INTERACTIONS OF SOIL MICRO-FLORA WITH *FUSARIUM SOLANI* (MART.) SACC., THE CAUSE OF DRY ROT OF POTATO TUBERS*

ANSAR AHMED QURESHI AND ABDUL GHAFFAR

Department of Botany, University of Karachi, Karachi

(Received July 23, 1965)

Fusarium solani was found to be associated with the dry rot of potato tubers. The fungus is a soil inhabitant. Apart from other factors, the effect of soil micro-flora on *F. solani* was studied to find a possible explanation for its persistence and accumulation in the soil.

Micro-organisms belonging to 13 genera of fungi, 2 of bacteria and to the genus *Streptomyces* were isolated from the soil of Karachi University Botanical Garden and identified.

The interactions of 59 isolates of fungi, 8 of actinomycetes and 10 of bacteria, respectively, with *F. solani* were studied on agar culture. These were grouped into 5 different types of reaction. With the exception of the unidentified *Penicillium* sp. none of the fungi, actinomycetes and bacteria inhibited the growth of *F. solani*. *Trichoderma viride*, *Cunnighamella echinulata*, *Monilia* sp. and *Rhizopus* sp. intermingled with the hyphae of *F. solani* but had no effect on it. *T. viride* which is known to coil around the hyphae of a number of organisms had no effect on *F. solani*. *F. solani*, however, grew over the isolates of *Alternaria* sp., *Curvularia* sp. and *Helminthosporium* sp.

Although the effect of pure antibiotics on *F. solani* was not studied, yet the attributes like the production of an antibacterial and antifungal substance "Javanicin" and "Oxyjavanicin" respectivety by *F. solani* and its tolerance of several txoins known to be produced by micro-organisms used in this study, suggest that *F. solani* can compete saprophytically with the normal soil micro-flora, persists and accumulates in the soil which ultimately becomes "disease sick".

Introduction

Fusarium solani (Mart.) Sacc., the cause of dry rot of potato tubers is a soil inhabitant.[1,2] The fungus seems to accumulate and persist in the soil making it "disease sick" since potatoes planted in virgin fields have been found to produce less infection of dry rot disease as compared to successive croppings in the same field.[3] Such similar observations have been made in case of *Fusarium lini* Bolley, the cause of wilt disease in flax.[4] *F. lini* which is unaffected by soil micro-flora accumulates and persists in the soil and due to continuous cropping of flax in the same field makes the soil "wilt sick". On the other hand *Helminthosporium sativum* Pamm., King, & Bakke, the cause of foot rot of wheat and barley is inhibited by soil micro-organisms and does not accumulate and persist in the soil.[4,5] However, there seems to be no report on the relation of soil micro-flora to the development of dry rot of potatoes and the accumulation of *Fusarium solani* in soil. In the present study, therefore, an attempt was made to find a possible explanation for the persistence of the fungus and its accumulation in the soil. Micro-organisms have been isolated from the soil and some of these have been used in experiments to study their interactions with *F. solani*.

The culture of *F. solani* used in this study was a fresh isolate obtained from a dry rot infected potato tuber var. Ultimus collected from the Karachi vegetable market. The optimum temperature for the growth of *F. solani* in culture is reported to be 28°C.[6] The interactions of *F. solani* with soil micro-flora were, therefore, studied at 28°C. Czapek-Dox agar medium** was used in order to have a uniform composition of the medium throughout the experiment.[7] This was also the medium used for the isolation of fungi, bacteria and actinomycetes from the soil.

Experimental

Isolation and Identification of Soil Micro-flora.— The microbial population of the soil was sampled to obtain isolates of the most prevalent group of organisms for studying their effects on the growth of *F. solani*. The soil used in the present study was garden loam from a piece of land then under the potato cultivation at the Botanical Garden Karachi University. The pH of the soil sample, was 5.8-6.4. At various intervals 5 different soil samples were collected in small sterilized glass tubes after removing an inch of the surface layer. Soil dilution plate technique as outlined by Waksman[8] was used. For the isolation of micro-organisms 2 g. of the soil was added to 18 ml. of sterilized distilled water, contained in a flask, to give an initial dilution of 1:10. This was then adjusted to a final dilution of 1:1000 for the isolation of fungi and 1:100,000 for bacteria and actinomycetes. From each of the prepared dilutions 1 ml. of the soil suspension was placed in sterlized

*Part of a thesis presented by Ansar Ahmed Qureshi, in partial fulfilment of the requirements for the degree of M.Sc., University of Karachi, July 1965.

**Composition of niedimm: $NaNO_3$, 2.0.g.; KH_2PO_4, 1.0.g.; Kcl, 0.5.g.; $MgSO_4.7H_2O$, O.5g.; $FeSO_4.7H_2O$, 0.01g.; Sucrose, 30.0.g.; Agar, 20.0g.; Distilled water, 1000 ml.

petri dishes. About 10 ml. of melted, cooled Czapek-Dox agar medium was poured in each petri dish and adequately dispersed by continuous shaking and rotating the plates before the agar solidified. The Czapek-Dox agar acidified to pH 4.4 by the addition of N/10 HCl was used for the isolation of fungi.[9] For the isolation of bacteria and actinomycetes the medium was adjusted to pH 7.2 with N/10 KOH. All plates were incubated at 28°C. Micro-organisms growing in isolated colonies on the plates were transferred to Czapek-Dox agar slants at pH 5.4, for fungi and at pH 7.2 for bacteria and actinomycetes. Amongst these isolates the following 13 genera comprising 29 different species of fungi[10–14] 2 genera of bacteria and the genus *Streptomyces* were identified.

Fungi.—*Alternaria* sp., *Aspergillus flavus* Link., *Aspergillus fumigatus* Fres., *Aspergillus niger* van Tieghem., *Aspergillus niveus* Bloch., *Aspergillus ochraceus* Wilhelm., *Aspergillus oryzae* (Ahlburg) Cohn, *Aspergillus rugulosus* Thom & Raper, *Aspergillus sydowii* (Bain & Sart) Thom & Church, *Aspergillus tamarii* Kita, *Aspergillus terreus* Thom., *Aspergillus terreus* Thom, var? *floccosus* Shih., *Aspergillus ustus* (Bainier) Thom & Church, *Aspergillus variecolor* (Bark & Br.) Thom & Raper *Cladosporium herbarum* Link ex Fries, *Cunninghamella echinulata* Thaxter, *Curvularia* sp., *Fusarium culmorum* (Smith) Sacc., *Fusarium solani* (Mart.) Sacc., *Fusarium* sp., *Helminthosperium* sp., *Monilia* sp., *Nigrospora* sp., *Penicillium funiculosum* Thom, *Penicillium variabile* Sopp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma viride* Pers. ex Fr., Sterile Hyphan: *Actinomycetes*: *Streptomyces* sp., *Bacteria*, *Bacillus* spp., *Pseudomonas* sp.

Interaction in Agar Culture.—(a) *With fungi*: (i) TYPES OF INTERACTION. The effect of various fungi, isolated from the soil as described above, on the growth of *Fusarium solani* was studied on the Czapek-Dox agar adjusted to pH 4.4. Plates containing about 10 ml. of the Czapek-Dox agar were inoculated at opposite sides 65-70 mm. apart with 5 mm. discs of inoculum from the actively growing edges of the 4—5 days old colonies of *F. solani* and a test fungus, respectively. The dishes were incubuted at 28°C and the rates of radial growth of the organisms towards one another were recorded daily. Final observations and the type of interaction were taken after six days.

When *F. solani* is growing on the Czapek-Dox agar in a pure culture, the mycelium at the edges of the colony is of uniform shape and the fungus fills the plate in 10 days. When it is growing with itself or with some other organism, it behaves differently depending on the organism with which it is associated. Porter [15] has described 5 conceiveable reactions that may occur when the mycelium of one fungus approaches that of another. The reactions of *F. solani* when growing opposite itself or other organisms fall into the 5 groups. They are exhibited in Figs. 1 and 2 and are explained as follows.

A. The hyphae of the two colonies, when they meet, intermingle with each other without any reaction. They are indistinguishable and there is no mark of separation. This condition is equivalent to Type A of Porter [15] and is exhibited when *F. solani* is grown with the same isolate of *F. solani*.

B. The two colonies approach one another and meet without overlapping. This type of reaction is represented when *F. solani* is grown opposite *Aspergillus ustus*.

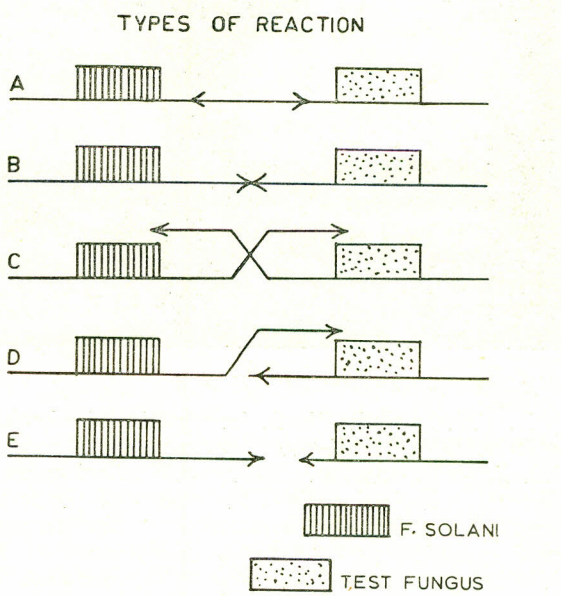

Fig. 1.—Diagramatic representation of the different types of reactions shown when *Fusarium solani* is grown with itself or with other fungi.

C. As in 'A' the hyphae of the two colonies intermingle and overlap each other, but the two organisms are clearly distinguishable, because of morphological differences between the two sets of hyphae, *e.g.*, *F. solani* growing opposite *Trichoderma viride*.

D. The growing margins of the two colonies meet, one of which is inhibited and becomes overgrown by the other. This type of reaction is similar to that of type 'B' of Porter [15] and is shown when *F. solani* is grown with *Curvularia* sp., the former growing over the latter.

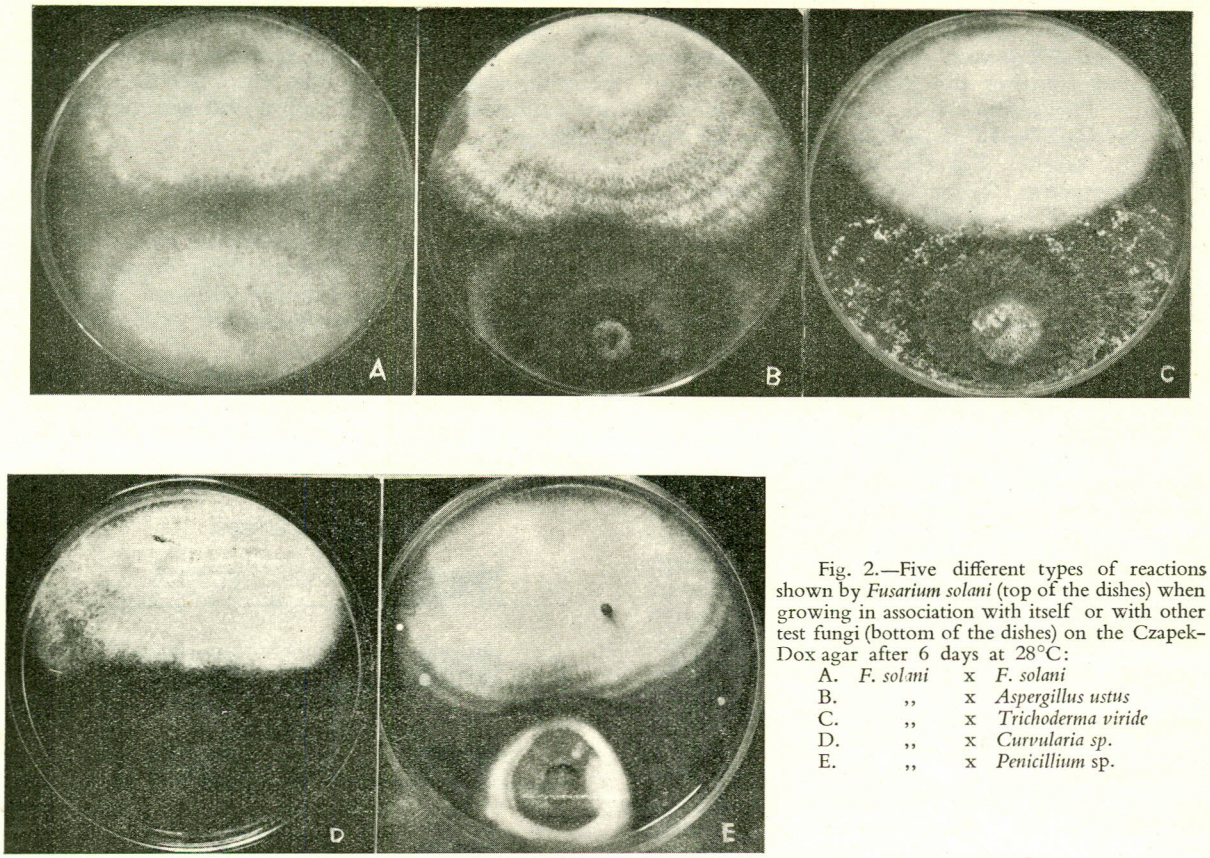

Fig. 2.—Five different types of reactions shown by *Fusarium solani* (top of the dishes) when growing in association with itself or with other test fungi (bottom of the dishes) on the Czapek-Dox agar after 6 days at 28°C:

A. *F. solani* x *F. solani*
B. ,, x *Aspergillus ustus*
C. ,, x *Trichoderma viride*
D. ,, x *Curvularia sp.*
E. ,, x *Penicillium* sp.

E. The growth of both the organisms is checked at a considerable distance leaving a clear zone of inhibition in between. Although *F. solani* growing faster is inhibited around the colony of the antagonist, the antagonist in 10-12 days' time shows a slow growth, upto 1-2 mm., in the zone of inhibition produced. This type is the same as 'E' type of reaction described by Porter[15] and occurs when *F. solani* is grown with an unidentified *Penicillium* sp. (cult. No. 32).

(ii) SCREENING TEST WITH SOIL FUNGI: The interaction of 59 isolates of fungi with *F. solani* in agar culture are presented in Table 1. The growth measurements are the averages of the figures from 2 replicates. These have been grouped according to the different types of reaction explained above. A number of microorganisms produced a 'B' type of reaction in which *F. solani* and the test fungus stopped growing when they met one another. In the 'C' type, shown in cultures with *Cunnighamella echinulata*, *Trichoderma viride*, *Monilia* sp. and *Rhizopus* sp., respectively, these fungi were found to overgrow and intermingle with the hyphae of *F. solani*. No coiling or penetration of the hyphae of one fungus into another was observed. In the 'D' type it was interesting to note that *F. solani* not only inhibited the growth of the test fungi, *viz.*, *Alternaria* sp., *Curvularia* sp. and *Helminthosporium* sp. but was also found to overgrow. The 'E' type of reaction was produced by the interaction of *F. solani* with only an unidentified species of *Penicillium* sp. (cult. No. 32). The growth of *F. solani* was completely inhibited and there was a clear zone of inhibition, 4 mm. wide, produced between the two organisms.

(b) *With Actinomycetes.*—The effect of actinomycetes (isolates of *Streptomyces* spp.) on the growth of *F. solani* was studied. The agar medium used was the Czapek-Dox agar adjusted to pH 7.2. About 10 ml. of the medium was poured into each petri dish and the *Streptomyces* isolates streaked on one side of the dish. A 5 mm. disc from the margin of an actively growing colony of *F. solani* was placed at a distance of about 50-55 mm. at right angles to the streaks. The plates were then incubated at 28°C and the rate of the growth of

TABLE 1.—INTERACTION OF 59 ISOLATES OF FUNGI WITH *Fusarium Solani* AFTER 6 DAYS ON THE CZAPEK-DOX AGAR AT 28°C.

Test Fungus	Radial growth (mm.)		Type of reaction	Zone of inhibition (mm.)
	F. solani	*Test fungus*		
Alternaria sp.	44	26	D	None
,, sp.	46	24	D	,,
,, sp.	38	32	D	,,
Aspergillus flavus	38	30	B	,,
,, ,,	39	30	B	,,
,, *Fumigatus*	37	32	B	,,
,, ,,	36	34	B	,,
,, *niveus*	44	24	B	,,
,, ,,	44	23	B	,,
,, *niger*	33	37	B	,,
,, ,,	38	32	B	,,
,, ,,	39	31	B	,,
,, *ochraceus*	41	31	B	,,
,, ,,	39	31	B	,,
,, *oryzae*	41	29	B	,,
,, ,,	39	31	B	,,
,, *rugulosus*	49	21	B	,,
,, *sydowii*	37	19	B*	,,
,, *tamarii*	40	30	B	,,
,, *terreus*	38	26	B	,,
,, ,,	36	32	B	,,
,, ,, var. *floccosus*	39	31	B	,,
,, *ustus*	38	18	B*	,,
,, ,,	43	18	B*	,,
,, *variecolor*	43	27	B	,,
,, sp.	43	23	B	,,
,, sp.	45	15	B*	,,
Cladosporium herbarum	42	28	B	None
Cunninghamella echinulata	22	48	C	,,
Curvularia sp.	32	38	D	,,
Fusarium culmorum	36	34	B	,,
,, *solani*	35	34	A	,,
,, sp.	34	38	B	,,
,, sp.	33	36	B	,,
Helminthosporium sp	35	37	D	,,
,, sp.	33	37	D	,,
,, sp.	34	35	D	,,
,, sp.	34	36	D	,,
Monilia sp.	22	45	C	,,
,, sp.	20	48	C	,,
Nigrospora sp.	40	28	B	,,
Penicillium funiculosum	44	24	D	,,
,, *variabile*	38	18	B*	,,
Penicillium sp.	42	18	B*	,,
,, sp.	43	14	B*	,,
,, sp.	43	12	B*	,,
,, sp.	42	29	B	,,
,, sp.	40	29	B	,,
,, sp.	45	18	B*	,,
,, sp.	46	10	B*	,,
,, sp.	36	33	B	,,
,, sp.	38	30	B	,,
,, sp.	38	19	B*	,,
Penicillium sp.	36	17	E*	4 mm
Rhizopus sp.	33	38	C	None
,, sp.	32	38	C	,,
Trichoderma viride	29	41	C	,,
,, ,,	28	42	C	,,
Sterile hyphae	44	23	B	,,
Fusarium solani. Control	56	—	—	—

*Colonies meet after=10 days.

F. solani was recorded daily. Final observations were taken after 5 days. The results are given in Table 2.

TABLE 2.—INTERACTION OF 8 ISOLATES OF *Streptomyces* SPP. WITH *F. solani* ON THE CZAPEK-DOX AGAR AFTER 5 DAYS OF GROWTH AT 28°C.

Test isolates	Radial growth of F. solani (mm.)	Zone of inhibition (mm.)
Control	55	0
Streptomyces sp. A_1	50	0
,, sp. A_2	53	0
,, sp. A_3	51	0
,, sp. A_4	54	0
,, sp. A_5	49	0
,, sp. A_6	52	3
,, sp. A_7	50	0
,, sp. A_8	51	2

Out of the 8 isolates of *Streptomyces* spp. tested, 6 of the isolates did not produce any zone of inhibition. Isolates, No. A_6 and A_8, however, produced a zone of inhibition of 3 and 2 mm., respectively, but later *F. solani* overgrew these isolates and was found to fill the plates.

(c) *With Bacteria.*—The interaction of 10 bacterial isolates belonging to the genera *Bacillus* and *Pseudomonas* with *F. solani*, respectively, is given in Table 3. The procedure adopted was

TABLE 3.—INTERACTION OF 10 ISOLATES OF BACTERIA WITH *F. solani* ON CZAPEK-DOX AGAR AFTER 5 DAYS OF GROWTH AT 28°C.

Test isolates	Radial growth of *F. solani* (mm.)	Zone of inhibition (mm.)
Control	54	—
Bascillus sp. B_1	20	0
,, sp. B_2	15	0
,, sp. B_3	10	0
,, sp. B_4	20	0
,, sp. B_5	12	0
,, sp. B_6	19	0
,, sp. B_7	12	0
Pseudomonas sp. B_8	40	0
,, sp. B_9	43	0
,, sp. B_{10}	38	0

the same as was used with the actinomycetes. None of the bacterial isolates produced a zone of inhibition. *F. solani* was found to grow over the

colonies of *Pseudomonas* spp. *Bacillus* spp., spreading faster were found to limit the growth of *F. solani*, however, no abnormality in the hyphae of *F. solani* was observed.

Discussion

The fact that soil cropped continuously to potatoes developed a "soil sickness" suggested that *F. solani*, the cause of dry rot of potatoes, persists and accumulates in the soil.[3] It also suggested that *F. solani* is capable of competing saprophytically with the normal soil microflora, although factors other than antibiosis may also influence its survival. The present investigation of the interactions of fungi, bacteria and actinomycetes with *F. solani* on agar plates provided comprehensive information to the effect that *F. solani* was not affected by soil microorganisms.

The interaction of 59 fungal isolates, respectively, with *F. solani* in agar culture showed different types of reaction. Of these the 'C', 'D' and 'E' types of reaction were outstanding. *Trichoderma viride*, *Cunninghamella echinulata*, *Monilia* sp. and *Rhizopus* sp. producing a 'C' type of reaction intermingled with the hyphae of *F. solani* but had no effect on it. It is interesting to note that *T. lignorum*, a synonym of *T. viride* (in the wide sense of Bisby)[16] which coils around the hyphae of *Phytophthora parasitica*, *Sclerotium rolfsii*, *Pythium* sp. and *Rhizoctonia solani*,[17] *Armillaria mellea* and *Polyporus schweinitzii*[18] and *Sclerotium cepivorum*[19] had no effect on the hyphae of *F. solani*. *F. solani*, however, grew over the isolates of *Alternaria Curvularia* and *Helminthosporium* spp. and produced the 'D' type of reaction.

With the exception of the unidentified *Penicillium* sp. producing an 'E' type of reaction, none of the fungi, bacteria and actinomycetes inhibited the growth of *F. solani*. A number of these fungi, *viz.*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. niveus*, *A. ochraceus*, *A. tamarri*, *A. oryzae*, *A. terreus*, *A. ustus*, *Trichoderma viride*., are known to produce antibiotics with antibacterial and/or antifungai properties. *Fusarium javanicum*, a synonym of *F. solani*, however, is reported to produce antibacterial and antifungal antibiotics known as *Javanicin* and *Oxyjavanicin* respectively.[20] The effect of pure antibiotics on *F. solani* was not studied. However, considering that microorganisms used in this study do produce antibiotics, the production of antibiotic toxins by *F. solani* and its tolerance of such toxins produced by other microorganisms would suggest that *F. solani* can compete saprophytically with normal soil microflora. It may be mentioned that Garrett[21] has also discussed these possibilities that influence the saprophytic ability of a fungus.

No experiment was carried out to study the interaction of micro-organisms with *F. solani* in soil. However, there are observations to substantiate that organisms which are antagonistic in plate culture also produce, to a greater or lesser degree, similar effects in soil.[4,19,22,23] It would, therefore, appear that whereas *F. solani* is unaffected by majority of the soil microorganisms studied, the unidentified *Penicillium* sp. may hold some promise in eliminating *Fusarium* infection from the soil.

There are several reports where antagonistic micro-organisms have been used in the control of plant diseases.[24,26] Of these Nikitina[26] found *Pseudomonas mycophaga* to reduce potato wilt disease caused by *F. oxysporum*. It would, therefore, be of interest to study this phenomenon in greater detail with the dry rot disease of potato.

Acknowledgement.—The authors are thankful to Dr. C. Booth of the Commonwealth Mycological Institute, Kew, Surrey, England for confirming the identification of *Fusarium solani* (Mart.) Sacc.

References

1. C.E. Foister, A.R. Wilson and A.E.W. Boyd, Nature, **155,** 793 (1945).
2. C. Booth and J.M. Waterston, *CMI Description of Pathogenic Fungi and Bacteria No.* 29 (1964).
3. A. Ghaffar and M.M Shaikh, Abs. 14th Pakistan Sci. Conf. (Bio. Sec.), B-10 (1962).
4. A.A. Anwar, Phythopath., **39,** 1005—1019 (1949).
5. A.W. Henry, Can. J. Res., **5,** 407–13 (1931).
6. C.C. Chi, Diss. Abs., **20,** 860—861 (1959).
7. L.E. Hawker, *Physiology of Fungi* (University of London Press, London (1950).
8. S.A. Waksman, *Principles of Soil Microbiology* London (1927).
9. J.H. Warcup, Trans. Brit. Mycol. Soc., **40,** 237—262 (1957).
10. J.C. Gilman, *A Manual of Soil Fungi* (The Iowa State College Press, Iowa, U.S.A., 1957).
11. F.E. Clements and C.L. Shear, *The Genera of Fungi* (H.W. Wilson Co., New York, 1931).
12. H.L. Barnett, *Illustrated Genera of Imperfect Fungi* (Burgess Publishing Co., Minnesota, U.S.A. 1955).
13. C. Thom & K.B. Raper, *A Manual of the Aspergilli*, U.S.A. (The Williams and Wilkins Co., U.S.A. (1945).

14. K.B. Raper and C. Thom, *A Manual of the Penicillia*, Baltimore, (The Williams and Wilkins Co., U.S.A. 1949).
15. C.L. Porter, Am. J. Botany, **11,** 168—188 (1924).
16. G.R. Bisby, Trans. Brit. Mycol. Soc., **23,** 149-168 (1939).
17. R. Weindling, Phytopath., **22,** 837—845 (1932).
18. R.S.C. Aytoun, Trans. Bot. Sec. Edin., **36,** 99-114 (1953).
19. A. Ghaffar, Ph. D. Thesis (University of Birmingham, 1960).
20. P.W. Brian, Bot. Rev., **17,** 357—430 (1951).
21. S.D. Gaett, *The Biology of Root Infecting Fungi*, Cambridge University Press, London, 1956).
22. R. Weindling and H.S. Fawcett, Hilgardia, **10,** 1—16 (1936).
23. W.E. Broadfoot, Can. J. Res., **8,** 545-552 (1933).
24. H.W. Florey, E. Chain, N.G. Heatley, M.A. Jennings, A.G. Sanders, E.P. Abraham and M.E. Florey, *Antibiotics*, Oxford University Press, London, (1949), vol. 1.
25. R.K.S. Wood and M.B. Tveit, Botan Rev., **21,** 441-492 (1955).
26. E.T. Nikitina, Abst. Rev. Appl. Mycol., **38**, 29 (1959).