SOME ASPECTS OF SOIL-BORNE FUNGAL DISEASES OF PLANTS

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INTRODUCTION

SOIL-BORNE fungal diseases of plants and their control have received considerable attention in recent years, particularly in the United States of America, Canada and the United Kingdom, both from the angle of physico-chemical factors of soil environment and microbiological. Indian workers have been contributing, though in a small measure, what might be called extensive observations on many of the field problems caused by soil-borne fungal infection in crop plants. However, an intensive academic study in the elucidation of the various factors, complicated though they are, that contribute towards a degree of intimacy with the actual mechanism of wilt has not been attempted in this country on any notable scale until recently. Those engaged in these investigations have read with interest a number of able reviews written on many aspects of soil-borne diseases notably by Garrett (1934, 1939, 1944, 1946), Waksman (1937, 1941, 1944), Garrard and Lochhead (1938), Weindling (1938, 1946), Porter and Carter (1938), D'Aeth (1939), Padwick (1942), Sanford (1946), Chupp (1946), Beach (1946), Walker (1946) and Daines (1946). No attempt is, therefore, made here to cover the ground already traversed. Instead, the results obtained by the author and his collaborators on some aspects of soil-borne diseases of plants is set out more from the point of view of stimulating future researches in India in a hitherto neglected, yet fascinating, field of research activity.

SAPROPHYTIC ACTIVITY OF CERTAIN SOIL FUNGI IN SOILS

(a) *Growth and Survival in Bare Soil* — Subramanian (1946 a, b), using the root burial technique of Sadasivan (1939), which was later used by Walker (1941), demonstrated that while *Fusarium vasinfectum*, the cotton wilt pathogen (prevalent in the cotton-growing tracts of Udamalpet, South India), makes no spread through bare soil in the absence of organic substratum, yet is able to do so when the microbiological factor of the soil ceases to operate on sterilization of soils collected from cotton wilt-infected fields. Subramanian considered that the fungus makes no extensive spread through unsterilized soil and, therefore, is *on a par* with the class of fungi that make no extensive spread through soil. On the other hand, Kovoor (1947), working with *Macrophomina phaseoli*, isolated from wilted cotton plants in another cotton-growing area at Kovilpatti, separated by over a hundred miles from the Udamalpet tract, observed that this fungus made free growth through unsterilized soils moderately rich in organic matter using the direct microscopic slide technique of Cholodny (1930). This confirmed the earlier observations made by Blair (1943) working with *Rhizoctonia solani* (= *Corticium solani*) of fungi making free growth in unsterilized soils. To this list was added *Pythium aphanidermatum*, an isolate from nursery bed seedlings of tomato, by Sadasivan (1947). Venkatram (1949) isolated a number of strains of fungi from the exterior and interior of cotton seeds. These fungi from the interior included *Macrophomina phaseoli*, *Rhizopus nodosus* and many strains of Aspergilli and Penicillia, although *F. vasinfectum* was isolated only from the exterior of such seeds. Many of the strains showed pathogenicity towards cotton, particularly *R. nodosus*, which occurred in two distinct strains — strain A and strain B — the former causing considerable pre-emergence wilt in cotton, whereas the latter did not. The most interesting part of Venkatram's observations is that *R. nodosus* like *M. phaseoli* falls under the category of fungi that make free growth in unsterilized soils (PLATE 1, FIGS. 1-3). The percentage infection on cotton seeds produced by *R. nodosus*, strain A, was not affected by sterilization of soils to eliminate the general microflora (TABLE I). Thus, it appears essential to evaluate the degree of susceptibility to microbial antagonism prior to estimating the saprophytic potentialities of soil-borne fungi causing wilts in plants. The work of Subramanian was confirmed by Sarojini (1948) and

TABLE I

Showing percentage germination and infection of cotton seeds sown in sterilized and unsterilized soils infected by different inocula of Rhizopus nodosus (*strain A*)

Inocula of the fungus	Percentage germination of seeds after 15 days		Total number of seedlings infected by the fungus	
	Sterilized series	Unsterilized series	Sterilized series	Unsterilized series
Infected root bits	47	54	3	2
Spore suspension	70	73	1	0
Agar discs	47	53	4	4
Cotton seed meal	33	30	8	6
Control	74	72	0	0

Thankam (1949), inasmuch as the saprophytic activity of *F. udum*, the wilt organism of *Cajanus cajan* and *F. vasinfectum* on cotton respectively, was not only controlled by microbial antagonism but also by the moisture holding capacity of soils. Thankam's experiments with the Cholodny slide indicated that whereas 5 per cent soil moisture content of soils was the minimum moisture requirement for germination of spores of *F. vasinfectum* in soils, increase in soil moisture with an optimum at 30 per cent was a necessary preliminary for increased percentage germination of the spores (Plate 1, Figs. 4-7). In air-dry soil, however, the spores showed no germination and retained their viability even after 250 days' incubation at laboratory temperatures of 28°-30°C. However, loss in viability of spores was evident after 60 days' incubation in soils at 5 per cent moisture, at the same temperature of incubation.

(b) *Colonization of Plant Material and Survival in Amended Soils* — Subramanian (1950) demonstrated that species of *Fusarium* including *F. vasinfectum* were the primary and dominant colonizers of various plant parts buried in moist " wilt-sick " soils from the Udamalpet cotton-growing tract. The Fusaria continued as a dominant colonizer for a period of 16 weeks on cotton root bits. He, therefore, concluded that *F. vasinfectum*, in view of its unlimited saprophytic potentialities, be included in the class of true *soil fungi* or *soil inhabitants*, a basic conception originated by Reinking and Manns (1934). Further, Subramanian observed that *Fusarium* colonization on roots was partially or completely inhibited by absence of moisture, excessive moisture, low temperature and organic manuring. On the other hand, colonization was favoured by the addition of lime to soil, but was not affected appreciably either by the nature of the crop or by seasons in which soils were collected. Experiments using wilt-sick soils from three different plots growing cotton, *Setaria* and onion, showed a spatial distribution of the fungus in soil profiles up to a depth of 36 in. Working on similar lines on the micro-ecology of the Udamalpet wilt-sick soils, Zachariah (1949) confirmed Subramanian's findings as regards the saprophytic status of *Fusarium* in those soils and added that *Macrophomina phaseoli* also behaved as a primary and dominant colonizer on both autoclaved and freshly excised surface sterilized root pieces buried in such soils. These two genera colonized equally well during the first few days after burying the root pieces in soils, after which the colonizing capacity of *M. phaseoli* showed a decline while *Fusarium* spp. remained the primary and dominant colonizer, irrespective of the period of incubation. Of equal importance is the record of *Fusarium solani* (Mart. pr. p.) App. et Wr. for the first time along with *F. vasinfectum* in cotton wilt-sick soils of the Udamalpet district. Indeed, in soil profile studies up to a depth of 36 in. both the fungi were present, and in experimental tests with cotton both species produced comparable pathogenicity.

More recent work by Thankam (1949) in this laboratory showed that the loss in viability of *F. vasinfectum* on the Cholodny slide was more rapid when organic nitrogen amendments were added to soil in the form of stable manure, groundnut cake and urea than when inorganic sources were administered; actually, sodium nitrate and ammonium sulphate increased the survival period over the control. Similar results denoting the superiority of organic nitrogen over inorganic were obtained by Radha (1949) with *Macrophomina phaseoli* both from the point of view of reducing longevity of the fungus in soils and its pathogenicity towards cotton. Somasundaram (1949), using inorganic nutrient solution in sand cultures, showed that in *F. vasinfectum* wilt of cotton, omission of nitrogen resulted in least wilt infection, whereas increase in nitrogen increased wilt. On the other hand, increasing the potassium content over the basic K-content (500 p.p.m.) of the nutrient solution used did not result in either a decrease or increase in wilt in the sterilized series, but in the unsterilized series wilt was decidedly less when potassium was increased in excess of the basic level. Doubling the potassium content over the basic level increased sporulation of *F. vasinfectum* in pure culture. This partly explains the increased percentage of wilt in sterilized soils by affording greater infection foci. Probably increased microbial antagonism to *F. vasinfectum* was responsible for decreased wilt percentage in the unsterilized soils. Somasundaram's investigations confirm results on record for *F. vasinfectum* wilt of cotton by Walker (1946), for *Verticillium albo-atrum* wilt of tomato by Roberts (1943) and for damping-off of seedlings of deciduous trees caused by *Rhizoctonia solani* and *Pythium ultimum* by Wright (1941) in so far as the effect of nitrogen and potassium in the matter of wilt increase and decrease respectively. The actual function of potassium in increasing microbial antagonism in unsterilized soils resulting in a decrease in wilt has hitherto not been explained so clearly by previous workers. It may be mentioned here that preliminary analysis of wilt-sick soil samples from Udamalpet and Kovilpatti cotton-growing areas undertaken by K. V. S. Pai in this laboratory (investigation still in progress) showed that while the total nitrogen of the Kovilpatti soils was 24 mg. per cent, that of the Udamalpet samples was more than double that figure, i.e. 49 mg. per cent on an air-dry soil basis. A word about the significance of these results may clarify the position regarding nitrogen in relation to wilt production. *F. vasinfectum* wilt has been recorded mainly in the past from the Udamalpet tract and not from the Kovilpatti fields. However, the water holding capacity, texture and other conditions of both the soils appear to be same or, at any rate, not very dissimilar. *Fusarium* species have been isolated from both the soils from time to time but the pathogenicity towards cotton has been established so far only in the Udamalpet isolates. Nevertheless, future work on the pathogenicity on cotton of the Kovilpatti isolates of *Fusarium* may result in establishing the presence of such pathogenic strains. In the context of the present findings on nitrogen content of these soils, it seems logical to view Somasundaram's results in juxtaposition with that of Pai: viz. that the increased wilt in the Udamalpet area is borne out by laboratory pot culture tests where higher wilt on cotton seedlings was recorded with increased nitrogen in sand cultures using the causal agent *F. vasinfectum*.

MICRO-ELEMENTS AS AFFECTING THE SAPROPHYTIC ACTIVITY OF SOIL FUNGI IN PURE CULTURES AND IN SOILS AND CONSEQUENT HOST RESPONSE IN WILT INCIDENCE

The first practical evidence of control of soil-borne fungal diseases of wheat caused by *Helminthosporium sativum*, *Curvularia ramosa*, *Fusarium culmorum* and *Rhizoctonia solani* by the application of zinc as zinc sulphate at rates of 15 to 30 lb. per acre came from Millikan (1938). The response in vegetative growth by many fungi in pure cultures and the essential nature of trace elements for growth of fungi in general has been known for well over three decades and the results of several workers has been ably reviewed by Foster (1939) and Steinberg (1939). Recent work in this laboratory by Yogeswari (1948) using three soil Fusaria, *F. vasinfectum*, the cotton wilt pathogen, *F. udum*, the causal agent of wilt in *Cajanus cajan*, and *F. moniliforme*, infecting paddy, showed that all three fungi responded well to various concentrations of boron, zinc and manganese added to Richard's liquid medium. Optimum levels for growth were low being 0·3 p.p.m. of boron, 0·05 p.p.m. of zinc and 0·2 p.p.m. of manganese for *F. vasinfectum*; 0·5 p.p.m. of boron, zinc and manganese for *F. udum* and 0·3 p.p.m. of boron, zinc and manganese for *F. moniliforme*. Higher concentrations of these trace elements, however, were not toxic. The point of importance noticed was that all three strains of the fungus yielded heaviest dry weights in nutrient media containing boron, but highest ash weights were recorded in nutrient media containing zinc. The author attributed the increased ash weight in the presence of zinc to accumulation in the fungal matrix of potassium and magnesium from the liquid

TABLE II

Showing the percentage colonization of Fusarium *on red gram stem pieces buried in infected soil as affected by the addition of micro-nutrient solutions (boron, manganese and zinc at* 20, 40 *and* 80 *p.p.m. levels) at monthly intervals*

MICRO-ELEMENT	LEVELS IN P.P.M.	PERIOD OF INCUBATION IN MONTHS			
		1	2	3	4
Boron	20	20	8	8	34
	40	16	25	66	84
	80	4	20	44	76
Manganese	20	26	35	77	92
	40	4	20	76	88
	80	16	37	84	92
Zinc	20	20	34	13	—
	40	12	8	—	—
	80	12	7	—	—
Control	—	100	100	100	100

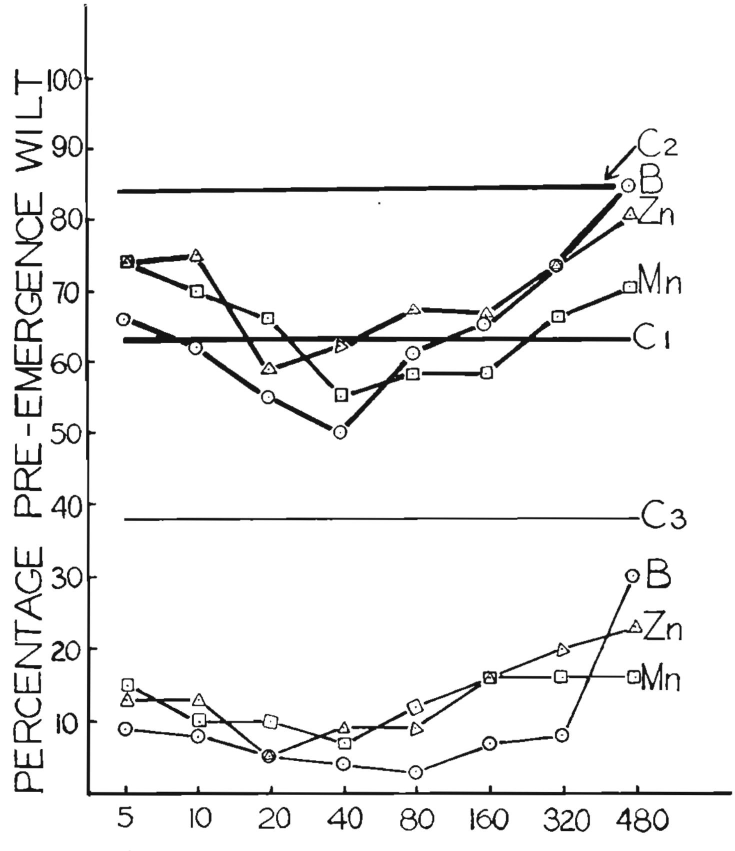
TABLE III

Showing percentage viability of F. udum *on red gram stubble buried in soil receiving micro-nutrient treatments*

MICRO-ELEMENT	LEVELS IN P.P.M.	PERIOD OF INCUBATION IN WEEKS							
		1	2	3	4	5	6	7	8
Boron	20	96	84	72	56	32	16	12	4
	40	96	80	76	56	36	12	—	—
	80	90	80	76	62	32	8	—	—
Manganese	20	90	84	76	64	36	20	12	12
	40	92	84	76	64	40	24	16	4
	80	90	80	72	60	40	20	16	8
Zinc	20	75	57	38	12	4	—	—	—
	40	76	48	32	8	—	—	—	—
	80	75	47	30	6	—	—	—	—
Control	—	100	100	96	88	84	80	76	76

culture substrate, and that selective absorption of the macro-elements takes place only in the presence of zinc and not boron. However, dry weight of the mycelial mats increased in the presence of boron only presumably due to accumulation of carbohydrates which were lost in the process of incineration. The work of Yogeswari has been extended further by Sarojini (1948) and Sulochana (1949), both working in this laboratory. Sarojini's results brought to the forefront an important aspect of micro-element amendment of wilt-sick soils, viz. the inhibition of the saprophytic capabilities of *Fusarium* infection present in gram wilt-sick soils when red gram stem pieces were buried in such soils (TABLES II & III).

It was observed that addition of boron and manganese brought about a decreased colonization at the end of the first month but gradually rose and towards the end of four months, except in the case of the 20 p.p.m. boron, the others showed a high percentage colonization, although still less than the control. Zinc, however, seemed to have an adverse effect on the colonization of *Fusarium*, and there was no colonization at the end of the third or fourth months in all the strengths of micro-element used although at 20 p.p.m. there was some colonization at the end of the third month. Similarly, Sarojini's results in the percentage viability of *F. udum*, when buried in garden compost soil in the form of laboratory colonized red gram stem bits are of considerable interest in that the most rapid disappearance of *F. udum* was brought about with the addition of zinc nutrient solutions, the fungus being exterminated from the colonized stubble within four to six weeks of burial in the soil as against the high percentage viability maintained by the fungus in the control soils.



TEXT-FIG. 1—Shows percentage pre-emergence wilt of *Cajanus cajan* in micro-element amended wilt-sick soils (causal agent — *Fusarium udum*). Top five bold lines represent micro-element amended November soils, bottom thin lines represent amended March soils along with their controls C_1, C_2 and C_3.

C_1: bare November soil; C_2: November soil + *F. udum* inoculum; C_3: bare March soil.

TABLE IV

Showing colonization of soil Fusaria and survival of Fusarium vasinfectum *colonized on root pieces in Udamalpet wilt-sick soil amended with micro-nutrient elements at 80 p.p.m. concentration*

MICRO-ELEMENT	INCUBATION PERIOD IN WEEKS															
	4				8				16				24			
	Treatment															
	A	A_1	B	B_1	A	A_1	B	B_1	A	A_1	B	B_1	A	A_1	B	B_1
Mn	100	100	92	100	80	100	72	100	56	100	64	100	56	100	40	100
Mo	84	100	100	—	72	100	100	60	32	100	100	64	32	100	100	60
Co	80	100	100	—	60	100	100	—	24	100	100	—	30	100	100	8
Zn	76	100	88	72	44	100	64	80	24	100	32	96	20	100	20	100
Al	52	100	100	—	40	100	100	—	20	100	100	—	8	100	100	12
B	52	100	80	88	48	100	60	84	20	100	28	100	12	100	12	100
Li	56	100	80	20	40	100	64	46	20	100	32	64	12	100	16	68
Ni	52	100	100	—	36	100	100	—	24	100	100	—	8	100	100	4
Control	100	100	100	32	100	100	100	40	100	100	100	75	100	100	100	78

A = Percentage colonization of root pieces by *Fusarium* spp.
A_1 = Percentage colonization by other fungi.
B = Percentage survival of *Fusarium vasinfectum* in artificially infected root pieces.
B_1 = Percentage colonization of secondary colonizers (in artificially infected root pieces with *F. vasinfectum*).

However, the most interesting results were obtained in the matter of percentage pre-emergence wilt of *Cajanus* in wilt-sick soils with the addition of micro-nutrient elements boron, manganese and zinc at various levels in pathogenicity trials (TEXT-FIG. 1).

Broadly speaking, micro-nutrient treated series on the whole yielded better results than the control by lowering the disease index to an appreciable extent and at the same time promoting plant growth. Of the treatments tried, manganese was the most beneficial followed by zinc and boron in the decreasing order of efficiency. It is interesting to note that at the levels used, manganese and zinc did not produce any apparent toxic symptoms even with the highest dosage used in the series. Sulochana (1949), working on other aspects of micro-nutrient elements and the behaviour of *F. vasinfectum* in soil, considerably expanded the list of micro-elements of Sarojini and studied the effect on colonization of the fungus on buried root pieces (TABLES IV-VII).

Of the micro-nutrient elements employed in the tests, lithium and molybdenum showed maximum inhibition in micro- and macro-conidial sporulation by *F. vasinfectum*. The practical application of this aspect, viz. the anti-sporulating effect produced by trace elements on soil fungi, cannot be properly assessed but it appears to be an important aspect worthy of consideration in future studies on soil fungi. The *summum bonum* of the observations made by Sulochana is that among the eight trace elements tried boron inhibits the saprophytic activity of *F. vasinfectum* by reducing both the percentage colonization on root pieces, as well as by reducing the percentage survival in colonized root-pieces at 80 p.p.m. concentration over a period of 24 weeks' incubation.

TABLE V

Showing effect of micro-nutrient elements on sporulation of F. vasinfectum

MICRO-ELEMENTS ADDED TO RICHARD'S MEDIUM	MICRO- AND MACRO-CONIDIAL NUMBERS IN MILLIONS					
	Micro-elements in p.p.m.					
	50	100	250	500	750	1000
Mn	11·2	8·6	6·30	5·6	5·23	5·2
Ni	7·33	4·1	0·53	—	—	—
B	6·6	5·8	5·13	4·43	3·83	3·06
Mo I*	6·3	6·3	5·8	1·33	0·13	0·065
Mo II†	2·76	1·9	0·53	—	—	—
Zn	6·0	4·1	2·5	2·05	1·6	0·9
V	5·4	5·2	5·13	2·63	2·03	1·4
Al	4·2	2·7	1·7	1·56	1·3	0·065
Li	2·48	0·003	—	—	—	—
U	4·45	4·31	3·67	3·3	2·5	2·3
Th	7·34	6·85	6·24	5·5	4·6	3·7
Co	4·88	4·2	3·75	2·6	1·03	0·65
Rb	9·5	6·5	6·35	5·6	5·2	5·01
Cd	1·6	0·95	0·8	0·65	0·02	0·005
Control	12·08					

* Mo I = Molybdenum trioxide.
† Mo II = Ammonium molybdate.

TABLE VI

Showing bacterial numbers in millions in 10 gm. of air-dry soil (Udamalpet wilt-sick soil) amended with micro-nutrient elements after 4 weeks' incubation

MICRO-ELEMENTS	MICRO-NUTRIENT ELEMENTS IN P.P.M.			
	50	100	200	400
Mn	156·2	237·4	126·3	105·0
Mo	75·9	70·7	51·3	25·9
Co	44·8	33·9	30·0	25·5
Zn	116·1	76·4	63·7	60·2
Al	63·3	41·0	30·5	18·1
B	117·8	120·0	96·2	80·7
Li	96·2	80·8	65·6	35·3
Ni	56·6	38·4	20·6	19·8
Control	76·4	—	—	—

TABLE VII

Showing percentage germination of micro- and macro-conidia of F. vasinfectum *in unsterilized " wilt-sick " soil amended with micro-nutrient elements after 48 hours' incubation (by using the Cholodny slide technique)*

MICRO-ELEMENTS	MICRO-NUTRIENT ELEMENTS IN P.P.M.			
	50	100	200	300
Mn	84	52	35	11
Mo	30	22	5	3
Co	58	45	44	18
Zn	23	20	8	2
Al	35	33	10	7
B	45	40	11	10
Li	30	20	16	8
Ni	25	25	23	11
Control	68	—	—	—

Comparing the results obtained in the general increase in the microbial activity brought about by increasing the bacterial numbers by the addition of micro-elements at various concentrations, manganese alone brings about maximum increase in bacterial numbers followed closely by boron (TABLE VI). Nevertheless, from the point of view of effective control of colonization and saprophytic activity of *F. vasinfectum* the addition of boron seems to be more beneficial than manganese. This is explainable since boron not only stimulates increase in bacterial numbers, though not to the extent of manganese, yet has the additional advantage over manganese in being able to produce toxic effects on *F. vasinfectum* spore germination (TABLE VII). The effectiveness of boron, therefore, in reducing the percentage survival and percentage colonization of *F. vasinfectum*, considerably over the control, is attributable to its ability to inhibit spore germination as well as to increase microbial antagonism in soil, which dual function is not attributable to the other elements tried.

SOIL BACTERIA AND CONTROL OF WILT CAUSED BY SOIL FUNGI

Sanford (1946) and Weindling (1946) have presented ample data to show that certain bacterial isolates from soils when cultured *in vitro* and added to fungus-infested soils check soil-borne wilts in plants to a considerable extent. Similar results have been obtained by various other workers. Recent work in this laboratory by Thankam (1949) afforded yet another example of biological control of this type. Several bacterial strains were isolated by her from the Udamalpet wilt-sick soils and these were cultured in artificial liquid broth before applying to pots growing a variety of cotton susceptible to wilt by *F. vasinfectum*. Her results (TABLE VIII) indicate that a considerable degree of protection was afforded by some of the bacterial strains against wilt caused by the fungus pathogen.

Indeed, bacterial strain I gave the largest measure of protection (PLATE 1, FIG. 8), its efficiency in checking post-emergence wilt being of a high order, while a small percentage of pre-emergence wilt did take place. It must, however, be stated that bacterial suspensions appeared to be more effective as inhibitors of the pathogenic progress of *F. vasinfectum* than their filtrates.

This aspect has been ushered into this general consideration of soil-borne fungal diseases of plants mainly with a view to stimulating work on these lines at a time when biological workers in many parts of the world are " antibiotic conscious ", although Weindling (1946) appears rather sceptical of the problem assuming a practical shape. To quote his words: " So far, practical application of biological control has been successful only when using an indirect approach, that is, soil modifications favourable for antagonistic action of the existing microflora. The direct approach, that is, applying specific organisms to soil or seed, has been mainly of theoretical interest."

In conclusion, it may be mentioned that emphasis is sought to be laid in this article on the necessity of further studying micro-element nutrition in relation to the complex problem of soil microflora as affecting the

TABLE VIII

Showing the effect of addition of bacterial suspensions and filtrates of five strains of bacteria to the soil on the pathogenicity of F. vasinfectum *to cotton*

	CONTROL HEALTHY	CONTROL INFECTED	BACTERIAL SUSPENSION					BACTERIAL FILTRATE				
			BI	BII	BIII	BIV	BV	BI	BII	BIII	BIV	BV
Percentage pre-emergence wilt	0	26	18	20	20	20	10	12	24	22	22	12
Percentage post-emergence wilt	0	78	0	15	12	30	27	0	66	67	62	50

saprophytic and parasitic activities of soil-borne fungi. That the results quoted here are largely the outcome of a narrow specialization in this field is obvious; but it is suggested that collaborative work applying spectroscopic methods of detection of these trace elements in fungal mats, residual solutions and soil substrates will repay itself, if planned on a large scale, both from the point of view of studying the interaction of the soil micro-organisms, bacteria and fungi, and the more important consideration of control of soil-borne diseases in its normal habitat — the soil.

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EXPLANATION OF PLATE 1

1. Showing free growth of *Rhizopus nodosus* in natural soil. × *ca.* 2.

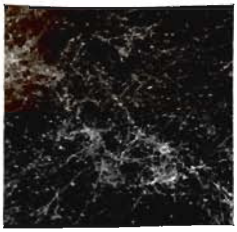
2. Showing cotton seed succumbed to pre-emergence infection by *R. nodosus*. *Ca.* nat. size.

3. Showing *R. nodosus* attacking cotton seedlings soon after emergence from soil. × *ca.* 3.

4-7. Showing germination of conidia of *Fusarium vasinfectum* on Cholodny slides buried in soils maintained at 5, 10, 20 and 30 per cent moisture levels respectively (note poor germination at 5 per cent and increasing germination with increasing moisture). × 160.

8. a-e, showing the effect of addition of suspension and filtrate of bacterial strain I to soil infected with *F. vasinfectum* and consequent control of wilt of cotton plants (photographed 6 weeks after sowing).

a = *F. vasinfectum* alone; b = pathogen plus filtrate of bacterial strain I; c and d = pathogen plus suspension of bacterial strain I; e = uninoculated control.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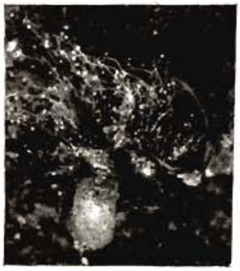
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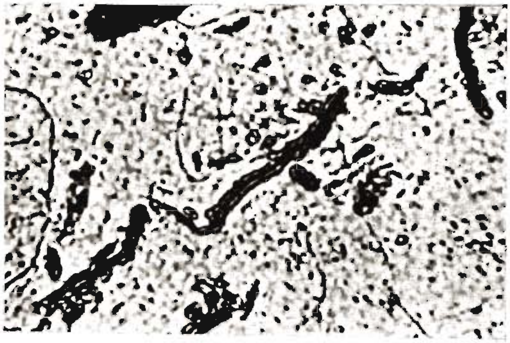
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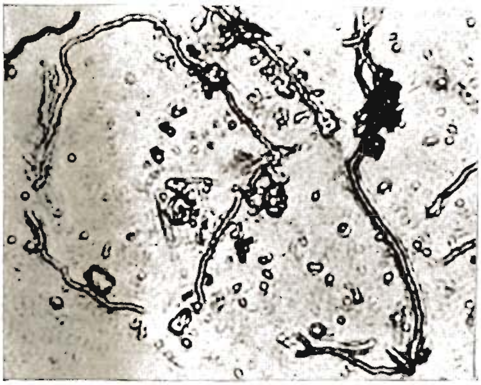
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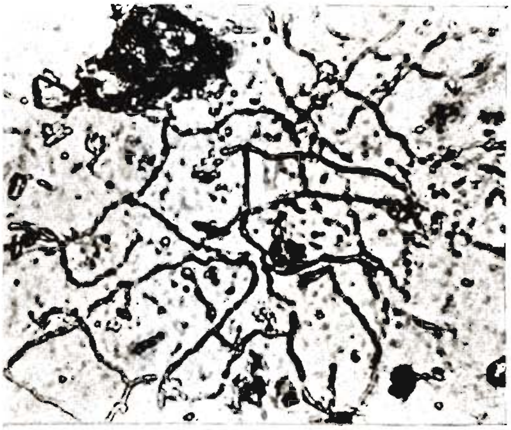
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