part A was immediately refiltered through a Seitz filter. This sterilized filtrate was supplemented with 1L of sterilized and cooled (40^{o}C) Czapek Dox Agar (CDA) medium from which about 15cm^3 was poured in each of the 50 replicate sterilized Petri dishes and allowed to solidify. Part B of the extract was

mixed with the ingredients of Czapek Dox medium for 1L and then mixed with distilled water to raise the volume to 1L. The medium was autoclaved for 20 minutes at 1.06 kg/cm^2 steam pressure at 121^{o}C and plates poured as above.

n. SELECTIVE ACTION OF FUNGICIDES ON SOME FUNGAL ISOLATES:

This was tried by using either the linear growth method (Brancato and Golding, 1953) and the dry weight method (Darby, 1960). For the linear growth, various dilutions of the fungicides used were incorporated into sterilized CDA medium. Five plates of each treatment were inoculated with 10mm hyphal disks of pure cultures of each test fungus and incubated at 27^{o}C. The colony diameters were measured daily for 7 days. Control treatments did not contain any fungicides in the medium.

In the fungicidal assay procedure of Darby (1960), sterilized malt extract broth containing different concentrations of fungicides was used. From the pure cultures of the fungi in the plates, 10mm hyphal disks of each test fungus was transferred aseptically to the flasks which were plugged with sterile cotton wool. The flasks were shaken and incubated in a shaking incubator at 50 rev/min for 9 days. Control treatments were without any fungicide. At the end of 9 days, the mycelia and spores were collected on known weights of filter paper, dried in an oven and weighed.

o. EFFECT OF T. HARZIANUM ON OTHER FUNGAL ISOLATES.

(i) ANTAGONISM IN CULTURE

In the laboratory tests, 6mm diameter of T. harzianum Rifai (MI 249180) were removed from the edge of a 6 - day old culture and placed on one side of a 90mm Petri dish containing 2% water agar (WA). Similar disks of other test fungi were placed on the opposite side of the Petri dish.

Each treatment was replicated 3 times. Cultures were observed daily to note any kind of antagonism. The following test fungi were used; F. solani, A. niger, F. oxysporum, A. tamarii, C. pallescens, A. ustus, A. carbonarius, B. theobromae, A. japonicus and Rhizoctonia spp. Control treatment was without T. harzianum.

(ii) GROWTH ON CELLOPHANE:

Cellophane disks, 9 cm in diameter, were made by cutting out a circular portion on folded sheets of commercial cellophane. The required number of disks were then placed singly in Petri dishes and autoclaved (Fleming and Smith, 1944). Afterwards, each disk was aseptically transferred with a forceps and laid on the surface of cold sterilized agar medium in another Petri dish.

Inoculum from a pure culture of T. harzianum was then placed on the centre of each cellophane disk and incubated at room temperature. After 3 days each disk was carefully removed from the surface of the medium and different fungi were inoculated on the plates and incubated as above. Controls were without T. harzianum on the cellophane disks.

p. CELLULOLYTIC ACTIVITIES OF ISOLATED FUNGI

The sole carbon source was carboxymethyl cellulose. A pH range of 4 - 8 was used either using 0.1N HCL or 0.1N NaOH to adjust to acidic or alkaline range. The poured plates were inoculated with fungal disks from the advancing edges of 4 - day old colonies. The linear growth and zone of clearing (if any) in each plate was measured daily.

The filter paper method (Park, 1976) was employed. The autoclaved flasks containing the filter paper medium were aseptically inoculated with 10mm hyphal disks of 4-day old pure cultures of the test fungi. There were four replicates.

Two sets were agitated in a shaker chamber at 50 rev/min while the remaining were kept still on a laboratory bench. At 12 days interval for 24 days, the filter paper strips were rinsed with distilled water to remove any adhering mycelia. The strips were dried in the oven at 80°C, placed in a dessicator to cool and weighed to constant weight. Percentage weight loss was then calculated.

RESULTS

SOIL SAMPLE:

The soil used throughout the study had the following characteristics: Sand 75%, Silt 16%, Clay 9%, N = 0.33% Cation Exchangeable Capacity 11.96 me/100g with pH value 6.8 (Table 2). It is a loamy sandy soil based on Salter and William's (1967) modified diagram.

EXPERIMENTAL PLANTS:

At two weeks, 'soko' had six leaves while okra had only three (Plate 1). Okra had more profuse root system than 'soko' and this contributed to the former supporting a greater population of microflora in the rhizosphere.

RELATIVE HUMIDITY:

The average relative humidity obtained in the course of this study was 75.29 Hm^{-26}. In the first and second weeks of measurements, the average was 90.25 and 92.0 respectively (Appendix 1). The lowest humidity of 52.0 was recorded in the 14th week of study.

RATE OF THIRAM DISAPPEARANCE IN SOIL:

The rate of disappearance of this fungicide was faster in unsterilized soil than the sterilized soil from the first week of assay. From the unsterilized, 57 µg/g of thiram was lost in the first week compared to 38µg/g from the sterilized sample. By the 28th day however, the difference in the residual fungicide in the two soil samples was quite small. On the 35th day thiram disappeared completely from both samples (Fig.3).

ESTIMATION OF SOIL MICROBIAL POPULATIONS:

Bacteria were found to be more abundant than fungi in the untreated soil. Thiram at minimum application rate of 100µg per gram of soil caused an immediate reduction in the number of bacteria and fungi. Benlate inhibited the growth of bacteria to an appreciable

TABLE 2: SOIL ANALYSES

	pH	Org C.	Total N	Av. P	Exchangeable Bases				Ex. Acidity	C.E.C.	Base Sat.	Extractable Micronutrient-				Moisture	Mechanical Analyses		
					Ca	Mg	Na	K				Mn	Fe	Cu	Zn		Sand	Silt	Clay
Lab Sample	H	%	%	ppm	me/100g	me/100g	me/100g	me/100g	me/100g	me/100g	%	ppm	ppm	ppm	ppm	%	%	%	%
796GS*	6.8	3.81	0.33	14.5	8.8	2.72	0.089	0.25	0.10	11.96	99.9	23.2	102.8	12.0	14.8	7.8	75	16	9

* GARDEN SOIL

C.E.C. Cation Exchangeable Capacity.

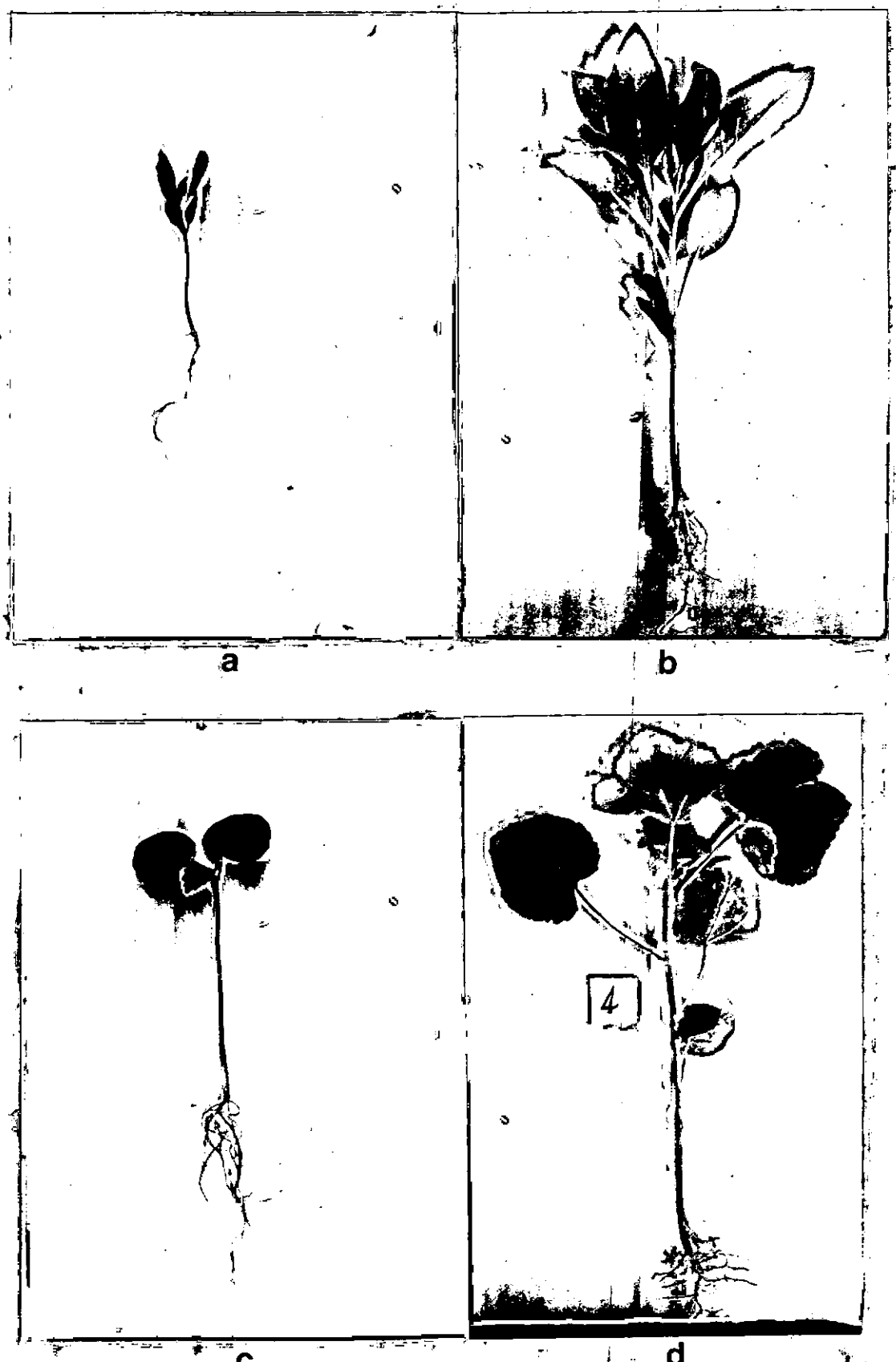
PLATE 1

a. C. ARGENTEA 2 - WEEK OLD

b. C. ARGENTEA 4 - WEEK OLD

c. H. ESCULENTUS 2 - WEEK OLD

d. H. ESCULENTUS 4 - WEEK OLD



a b c d

FIG. 3 CONCENTRATION OF THIRAM IN SOIL AMENDED WITH 100 µG/ML OF THE FUNGICIDE

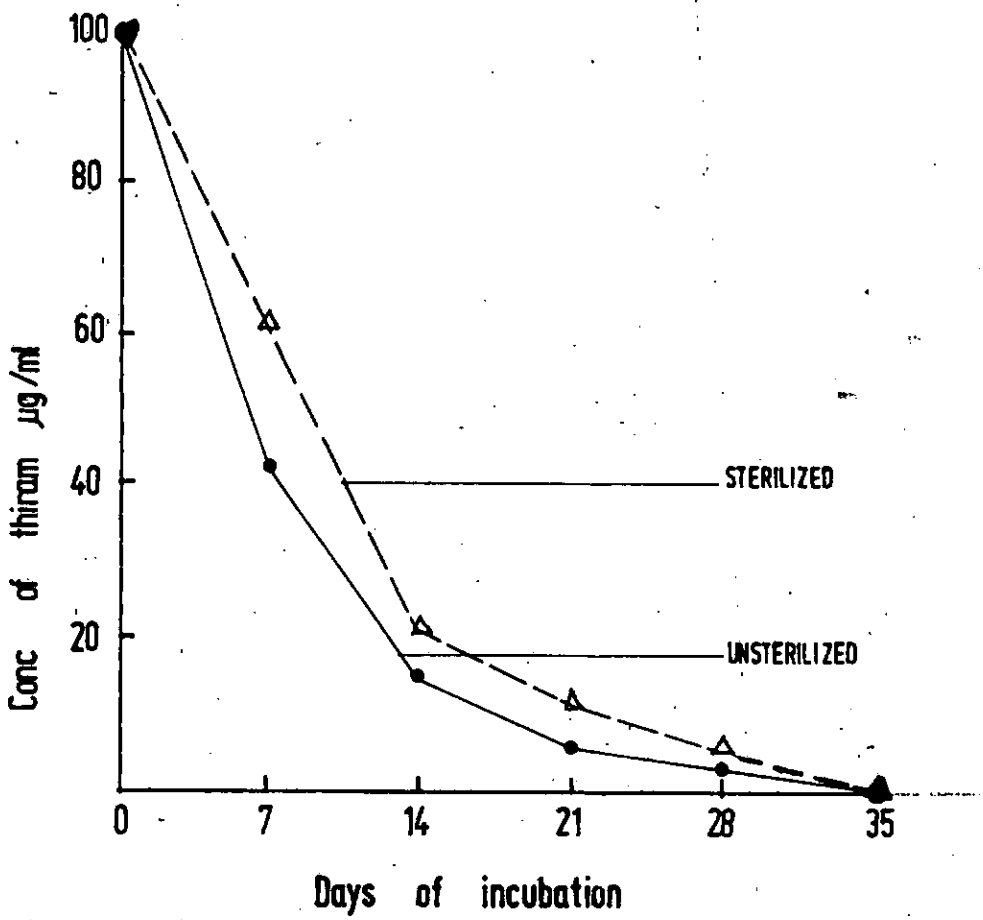

100
80
60
40
20
Conc of thiram µg/ml
STERILIZED
UNSTERILIZED
0
7
14
21
28
35
Days of incubation

FIG 3

extent, but had no effect on the fungal population. Bestan had no stimulatory or inhibitory effect on bacteria and fungi (Table 3).

The total numbers of fungi and bacteria isolated from the untreated garden soil, rhizosphere and rhizoplane of the test crops at different stages of growth are as shown (Tables 4&5). The following fungi were isolated: Aspergillus funiculosus Thom, A. carbonarius Bain, A. nidulans Winter, A. fumigatus Fresenius, A. niger van Tieghem, A. flavus Link ex Fries, A. tamarii Kita, A. ustus Banier A. sydowii Thom and Church, A. terreus Thom, A. japonicus Saito, A. flavipes. Thom and Church, A. quercinus Boedijn, Curvularia pallescens Boedijn, Cladospora cladosporoides de Vries, Paecilomyces variotii Bainier, P. lilacinum Samson, Trichoderma longibranchiatum Rifai, T. harzianum Rifai, T. koningii Oud, Fusarium equiseti Sacc, F solani Sacc F. oxysporum Schlecht, Gliocladium roseum Bain, Penicillium aculeatum Raper and Fennell, P. citrinum Thom, P. vinaceum Gilman and Abott, P. wortmannii Klocker, P. funiculosum Thom, P. janthinellum Biourge. P. jensenii Zaleski, Rhizoctonia spp Donk, Myrothecium cinctum Sacc, Scolecobasidium constrictum Abbott Botryodiplodia theobromae Pat and sterilia mycelia.

Pre-emergence treatment of the seeds with fungicides sown in sterilized and unsterilized soil showed that thiram was most effective in reducing mycoflora. The % germination of okra and 'soko' seeds treated with thiram was higher than those treated with benlate, brestan and the untreated (Table 6). Occurrence of mycoflora associated with ungerminated seeds after fungicidal treatment is as shown (Tables 7 & 8). Growth was much greater in unsterilized than in sterilized soil.

TABLE 3: Comparative Numbers of Fungi and Bacteria per gram of air-dried Soil following Fungicide Treatment.

Soil treatment	Amt. of pesticide µg/g of soil	No. of bacteria	No. of Fungi
Control	0	2.1×10^6	1.1×10^4
Thiram	100	2.6×10^4	4.5×10^3
Benlate	450	0.8×10^5	2.5×10^4
Brestan	800	1.7×10^6	2.2×10^4

TABLE 4: FUNGAL ISOLATES FROM THE RHIZOSPHERE AND RHIZOPLANES OF A. ESCULENTUS AND C. ARGENTEA.

SOIL		TOTAL NO. OF FUNGAL ISOLATES									
	wk	2	4	6	8	10	12	14	16	18	20
A. esculentus	rhizosphere	14	10	10	19	12	10	9	18	9	7
	rhizoplane	6	5	10	8	5	5	4	4	3	2
C. argentea	rhizosphere	12	7	8	7	6	6	5	17	14	13
	rhizoplane	5	9	6	7	2	4	2	3	3	3

TABLE 5: BACTERIAL ISOLATES FROM THE RHIZOSPHERE OF A.ESCULENTUS AND C. ARGENTEA

SOIL	TOTAL NO. OF BACTERIAL ISOLATES									
WK	2	4	6	8	10	12	14	16	18	20
A. esculentus										
non-rhizosphere	$1x10^4$	$09x10^4$	$63x10^4$	$75x10^4$	$28x10^4$	$23x10^4$	$28x10^4$	$20x10^4$	$20x10^4$	$15x10^4$
rhizosphere	$12x10^4$	$10x10^4$	$12x10^4$	$70x10^4$	$24x10^4$	$15x10^4$	$12x10^4$	$18x10^4$	$21x10^4$	$17x10^4$
C. argentea										
non-rhizosphere	$1x10^4$	$04x10^4$	$24x10^4$	$90x10^4$	$26x10^4$	$21x10^4$	$15x10^4$	$14x10^4$	$15x10^4$	$13x10^4$
rhizosphere	$20x10^4$	$08x10^4$	$61x10^4$	$26x10^4$	$27x10^4$	$22x10^4$	$16x10^4$	$33x10^4$	$31x10^4$	$26x10^4$

TABLE 6 % GERMINATION OF SEEDS TREATED WITH FUNGICIDES
(3 replicates)

Fungicide	% Germination of Okra		% Germination of Soko		Vigour of Seedling Okra	Soko
	US	S	US	S		
Thiram	60	10	65	10	+++	++++
Benlate	20	10	10	5	++	++
Brestan	20	10	20	5	++	++
No. fungicide	50	0	50	0	++	++

US, unsterilized soil, S, sterilized soil.

++ Normal, +++ Good ++++ Excellent

TABLE 7: OCCURRENCE OF MYCOFLORA ASSOCIATED WITH UNGERMINATED "SOKO" SEEDS TREATED WITH FUNGICIDES AFTER 9 DAYS:

FUNGI RECORDED	CONTROL		BENLATE		BRESTAN		THIRAM	
	US	S	US	S	US	S	US	S
A. niger	19	8	15	2	18	6	11	1
A. flavus	10	3	9	1	10	2	9	2
A. fumigatus	11	0	4	0	10	0	8	0
A. japonicus	1	0	0	0	1	0	0	0
A. terreus	2	0	1	0	2	0	2	0
A. nidulans	2	0	1	0	2	0	2	0
P. citrinum	5	0	0	0	5	0	3	0
F. oxysporum	1	0	0	0	1	0	1	0
Rhizoctonia solani	1	0	0	0	1	0	1	0
C. cladosporoides	4	1	1	0	4	2	2	0
B. theobromae	3	0	1	1	2	1	1	0
A. tamarii	6	0	2	0	4	1	3	1
Sterilia mycelia	2	1	1	0	2	2	1	0

US - Unsterilized Soil

S - Sterilized Soil

TABLE 8: OCCURRENCE OF MYCOFLORA ASSOCIATED WITH UNGERMINATED OKRA SEEDS TREATED WITH FUNGICIDES AFTER 9 DAYS.

FUNGI RECORDED	CONTROL		BENLATE		BRESTAN		THIRAM	
	US	S	US	S	US	S	US	S
A. niger	19	8	17	1	18	5	10	0
A. flavus	10	3	8	1	10	2	1	1
A. fumigatus	11	0	2	0	8	0	6	0
A. japonicus	1	0	1	0	0	0	1	0
A. terreus	2	0	1	0	1	0	2	0
A. nidulans	2	0	1	0	2	0	0	0
P. citrinum	5	0	0	0	5	0	2	0
F. oxysporum	1	0	0	0	0	0	0	0
R. solani	1	0	0	0	0	0	0	0
C. cladosporoides	4	1	1	0	3	2	1	1
B. theobromae	3	0	1	0	2	1	1	0
A. tamarii	6	0	2	1	3	2	2	1
Sterilia mycelia	2	1	1	1	2	1	1	1

US - Unsterilized Soil

S - Sterilized Soil

No sugars were detected chromatographically in the root exudates. The amino acids derived from their Rf values (Table 9) were alanine and glycine from okra while 'soko' contained aspartic acid, glutamine and an unidentified amino acid (with Rf value 0.5048).

GROWTH OF SOME FUNGAL ISOLATES ON AGAR MEDIA SUPPLEMENTED WITH ROOT EXUDATE:

Studies carried out showed that the two root exudates when incorporated into czapek Dox Agar, encouraged the growth of some soil fungi. The filtered medium encouraged better growth in all cases (Figs. 4-6) than the autoclaved ones (Figs 7-9). The growth of S. constrictum (Figs 4 & 7) was favoured by okra exudate. In the filtered samples, there was better growth in soko exudate agar (CA) than in CDA and okra exudate agar (HDA) from the first through the fourth day of growth, thereafter the colony diameters became the same. The growth of F. oxysporum was also encouraged by CA and CDA. (Figs. 4&7). The growth of B. theobromae, T. harzianum, C. pallescens (Figs 5&8) and M. cinctum (Figs 6&9) were obviously stimulated by HDA.

ESTIMATION OF MICROBIAL POPULATION FROM PRIMARY ROOT:

A high fungal count was obtained from soil samples collected between 0 and 3cm from the primary roots of both vegetables. (Figs 10&12). Bacterial population in this region was very low for okra seedling while it was fairly high in 'soko' (Figs 11&13) Besides the influence of these populations in the immediate vicinity of their roots, there were fluctuations with increase in distance away from the primary root. The highest bacterial count was obtained for okra between 24&27cm while it was between 12 and 15 cm for 'soko'.

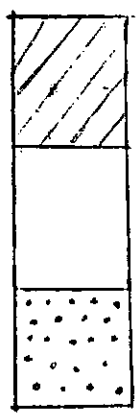
BIOASSAY OF AMINO ACID CONSTITUENTS:

The Rf values of the reference amino acids and those detected in the root exudates were calculated.

TABLE 9

AMINO ACIDS	Rf VALUES	OKRA	'SOKO'
ALANINE	0.4097	0.3916	ABSENT
ARGININE	0.3134	ABSENT	ABSENT
ASPARAGINE	0.2966	ABSENT	ABSENT
ASPARTIC ACID	0.2326	ABSENT	0.2259
CYSTEIN	0.2052	ABSENT	ABSENT
GLUTAMINE	0.3333	ABSENT	0.3365
GLUTAMIC ACID	0.3497	ABSENT	ABSENT
GLYCINE	0.3125	0.3125	ABSENT
HISTIDINE	0.2674	ABSENT	ABSENT
LEUCINE	0.7465	ABSENT	ABSENT
LYSINE	0.2013	ABSENT	ABSENT
PROLINE	0.4479	ABSENT	ABSENT
SERINE	0.4115	ABSENT	ABSENT
THREONINE	0.4104	ABSENT	ABSENT
TYROSINE	0.6500	ABSENT	ABSENT
VALINE	0.6250	ABSENT	C_{10}(0.5048)

FIG. 4 EFFECT OF ROOT EXUDATES (FILTERED) ON LINEAR GROWTH OF P. CITRINUM, S. CONSTRICTUM AND F. OXYSPORUM.



CDA - Czapek Dox Agar
HDA - Okra Exudate Agar
CA - Soko Exudate Agar

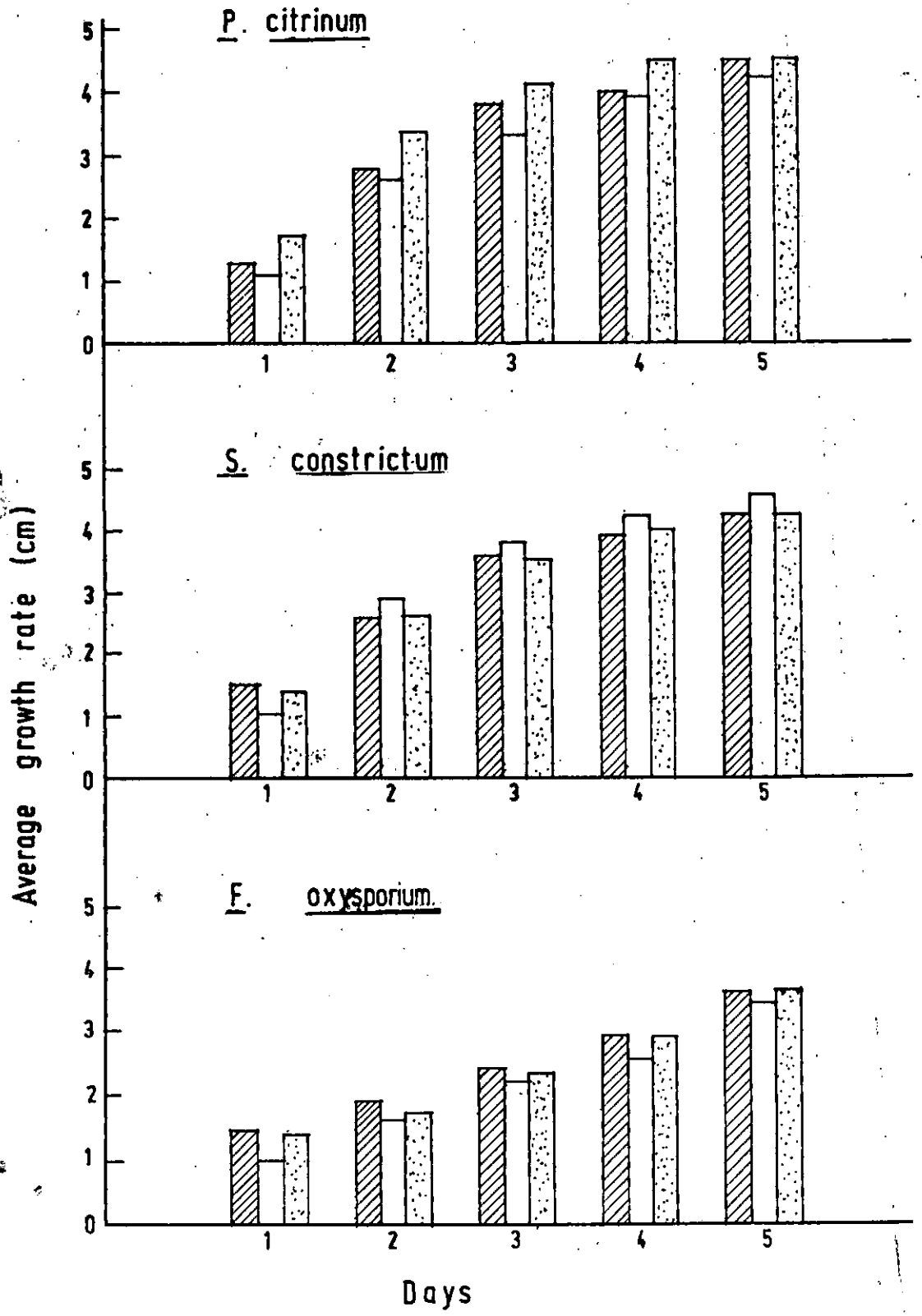
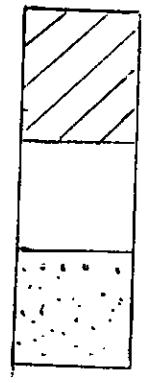

P. citrinum
S. constrictum
F. oxysporium.
Average growth rate (cm)
Days
0
1
2
3
4
5

Fig 4

FIG. 5: EFFECT OF ROOT EXUDATES (FILTERED) ON LINEAR GROWTH OF *B. THEOBROMAE*, *T. HARZIANUM* AND *C. PALLESCENS* .



CONTROL

HDA

CA

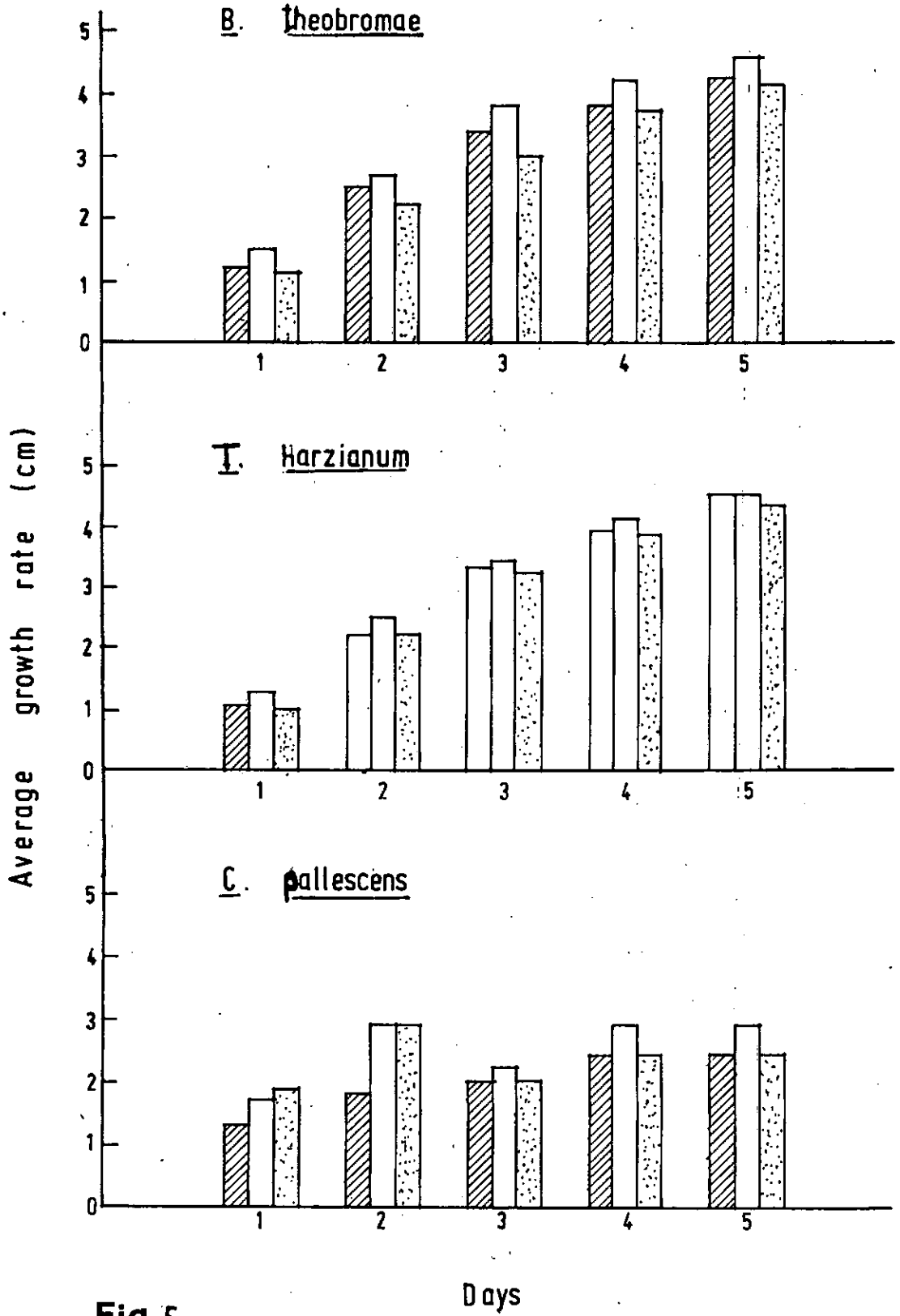
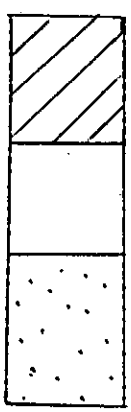

B. theobromae
T. harzianum
C. pallescens
Average growth rate (cm)
Days
0
1
2
3
4
5

Fig 5

FIG. 6: EFFECT OF ROOT EXUDATES (FILTERED) ON LINEAR GROWTH OF M. CINCTUM.



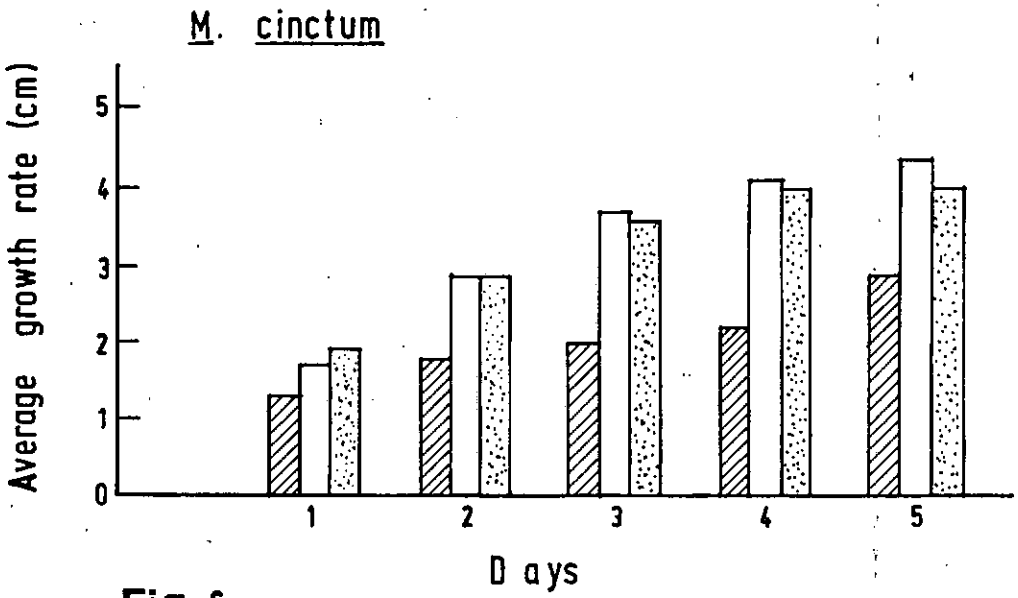
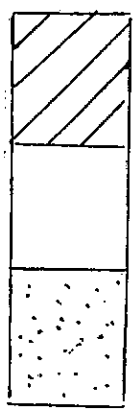


Fig 6

FIG. 7: EFFECT OF ROOT EXUDATES (AUTOCLAVED) ON LINEAR GROWTH OF P. CITRINUM, S. CONSTRICTUM AND F. OXYSPORUM.



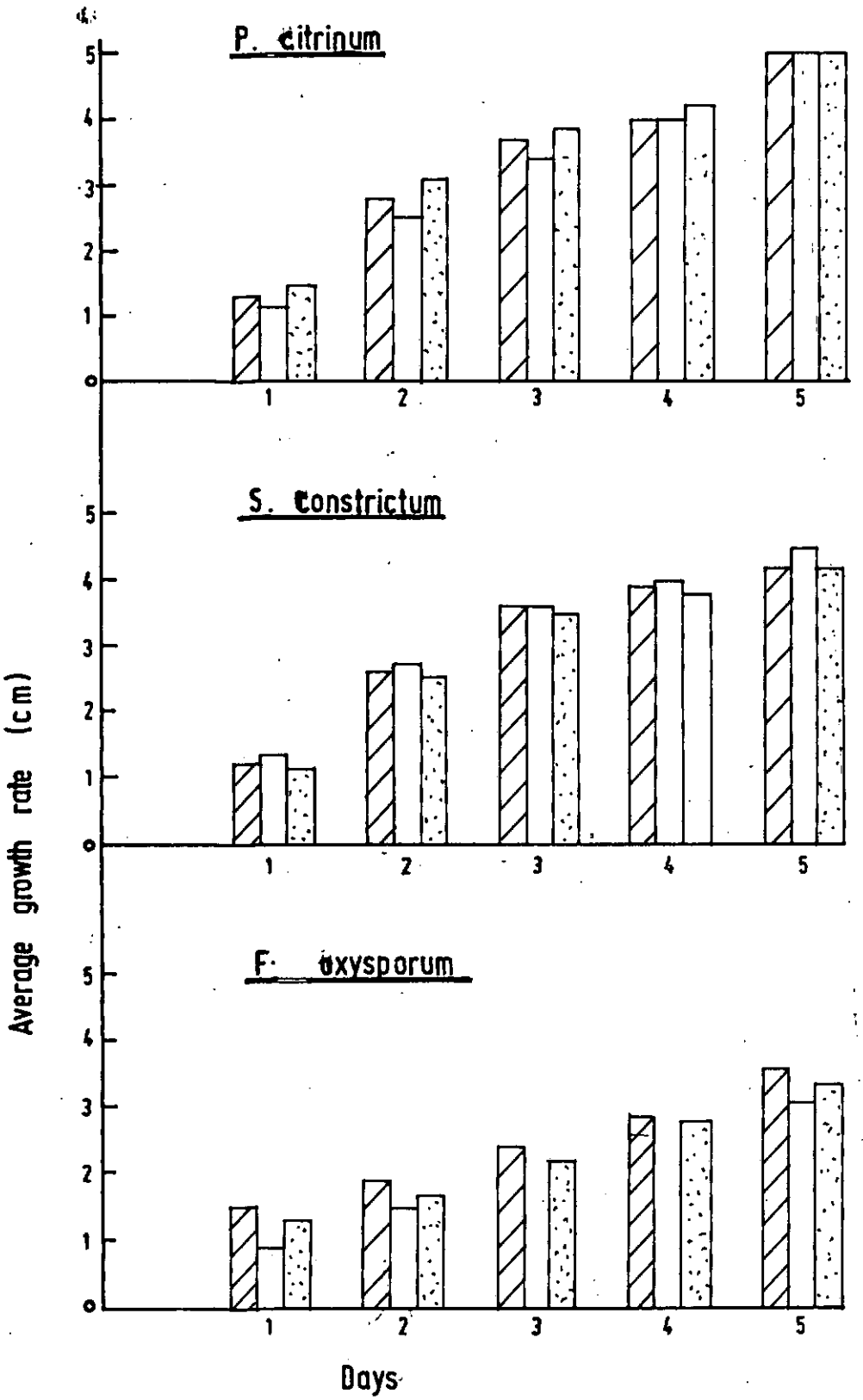

P. citrinum
S. constrictum
F. oxysporum
Average growth rate (cm)
Days
0
1
2
3
4
5

Fig 7

FIG. 8: EFFECT OF ROOT EXUDATES (AUTOCLAVED) ON LINEAR GROWTH OF *B. THEOBROMAE*, *T. HARZIANUM* AND *C. PALLESCENS*.

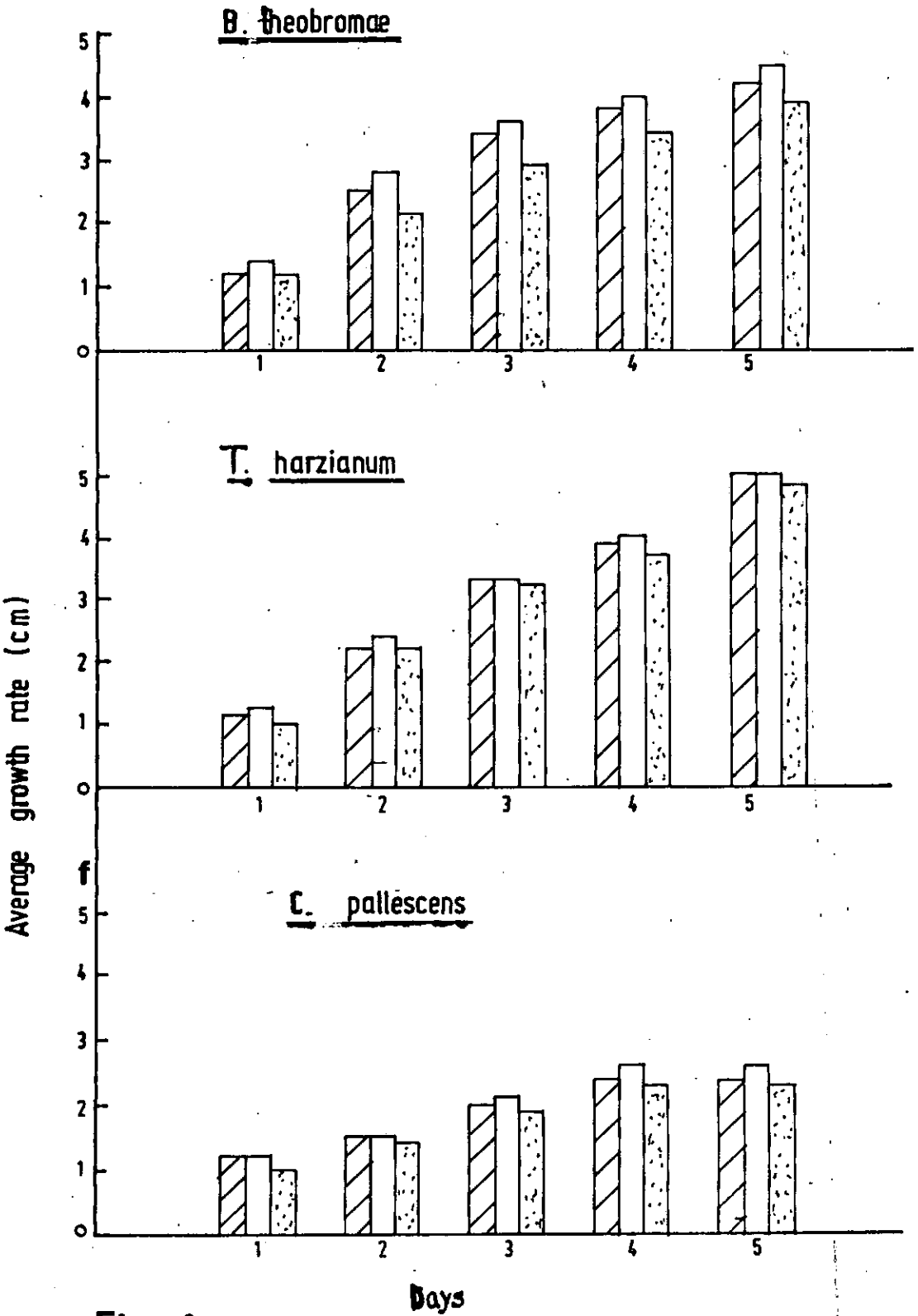
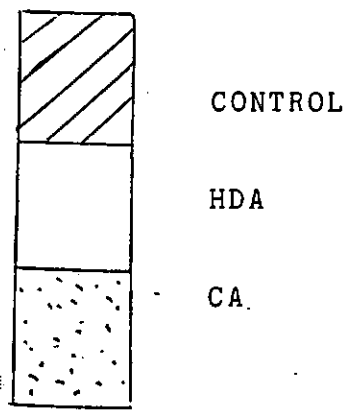

B. theobromae
T. harzianum
C. pallescens
Average growth rate (cm)
Days
0
1
2
3
4
5

Fig. 8

FIG. 9: EFFECT OF ROOT EXUDATES (AUTOCLAVED) ON LINEAR GROWTH OF M. CINCTUM.



M. cinctum

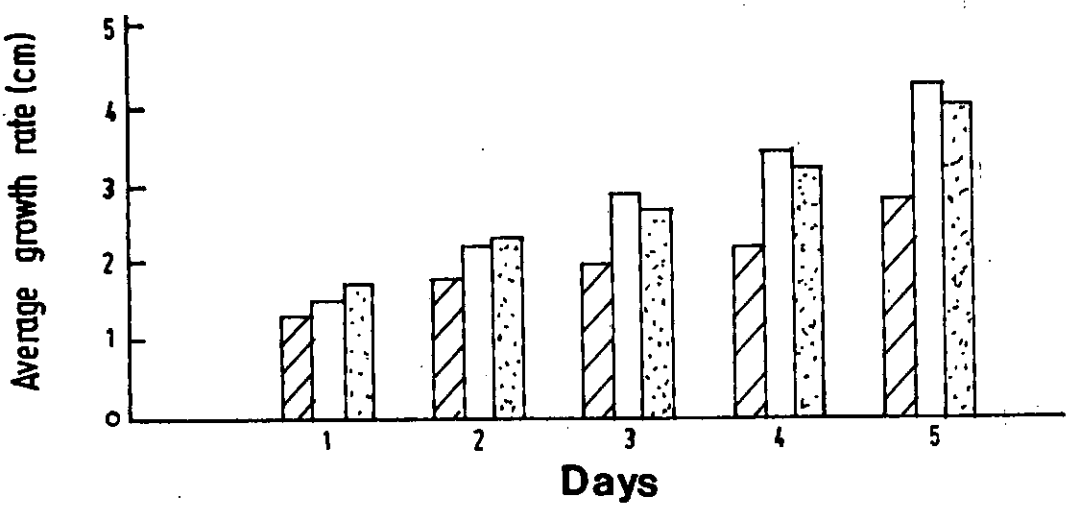


Fig 9

FIG. 10: CHANGES IN THE NO. OF FUNGAL POPULATION WITH DISTANCE FROM PRIMARY ROOT SURFACE OF TEN DAY OLD OKRA SEEDLING.

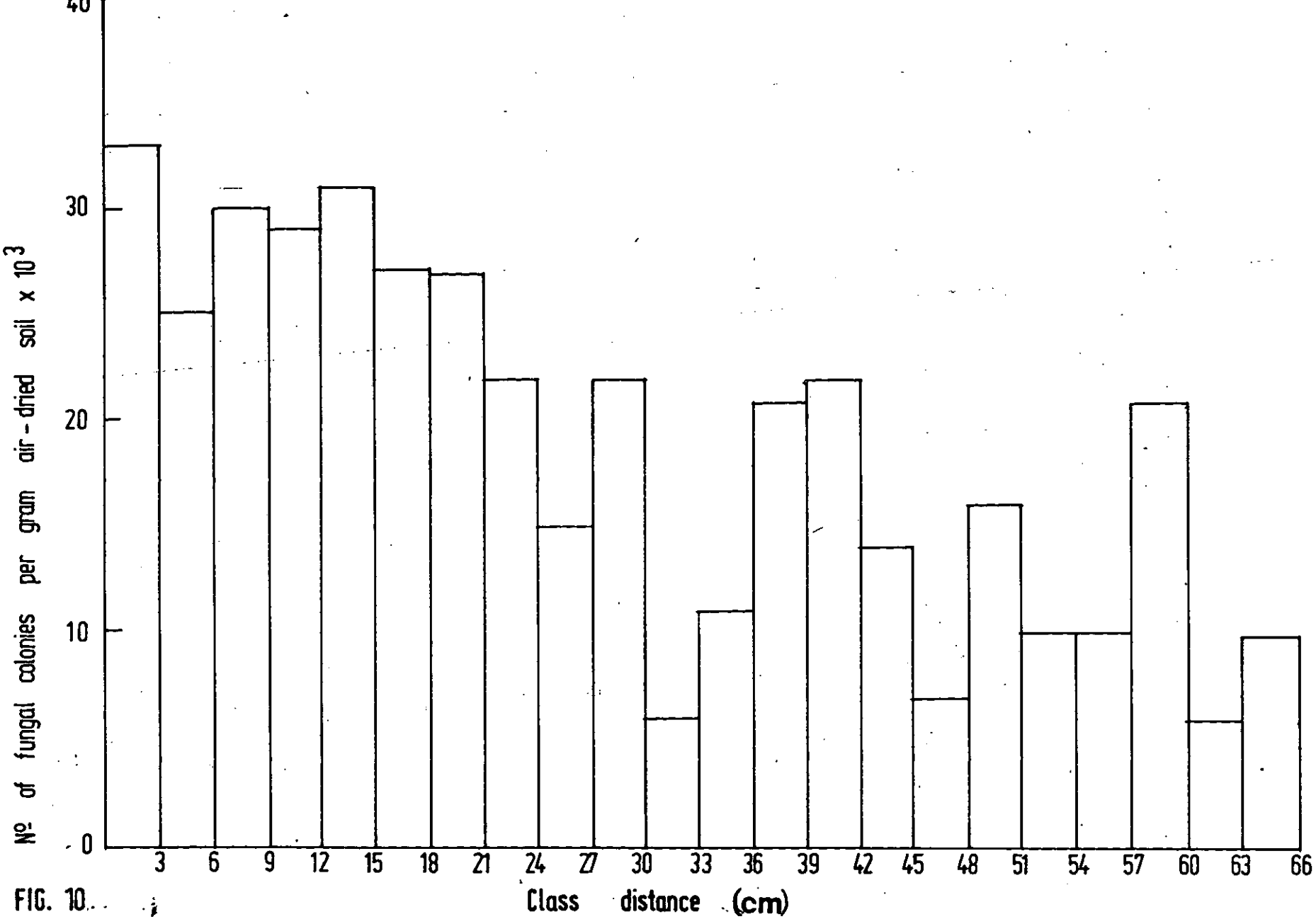

40
30
20
10
0
№ of fungal colonies per gram air-dried soil x 10^3
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
60
63
66
Class distance (cm)

FIG. 10

FIG. 11: CHANGES IN THE NO. OF BACTERIAL POPULATION WITH DISTANCE FROM PRIMARY ROOT SURFACE OF TEN-DAY OLD OKRA SEEDLING.

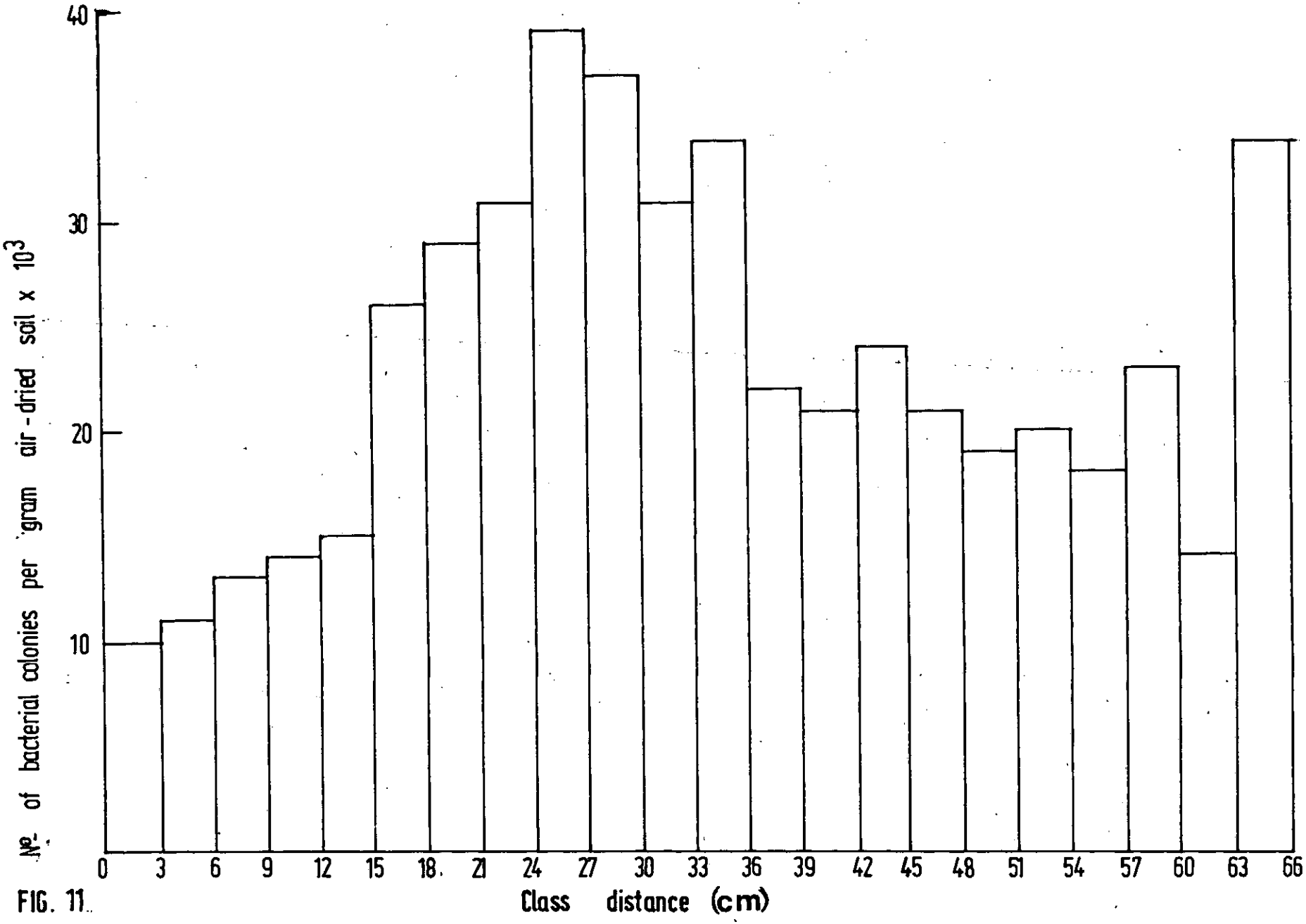

40
30
20
10
Nº. of bacterial colonies per gram air-dried soil x 10^3
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66
Class distance (cm)

FIG. 11.

FIG. 12: CHANGES IN THE NO. OF FUNGAL POPULATION WITH DISTANCE FROM PRIMARY ROOT SURFACE OF TEN- DAY OLD SOKO SEEDLING.

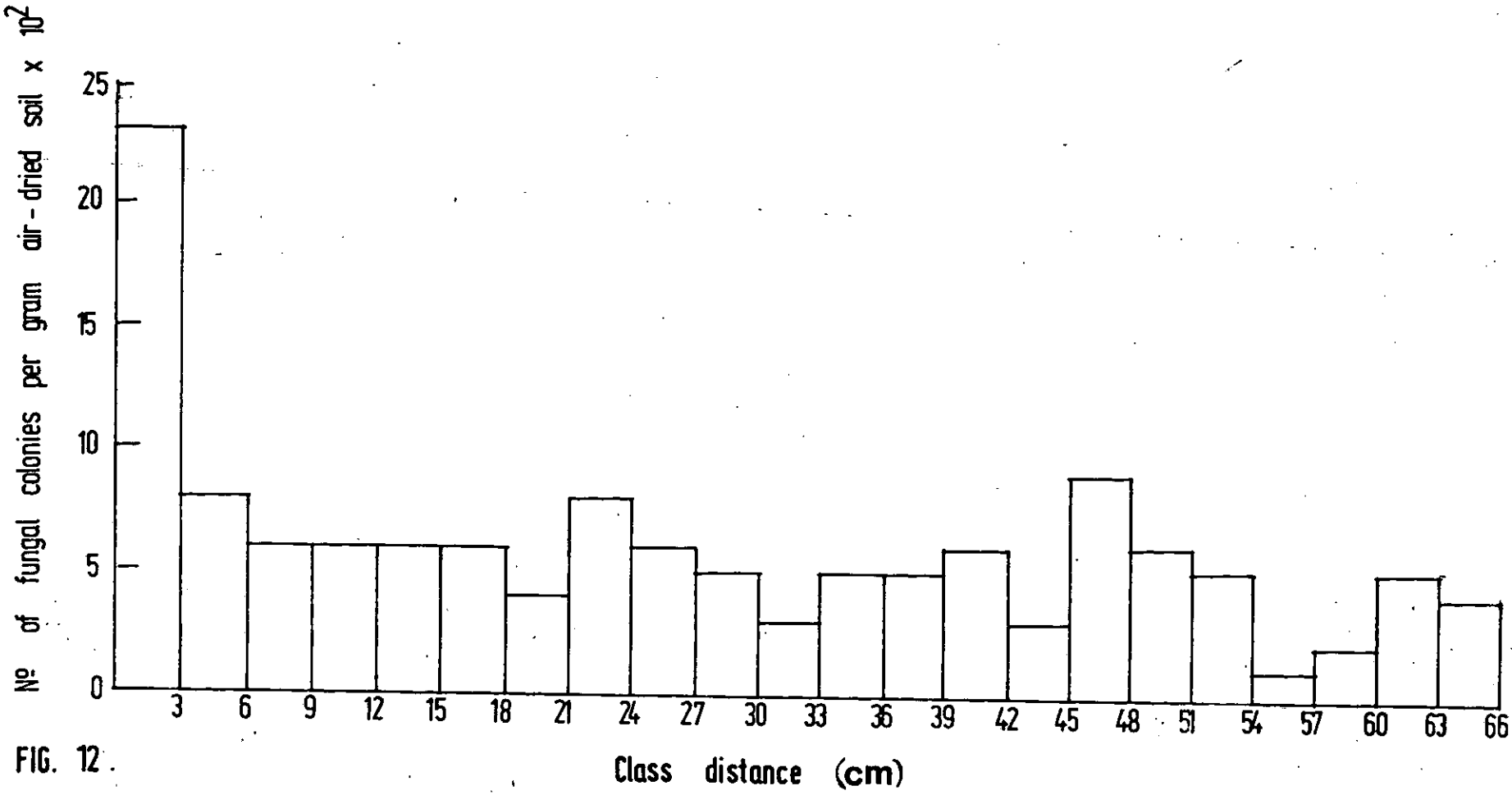

№ of fungal colonies per gram air-dried soil x 10^2
25
20
15
10
5
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
60
63
66
Class distance (cm)

FIG. 12.

FIG. 13: CHANGES IN THE NO. OF BACTERIAL POPULATION WITH DISTANCE FROM PRIMARY ROOT SURFACE OF TEN-DAY OLD SOKO SEEDLING.

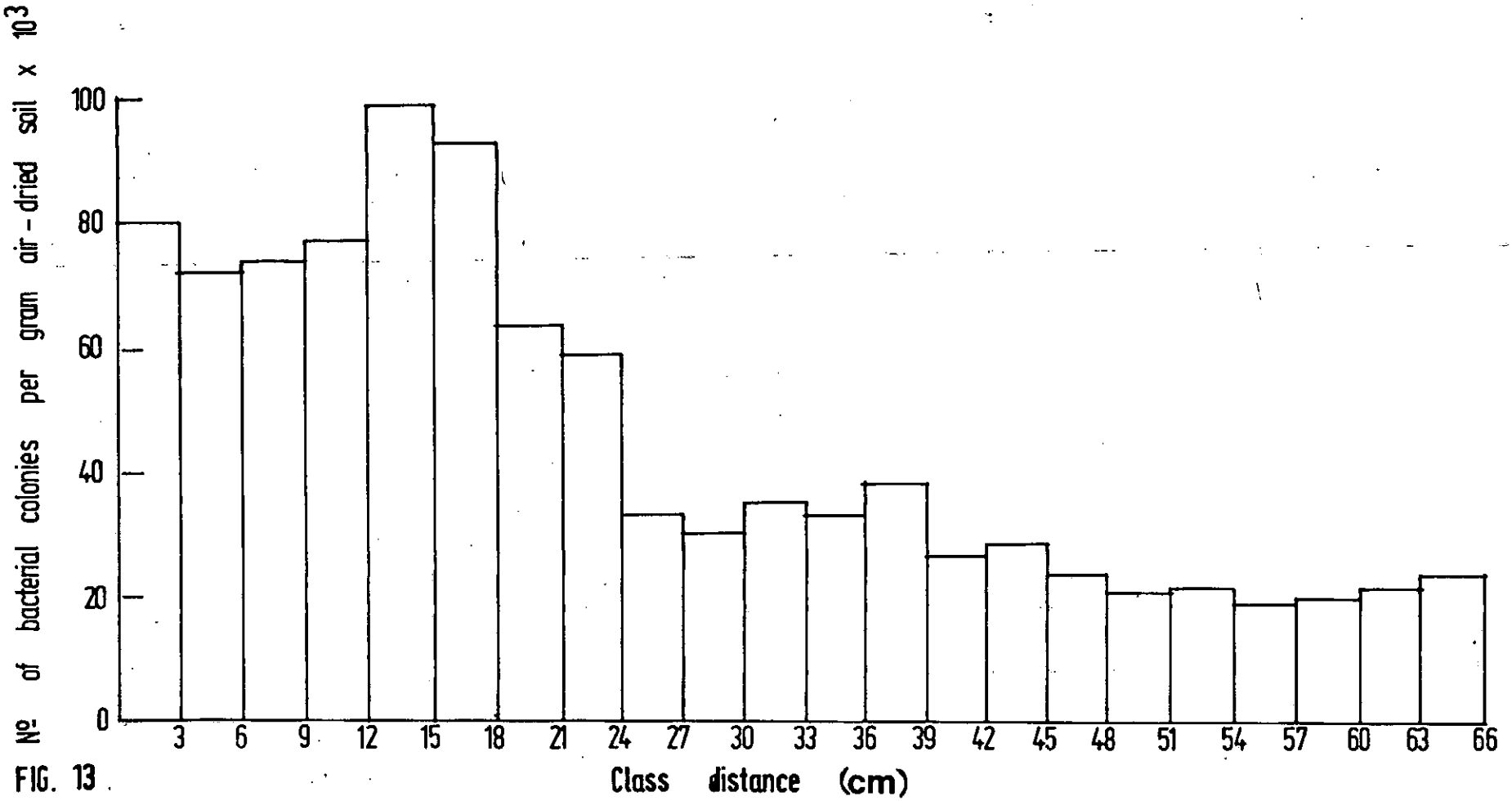

Nº of bacterial colonies per gram air-dried soil x 10^3
100
80
60
40
20
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
60
63
66
Class distance (cm)

FIG. 13

EFFECT OF FUNGICIDES ON SOME ISOLATED FUNGI

Thiram, benlate and brestan were all effective in reducing the growth of fungi on solid medium but benlate was the most effective. It stopped the growth of B. theobromae completely at 12.5ppm, A. tamarii at 25 ppm and F. solani at 6.2ppm (Table 10). In liquid medium, the fungi could only tolerate lower concentrations of the fungicides (Table 11)

CELLULOSE DECOMPOSITION; EFFECT OF TEMPERATURE AND pH.

There was no observed growth of any of the fungi at 10° and 20° C. At 30°C however, all the test fungi grew on both CDA and CMC (Table 12). There was very slight growth of A. japonicus at 40°C.

The pH that favoured cellulose decomposition by these fungi was between 6.5 and 8.5. B. theobromae grew best at pH 8.5 while A. japonicus and T. harzianum were favoured by pH 7.0 (Table 14)

GROWTH OF FUNGI ON FILTER PAPER MEDIUM

Filter paper (example of native cellulose) was used for cellulolytic activity. Measure of activity was assessed as % weight loss of filter paper (Table 15) A. japonicus, A. flavus and A. sydowii caused more weight loss of filter paper than the other fungi. They utilized the filter paper more efficiently in shaken cultures than those kept standing on the laboratory bench. The pattern of degradation of the filter paper by A. japonicus and B. theobromae was scattered while A. niger attacked from the edges.

EFFECT OF T. HARZIANUM ON OTHER FUNGAL ISOLATES.

T. harzianum inhibited the growth of R. solani (Plate 2). It had no apparent effect on any of the other fungi (plates 3&4). The test fungi P. variotii, F. oxysporum, A. tamarii, C. pallescens, A. carbonarius, A. ustus, B. theobromae and A. japonicus grew together with T. harzianum without showing any zone of demarcation. After colonies met, the mycelium from each colony

TABLE 10:

SELECTIVE ACTION OF FUNGICIDES ON SOIL FUNGI AT 12.5PPM AFTER 7 DAYS

Fungus	Linear growth (mm) on Solid medium			
	Control	Thiram	Benlate	Brestan
B. theobromae	45	4.10	5.0	42.5
A. tamarii	21.5	7.0	7.0	10.5
F. solani	19.5	19.0	5.0	19.5
F. equiseti	45.0	26.0	17.0	22.0

TABLE 11

EFFECT OF FUNGICIDES ON FUNGAL ISOLATES

AT 12.5ppm AFTER 7 DAYS

	Mycelial dry wt (mg) on liquid medium			
Fungus	Control	Thiram	Benlate	Brestan
B. theobromae	0.173	0.025	0.032	0.096
A. tamarii	0.128	0.038	0.030	0.059
F. solani	0.096	0.038	0.029	0.045
F. equiseti	0.180	0.133	0.040	0.031

TABLE 12: MEAN GROWTH RATE (MM) OF FUNGAL ISOLATES ON CDA AND CMC AT RT FOR 6 DAYS.

FUNGUS	CDA	CMC
A. flavus	15.5	13.6
A. niger	17.8	12.6
A. japonicus	13.1	9.3
A. sydowii	15.0	9.5
A. tamarii	16.0	9.5
A. terreus	16.1	7.6
A. fumigatus	15.6	10.3
A. ustus	10.3	5.7
P. variotii	24.3	17.5
T. koningii	17.9	12.1
T. harzianum	31.6	17.7
B. theobromae	33.1	18.6
R. solani	11.6	8.3

TABLE 13: ABILITY OF SOME FUNGI TO UTILIZE CMC AS SOLE C SOURCE AS INDICATED BY ZONE OF CLEARING AROUND FUNGAL COLONIES:

FUNGUS	DEGREE OF CLEARING					
DAYS	1	2	3	4	5	6
A. niger	-	-	-	+	+	++
A. sydowii	-	-	-	-	+	+
A. tamarii	-	-	-	-	+	+
A. japonicus	-	-	-	-	-	+
P. variotii	-	-	-	-	+	+

\- No clearing;

\+ Slight clearing;

++ Good clearing.

TABLE 14: EFFECT OF DIFFERENT pH ON CELLULOSE DECOMPOSITION AT RT AFTER 6 DAYS

FUNGUS	MEAN DIAMETER (MM)											
	CDA						CMC					
	4.5	5.5	6.5	7	7.5	8.5	4.5	5.5	6.5	7	7.5	8.5
A. flavus	10	10	30	44	44	44	12	25	40	40	40	40
A. niger	12	15	30	44	44	44	11	14	18	36	36	36
A. japonicus	10	12	17	34	30	30	13	15	30	24	17	15
A. sydowii	10	14	14	40	42	42	10	25	27	30	35	35
A. tamarii	10	17	20	44	50	50	10	10	25	24	30	30
A. terreus	10	12	20	34	36	36	10	10	12	20	22	22
A. fumigatus	10	15	20	40	40	42	10	10	18	24	24	26
A. ustus	10	10	21	28	32	25	10	12	12	14	19	13
P. variotii	10	24	39	62	64	50	10	20	35	60	60	40
T. koningii	10	27	38	46	46	46	10	20	27	30	30	30
T. harzianum	12	30	45	80	80	80	10	28	35	46	46	46
B. theobromae	15	35	52	90	90	90	10	34	36	50	50	55
R. solani	10	12	20	30	30	30	10	12	17	24	24	24

TABLE 15: GROWTH OF FUNGI ON FILTER PAPER AS % WT. LOSS AFTER 24 DAYS

	FUNGUS	% WT. LOSS
SHAKE CULTURES	*A. flavus*	16.0
	A. niger	13.6
	A. japonicus	38.5
	A. sydowii	16.6
	A. tamarii	8.9
	A. fumigatus	7.5
	P. variotii	6.7
	T. harzianum	7.4
	B. theobromae	4.5
STANDING CULTURES		
	A. flavus	14.0
	A. niger	12.5
	A. japonicus	30.0
	A. sydowii	14.2
	A. tamarii	6.6
	A. fumigatus	7.0
	P. variotii	5.5
	T. harzianum	7.0
	B. theobromae	2.5

PLATE 2: GROWTH OF PURE CULTURES OF R. SOLANI (R) AND T. HARZIANUM (T)

NOTE: OLDER COLONY OF 'R' WITH FEW YOUNG COLONIES AROUND IT DUE TO DISPERSED SPORES DURING INOCULATION.

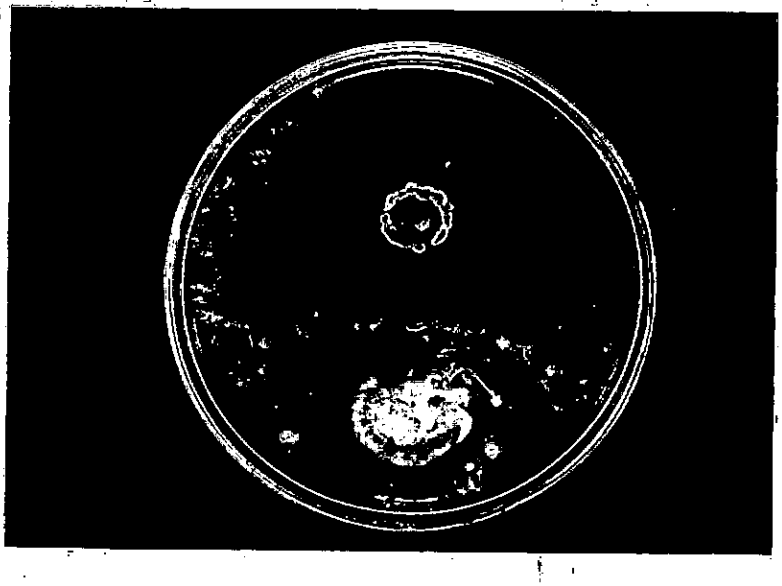


PLATE 3: PURE CULTURES COLONIES OF

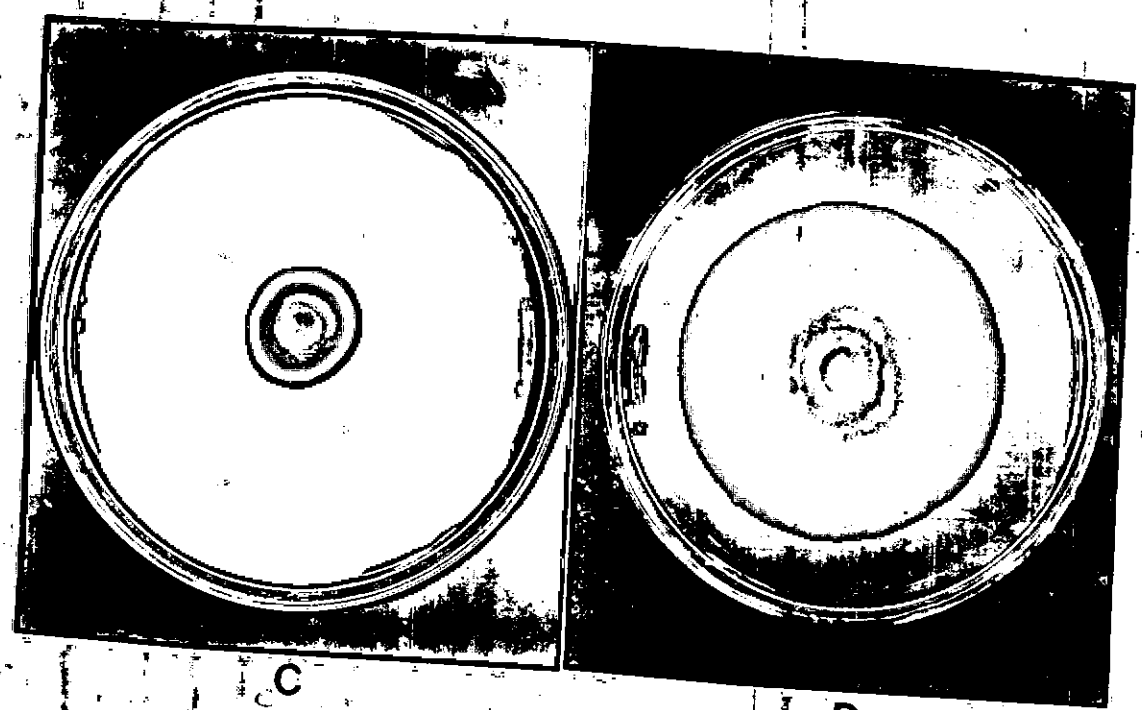
A - *T. harzianum*

B - *B. theobromae*

C - *A. ustus*

D - *P. variotii*

A B



C D

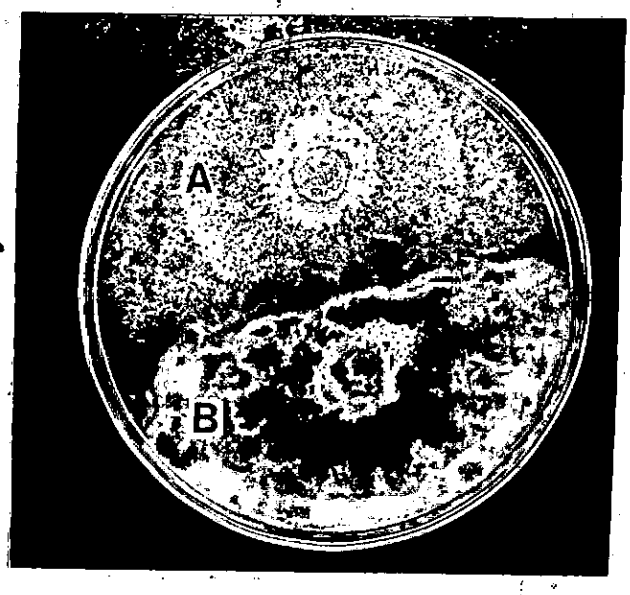
ANTAGONISM IN CULTURE:-

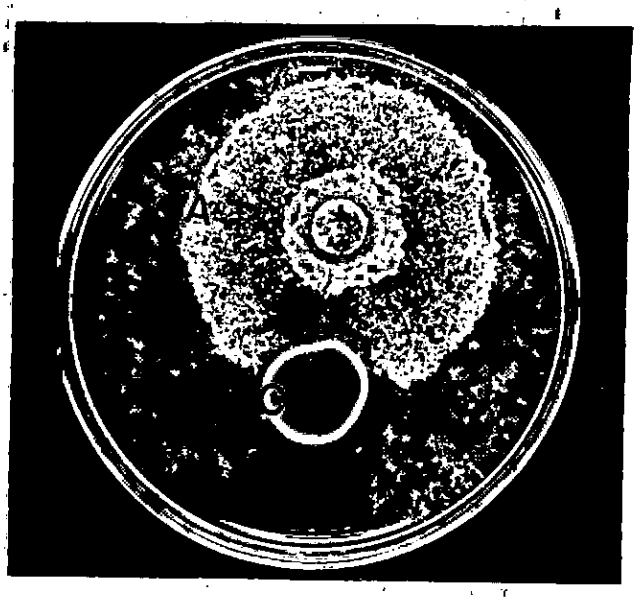
PLATE 4: GROWTH OF

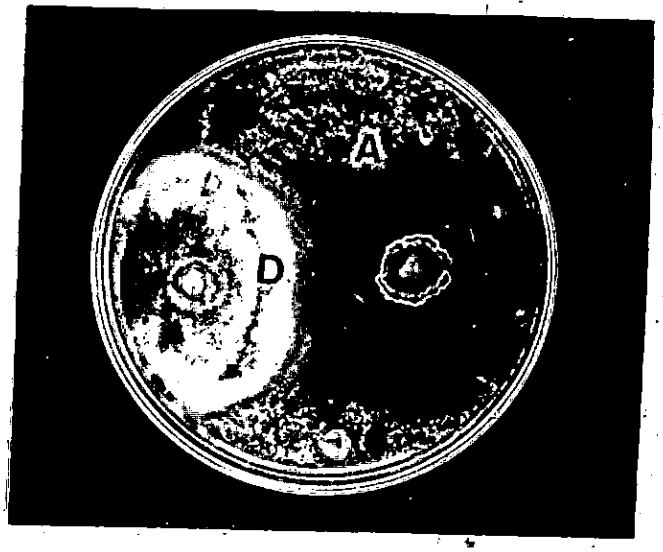
A + B - *T. HARZIANUM* AND *B. THEOBROMAE*

A + C - *T. HARZIANUM* AND *A. USTUS*

A + D - *T. HARZIANUM* AND *P. VARIOTII*


A
B


A
C


A
D

grew into areas that had already been occupied by the fungus placed on the opposite side of the plate.

CELLULOLYTIC ACTIVITIES OF FUNGI:

The mean growth rates of some isolated fungi grown on CDA and CMC are as shown (Table 12). A. niger, A. sydowii, A. tamarii and P. variotti all utilized CMC as sole carbon source as indicated by the zone of clearing around their colonies (Table 13).

DISCUSSION

The soil used throughout this study is loamy sandy based on Salter and Williams (1967) modified diagram.

The high relative humidity recorded in the first two weeks of study contributed to the large number of fungi isolated from the non-rhizosphere soil. Most fungal pathogens are dependent on the presence of free moisture on the host or on the presence of high relative humidity in the atmosphere only during germination of their spores. They become independent once they can obtain nutrients and water from the host. Some pathogens however, such as those causing late blight of potato and the downy mildews, require at least high relative humidity in the environment throughout their development. In these diseases, the growth and sporulation of the pathogens and also the production of symptoms come to a halt as soon as dry, hot weather sets in and resume only after a rain or after the return of humid weather (Agrois, 1969b).

This was observed during this study as there was a decrease of both bacterial and fungal populations during the dry season (12th-20th week). This was due to the low moisture content in the soil necessitating the bacterial and fungal organisms to go into dormancy by forming slerotia, spores, endospores and conidia. Most bacterial diseases and also many other diseases of young tender tissues are particularly favoured by high moisture or high relative humidity. Seasonal changes in the rhizosphere mycoflora in response to environmental factors have been reported (Eggleton, 1938; Rouatt et al; 1963).

Plate 1 shows two different stages of growth of 'soko' and okra. The two vegetables encouraged different microflora in their rhizosphere. It was observed that okra had more profuse root system. Root hairs are known to produce exudates

so it was not surprising therefore that okra supported a greater population of microflora in its rhizosphere than Soko. Plants generally of the same age could encourage different population counts because of different growth rates and maturity periods.

Thiram disappeared from the soil after the 35th day (Fig.3). The fact that it could be assayed is of great value in studying the mode of action in soil in relation to the microflora. The nature of the curve representing the decline of thiram in soil with time suggested that more than one process was involved. The rate of disappearance was faster in unsterilized than in sterilized soil suggesting microbial involvement in its disappearance. This was so because soil microorganisms are often the sole agents involved in the removal of harzardous pesticides used against various pests and diseases of crop plants (kearney and kaufman, 1976).

This study has shown that thiram is relatively unstable in agricultural soil. It is clear, from the results that thiram can provide protection for seedlings against soil-borne pathogens for a considerable time after it had been reduced to levels that are not toxic to other organisms. Thiram was selected because of its proven value for combatting damping-off disease of certain vegetables (Taylor and Rupert, 1946; McKeen, 1950; Odeyemi, 1979).

Because of the practical significance of the pathogen-microflora interaction, many attempts have been made to modify the microbiological equilibrium in the hope of controlling specific plant pathogens. The alteration in the rhizosphere microflora and soil enzymes have been reported upon the application of various chemicals such as fungicides (Vrany et al; 1962).

ESTIMATION OF SOIL MICROBIAL POPULATIONS

The result of the soil samples tested showed that bacteria were found to be more abundant than fungi in the soil. This is probably due to the ability of bacteria to decompose a variety of natural substrates and their capacity to grow spontaneously. The bacterial and fungal populations in the untreated soil samples did not show any fluctuation since the soil maintained its physical and chemical properties under laboratory conditions (Table 2). It is apparent that thiram treatment reduced the number of fungi to about one third compared to the fungal population in the control soil (Table 3). Bollen (1961) discovered that thiram was extremely toxic to microorganisms above 31 ppm. Odeyemi and Alexandar (1977) also found that thiram underwent rapid biotransformation to form a fungicide, dimethylamine (a carcinogenic precusor) and carbon disulphide, a cytotoxic and fungicidal compound. Benlate inhibited the growth of bacteria to an appreciable extent, but had no effect on the fungal population. Brestan had no stimulatory or inhibitory effects on bacteria and fungi because it is less toxic to microorganisms.

The concentrations of some of these fungicides might be too low to exert any inhibitory or stimulatory effects on microbial populations. This reasoning agrees with the works of Johnen and Drew (1977), Domsch and Paul (1974) who discovered that normal application rates of herbicides did not exert any significant change on microbial populations in the soil. Moreover herbicides are known to inhibit the Hill reaction, that is, the evolution of oxygen in the presence of living chloroplasts and a suitable hydrogen acceptor. Since microorganisms do not have chlorohyll and living cloroplasts, their inability to exert any toxic effects is not surprising. The definition of fungicides as fungus-killing substances is somewhat oversimplified. Fungicides can be considered as inoculum - reducing substances, which stop the fungus from

forming viable spores or stop spores from germinating, or stop germ tubes or other mycelia from infecting the host plants. In all these ways, they reduce inoculum.

Aspergillus was the most largely represented genus. Their dominance justifies the observation made by Jensen (1931) that Aspergilli are a group of somewhat thermophillic organisms able to withstand high temperatures. On the whole, 13 species were isolated as compared to 7 of Penicillium. The readily sporulating genera appeared in large numbers on the agar plates because each individual spore might have given rise to a colony. It is therefore not surprising that fungi sporulating profusely like Aspergillus and Penicillium species were isolated frequently.

The total fungal population in the rhizosphere remained high up to the 10th week after which they became repressed. There was no significant increase on the rhizoplane population of both vegetables studied from the first to the fourth week. The microbial population however increased thereafter up to the 10th week. The observation that fungus units in the rhizosphere occur to a large extent in the vegetative state (Agnihothrudu, 1955) is relevant here since the reproductive phase commenced at about the 10th week in the present experiments. Bacterial count was also affected in like manner.

ESTIMATION OF POPULATION FROM PRIMARY ROOT:

A high fungal count was obtained between 0 and 3cm from the primary roots of both vegetables (Figs 10 & 12). It is now well established that a wide range of organic compounds is released by the roots of plants. Odunfa and Oso (1979) found a high proportion of Rhizopus, Mucor, Pythium and a few other species of phycomycetes on the rhizoplane of plant roots. They concluded that these fungi were frequently associated with the utilization of sugars which are known to be exuded more abundantly at the seedling stage of plant growth. It is not surprising, therefore, that the fungal population in the immediate vicinity of the root was high. There was a general

increase of the fungal population in both vegetables up to a distance of between 27 and 30 cm; thereafter there were fluctuations.

Bacterial count in the immediate vicinity of both vegetables was comparatively low (Figs 11&13). A high population was however obtained between 24 & 27 cm for okra while for 'Soko' it was between 12 and 15 cm. The mean of the means of the total counts of fungi and bacteria associated with the two vegetables differed significantly as the dimension of sampling increased. F value was significant at P =0.01.

The decreased relative incidence of bacteria in the rhizosphere may be explicable on the assumption that these organisms are more closely associated with breakdown of humus residues than of the fresh organic material present at the root surface.

The flora of the rhizosphere is affected by a number of factors. Proximity of the soil sample to the root is particularly important. One of the most important considerations in the study of the microorganisms of the rhizosphere is obviously that concerned with the extent of the zone of root influence. The effects of the root growth and physiology diminished with distance from 0 cm to 66 cm hence the fluctuation in the microfloral count.

Congregation of microorganisms on root surfaces and preferential stimulation of specific types or groups seem to be intimately related to the supply of inorganic and organic nutrients at this root-soil interface. With increased supply of nutrients from plants in the rhizosphere, fungi and bacteria capable of utilizing inorganic or simple organic nutrients would to expected to multiply. The growth rate of microorganisms at each point in soil can be assumed to be controlled by the concentration of soluble Organic substrate and its diffusion through the soil (Newman and Watson, 1977).

At pH 6.8 (Table 2), it was not surprising that the soil harboured more bacteria than fungi as that pH favoured organisms which can tolerate some degree of alkalinity. It is important to

remember however that a sufficient degree of acidity in the soil solution can itself reduce the percentage of spores germinating in it. Some diseases are most serious in areas whose pH favours the particular pathogen.

Some fungi and bacteria may have been present in the soil used but were not isolated due to environmental factors and isolation techniques. Some spores might not have germinated due to exogenous dormancy imposed upon them by soil fungistasis.

ROOT EXUDATE STUDIES:

For the collection of root exudates, sterile sand was used although this introduced artifacts. It is closer to reality than solution culture systems which enable one to isolate effects of soil physical and chemical variables from those of other soil microorganisms. It has been suggested that greater scretion occurs in soil than in solution. Barber and Gunn, (1974), Barber and Martin, (1976), reported that exudation of organic materials from cereal roots was doubled when the roots were grown on a solid medium rather than in solution. Burke, Holmes and Baker (1972), also concluded that root exudation increased with greater mechanical resistance.

The results (Table 9) indicate that living plant roots release some quantities of organic materials into soil. Not merely does growth of the roots in soil increase the amount of material released but microorganisms also appeared to stimulate the process. No distinction can be made between substances exuded by roots as diffusable, soluble compounds and non-diffusable mucilaginous substances or indeed as sloughed cells. However, since all three types of material are present and might be expected to modify the environment, in agreement with the definition of Rovira (1969), they are collectively regarded as exudate.

It was of great advantage to have used acid-washed sterile sand because non-sterile sand or sand-soil mixtures supporting plant growth are known to contain a number of amino acids including

glutamic acid, aspartic acid, proline, leucine, alanine, cystein, glycine, lysine and phenylalanine. Exudation which is a physiological process should not be expected to proceed at uniform rate in the growing plant. Rovira (1959) demonstrated that exudation was greatest during the first few weeks of growth; hence exudates were collected at 5 days interval for 20 days.

ROOT EXUDATE CONSTITUENTS:

No sugars were detected chromatographically. They might have been produced by the roots in amounts too small to be detected. Absence of sugars in the root exudates was not surprising because fungi frequently associated with the utilization of sugars were not isolated from the rhizoplane of the plant roots.

The amino acids derived from the root exudates of okra were alanine and glycine (Table 9) while 'soko' contained aspartic acid, glutamine and an unidentified amino acid (with Rf value 0.5048). The difference in the types of amino - acids produced may have arisen from one of many reasons. The plants may exude different amino-acids as a physiological expression of their genetic differences.

Root exudation is a phenomenon common to all higher plants, for a plant which did not exude substances would have no rhizosphere except for that resulting from stimulatory effect of thigmotropism.

Exudates may inhibit or stimulate plant pathogens by supporting an antagonistic or mutualistic microflora. It is suspected that there may be in the rhizosphere of these two plants an element of the microflora which is antagonistic or stimulatory to any given pathogen. Its significance in inhibiting disease development would depend to a large extent on whether or not the chemical or biological environment of the rhizosphere was favourable for its growth and multiplication. If the nature of the exudates and environment preferentially favoured the development of antagonists rather than the pathogen, their activities might result in the suppression of disease.

Root exudates from okra supported more growth of fungi in the

laboratory(Figs 4,5 & 6). It was not surprising therefore that more fungi were isolated, from its rhizosphere than 'soko' rhizosphere. Perhaps the greatest obstacle in evaluating the influence of plant exudates on pathogenesis and saprophytic activities of root-infecting fungi is the difficulty in experimenting under natural conditions with the resultant establishment of natural relationships. Thus, study was made under artificial conditions of controlled laboratory experimentation. The phenomenon of root exudation affected a vast variety of interaction in the rhizoplane-rhizosphere length. This was obvious from the results (Figs 10-13).

It was found that the filtered exudates encouraged better growth of fungi in most cases than the autoclaved ones. Autoclaving might have destroyed some properties of these exudates. From the calculated values of F, the means of the linear growth of fungi in the three series of exudate - incorporated agar did not differ significantly even at 1% probability.

ACTION OF FUNGICIDES ON SOME ISOLATED FUNGI:

Aspergillus tamarii Kita was the most susceptible to the fungicides used. Although, the three fungicides were effective in reducing the growth of fungi on solid medium, the most effective was benlate. The nature of the hyphal wall may be responsible for these various responses although permeability is not necessarily related to wall thickness. A thin wall may be as impermeable as a thick one.

In the fungicidal assay by the shaker flasks technique, the flasks were loosely plugged and transferred to a shaking incubator. The ease with which the apparatus was obtained, elimination of aseptic techniques and better control of conditions such as pH, aeration and nutrition afforded by this technique recommend the method for routine studies of fungicides.

Pre-emergence treatment of the seeds showed that thiram was most effective in reducing mycoflora. The percentage germination of okra and 'soko' seeds treated with thiram was higher than

those treated with benlate, brestan and the untreated (Table 6). It is generally assumed that most seed - dressing fungicides control damping-off disease by direct fungicidal action on the pathogen. In laboratory studies, the three fungicides, inhibited fungus growth to varying degrees. This may be explained by the following possibilities: different degrees of fungitoxicity, different degrees of percentage active ingredients, different degrees of solubility in water and the fungicide may be degraded, oxidized or hydrolyzed to a non-effective compound, before the seeds had germinated.

Mycoflora present on or in seeds may result in prolonged dormancy, reduced emergence or vigour of the seedlings due to mycotoxins (Grewal and Mehendra, 1965). Occurrence of mycoflora associated with ungerminated seeds after fungicidal treatment was also estimated (Tables 7 and 8). Growth was much greater in unsterilized than in sterilized soil. This may reflect the microbial stimulation of seed and the root growth in unsterilized soil.

EFFECT OF T. HARZIANUM ON OTHER FUNGAL ISOLATES.

T. harzianum inhibited the growth of R. solani only, of all the fungi grown together with it. Water agar was used for the growth of T. harzianum because it provided no nutrients. Cellophane was used because Fleming and Smith (1944) stated that "if disks of paper or cellophane were placed on the surface of a solid medium, the elements necessary for growth diffused through the disks".

There is an indication that some fungi under favourable conditions may play a beneficial role in the soil, reducing plant diseases by attacking soil-borne pathogenic fungi.

In the past, antagonists usually were selected for ability to inhibit a pathogen under pure culture conditions (Baker and Cook, 1974). One of the reasons for failure of Trichoderma to inhibit other fungi is probably that the environmental conditions

in agar are not related to those in the soil. The application of antagonists in plant pathology should be successful in controlling the disease of damping - off if the antagonists are properly selected and administered. Successful control of diseases with biological agents is dependent upon practice, the biology of the pathogen and the method used for selection and application of the antagonist.

CELLULOLYTIC ACTIVITIES OF FUNGI:

Most of the test fungi (Table 12) utilized CMC confirming the production of C_x cellulase by them while ability to utilize insoluble cellulosic substance indicate presence of C_1 cellulase. A. niger, A. sydowii, A. tamarii and A. japonicus all utilized CMC (Table 13). Olutiola (1976); Reese and Levinson 1952; Rosenberg (1978) all found that Aspergillus species produce cellulase.

In the filter paper medium, A. japonicus, A. flavus and A. sydowii caused more weight loss of filter paper than the other fungi (Table 15) suggesting the presence of C_1 cellulase. Thus the three fungi were able to incorporate the mineral salts in the basal medium thereby showing improved cellulolytic activities. They utilized the filter paper more efficiently in shaken cultures than those kept standing on the laboratory bench.

The shaken and standing cultures showed positive correlation. The calculated correlation coefficient, r, was 0.9929 while the tabulated value for 8 degrees of freedom (n-1) and p = 0.1 was 0.549. Since they are highly correlated, there is probably only a 1% chance of being wrong. Aeration governs the composition of active flora. Because of the energetics of anaerobic processes, the rate of cellulose metabolism in environments lacking good supply of oxygen is significantly reduced by comparison with aerated habitats.

An organism is considered to be cellulolytic if it brings about a 10% loss in tensile strength of cotton strips over a

period of two weeks under the test conditions (Reese and Downing, 1951). The ability of microorganisms to use native cellulose as a substratum is more restricted.

CELLULOSE DECOMPOSITION: EFFECT OF TEMPERATURE AND pH.

At room temperature, the hydrolysis of CMC was best favoured by pH 6.5 - 8.5 (Table 14). In environments of neutral to alkaline pH, it was obvious the fungi grew and liberated the appropriate enzymes for the hydrolysis of the polysaccharide.

The soil usually provides a buffering action around plant residues and while the pH on the surface of the material could be very low, that of the bulk soil may be slightly higher and conducive for fungal attack.

From the results obtained in this work, it can be suggested that different plant species favour the development of a specific rhizosphere mycoflora. It was found that the root zone of the healthy plants had a lot of organisms that could invade the plant and do extensive damage but these were checked by other inhabitants of the same underground ecosystem.

Plant pathologists need not be told that pathogens infecting roots constitute a threat to the welfare of economic plants. Not all pathogenic plant organisms are however detrimental to the plant.

REFERENCES

Adati, M. (1976) Untersuchungen iiber die Rhizosphare des pflanzen. Jour. Soc. Trop. Agr. (Taiwan) 11: 57-65.

Adeniyi, M.O. (1966) Role of fungi associated with brown rot of tomato in Colletotrichum infection. Nigerian Agricultural Journal 3, 4-31

Agnihothrudu, V.(1955) Naturwissenschaften 42: 515-516

Agrois, G.N. (1969a) Plant diseases caused by fungi 209pp. In Plant Pathology - Agrois, G.N.Academic Press, New York, San Francisco, London.

Agrois, G.N.(1969b). Effect of environment on development of infectious plant diseases. 166pp. In Plant Pathology - Agrois G.N. Academic Press, New York, San Francisco, London.

Alexander, M. (1977). Introduction to Soil Microbiology. 2nd edition, John Wiley, New York and London.

Allen, M.C. and Haensler, C.M. (1935). Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. Phytopathology 25: 244 - 252

Anon, (1976). Farm Chemicals Handbook, 1976. Meister Publishing Co; Willoughby, Ohio 44077.

Anwar, A.A. (1949). Factors affecting the survival of Helminthosporium sativum and Fusarium lini in soil. Phytopathology 39: 1005-1019

Baker, K.F. and Cook R.J. (1974) Biological Control of Plant Pathogens. W.H. Freeman and Company, San Francisco 433p.

Barber, D.A. and Gunn, K.B. (1974). The effects of mechanical forces on the exudation of organic substances by roots of cereal plants grown under sterile conditions. New Phytol. 73: 39 - 45.

Barber, D.A. and Martin, J.K. (1976) The release of organic substances by cereal roots into soil. New Phytol 76: 69 - 80.

Barea, J.M. and Brown, M.E. (1974). Effects of plant growth produced by Azobacter paspale related to synthesis of plant growth - regulating substances. J. appl. Bact; 37: 583.

Barnett, H.L. and Binder, F.L. (1973) The fungal host-parasite relationship. Ann. Rev. phytopathol II: 273 - 292

Bollen, W.B. (1961) Interactions between pesticides and soil microorganisms. Ann.Rev. of Microbiology. 15: 69 - 92.

Brancato, E.P. and Golding, N.S. (1953). The diameter of the mold colony as a reliable measure of growth. Mycologia 45: 848 - 864.

Burke, D.W. Holmes, L.D. and Barker, A.W. (1972) Distribution of F. Solani f sp. phaseoli and bean roots in relation to tillage and soil composition. Phytopathology 62: 550 - 554

Buxton, E.W. (1962) Root exudates from banana and their relationship to strains of the Fusarium causing Panama wilt. Ann. Appl. Biol. 50: 269 - 282.

Clark, F.E. (1949) Soil microorganisms and plant roots. Advances in Agron 1:241 - 288

Clarkson, T.W. and Rishbeth. J.(1980). In "Microbial Control of Insects, Mites and Plant Diseases" (ed. H.D. Burges), Vol.2 Academic Press, London.

Darby, R.T. (1960) Fungicide assay by spore germination in shaker flasks. Appl. Microbiol. 8: 146-148.

Domsch, K.H. and Paul, W. (1974) Stimulation and experimental analysis of the influence of herbicides on soil nitrification Archives of Microbiology. 97 (4): 283 - 301

Eggleton, W.G.E. (1938). The influence of environmental factors on number of soil microorganisms. Soil Sci. 46: 351 - 363.

Esterman, E.F. and McLaren, A.O.(1961). Contributions of rhizoplane organisms to the total capacity of plants to utilize organic nutrients. Pl. Soil 15: 243 - 260

Ezedima, F.O.C. (1960) Reports on cowpea (Vigna spp.) in Nigeria before 1960. Federal Department of Agricultural Research, Ibadan, Nigeria. Library Service Publication.

Fleming, A. and Smith G. (1944), Some methods for the study of moulds. Trans Br. Mycol. Soc. 27: 13 - 19

Giha, O.H. (1976). Fungi associated with foot and root rot diseases of irrigated wheat in the Northern States of Nigeria. Pest Articles and News Summaries 22: 479 - 487.

Goldsworthy, M.C. Green E.L. and Smith, M.A. (1943) Fungicidal and Phytocidal properties of some metal dialkyldithiocarbamate. J. Agr. Res. 66: 277.

Gottlieb, D.1 and P. Siminoff, (1952) The production and role of antibiotics in soil. II. Chloromycetin Phytopathology. 42: 91-92.

Gottlieb, D; Siminoff, P. and Martin, M.M. (1952) The production and role of antibiotics in soil IV. Actidione and Clavacin. Phytopathology 42: 493 - 496.

Grewal, J.S. and Mehendra (1965) Seed mycoflora. I. Seed fungi of Ragi (Eleusine coracana Goertn), their distribution and control. Indian Phytopath. 18: 33 - 37

Harder, Y; Chet, I; and Y. Henis. (1979). Biological control of Rhizoctonia solani damping-off with wheat bran culture of Trichoderma harzianum. Phytopathology 69: 64 - 68

Hiltner L. (1904) Arb. deut. Landwirtsch - ges. Osterreich 98, 57-78

Hislop, E.C.(1976) In "Microbiology of Aerial Plant Surfaces" (ed. C.H. Dickinson and T.F. Preece), 41 - 74pp. Academic Press, London.

Horst, R.K. and Williamson, C.E.(1974) New York State Insecticide, Fungicide and Herbicides recommendations. Cornell University. Ithaca. New York.

Jay, J.M.(1978) Modern Food Microbiology 2nd edition. D. Van Nostrand Company New York, Toronto. London. 479 pp.

Jensen, H.L. (1931) — The fungal flora of the soil. Soil Sci. 31: 123 - 158.

Johnen, B.G. and Drew, E.A. (1977) — Ecological effects of pesticides on soil microorganisms. Soil Sci 123 (5): 319 - 324.

Katznelson, H. and Sirois, J.C. (1961) — Auxin production by species of Arthrobacter. Nature (London) 191: 1323-1324.

Kearney, P.C. and Kaufman, D.D. (1976) — Herbicides: Chemistry, degradation and mode of action. Vol.2: 2nd edition Marcel Dekker, Inco. New York.

Keppel, G.E. (1956). — Report on TMTD. J.Assoc. Offic. Agric. Chem.; 39: 709 - 712

Le Clerg, E.L. (1964) — Grop losses due to plant diseases in the U.S. Phytopathology 64: 1309 - 1313.

Lundergardh, H. (1945) — Absorption, transport and exudation of inorganic ions by the roots. Arkiv. Botanik 32A (12): 1 - 139.

McDonald, D. (1968) — Soil fungi and fruit of groundnut (Arachis hypogea) Samaru Miscellaneous Paper No. 28. Institute of Agricultural Research A.B.U. Zaria, Nigeria.

McDonald, D. (1069) — Influence of the development of groundnut on soil mycoflora in Nigeria. Trans. Br. Mycol. Soc., 53: 393 - 406.

McDonald, D. (1970a) — Fungal infections of groundnut after maturity and during drying. Trans.Br. Mycol. Soc., 54: 461 - 472.

McDonald, D. (1970b) — Fungal infection of groundnut before harvest. Trans. Br. Mycol. Soc. 54: 453 - 460.

McKeen, C.D. (1950) — Arasan as a seed and soil treatment for the control of damping - off in certain vegetables. Sci. Agric. 30:261-271

McManus, M.A. (1960) — Certain mitotic effects of kinetin, G.A. I.A.A. and nucleic hydrazide on the root of Allium cepa L. Nature (London) 185: 44 - 45.

Michael, A.H. and Nelson, P.E. (1972) Antagonistic effect of soil bacteria on Fusarium roseum 'Culmorum' for carnation. Phytopathology 62: 1052 - 1056.

Newman, E.I. and Watson, A. (1977) Microbial abundance in the rhizosphere: A computer model. Pl. Soil. 45: 17-56

Odeyemi, O. (1979) Persistance of arasan (pesticide) in aquatic ecosystems in Nigeria. Prog. Wat. Tech. 1979, Vol. II, No.6, pp. 95 - 102:Pergamon Press. Printed in Great Britain.

Odeyemi O and Alexander M. (1977) Resistance of Rhizobium strains to phygon, spergon and thiram Appl. Environ. Microbiol. 33,784-790.

Odunfa, V.S.A. (1978) Root exudation in Cowpea and Sorghum and the effect on spore germination and growth of some soil fusaria. New Phytol 80:607-612.

Odunfa, V.S.A. and Oso, B.A. (1979). Fungal populations in the rhizosphere and rhizoplane of cowpea. Trans Br. Mycol. Soc. 73 (1): 21 - 26

Oke, O.L. (1966) Chemical studies on the more commonly used leaf vegetables in Nigeria. J. West Africa Sci. Assoc. II: 42

Okigbo, B.E. (1975) Farming systems for the production of fruits and vegetables pp 48 - 62. In 1st Natural Seminar on Fruits and vegetables. O.O. Ojehomon, P.A. Town and K. McLean, eds) Ibadan, Nigeria.

Olutiola, P.O. (1976) Cellulase enzymes in culture filtrates of Aspergillus flavus, Trans Br. Mycol. Soc. 67 (2): 265 - 268.

Ordish, G. and Mitchell, J.F. (1967) World fungicide usage. In D.C. Torgesson (ed). Fungicides. Academic Press. New York. 39 - 62 pp.

Park, D. (1976) Cellulose decomposition by a pythiaceous fungus. Trans. Br. Mycol. Soc. 66 (1) 65 - 70

Redfield, R.R. (1935) Two dimensional paper chromatographic systems with high resolving power for amino acids. Biochem. Biophys. Acta. 10: 344.

Reese, E.T. and Downing, M.H. (1951) Activity of the Aspergilli on Cellulose, cellulose derivatives and wool. Mycologia 43: 16.

Reese, E.T. and Levinson, H.S.(1952) A comparative study of the breakdown of cellulose by microorganisms. Physiol. Plant. 5: 345 - 366

Rhodes, M.J.C.(1978) Keeping quality of fruits and vegetables. Nutrition and Food Science, 52: 20.

Rosenberg, S.L.(1978) Cellulose and ligno-cellulose degradation by thermophilic and thermotolerant fungi. Mycologia 70: (1) 1 - 72.

Rouatt, J.W. Peterson, E.A. Katznelson H, and Henderson, V.E. (1963). Microorganisms in the root zone in relation to temperature. Can J. Microbiol. 9: 227 - 236

Rovira, A.D. (1959) Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plant, light, temperature and calcium on exudation. Pl. Soil 11: 53 - 64

Rovira, A.D. (1969) Diffusion of Carbon compounds away from wheat roots. Aust. J. Biol. Sci. 22: 1287.

Salter, P.J. and J.P. Williams (1967) The influence of texture on the moisture characteristics of soil. J.Soil. Sci. 18: 174 - 81.

Schreven, D.V.: D.J. Lindenberg and A. Koriddon (1970) Effect of several herbicides on bacterial populations and activity and the persistence of these herbicides in soil. Plant and Soil. 33:513 - 532

Schroth, M.N. and Snyder, W.C. (1961) Effect of host exudates on chlamydospore germination of the bean root rot fungus, Fusarium Solani f. Phaseoli. Phytopathology 51: 389 - 393.

Starkey R.L.(1932) — The application of available methods to studies of certain factors associated with changes in the soil population. Proc. 2nd Int. Congr. Soil Sci. Leningrad - Moscow III 248 - 259.

Sulochana, C.B. (1962) — Amino acids in root exudates of cotton Pl. Soil. 16: 312 - 326

Taylor, C.F. and Rupert, J.A. (1946) — A study of vegetable seed protectants. Phytopathology 36: 726 - 749.

Thonner, F. (1962) — The flowering plants of Africa. Wheldon and Wesley Ltd. and Hafner Publishing Co. Codicotes - Herts, New York. 647 pp.

Trevelyan, W.E.; Procter, D.P. and Harrison, J.S. (1950) — Detection of sugars on paper chromatograms. Nature, London, 166: 444 - 445

Vrany, J; V. Vancura and Macura, J. (1962) — The effect of foliar applications of some readily metabolised substrate growth regulators and antibiotics on rhizosphere microflora. Foliar microbiol. Praque 7: 61 - 70.

Warcup, J.H. (1950). — The Soil-Plate method for isolation of fungi from soil. Nature 166: 117-118.

Weindling, R. and H.S. Fawcett, (1936). — Experiments in the control of Rhizoctonia damping-off of citrus seedlings. Hilgardia 10: 1 - 16.

Westeijn, G. (1967) — Symptomatology and incidence of some root diseases of cocoa (Theobromae cocoa L.) in Nigeria. Nigeria Agricultural Journal 4: 60 - 63

White, A.A. and Hess, W.C. (1956) — Paper chromatographic detection of sugars in normal and dystrophic human urines. Arch. Biochem. Biophys. 64: 57 - 66.

APPENDIX 1

% RELATIVE HUMIDITY (HM^{-26})

WK	AV. % RH
1	90.25
2	92.0
3	71.0
4	73.0
5	72.0
6	69.0
7	72.0
8	80.0
9	63.0
10	70.0
11	73.0
12	72.0
13	69.0
14	52.0
15	79.0
16	80.0
17	81.0
18	88.5
19	82.0
20	87.0

(APPENDIX 2) DATA FOR FIG. 3

RATE OF THIRAM DISAPPEARANCE IN SOIL

DAYS OF INCUBATION	CONC. OF ARASAN - µG/G	
	UNSTERILIZED SOIL	STERILIZED SOIL
0	99.0	99.0
7	42.0	61.0
14	15.0	21.0
21	6.0	12.0
28	3.0	5.0
35	0.0	0.0

APPENDIX 3 (DATA FOR FIG.4 - 6)

LINEAR GROWTH OF FUNGI ON CDA AND EXUDATES FOR OKRA AND 'SOKO'

FUNGUS	TREATMENTS (SEITZ FILTERED)			
P. citrinum	DAYS	CDA	HDA	CA
	1	1.30	1.10	1.70
	2	2.80	2.70	3.40
	3	3.75	3.30	4.10
	4	4.0	3.90	4.50
	5	4.5	4.20	4.50
S. constrictum				
	1	1.15	1.50	1.20
	2	2.60	2.90	2.60
	3	3.55	3.80	3.50
	4	3.90	4.20	3.95
	5	4.20	4.50	4.20
F. oxysporum				
	1	1.50	1.00	1.40
	2	1.90	1.60	1.70
	3	2.40	2.2	2.30
	4	2.90	2.50	2.90
	5	3.60	3.40	3.60
B. theobromae				
	1	1.20	1.50	1.10
	2	2.50	2.70	2.20
	3	3.40	3.80	3.0
	4	3.80	4.20	3.7
	5	4.20	4.50	4.1

	DAYS	CDA	HDA	CA
T. harzianum				
	1	1.05	1.25	0.95
	2	2.20	2.40	2.10
	3	3.25	3.25	3.15
	4	3.90	3.95	3.70
	5	4.50	4.5	4.20
C. pallescens				
	1	1.25	1.30	1.20
	2	1.45	1.53	1.40
	3	2.0	2.10	1.90
	4	2.40	2.60	2.30
	5	2.40	2.60	2.30
M. cinctum				
	1	1.30	1.50	1.70
	2	1.80	2.20	2.30
	3	2.0	2.90	2.70
	4	2.18	3.40	3.20
	5	2.8	3.40	4.0

APPENDIX 4 (DATA FOR FIG. 7-9)

LINEAR GROWTH (CM) OF FUNGI ON CZAPEK DOX AGAR (CDA) AND EXUDATES OF OKRA (HDA) AND 'SOKO' (CA). 4 REPLICATES.

FUNGUS	TREATMENTS (AUTOCLAVED)		
	DAYS CONTROL		
P. citrinum	DAYS	CDA	HDA	CA
	1	1.30	1.10	1.50
	2	2.80	2.50	3.10
	3	3.75	3.43	3.90
	4	4.0	3.95	4.20
	5	4.50	4.50	4.50
S. constrictum				
	1	1.15	1.30	1.10
	2	2.60	2.70	2.50
	3	3.55	2.63	3.45
	4	3.90	4.0	3.75
	5	4.20	4.50	4.15
F. oxysporum				
	1	1.50	0.90	1.30
	2	1.90	1.50	1.70
	3	2.40	2.10	2.20
	4	2.90	2.60	2.80
	5	3.60	3.1	3.40
B. theobromae				
	1	1.20	1.40	1.0
	2	2.50	2.80	2.1
	3	3.40	3.60	2.9
	4	3.80	3.95	3.4
	5	4.20	4.50	3.9

	DAYS	CDA	HDA	CA
T. harzianum				
	1	1.05	1.25	0.95
	2	2.20	2.40	2.10
	3	3.25	3.25	3.15
	4	4.90	3.95	3.70
	5	4.50	4.50	4.20
C. pallescens				
	1	1.25	1.30	1.20
	2	1.45	1.53	1.40
	3	2.00	2.10	1.90
	4	2.40	2.60	2.30
	5	2.40	2.60	2.30
M. cinctum				
	1	1.30	1.50	1.70
	2	1.80	2.20	2.30
	3	2.0	2.90	2.70
	4	2.18	3.40	3.20
	5	2.8	4.30	4.0

APPENDIX 5 (DATA FOR FIGS. 10 - 13)

TOTAL BACTERIAL AND FUNGAL COUNTS IN 0.1ML OF AIR-DRIED SOIL FOR OKRA AND 'SOKO' (3 REPLICATES).

CLASS DISTANCE (cm)	BACTERIA x 10^3		FUNGI x 10^2	
	OKRA	SOKO	OKRA	SOKO
0 - 3	10.0	80	33	23
3 - 6	11	72	25	8
6 - 9	13	74	30	6
9 - 12	14	77	29	6
12 - 15	15	99	31	6
15 - 18	26	93	27	6
18 - 21	28	64	27	4
21 - 24	31	59	22	8
24 - 27	39	34	15	6
27 - 30	37	31	22	5
30 - 33	31	36	6	3
33 - 36	34	34	11	5
36 - 39	22	39	21	5
39 - 42	21	27	22	6
42 - 45	24	29	14	3
45 - 48	21	24	7	8
48 - 51	19	21	16	6
51 - 54	20	22	10	5
54 - 57	18	19	10	1
57 - 60	23	20	22	2
60 - 63	14	22	6	5
63 - 66	34	24	10	4