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SOME ASPECTS OF STUDIES ON SOIL FUNGI*

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I express my heartfelt gratitude to the members of the Indian Botanical Society for having elected me as President. I have chosen the subject of Soil Fungi for my address, not only because of the interest I have in it but also because I feel that in India this subject has not received the attention it deserves. The work on soil fungi has been reviewed earlier by Brierley (1923), Gilman and Abbott (1927), Gilman (1945) and Chesters (1949). In this review I wish to present to the members a brief account of the earlier work and a survey of the recent developments in the study of soil fungi with special reference to the work done in India.

Taxonomic Work

A commencement of a study of soil fungi was made as early as 1886 when Adametz in Germany isolated several species of fungi in the course of his biochemical studies on soils. This work was, however, not followed till in 1902 Oudemans and Koning isolated and described 45 species of soil fungi from Holland, the majority of which were new to science. This was soon followed by a flood of activity in the studies of soil fungi and a large number of contributions came forth from different parts of the world. The well-known monographic works on Mucorales by Hagem in Norway and Lendner in Switzerland appeared in 1908. Dale (1912) in England studied the fungi from several kinds of soils. Almost contemporaneously, Jensen (1912), Goddard (1913) and Waksman (1916) published accounts of soil fungi from various parts of U.S.A. The studies of Waksman were the most outstanding. He isolated more than 200 species. Other notable workers have been Werkenthin (1916), LeClerg and Smith (1928), Jensen (1931) and Cobb (1932). With the studies of Harvey (1925) a new technique for isolating aquatic forms from soils was introduced. He isolated a number of aquatic moulds of the Saprolegniaceæ by placing little bits of soil in water and adding boiled hemp seeds as baits. Several other

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workers followed this method and it is now well established that soil is a natural habitat for these fungi.

In India, Butler (1907) was the first to pay attention to soil fungi. He isolated species of *Pythium* and some chytridiaceous fungi from soil. Thakur and Norris (1928), and Chaudhuri and Sachar (1934) described the soil fungal flora of some Madras and Punjab soils respectively. Galloway (1936) studied the soil fungi of various parts of Northern India especially from the Himalayan hills. Other important workers have been Hukumchand (1937) who studied the fungal flora of Lahore soils, Ghatak and Roy (1939) who listed the fungi isolated from a paddy field, and Dutta and Chaudhury (1944) who worked on Lahore soils. Sadasivan, his students and collaborators have done extensive work on tropical cultivated soils in relation to soil-borne Fusarioses. Their studies on the wilt organisms and the metabolites in their hosts have revealed many new experimental facts of fundamental importance. These results have been recently reviewed by Sadasivan and Subramanian (1954). More recently Ravi Prakash and Saksena (1952) have studied the decomposition of paddy and bajra straws by soil fungi, Saksena and Mehrotra (1952) have worked out the soil fungi of Allahabad, Saksena and Murty (1953), Saksena (1953 *b*, 1954) and Shetye (1954) have investigated the fungal flora of the forest soils of Sagar and its adjoining area.

Much of the work cited above is predominantly confined to taxonomic studies and it is generally the agricultural soils which have been dealt with having a few exceptions of such natural soils as forest and prairies. The soils in agricultural lands are constantly disturbed by various tillage operations, manuring, etc., and hence in spite of their great economic importance, these soils are less suited for understanding the fungi in their natural and undisturbed state. The study of soil fungi of forest and grasslands assumes a special significance in this context and should receive greater attention than has been its share so far. These soils have acquired an equilibrium with the physical, chemical and biological conditions of the environment and are the natural habitats of the soil fungi. Ling-Young (1930) has pointed out that to obtain a correct idea of the soil fungi in their natural endemic conditions the soils from such areas as forests, prairies and hill tops should be examined.

As in all other groups of Botany, in fungi too, the study of ‘function’ is gaining greater importance than that of ‘structure’, although it is well known that the study of one divorced from the other may not only be barren but also futile. A vigorous search in the soil is apt to reveal new species of unique importance which may go a long way in understanding the structural sequences in phylogenetic considerations. *Actinoplanes philipinensis* Couch and *Saksenæa vasiformis* Saksena may be cited as examples. Besides, the new forms may provide fresh and suitable material in understanding the riddles and complexities of such other branches of study as Genetics and Physiology. The classical works on *Neurospora* by Dodge and others and on *Melanospora destruens* Shear by Hawker are the glaring examples.

Another remarkable fact which emerges out of these floristic studies is the difference in the microflora of different regions especially of the deeper layers. The spatial distribution under subterranean conditions is slow and radically different from that which exists in ordinary aerial or sub-aerial environments. A fungus producing a mass of spores in air has a rapid chance of dispersal through the air currents, water and other physical and biotic agencies. The situation in the under-world is more circumscribed. The fungus either moves slowly as a result of vegetative growth of the hyphæ or, if sporulating, the spores are carried by the undercurrents of water or by tiny motile creatures which act as vehicles of dispersal. The resistance and obstruction offered by the soil is considerable and the rate of dispersal is fairly low. It will be an interesting study to work the dispersal rates of different species of fungi in soils especially at different depths and the mechanisms they employ.

Isolation of Soil Fungi

Methods for the isolation of soil fungi deserve special consideration. Every new method which is devised has its own special value and gives information of a particular nature which cannot be easily derived by other means.

Soon after the earliest reports of fungi from soils, many mycologists began to throw doubts on the existence of a true fungal flora in them. The commonest method of isolating fungi has been by agar platings modified by various workers to suit their particular points of view. A common objection against this is that in spite of the most scrupulous care there always remains a chance that some fungus spores from air may find their way in the dishes and on being lodged on the medium, form colonies. The other limitation of this method is that the media used are selective in their action and many fungi may not find the conditions suitable for their growth and may thus get eliminated. This second objection is much more valid since comparative studies by agar plate method and the so-called direct microscopic methods have revealed that it is usually a fraction of the total soil micro-population which gets reflected in the agar plates or culture flasks (Waksman, 1952). However, there is one great advantage in the agar plate method in that here only living organisms are counted.

To meet the above objections, methods have been devised to study the soil by microscopic examination without taking resort to culture. These methods are usually referred to as the 'direct methods'. A commonly employed method is the preparation of a sort of smear of the soil on a slide. A suspension of the soil is made in some dilute fixative and a few drops of it are spread on a clean slide which is then dried, stained and examined under the microscope (Conn, 1922). Various modifications of this method have been employed to suit the different types of micro-organisms. The one used by Jones and Mollison (1948) deserves special mention. In this, a measured small quantity of soil is mixed up in molten agar gel. Small drops from this are placed on the slides, spread in thin films and allowed to solidify.

Staining is done with acetic-aniline blue followed by dehydration in alcohol and mounting is done in euparol. By counting the number of organisms in a measured area of the film a quantitative estimation is obtained. This method has been usually employed with the various classes of micro-organisms—fungi, bacteria and actinomycetes.

Cholodny (1930), however, pointed out that it is not possible to obtain an exact picture of the micro-organisms by any of these methods. Since the shaking of the soil with water disturbs it in a manner that its original state is lost. Rossi (1928) and Cholodny (1930) devised a method commonly known as 'contact-slide' or 'soil-plate' method. This consists of making a narrow slit in the soil by means of a sharp knife and inserting a clean slide into the slit. The soil from the two slides is gently pressed so that it comes in contact with the slide from both the sides and remains in the natural state. The slide is left in position for 1 to 3 weeks. It is then removed and cleaned on one side. The preparation is fixed, stained with phenol erythrosin and finally washed, dried and examined. The method has succeeded in giving a more natural picture of the organisms. It has, however, its own limitations. Often the specific identifications are difficult to obtain and it gives no idea about the function or special characteristics of the organisms.

Another important direct method has been developed by Kubiena (1932). He used a special microscope for the examination of soil in an undisturbed condition. There is a special device of surface illumination attached to the microscope and the organisms can be seen in their natural state. It is possible to pick up the bigger organisms with the help of micro-manipulators and to study them after suitable preparation. The method, however, has not been used extensively and only limited information is available. For the measurement of colonisation and survival of soil Fusaria, Sadasivan (1939), Walker (1941), Subramanian, (1946, 1950) and Zachariah (1949) have found the root burial technique of great help. But this technique does not give a quantitative picture of the distribution of spores, mycelia, etc., in various regions in the soil profile with or without crop plants (Stover, 1953). Among others the 'immersion tube method' of Chesters (1940) may be mentioned. He has employed tubes with capillary orifices in their lower parts. Such tubes are filled with sterilised medium and are immersed in soil for incubation. According to Chesters (1949) "the species of fungi which are isolated from such tubes usually possess a widely spreading mycelium and grow actively in the soil. Although this method does not permit visual inspection of the soil for active mycelium there is little doubt that living hyphæ must be present in the soil adjacent to the tube before the species can be isolated".

It will be clear from the aforesaid that different methods have their own specific merits and none is perfect in all respects. It may be mentioned here that in spite of several 'direct methods' which are available now the age long 'dilution plate method' still remains a potent tool of investigating the soil microflora and it is by this method that a mass of information on this subject has been gathered. A comparison of the 'dilution plate method' with the 'direct methods' has been made by Jensen (1931) in his extensive studies on Danish soils. He made a statistical analysis of his results and came to the conclusion that the information obtained by the 'plate method' on the quantitative occurrence of various fungi was not a matter of chance but it was a true representation of facts. In my opinion the information should be obtained by as many methods as possible in order to get a correct picture of the fungal flora.

Ecological Aspects

Though studies of soil fungi have been engaging the attention of a large number of workers, the ecological factors which govern their distribution have not been seriously studied till recently. In the earlier works it has been recognised that the bulk of the fungal flora in soil is confined to the upper layers. Some fungi habitually occur in deeper layers such as *Zygorrhynchus* (Waksman, 1916) which is present at 12 to 30 inches depth and species of *Fusarium* were found up to a depth of 38 inches (Rathbun, 1918).

There has been considerable difference of opinion regarding the species of fungi which are found to occur in different types of soils. Werkenthin (1916) and Brierley (1923) could not find any substantial difference between the cultivated and uncultivated soils. It is generally agreed that the Mucorales and species of Penicillia widely occur in the soils of northern latitudes whilst species of Aspergilli are dominant in the warmer regions. Certain species are dependent on the reaction of the soil, for example, Jensen (1931) reported Penicillia and *Trichoderma* common in acid soils and *Mycogone nigra* (Morgan) Jensen and *Coccospora agricola* Goddard in alkaline soils. The genus *Trichoderma* is usually found to occur in wet and water-logged soils. With other common species there are conflicting findings on the nature of their habitat.

Recently, Warcup (1951) has studied the fungal flora of five different kinds of natural undisturbed grassland soils at Lakenheath Warren, England, in great detail. He recorded the frequency of occurrence of different species at different horizons and gathered very valuable data. The commonest genera isolated by him have been *Penicillium, Mortierella, Absidia, Cephalosporium, Fusarium, Gliocladium, Gliomastix, Mucor, Thielavia, Trichoderma* and *Zygorrhynchus.* The fungi show different distributions in the five soils and two large groups could be distinguished: those common in the acid soils and those occurring in alkaline soils. Warcup's work has been unique in the sense that the technique for quantitative analysis of different flora was applied for the first time. Recently Tresner *et al.* (1954), who investigated the distribution of soil micro-fungi of the hardwood forest of Wisconsin, have used the technique of phytosociological analysis in the same manner as it is done in the case of higher vegetation. It was possible to apply such denotations as 'dominant species', 'pioneer species', 'climax species', etc., after determining the frequency and relative density of different species in different soil types. Obviously, this enables one to get a more correct picture of the micro-organisms residing in the soil. They also gave the relative size of accompanying bacterial and actinomycetous populations. This line of approach offers great possibilities since we are able to probe deeper into the meaning of the distribution of various species.

Almost simultaneously, Saksena (1954) has worked out the fungal flora of forest soils of Sagar. Incidentally the technique applied has been almost the same as followed by Tresner *et al.*, though the terrain involved and the climatic conditions, etc., are entirely different and so the findings. Along with the analysis of the surface vegetation and the fungal flora the soil characteristics, both physical and chemical, were examined in detail. An exhaustive data has been collected on such items as the surface geology, physical characteristics, moisture content, water-holding capacity, hydrogen-ion concentration, organic matter, nitrogen content, exchangeable bases, carbonates and phosphates. Of the characters studied six were found to be specially significant which appear to govern the distribution of fungi, *viz.*, the surface cover, water-holding capacity, organic matter, hydrogen-ion concentration, exchangeable calcium and the nitrogen content. The quantitative abundance of fungi in different horizons of profiles were studied. In general, fungi were found to be more abundant in the upper layers than down below but exceptions to this did occur, sometimes, in one soil type, which was badly exposed to summer drought as having "pioneer conditions". The analysis of the phytosociological data reveals that there were species which could be termed as "pioneer species," *e.g.*, *Penicillium funiculosum* Thom and *Aspergillus fumigatus* Fresenius which occurred only in these dry conditions. On the other hand there were species such as species of *Gliocladium* and *Cunninghamella* which appeared only in soils of mature forests and hence called "climax species". Such 'climax' and 'pioneer' species can be regarded as good indicators of soil conditions. Of course, there were a host of other fungal species which did not show such rigid distributional patterns but had greater ecological tolerance with irregular patterns of distribution. This situation is found to be analogous to what is found in the distribution of species of higher plants constituting the forest in the investigated area.

The aforesaid work of Warcup, Tresner *et al.* and Saksena opens up a new approach to the study of soil micro-organisms. Valuable information on the occurrence and distribution of species can be elicited by these methods and important conclusions can be drawn. These ecological studies supplemented with the information on the physiological behaviour by cultural studies would throw a fresh light on the associative relationship of fungi and other organisms as they exist in nature. It is expected that more workers will follow this method to gather information from different types of soils which will enable us to make comparisons on a large scale.

The Biological Balance in Soil

In recent years few scientific discoveries have appealed to the public imagination as much as the discovery of penicillin. It has been aptly called the "wonder drug" and countless lives have been saved by its use. Its vast therapeutic importance has overshadowed the fact that it is but one of the many antibiotic substances which are secreted by the micro-organisms in order to maintain an equilibrium in nature. To appreciate the role of antibiotics in nature one has to turn to the natural habitat of these organisms which normally, in most cases, is soil. The upper layers of most soils are very rich in micro-organisms both in quantity and variety. Obviously there is a very keen competition between the organisms for food. If an organism is able to secrete a substance which is inimical to the growth of other organisms thus forming a sort of protective fence all-round, it is able to succeed better than others which are unable to do so. If the mould flora of a soil rich in organic matter is examined it is found to consist of species which are known to produce such antibiotic substances as penicillin, gliotoxin, patulin or viridin. The exact relationship between the different fungi and other organisms in the soil is to a large measure still unknown. Although most of the antibiotic substances that have been studied so far are largely active against bacteria, some have also been found to exert a marked effect upon fungi. Millard and Taylor (1927) showed that potato-scab caused by *Actinomyces scabies* (Thax.) Gussow was arrested if the field received large amount of organic matter by green manuring. It was found that under these conditions the growth of other saprophytic species of *Actinomyces* was favoured which suppressed the pathogenic species. Further, it has been reported that Fusaria are unable to make free spread in unsterilised soils. Evidence indicates that any change brought about in microbial numbers either by addition of an active micro-population from pure cultures or by increasing soil fertility by trace element amendments (Thankam, 1949; Sadasivan, 1950, 1951; Sulochana, 1952, 1952 *a*) brings down the survival and saprophytic activity of Fusaria in soils. It has further been pointed out by Sadasivan and Subramanian (1954) that the longevity of *Fusarium solani* and possibly other Fusaria also in soils seems to depend on the associative microfloras with which they occur. Experiments in America have shown that in the absence of effective antagonism from associated saprophytes the incidence of the scab is severe.

The antagonistic action of *Trichoderma lignorum* (Tode) Harz. and *Gliocladium fimbriatum* (Gilman and Abbott) on such parasitic fungi as *Rhizoctonia solani* Kuehn, *Sclerotium rolfsii* Sacc. and *Phytophthora parasitica* Dastur was demonstrated by Weindling (1932, 1934, 1938). Allen and Haensler (1935) applied cultures of *Trichoderma* to the soil and demonstrated that the incidence of damping off was checked. Work on similar lines has been carried out by Vasudeva and his collaborators (Vasudeva, 1949). In the case of *Rhizoctonia bataticola* and *R. solani* the causal organisms of root-rot disease of cotton, he showed that the presence of *Trichoderma lignorum* and *Aspergillus niger* in the inoculum greatly retarded the growth of the pathogens. The hyphæ of *T. lignorum* showed a dissolving effect on the hyphæ of these parasitic forms (Vasudeva and Sikka, 1941). Garrett (1934, 1938), Porter and Carter (1938) and Weindling (1938) have reviewed the work done on the role which the microflora of the soil play in keeping down the incidence of the parasitic organisms in the soil. This field of research is full of many possibilities. The phenomenon of associative and antagonistic effect is obviously complex and needs investigation by the mycologists, bacteriologists and biochemists.

EPILOGUE

The study of soil fungi is a complex science and needs investigation from several view-points. Taxonomically there are a large number of fungi awaiting discovery, especially from the deeper layers which are more restricted in their distribution. Ecologically, we are yet to understand and analyse the complexity of factors which govern the occurrence and distribution of various species. The situation here is more complex and difficult than in the case of ecology of higher plants because of the greater changes and variety in the subterranean micro-climates which are more difficult to assess and analyse. The associative and antagonistic effects of various classes of micro-organisms add further problems to the already bewildering complexities. Though these studies of soil fungi bristle with difficulties yet the economic and academic aspects, such as the role of fungi in the decomposition of plant debris, the production of antibiotics, the occurrence of various soil-borne plant pathogens and above all the challenging complexities encountered in all these phases of study should attract a galaxy of enthusiastic and energetic investigators working in teams.

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