SOIL CONDITIONS AND ROOT DISEASES

VIII. Rhizosphere Microflora of Some of the Important Crop Plants of South India

By V. Agnihothrudu

(University Botany Laboratory, Madras)

Received November 7, 1952

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

In recent years considerable attention has been paid to the study of the "rhizosphere" of crop plants, particularly in relation to soil-borne diseases of plants (Timonin, 1940, 1941, 1947; Lochhead, *et al.*, 1940; Hildebrand and West, 1941; West and Hildebrand, 1941; Harper, 1951). The results of investigations so far reported on this subject indicate a fruitful line of work and appeared to be important. During the past few years the problem of soil conditions in relation to root diseases has received attention from workers in this laboratory and as a part of this general programme of research, the study of the rhizosphere microflora of certain crop plants was undertaken, being a preliminary to a better understanding of the problems of infection, disease resistance, etc. The results of the study are presented in this paper.

Materials and Methods

Infected soil from the laboratory garden plot was passed through a 1/16″ mesh sieve and filled into earthenware pots. Twenty seeds per pot were sown. A series of unplanted pots served as control. At each sampling 10–20 plants were taken from each series for rhizosphere analysis. The block of soil surrounding the plant roots was carefully cut out, gently crushed with as little of tearing of roots as possible. The superfluous soil was removed by gently tapping the root system. The tops of the seedlings were cut off and roots with the soil attached deposited into a sterile Erlenmeyer flask with 100 ml. of sterile tap water and suitable dilutions were prepared. In fully grown plants only the representative portions of the root system were selected (Timonin, 1940). The first dilution was shaken by hand for 5 minutes and the subsequent dilutions 1 minute each which were prepared on the basis of 1 : 10, *i.e.*, each higher dilution made by taking 10 ml. of lower dilution and adding to 90 ml. of sterile water. The numbers of micro-organisms were estimated by plating method (Brierley, *et al.*, 1927).

Counts were made on 4 replicate plates and average counts recorded. Modified Horne and Mitter's medium (pH_{4-5}) was used for fungi, and for bacteria and actinomycetes Thornton's medium (pH_{6-8}). Fungal counts were taken on the 5th and the 7th days, bacteria on the 7th and the 10th days, actinomycetes after 11 days of incubation at laboratory temperature (29–31° C.). The dilutions of control soil were made on 10 g. basis.

The weight of the rhizosphere soil was determined by removing the roots from the original flask and draining off its contents completely into a previously weighed Petri dish. The water was evaporated on a water-bath and the soil residue dried to constant weight in an oven at 105° C. The weight of the soil lost in the first dilution from the original flask was determined by evaporating 10 ml. of the first dilution on a watch-glass and this was added to the weight of the rhizosphere soil every time. From this final weight of the rhizosphere soil the dilution coefficient was computed. The numbers of fungi, bacteria and actinomycetes are expressed on the basis of 1 g. moisture-free soil. All the results were statistically analysed.

EXPERIMENTAL

Representative plants from four different families, namely, Leguminoseæ: pigeon-pea (*Cajanus cajan*); sann-hemp (*Crotalaria juncea*) ; cluster bean (*Cyamopsis tetragonoloba*); french bean (*Phaseolus vulgaris*); Pedalineæ: sesame (*Sesamum indicum*); Malvaceæ: K.2 cotton (*Gossypium arboreum*, v. *neglectum* forma *indica*) and Gramineæ: sorghum (*Sorghum dochna*, v. *irungu*) were selected for rhizosphere studies which extended over a period of three months. All seeds were sown after surface sterilization with 1 : 1000 aqueous mercuric chloride. Cotton seeds were delinted with concentrated sulphuric acid. Sampling for rhizosphere analysis was done at 15, 31, 46, 61, 77 and 93 days after complete germination. Of the plants studied except pigeon-pea, cotton and *Sorghum*, in the remaining flowering and pod-setting occurred during the estimations. In french bean and cluster bean flowering was observed about 30 days after germination whereas in sesame and sann-hemp it was recorded after 40 and 62 days respectively.

RESULTS

Quantitative Nature of the Rhizosphere Microflora

1. Significantly higher numbers of bacteria, fungi and actinomycetes were recorded in the rhizosphere than in the control soil. A decreasing order of 'rhizosphere effect', *i.e.*, the rhizosphere: control soil ratio was noted as follows: bacteria > fungi > actinomycetes (Tables I, II and III; Text-Fig. 1).

TABLE I

Numbers of Fungi, Bacteria and Actinomycetes in the Rhizosphere

Plants	Fungi*						Bacteria*						Actinomycetes*					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	61	77	93
Pigeon-pea ..	488	415	575	475	305	280	1435	1783	1873	1993	2230	2380	2·3	2·5	4·0	5·5	5·0	6·0
Cluster bean ..	145	175	295	190	298	405	998	1315	1233	1040	900	830	4·0	4·3	5·0	6·8	7·0	8·0
Cotton ..	130	225	350	303	230	223	743	833	998	1058	1250	1350	4·0	3·5	4·0	5·0	5·0	7·3
Sorghum ..	440	370	413	400	308	270	720	858	903	1003	1230	1345	8·5	8·8	9·8	10·8	10·0	12·0
Sesame ..	118	150	248	180	283	353	493	835	943	723	693	553	5·0	4·0	6·8	5·3	7·0	8·0
French bean ..	288	315	410	433	493	595	1320	1475	1390	1048	883	1033	1·5	3·8	4·5	6·3	8·3	10·3
Sann-hemp ..	385	430	395	233	380	490	1248	1370	1670	2450	2040	1930	3·5	5·0	6·5	5·3	6·0	5·5
Control soil ..	55	55	65	60	60	60	7·0	7·0	10·0	9·0	10·0	10·0	1·5	1·3	1·8	2·0	1·8	1·8

* Fungi in thousands, Bacteria and Actinomycetes in millions per 1 g. moisture-free soil.

TABLE I

Numbers of Fungi, Bacteria and Actinomycetes in the Rhizosphere

Plants	Fungi*						Bacteria*						Actinomycetes*					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	61	77	93
Pigeon-pea ..	488	415	575	475	305	280	1435	1783	1873	1993	2230	2380	2·3	2·5	4·0	5·5	5·0	6·0
Cluster bean ..	145	175	295	190	298	405	998	1315	1233	1040	900	830	4·0	4·3	5·0	6·8	7·0	8·0
Cotton ..	130	225	350	303	230	223	743	833	998	1058	1250	1350	4·0	3·5	4·0	5·0	5·0	7·3
Sorghum ..	440	370	413	400	308	270	720	858	903	1003	1230	1345	8·5	8·8	9·8	10·8	10·0	12·0
Sesame ..	118	150	248	180	283	353	493	835	943	723	693	553	5·0	4·0	6·8	5·3	7·0	8·0
French bean ..	288	315	410	433	493	595	1320	1475	1390	1048	883	1033	1·5	3·8	4·5	6·3	8·3	10·3
Sann-hemp ..	385	430	395	233	380	490	1248	1370	1670	2450	2040	1930	3·5	5·0	6·5	5·3	6·0	5·5
Control soil ..	55	55	65	60	60	60	7·0	7·0	10·0	9·0	10·0	10·0	1·5	1·3	1·8	2·0	1·8	1·8

* Fungi in thousands, Bacteria and Actinomycetes in millions per 1 g. moisture-free soil.

TABLE III

Table of Significance

Age of plant in days	Fungi	Bacteria	Actinomycetes
15	Pp, Sor, Sh, Fb, Cb, Cot, Ses, C	Pp, Fb, Sh, Cb, Cot, Sor, Ses, C	Sor, Ses, Cb, Cot, Sh, Pp, Fb, C
31	Sh, Pp, Sor, Fb, Cot, Cb, Ses, C	Pp, Fb, Sh, Cb, Sor, Cot, Ses, C	Sor, Sh, Cb, Ses, Fb, Cot, Pp, C
46	Pp, Sor, Fb, Sh, Cot, Cb, Ses, C	Pp, Sh, Fb, Cb, Cot, Ses, Sor, C	Sor, Ses, Sh, Cb, Fb, Pp. Cot, C
61	Pp, Fb, Sor, Cot, Sh, Cb, Ses, C	Sh, Pp, Cot, Fb, Cb, Sor, Ses, C	Sor, Cb, Fb, Pp, Sh, Ses, Cot, C
77	Fb, Sh, Sor, Pp, Cb, Ses, Cot, C	Pp, Sh, Cot, Sor. Cb, Fb, Ses, C	Sor, Fb, Cb, Ses, Sh, Pp, Cot, C
93	Fb, Sh, Cb, Ses, Pp, Sor, Cot, C	Pp, Sh, Cot, Sor, Fb, Cb, Ses, C	Sor, Fb, Ses, Cb, Cot, Pp, Sh, C

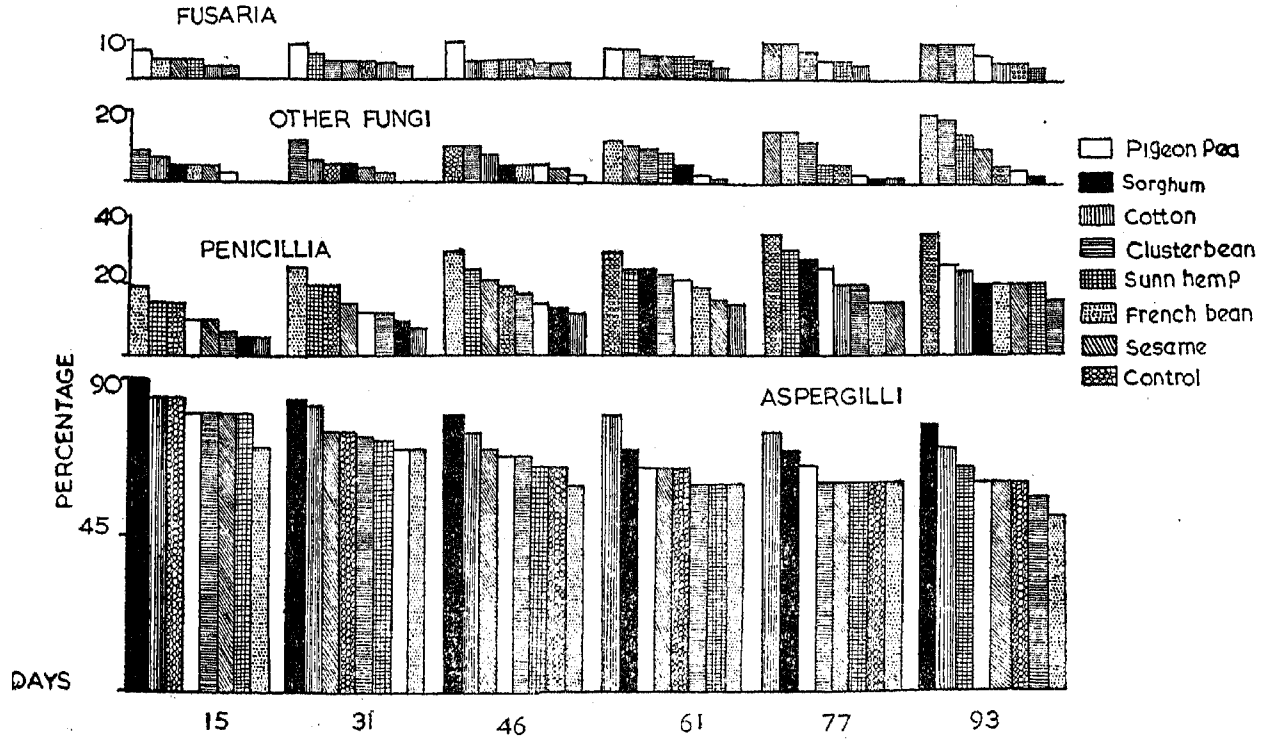
Pp = Pigeon pea
Sor = Sorghum
Sh = Sann-hemp
Fb = French bean
Cb = Cluster bean
Cot= Cotton
Ses = Sesame
C = Control soil

3. A decrease in fungal numbers was recorded with advance in age of the plants but in flowered plants (french bean, cluster bean, sesame and sann-hemp) an increase in numbers was recorded after flowering. In the unflowered plants the bacteria increased in numbers with the age of the plant but, in the flowered plants this increase continued up to the time of flowering only followed by a fall in the pod setting stages of the plants (Tables I and III; Text-Fig. 1).

4. *Sorghum* had consistently higher numbers of actinomycetes in the rhizosphere than in the case of the other plants studied. Although in the seedling stages no significant differences between rhizosphere and control soil numbers of actinomycetes were seen, with the advance in age of the plants the stimulative effect of the root was evident (Tables I and III; Text-Fig. 1).

Qualitative Nature of the Rhizosphere Fungal Flora

(*a*) *Fungi in the Dilution Plates.*—In order to make a critical study of the nature and abundance of various fungi occurring in the rhizosphere of different plants, the fungal colonies in the dilution plates were carefully examined and identified to the genus. The approximate numbers and percentage occurrence of some of the more predominant soil fungi, namely, *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., were calculated from the dilution plates and the data presented in Table I, Text-Fig. 2.



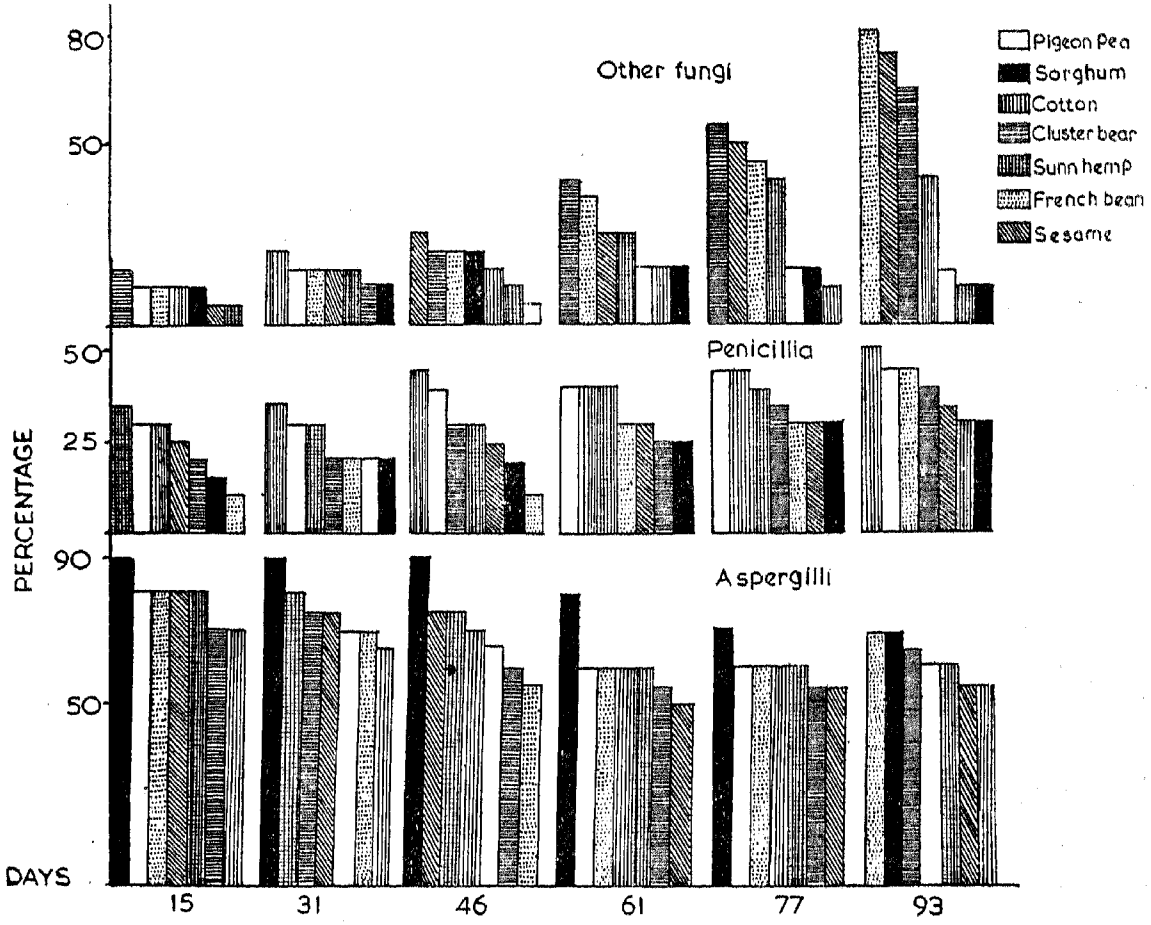
TEXT-FIG. 2. Showing the percentage occurrence of Aspergilli, Penicillia, Fusaria and other fungi in the dilution plates.

(*b*) *Fungi from Root Plantings.*—The roots of the plants used in the dilution were removed, washed in several changes of sterile tap water and plated on modified Horne and Mitter's medium. The percentage occurrence of various fungi was recorded and expressed on twenty root-bit basis (Text-Figs. 3 and 4).

Conclusions

1. *Aspergilli.*—This is by far the most predominant fungus in the rhizosphere and highest percentage was recorded in *Sorghum* and cotton (Table IV; Text-Figs. 2 and 3).

2. In general both in the control and rhizosphere soils, decrease in numbers and percentage occurrence of Aspergilli was recorded towards the final estimation except in flowered plants where an increase at the end was registered (Table IV; Text-Fig. 2).



Text-Fig. 3. Showing the percentage occurrence of Aspergilli, Penicillia and other fungi in the root platings.

TABLE IV

Numbers of Aspergilli, Penicillia and Other Fungi in the Rhizosphere

Plants	Aspergilli						Penicillia						Other Fungi					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	51	77	93
Pigeon pea ..	390	294	398	312	201	167	50	63	106	120	87	74	10	21	13	5	2	20
Cluster bean ..	120	133	204	114	180	220	12	21	54	45	60	60	13	22	30	20	36	76
Cotton ..	204	263	263	240	173	154	67	42	42	45	46	55	8	0	27	0	0	3
Sorghum ..	396	315	328	280	217	210	22	37	62	100	87	54	22	18	23	20	4	5
Sesame ..	96	113	175	117	168	210	12	24	55	32	42	70	4	6	10	20	42	35
French bean ..	203	224	246	258	300	300	58	80	123	86	75	120	15	2	20	51	75	120
Sann-hemp ..	312	314	260	138	228	308	58	86	100	58	114	98	0	0	20	20	19	30
Control soil ..	47	41	39	36	36	36	8	11	13	18	21	21	0	0	8	0	3	3

Fungi in thousands per 1 g. of moisture-free soil.

3. Unlike Aspergilli, Penicillia show a gradual increase in the numbers and percentage with the advance in age of the plant (Table IV; Text-Fig. 2).

4. The changes in the total fungal numbers roughly correspond with the changes observed in numbers and percentage occurrence of Aspergilli and Penicillia (Tables I and IV).

5. Although Fusaria were very rarely observed in the control soil dilutions, they were regularly encountered in the rhizosphere dilutions. Highest numbers and percentage occurrence was recorded in the case of pigeon-pea whereas it was almost absent in *Sorghum* (Table V; Text-Figs. 2 and 4).

6. *Macrophomina phaseoli* was not obtained in dilution plates. Highest percentage occurrence was recorded in the seedling stages of plants but in french bean and cluster bean a second increase was observed in the final estimation (Text-Fig. 4).

7. *Neocosmospora vasinfecta* was very rarely obtained in the dilution plates. Highest percentage was recorded on the roots of pigeon-pea.

Both *M. phaseoli* and *N. vasinfecta* were absent in the case of *Sorghum* (Text-Fig. 4).

TABLE V

Fusaria in the Rhizosphere

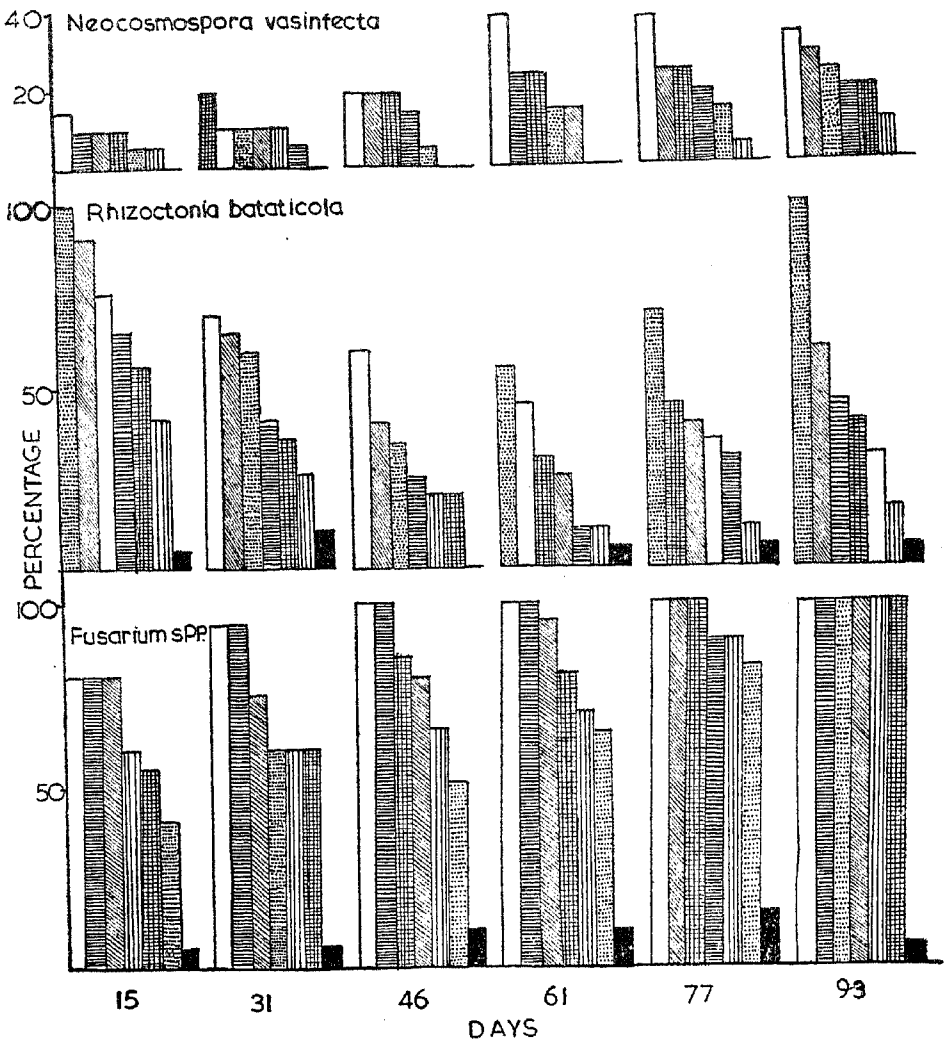
Plants	Thousands in 1 g. of moisture-free soil						Percentage occurrence in dilution plates						Percentage occurrence in root platings					
	Age of the plant in days						Age of the plant in days						Age of the plant in days					
	15	31	46	61	77	93	15	31	46	61	77	93	15	31	46	61	77	93
Pigeon pea ..	40	42	58	38	15	20	8	10	10	8	5	7	80	95	100	100	100	100
Cluster bean ..	5	9	12	11	24	40	3	5	4	6	8	10	80	95	100	100	90	100
Cotton ..	4	18	18	15	13	11	3	4	5	3	4	5	60	60	65	70	90	100
Sorghum ..	0	0	1	2	1	1	0	0	0·23	0·5	0·3	0·37	5	5	10	10	15	5
Sesame ..	6	8	10	11	28	35	5	5	4	6	10	10	80	75	80	95	100	100
French been ..	15	9	20	34	5	60	5	3	5	8	10	10	40	60	50	65	80	100
Sann-hemp ..	2	26	20	13	19	20	5	7	5	6	5	4	55	60	85	80	100	100
Control soil ..	0	3	3	3	0	3	0	5	5	5	0	5	..	..	..	..	..	..

8. *Other Fungi.—Alternaria, Stemphylium, Curvularia, Helminthosporium, Trichoderma, Mucor, Rhizopus, Cunninghamella, Phoma, Diplodia, Chætomium,* etc., were often recorded in the rhizosphere dilutions and root plantings. In the unflowered plants the occurrence of these fungi was irregular but a definite increase in numbers and percentage occurrence was recorded in the final estimation of the flowered plants (Table IV; Text-Figs. 2 and 4).

DISCUSSION

Significantly greater accumulations of fungi, bacteria and actinomycetes were recorded in the rhizosphere than in the control soils (Tables I, II and III; Text-Fig. 1), confirming the observation reported by many of the previous workers (Starkey, 1929, 1931, 1938; Gräf, 1930; Timonin, 1940; Krassilnikov, 1945; Katznelson, 1946). Leguminous plants, namely, pigeon-pea, french bean, cluster bean and sann-hemp had highest numbers of bacteria in the rhizosphere than the non-legumes, an observation similar to that of Starkey (1931) in his studies on the influence of the development of higher plants upon the micro-organisms of the soil (Tables I, II and III; Text-Fig. 1).

Increase in bacterial numbers with the age of the plants was recorded. In the flowered plants the increase continued up to the time of flowering



TEXT-FIG. 4. Showing the percentage occurrence of *Rhizoctonia bataticola* (*Macrophomina phaseoli*), *Neocosmospora vasinfecta* and Fusaria in the root platings.
(Index same as in Text-Fig. 3)

followed by a fall during pod setting stages of plants, but in the unflowered plants the increase continued up to the final estimation (Table I; Text-Fig. 1). The behaviour of the fungal numbers on the other hand was different. The numbers in the flowered plants in general continued to decrease up to the time of flowering followed by an increase (Table I; Text-Fig. 1). The observed maximum 'rhizosphere effect' during the active vegetative growth was also reported by Starkey (1938), Gräf (1930), Katznelson (1946), Krassilnikov (1945). Gräf (1930) in her studies on the rhizosphere microflora noted an increase in the total numbers of micro-organisms during the active growth of the plants followed by a decline during the ripening period

and a further decline after the harvest of the crop. The decrease in fungal numbers recorded prior to flowering was not reported by any of the previous workers, although Krassilnikov (1945) reported a gradual increase in the fungi after the maturity of the plant.

The actinomycete population of the soil was not much influenced by the roots in the early stages of plant growth but with the advance in age of the plant significant differences were obtained which may probably be due to the slow growth rate of actinomycetes (Tables I and III; Text-Fig. 1). Although the numbers of actinomycetes in the rhizosphere are greater than the fungi, the rhizosphere: soil ratios are not as much as fungi (Tables I and II). Despite the fact that the root effect is not so pronounced in fungi and actinomycetes, it is not improbable that their activity is as much modified as bacteria.

Rhizosphere effects with fungi have not attracted the same attention as those concerning bacteria and there are comparatively few records concerning the qualitative nature of the fungi (Thom and Humfeld, 1932; Timonin, 1940, 1941; Katznelson, Lochhead and Timonin, 1948). The percentage occurrence of Aspergilli in the rhizosphere and control soils was the same, but in the case of Penicillia lower percentage occurrence in the rhizosphere as compared to the control soil was observed (Text-Fig. 2). In general the changes in the total fungal numbers of the rhizosphere roughly correspond with the quantitative changes recorded in Aspergilli and Penicillia (Tables I, IV and V; Text-Figs. 1, 2 and 3).

Fusarium spp. also constituted one of the important genera in the rhizosphere fungal population. More than 50 per cent. of all the plant roots yielded *Fusarium* spp. except *Sorghum* (Table V; Text-Fig. 4). Although this fungus was abundant on the root surface comparatively lower numbers were obtained in the dilution plates (Table V; Text-Figs. 2 and 4).

Macrophomina phaseoli and *Neocosmospora vasinfecta* were not obtained in dilution plates. Highest percentage occurrence of the former was recorded during the seedling stages followed by a fall with the advance in age of the plants, while the percentage occurrence of the latter fungus increased with the age of the plant and the highest percentage was recorded in pigeon-pea on which it has recently been proved to be pathogenic by Sarojini (1952) (Text-Fig. 4). Similarly highest percentage occurrence of *Macrophomina phaseoli* was observed on the roots of french bean which has been proved to be most susceptible to this fungus (John Chinnayya, 1951). The predominance of this fungus on the root surface of plants in early stages of growth suggests that it may be a seedling pathogen.

The higher numbers of fungi found in estimations of flowered plants can be attributed to the preliminary stages of root colonization by soil saprophytes following decline in plant growth (Table IV; Text-Figs. 2 and 3). It may be that the common saprophytes of the soil find the root region uninhabitable as long as the plant growth is active but with the advent of senility they begin their invasion or colonization.

SUMMARY

1. Greater accumulations of fungi, bacteria and actinomycetes in the rhizosphere than in the control soils were recorded.

2. A decreasing order of 'rhizosphere effect' as follows was noticed: bacteria > fungi > actinomycetes.

3. Maximum 'rhizosphere effect' on bacteria was noticed during the time of flowering. Increase in fungal numbers after flowering was recorded.

4. Changes in the total fungal numbers correspond with the changes in numbers of Aspergilli and Penicillia, both in the dilution plates and the root platings.

5. The absence of certain predominant fungi like *Fusarium* spp., *Macrophomina phaseoli*, *Neocosmospora vasinfecta* in the rhizosphere dilutions emphasizes the necessity of assessing the physiological state in which they are extant in the rhizosphere.

6. *Sorghum* stands unique in that its rhizosphere had highest numbers of fungi among the non-legumes and highest numbers of actinomycetes among all the plants studied. The potential root pathogens so often encountered in the rhizosphere of other plants were absent in the case of *Sorghum*.

ACKNOWLEDGEMENTS

I am deeply indebted to Professor T. S. Sadasivan and Dr. C. V. Subramanian for critically reading the manuscript and thankful to Dr. E. John Chinnayya for assistance in the statistical analysis of the data. I thank the University of Madras for award of studentship during the tenure of which this investigation was conducted.

REFERENCES

Brierley, W. B., Jewson, S. T. and Brierley, M. .. "The quantitative study of soil fungi," *Proc. International Congress of Soil Science*, 1927, **3**, 24.

Gräf, G. .. "Über den Einfluss des pflanzenwachstums auf die Bakterien im wurzelbereich," *Centbl. Bakt.*, II, 1930, **82**, 44–69.

Harper, J. L. .. "Studies in the resistance of certain varieties of banana to panama disease, II. The Rhizosphere," *Plant and Soil*, 1950, 2, 383–94.

Hildebrand, A. A. and West, P. M. "Strawberry root rot in relation to microbiological changes induced in root rot soil by the incorporation of certain cover crops," *Canad. J. Res. C.*, 1941, **19**, 183–98.

John Chinnayya, E. .. "Soil conditions and wilt diseases in plants with special reference to *Rhizoctonia* spp. on cotton," 1951 (Thesis approved for the Ph.D. degree of the Madras University).

Katznelson, H. .. "The rhizosphere effect of mangels on certain groups of soil micro-organisms," *Soil Sci.*, 1946, **62**, 343–54.

———, Lochhead, A. G. and Timonin, M. I. "Soil micro-organisms and the rhizosphere," *Bot. Rev.*, 1948, **14**, 543–87.

Krassilnikov, N. A. .. "Microflora of soils as influenced by plants," *Microbiology* (U.S.S.R.), 1945, **13**, 187–98, (English summary).

Lochhead, A. G., Timonin, M. I. and West, P. M. "The microflora of the rhizosphere in relation to resistance of plants to soil-borne pathogens," *Sci. Agr.*, 1940, **20**, 414–18.

Sarojini, T. S. .. "Soil conditions and root diseases. Fusariose disease of *Cajanus* with special reference to *Neocosmospora vasinfecta* Smith" (in press).

Starkey, R. L. .. "Some influences of the development of higher plants upon the micro-organisms in the soil, I. Historical and introductory," *Soil Sci.*, 1929, **27**, 319–34.

——————— .. "Some influences of the development of higher plants upon the micro-organisms in the soil, II. Influence of the stage of plant growth upon abundance of organisms," *ibid.*, 1929, **27**, 355–79.

——————— .. "Some influences of the development of higher plants upon the micro-organisms in the soil. III. Influence of the stage of plant growth upon some activities of the organisms," *ibid.*, 1929, **27**, 248–59.

——————— .. "Some influences of the development of higher plants upon the micro-organisms in the soil, IV. Influence of proximity to roots on abundance and activity of micro-organisms," *ibid.*, 1931, **32**, 367–93.

——————— .. "Some influences of the development of higher plants upon the micro-organisms in the soil, VI. Microscopic examination of the rhizosphere," *ibid.*, 1938, **45**, 207–49.

Thom, C. and Humfeld, H. .. "Notes on the association of micro-organisms and roots," *ibid.*, 1932, 34, 29–36.

Timonin, M. I. .. "The interaction of higher plants and micro-organisms, II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases," *Canad. J. Res., C.*, 1940, **18**, 444–56.

——————— .. "The interaction of higher plants and micro-organisms, III. The effect of by-products of plant growth on the activity of fungi and actinomycetes," *Soil Sci.*, 1941, **52**, 395–412.

——————— .. "Microflora of the rhizosphere in relation to manganese deficiency disease of oats," *Soil Sci. Soc. Amer. Proc.*, 1947, 284–92.

West, P. M. and Hildebrand, A. A. "The microbiological balance of strawberry root rot soil as related to the rhizosphere and decomposition effect of certain cover crops," *Canad. J. Res., C.*, 1941, **19**, 199–210.