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PERSPECTIVES OF MICROBIAL INTERACTIONS*

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The interactions among microbes, particularly those living in soil and on the leaf surface are presented in the present write-up. The microbial interactions viz., fungi vs. fungi, fungi vs. bacteria, fungi vs. nematodes, fungi vs. mycorrhizas, fungi vs. soil amoebae, virus vs. virus etc. are highlighted with their biological significance towards control of plant diseases. The activities of microbes in the soil and on other substrates have been studied by different workers with specific techniques and this kind of study opens a wide scope for revealing the secret of the nature and for ameliorating the plant diseases biologically instead by application of toxic chemicals that cause harmful effects on health of humans and other animals. Hence application of microbes in the service of mankind in this respect opens bright future for joining various scientists including microbiologists, biotechnologists, plant pathologists etc. for exploring many microbes from soil and other substrates for usage in biocontrol of plant diseases.

Fellow Botanists, ladies and gentlemen

I welcome you all to the deliberations of the 19th Indian Botanical Society Conference, 1996 being held at the holy city of Hardwar where the pious Bhagirathi flowing through the long way of the Himalayan range descends and touches the plains, and is named here as the Ganga. I am conscious of the honour done to me by the Executive Council of the Indian Botanical Society unanimously electing me to the Presidentship of the society for 1996-97. On this occasion I remember my reverend teacher, the late Dr. R.Y. Roy, Ph.D. (London), D.I.C. under whose able guidance I learnt the soil mycology, plant pathology and microbial ecology. I express my deep sense of gratitude to him. I am grateful to Professor R. Misra, Ph.D. (Leeds), F.N.A., F.W.A. who encouraged me for taking the liberty to discuss the ecological aspects of soil microorganisms.

My first experience with fungi inhabiting soil started about forty years ago when I took research work on them with the ecological touch. There was lack of enough facility in the laboratory then as usually the laboratories of to-day have; so my initial research in soil mycology started amidst constraints with meager facilities. My three year-stay as a research student in the laboratory at the Botany Department, B.H.U. was mixed with rays of optimism and pessimism about fate of the results obtained during the research period. But a corpus of literature existing on soil mycology including techniques and methodology then published by various workers in different

parts of the world encouraged me as the time elapsed during research period. I was most encouraged by the work of S.D. Garrett of Cambridge University whose published work is monumental in the field of soil mycology and pathogenic root fungi distributed in different books and journals either written by him or having been quoted by other workers in these fields. His four original books on soil mycology (Garrett 1944, 1956, 1963 1970) are the sources of encouragement to the future research workers and post-graduate students who wish to opt for soil mycology and root disease fungi as the research career. My association with him as his research associate at the Cambridge during 1965-66 was pleasant with most intellectually stimulating and learning experiences. Garrett was a dominant figure of international repute in these subjects. I profited much with him about the subject with a new line of work in the field of soil fungi and soil microbiology for production and detection of antibiosis by fungi existing in the composite inocula of soil. It was a simple experimental evidence on agarplates in which a pinch of soil particles were inoculated and operated timely from 24 hr to 120 hr for intensity of production of staling products toxic to other microorganisms of soil origin preventing others to appear on agar discs at the reverse side and establishing a kind of competition- a microbial competition and interactions for existence on the staled agar discs on which only a few fungal species belonging to *Penicilium* and *Trichoderma* could colonize. The observations and results were interpreted in a mathematical model, the findings of significance in the

field of soil microbiology, microbial toxicology and antibiotics leading to the future prospects of discovery of competent microorganisms for production of these myco/microbial toxins (Dwivedi and Garrett, 1968). My association with another laboratory at the Department of Soil Microbiology, Rothamsted Experimental Station, U.K. was an occasion to be associated with research group of Dr. P.S. Nutman, a leading scientist on *Rhizobium* biology to explore the possibility of effects of soil microbes other than rhizobia on nodule development in leguminous plants. During interaction of this kind I learnt techniques for studying process of nodulation in *Trifolium in vitro* and interaction of some rhizosphere fungi on nodule development. Leguminous plants grow in soil and rhizobial nodules develop in roots facing several adverse soil factors including microbial interactions which is a fascinating and unexplored area giving enough scope to be discovered by future researchers of wide applicability in agriculture. The discovery of this kind had earlier been made by Lim (1961) who showed that certain root surface fungi e.g. species of *Verticillium* consistently decreased infection of *Rhizobium* without affecting the number of *Rhizobium* in the rhizosphere; where as other fungi were either stimulatory e.g. species of *Paecilomyces* or had no effect. The cause of these interactions are not known; interactions between nodule bacteria and other soil microorganisms have been very little studied and open a wide scope to explore this aspect in future.

The interactions of microorganisms in the soil environment is a hidden mystery of the nature and my attempt to continue this work increased due to some available literature and results of my own work. I joined the Department of Plant Pathology, Waite Agri. Research Institute, University of Adelaide, S. Australia during 1977-78 to continue the interaction work among microbial population of soil. About a few years earlier there was a problem with wheat crop in S. Australia due to infection by take-all fungus (*Gaeumannomyces graminis tritici*) which caused hay-die disease; but after repeated cultivation of the crop in the same field the disease declined automatically. I considered this problem in deep and planned to work on the microbiology of the soil of such fields. After repeated culturing and isolating various microbes. I came across a kind of microbe which had a fascination for colonizing hyphae of the above pathogen. Isolation of the pathogen on agar plate was an

easy task which was done accurately and easily, but isolation of the microbe colonizing hyphae of the pathogen was a difficult and cumbersome task because it could not colonize directly on the agar discs from soil or root inocula. Employing a special technique of my own, I could succeed in isolating and growing the microbe in agar plates with pre-grown colony of *Arobacter aerogenes*, a saprophytic soil bacterium as a substrate. The organism could grow on the pre-grown colony of this bacterium which was later isolated in pure form and identified as soil amoebae. The idea struck to me as why the disease declined automatically after repeated monoculture of the wheat crop in the same field. Perhaps amoebae isolated by me might be instrumental to this cause as they grow on other microbes as food substrates and there was some possibility of discovery of parasitic nature of the amoebae on hyphae of the pathogen destructing the hyphae by their parasitic activities, thereby declining the population of the hyphae or the propagules of the pathogen. This idea mooted in my mind encouraged me to proceed further with this nature of interactions of soil microbiology and plant pathology; and so I finally decided to take the risk implied in my mind towards this problem. I present below the address after giving a frank autobiographical account of my early start and difficulties in research, later developed getting initiation of the encouragement from the Scientists like Drs. S.D. Garrett, P.S. Nutman, R.M. Jackson, J.H. Warcup etc., the outcome of which makes the subject matter of this address.

Soil is an abode of a large number of living components i.e. microorganisms including fungi, bacteria, protozoa, nematodes, viruses, amoebae etc. interacting with one another therein.

Scientifically the most important development of the last one decade has been the integration of molecular biology into actually all the traditional areas of biology of pathogens, disease epidemiology, genetics, breeding for resistance, host-pathogen interactions, microbial interactions with pathogens, pathogen-pathogen interactions, biocontrol, chemical control and other subject areas of the field. It is a natural phenomenon that when there is a huge population of organisms either of individuals or of mixed kind, there will be a competition for existence and survival in the soil environment. The tool of competing ability provided naturally to the microbes is with organic chemi-

cals, the enzymes and toxins, being continuously excreted by the organs of the microbes. The physical aspect of competing ability of them is provided with the mantle of hyphae forming strong protective organ known as the rhizomorph or sclerotia which have the strong hold physically, not allowing any other interacting microbe to invade the domain of pre-existing organism/organisms. It is a difficult assumption in the soil environment as how a mixed population of an array of microbes survive. Perhaps the fungi are the strongest competitors than are bacteria, protozoa and nematodes because of their being equipped well with enzyme secreting ability and physical structure. Some of the organisms must be forming pockets of population of a large number of vegetative organs in soil and there must be continuous competition for survival. The nutrient rich in energy available to them from decomposition of plants and animals residues are the sources of survival. During decomposition there is again interaction and a competition for colonization of the substrates and due to accumulation of a large number of microbes either of individual kind or of composite ones, there is secretion of toxic metabolites i.e. mycotoxins, bacterial toxins, enzymes, ethane, aldehyde, alkanes, CO₂, ammonia etc. that cause impediment of growth of microbes. During these activities and interactions only those microbes are able to survive that have the best ability of competition during interactions for survival and persist longer in soil as the soil inhabitants. The soil microbes survival has been categorized into (i) saprophytic competitors on decaying substrates (ii) survival as saprophytic on decaying litter or on weeds infected during parasitic phase, (iii) resting bodies like oospores, chlamydospores and sclerotia, (iv) survival on living roots or on other susceptible living plants, (v) as parasites on living roots showing no disease symptoms and (vi) as mycoparasites living inside other hyphae by interaction and penetrating, and finally establishing inside (Garrett, 1955). These microbes survive by getting nutrients excreted from living roots in the form of sugars, amino acids, vitamins, proteinaceous compounds, organic acids etc. Site of exudation process has been recognized using plants pre-supplied with ¹⁴CO₂, method of estimation of released radio-active carbon compounds, by collecting exudates in filter paper and scanning for radio activity. It has been discovered that the effects of antibiosis from toxic metabolites or from direct parasitism of the associated microorganisms on deve-

lopment of root diseases are the controlling factors for interaction. *Rhizoctonia* faces adverse interaction with *Actinomyces scabies* during its parasitic phase in unsterilized soil as reported by Sanford (1923, 1926). Similarly interactions of hyphae of 'take-all' fungus with *Pseudomonas fluorescence*, demonstrated by Rovira and Campbell (1975), led to the destruction of hyphae of the parasite. Implications of heterolysis and autolysis of hyphae of parasites are the cause of interactions either by by-products of other microbes or by self-secretion of enzymes during growth phase condition in soil environment. In continued study of interactions for intraspecific fungal competition for substrate colonization, Rao (1959) and Wastie (1961) reported the colonization of nutrient agar plates from mixed inocula of soil. The degree of success attained by any fungal species in competitive saprophytic colonization of a substrate due to interactions is determined by (i) its inoculum potential at the surface of the substrate and (ii) its competitive saprophytic ability for the particular substrate and under the particular set of environmental conditions. Competitive saprophytic ability shown by a particular fungus can vary from one type of substrate to another, and hence is a substrate specific (Garrett, 1963 p. 120).

The assessment of competitive saprophytic ability, which is the result of microbial interactions during successful colonization of substrates by a pathogen, was done in laboratory by Butler (1953) and Lucas (1955) for *Curvalaria ramosa*, *Fusarium culmorum*, *Helminthosporium sativum* and *Gaeumannomyces graminis* by a specific technique of burying the substrate units to be colonized in a series of inocula and soil mixtures with chosen microbe. Here the microbial interactions in soil decide the fate of the successful pathogen/pathogens for colonization of the substrates.

Interactions among different fungi: Many soil inhabiting fungi interact by being the parasites or the hosts. The mycoparasites penetrate the host hyphae and get nourishment from them. *Trichoderma lignorum* has been reported to parasitize a number of soil borne fungi when cultivated on agar plates - thereby showing its habit of being mycoparasite in the soil environment as reported by Weindling (1932) who suggested for control of certain pathogenic fungi by introducing this mycoparasite in soil. Interactions of *Trichoderma* species and *Gliocladium virens* with plant parasites of soil origin have been presented in details

by Papavizas (1985). Similarly *Coniothyrium minitans*, *Laetisaria arvalis*, *Pythium nunn*, *Talaromyces flavus* and *Sporidesmium sclerotiorum* have been discussed for their interactions and potentiality for control of the diseases caused by *Sclerotinia*, *Pythium* & *Rhizoctinia* (Adams, 1990). While studying interactions with fungistatic growth products of microbes diffused in agar discs towards tolerance of some pathogenic fungi expressed by growth rate on such agar discs *vis-a-vis* growth rate on virgin agar, Wastie (1961) expressed the effects of interactions of fungitoxic substances in numericals of reduction factor with 14 fungi (Cf. Garrett, 1970, p. 132). The outcome of the struggled interactions between the inoculant fungus and the other soil fungi in the inoculum soil-agar discs is decided before any fungistatic concentration of fungal growth products has had time to accumulate in the agar.

The antagonistic interactions between *Sphacelotheca fuliginea*, causing powdery mildew of plants, and yeasts, and other fungi have been reported by some workers leading to inactivation of the mildew pathogen. Blackman and Follema (1982) have reported inactivation of *S. fuliginea* by epiphytic yeasts, *Tilletiopsis minor*- another yeast caused destruction to *S. fuliginea* on cucumber leaf (Hoch and Provvidenti, 1979) the hyperparasite- *Ampelomyces quisqualis* interacted and controlled *S. fuliginea* on cucumber (Jarvis and Slingsby, 1977). *Stephanoascus flocculosus* and *S. rugulosus* have been reported to inactivate and kill *S. fuliginea* on leaf disks of cucumber (Jarvis *et al.*, 1989). The antagonistic interaction between this powdery mildew fungus and other fungi is conditioned by environmental factors i.e. humidity of the atmosphere, temperature, morphological features of the leaf surface and retention of water in the epidermal cells of plants. The nature of interactions may be in the form of penetration of hyphae in the fungus-host hyphae in case of *Ampelomyces quisqualis* and utilization of nutrient of the host hyphae; while in other cases secretion of substances that kill the host cells and then utilization of nutrient released from them.

The interactions between *Coniothyrium minitans*- a mycoparasite and *Sclerotia* of *Sclerotinia sclerotiorum*, *Rhizoctonia tuliparum*, *Sclerotium delphinii* and *Typula incarnata* on conidial germination, germ tube and mycelial growth of *C. minitans* were studied by Whipps *et al.* (1991) who reported that

both live and autoclaved sclerotia of all these species except *S. delphinii* stimulated conidial germination and germ tube growth of *C. minitans*. Similarly Bradshaw *et al.* (1991) reported interactions between *Pythium oligandrum* and the fungal pathogens causing foot rot of peas. *P. oligandrum* showed antagonistic interaction towards *F. solani*, *F. sp. pipi*, *Phoma medicaginis* var. *pinodella* and *Mycosphaerella pinodes*, the three major foot rot pathogens of peas under the range of *in vitro* conditions. The necrotrophic parasitism by *P. oligandrum* was recorded due to production of a volatile antibiotic which reduced growth of *P. oligandrum* killed both *M. pinodes* on oxoid-corn meal agar. *P. oligandrum* killed both *M. pinodes* and *P. medicaginis* in dual culture plates. Lysis was the predominant interaction parameter observed (Lewis, 1988). The initial hyphal interactions on cellophane resulted in lysis of hyphae of pathogen. During studies of interactions between fungi, Keay and Brown (1989) reported that *Trichoderma* spp. markedly inhibited the growth of *Psilocybe semilanceata*, while the latter significantly suppressed the growth, in dual culture, of many of the fungi screened from the rhizosphere of grasses. Their interaction study was performed on malt agar by inoculating individual rhizosphere fungi and *P. semilanceata* at a constant distance of 2.5 cm. from the edge of the Petri dish with mycelial disc of 4 mm diam. cut from the leading edge of a colony of *P. semilanceata*. The latter inhibited many fungi and there was mutual inhibition between *P. semilanceata* and *Penicillium* sp., and the related *Byssoschlamys nivea* which are known to produce antibiotics. The growth of some other fungi viz., *Fusarium* spp., and *Sepedonium chrysospermum* whose colonies later intermingled with *P. semilanceata* showing thereby interactions between hyphae due to production of antibiotics by all fungi. The production of toxic substance of hallucinogenic nature and also of phenolic nature by *P. semilanceata* may be responsible for inhibition of growth of other fungi. Arora and Dwivedi (1980) reported hyphae of fusaria penetrating in those of *Rhizoctonia solani* and interacting with them. Dubey and Dwivedi (1986) have reported penetration of hyphae of fungal pathogen (*F. solani*) in those of saprophytic fungi as a mode of survival in absence of plant hosts and due to interactions after penetration/vacuolation and destruction of hosts hyphae occurred. Interaction of hyphae of *Sclerotium rolfsii* causing foot-rot of barley and hyphae of saprophytic fungi have been

reported by one of my students (Singh, 1987).

Interactions between 'sclerotia of *Rhizoctonia tuliparum* and soil microorganisms have been studied in detail by Gladders and Coley-Smith (1980). It is an established fact that sclerotia survive for longer period under adverse conditions and there appears to be variations in the longevity of sclerotia in those of different genera. The air dried and wetted sclerotia excrete nutrients and the latter support the growth of micro-organisms in the soil environment. Some of the microbes invade the sclerotia and reduce the viability for germination. The air dried sclerotia of *Rhizoctonia tuliperum* were readily invaded by some fungi inhabiting soil i.e. species of *Mucor*, *Fusarium*, *Trichoderma* etc. The invasion of sclerotia by soil microbes is influenced not only by the quantities of materials leaked by sclerotia but also by their nature. Thus the interacting soil microorganisms with sclerotia play an important role in their existence in soil, and stronger are the antagonists, lesser would be ineffectiveness of sclerotia for invasion of the host. The sclerotia of *R. tuliparum* when wet may be invaded by soil fungi and less colonized by bacteria due to presence of pyrone antibiotic. Interactions between *Nectria inventa* and each of 14 fungi screened from rapeseeds were studied by Tsuneda and Skoropad (1980). Hyphae of *N. inventa* grew towards susceptible fungi and on contact with host hyphae, they formed appressorium-like bodies and hyphal coils. Granulation of cytoplasm of susceptible fungi was the first visible effect, later vacuoles formation occurred and the host hyphae became empty. Hyphae of *Pythium acanthophoron* grow as mycoparasite in side the hyphae of *Fusarium solani* and *Pythium myriotylum* and other fungi (Lodha and Webster, 1990) and due to this interaction nutrient of the host hyphae is depleted. Dwivedi (1992) reported inhibition of the growth of *Fusarium oxysporum* f. sp. *psidii* and *F. moniliforme*, the causal agents of guava wilt by *Trichoderma* spp. during study of colony interactions *in vitro*. Dwivedi (1993) further studied interactions between the colonies of *F. oxysporum* f. sp. *psidii* and those of some antagonistic soil fungi, and *Streptomyces chibaensis*; and found inhibition of growth of the former ones by the colonies of the latter. Dwivedi *et al.* (1993) tested antagonistic activities of *Trichoderma* spp. against *F. oxysporum* f. sp. *lini* and *F. oxysporum* f. sp. *lycopersici* with high percentage of inhibition of colonies *in vitro* studies, while *Aspergillus flavus* inhibited the colony growth of these pathogens

with less percentage of inhibition compared with *Trichoderma* spp.

The life in soil is highly diverse and consists of interacting population of microorganisms and soil fauna, and their activities affect physical, chemical and biological characteristics of soil. The microfloras catabolize organic matter, mineralize and immobilize soil nutrients. The microfauna are responsible for regulation of bacterial and fungal population and alter nutrient turn-over, mesofauna regulate fungal and microfaunal populations and fragment plant residues; while macrofauna fragment residues of plants on which microbial activity is stimulated (Hendric *et al.*, 1990). It is estimated that in a fertile soil the soil biota may have a biomass of 20 t ha⁻¹ with forms ranging from microscopic bacteria to earthworms (Wallwork, 1976; Kee and Oanjgrst, 1992). The soil microorganisms such as bacteria, fungi, actinomycetes, algae and protozoa are of great taxonomic and functional diversity, and during their functional diversity, the interactions play a much important role in establishing either a close relationship with other microbes giving benefit to each other or disturb the coherent populations of other microbes by secreting toxic substances or other secondary metabolites.

Microbial interactions with mycorrhizas:

Mycorrhizal fungi are considered to be the agents to interact with the pathogen of roots and this kind of study has emerged on strong footing during the last two and half a decades. Some of the workers are hopeful for bright future of such interactions for control of root pathogens (Papavizas and Lumsden, 1980). Interactions of *Glomus fasciculatum* and *Phytophthora* sp. causing root-rot of a few crop plants were studied by Davis *et al.* (1978) and they found control of the disease to some extent. Similarly disease severity of cotton caused by *Thielaviopsis basicola* declined due to inoculation and interactions of *G. fasciculatus* (Schoenbeck and Dehne, 1977). Development of sclerotia of *Sclerotium rolfsii* reported by Krishna and Bagyaraj (1983) on roots of groundnut was less in presence of *G. fasciculatus*. Favourable response of result of interactions of *Macrophomina phaseolina* causing root disease of soybean and mycorrhizal fungus *G. mosseae* to decline the disease was reported by Zambolin and Schenck (1983). Even nematodes have interactions with the mycorrhizal fungus in case of crop plants like oat, groundnut,

soybean and tomato (Silora and Schoenbeck, 1975; Hussey and Roncadori 1982; Kellan and Schenck, 1980). Thus the role of mycorrhizal fungi on decline of root diseases may be attributed to the fact that interactions among them cause adverse effects due to which disease decline biologically by (i) development of hyphal mantle around root by mycorrhizal fungi debarring from entry of the pathogen, (ii) diseases encouraged by deficiencies of minerals are compensated by supply of minerals by mycorrhizal fungal hyphae, (iii) production of antibiotics by mycorrhizal fungi that affect growth and development of pathogens, thereby decline the disease. The interactions of mycorrhizal fungi and pathogenic fungi open a wide scope for future studies in the agro-and forest ecosystems. One of my research students, Leela (1991), while working on microbial ecology of pea rhizosphere, found much of interactions between mycorrhizal fungi and *F. pisi* and regard to reduced intensity of disease development.

The more befitting examples of interactions of microbe- to microbe are found in connection with spore germination of certain fungi due to interactions of other fungi. Spores of certain fungi do not germinate without interaction of bacteria or other fungi. Spore germination of ectomycorrhizal hymenomycetes is difficult to achieve in the laboratory conditions. The spore germination of species of *Boletus*, *Amanita* and *Tricholoma*- the ectomycorrhizal fungi, was achieved by using a living yeast i. e. *Torulopsis sanguinea* or *Rhodotorula glutinis* as a stimulator (Fries, 1943). Similarly induction of germination of basidiospores of *Pisolithus tinctorius*, *Rhizopogon roseolus* and *Suillus granulatus* has been reported in presence of the yeast - *Rhodotorula glutinis* (Lamp and Richards, 1974). Spores of lactarius species were stimulated to germinate by *Ceratocystis fagacearum* (Oort, 1974). It is to be understood that spore germination of ectomycorrhizal fungi in laboratory is difficult and the role of microorganisms as stimulants deserves further work in this direction. Ali and Jackson (1989) assayed different isolates of bacteria and fungi screened from sporophores, mycorrhizal sheaths and soil for their ability to induce germination of spores of ectomycorrhizal fungi; and some success was achieved in spore germination. *Pseudomonas stutzeri* was active in stimulating germination of spores of *Hebeloma crustuliniforme* within three days, while *Corynebacterium* isolates stimulated spore germina-

tion after two weeks incubation. Spores of *Paxillus involutus* were stimulated by two isolates of *Corynebacterium* and *Arthrobacter*. Azcon-Anguiller *et al.* (1986) reported the effects of several microbes on the spore germination of *Glomus mosseae* and on subsequent development of its hyphae. Soil bacteria *Corynebacterium* and *Pseudomonas* also induced spore germination of the same mycorrhizal fungus. The colonization of mycorrhizal hyphae by soil bacteria gives support to the concept that such bacteria have affinity with hyphae due to nutrient availability exuded from fungal organs and may induce germination of spores of such fungi. Hopefully the problem of *in vitro* culture of mycorrhizal fungi would be solved by application of bacteria/fungi as germination stimulators.

Interactions between nematodes and other microorganisms: Nematodes living in soil cause root diseases of variable intensity depending upon the inoculum potential of the pathogen in soil which is governed by edaphic factors. They attack plant roots either through wound caused on root due to mechanical injury or through the soft tissues due to attack of other type of pathogens viz., fungi bacteria and actinomycetes by the activity of enzymes. Fungi interact with nematodes by attacking through adhesive mechanism or by ring formation around the hyphae. They secrete toxic substances due to which nematodes are paralysed and finally killed. Zopt (1988) was the pioneer to discover fungi parasitizing and destructing nematodes. The population of nematodes declines due to attack of predaceous fungi in soil and so the nematode disease of plants are ameliorated. *Arthrobotrys robusta* can interact with *Ditylenchus myceliophagus* and *Meloidogyne*. Some of the species of *Fusarium*, *Cephalosporium* and *Dactyella oviparasitica* infect nematode eggs. *Paecilomyces lilacinum*, *Acremonium bacillosporium* and *Helicoon farinosum* interact with eggs of nematodes and infect them causing decline of their population. *Verticillium chlamydosporium* is reported as potent interacting fungus parasitizing eggs of *M. arenaria* (Morgan-Jones, *et al.*, 1981). *Penicillium anatolium* interact with *Globodera rostochiensis*; *Gliocladium* sp., *Trichocladium* sp., *Trichurus* sp. and *Drechslera* sp. interact with cysts of *Glodera pallida* and later cause damage to them (Gonzales *et al.*, 1984). Interactions between root-knot nematodes and *Fusarium* wilt causing fungus have been reported by Mai and Abawi (1987). Nematodes increased the disease incidence and severity of the wilting on *Fusarium*

susceptible and *Fusarium*-tolerant crops. *Nematophthora gynophila*, a nematode (*Heterodera* sp.) and due to interaction female nematodes fail to form cysts, and their bodies replaced by a mass of resting spores (Kerry and Crump, 1980) in addition to fungi, certain bacteria also interact with nematodes and cause destruction to their eggs and cysts. Some of such bacteria are *Pseudomonas denitrificans* (Adamo and Eicheumuller, 1963), *Clostridium pasteuriarum* (Banage, 1965), *Pasteuria penetrans* (Stirling and Wachtel, 1980) etc.

Interactions of *Heterodera rostochiensis* with *Rhizoctonia solani* and *Colletotrichum atramentarium* have been discovered with tomato plants. There appears to be an inter-relationship between the latter two fungal pathogens when they grow together. Further, root decay in lemon caused by *Fusarium solani* is increased when *Tylenchulus semipenetrans* is also present (O' Bannon *et al.*, 1967). The relationship with wilt diseases caused by fusaria and nematode has been of significance as reported by several workers. The first report in this direction was by Atkinson (1892) who dealt with *Fusarium* wilt of cotton and the effect of root-knot nematodes on the appearance of this disease. He noted repeatedly that infection by root-knot nematodes increased the severity of *Fusarium* wilt. Pathak, one of my students (1979) and Pathak and Dwivedi (1980) reported the enhanced wilting of tomato plants due to *Fusarium oxysporum* F. sp. *lycopersici* when nematode infection on root system was severe and plants dried up completely. The influence of nematode on fusarial wilt is sometime so severe that wilt control in crops is done by controlling nematodes. *Pratylenchus* spp. are sometimes influenced by fungi. Presence of *Verticillium* encourages the enhancement in reproduction by *Pratylenchus* and root invasion by the fungus can cause the plant more attractive to the nematode (Mountain and McKeen, 1962).

The factors influencing severity of diseases caused by interaction of fungi nematodes have been studied by some workers. The biochemical changes in plants due to nematode infection are yet to be discovered in future, but chemical changes have been found in plants due to such events. Changes have been reported with the infection by *Meloidogyne* sp. Free amino acids in galls by this nematode increase up to 100-700% in comparison with healthy tissues. Auxins have also

been reported in nematode-infected tissues by Viglierchio and Yu (1968). Some unknown growth-promoting and inhibiting substances have been reported by Bird (1962). The changes in physiology of the hosts due to interactions of fungi and nematodes in infected tissues are due to changes in chemical contents of hosts. Proneness of the host to diseases as influenced by primary disease has been discussed by Gaimann (1950) and his this assumption appears to be true to the nematode-fungus disease complexes. The infectious primary diseases not only overcome the hosts' resistance to penetration, but also break down the hosts' resistance to further spread of the subsequent pathogen, and evidences support this in some nematode-fungus wilt diseases (Powell, 1971). Singh and his associates (1996) tested some plant parasitic nematodes and a non-parasitic one for their susceptibility to the fungus *Catanaria anguillulae*, belonging to Blastocladales. The interactions of non-motile juveniles of *Anguina tritici*, *Seinura* sp. and *Xiphinema basiri* were highly susceptible. The fungus caused paralysis of invaded portion of the body of *X. basiri*, *Seinura* sp., *H. javanica* and *H. cajani*. The sporangium formation in these species occurred after death of nematodes. Thus the fungus interacts with nematodes causing damage of their population thereby declining population of nematodes to reduce disease incidence on plants.

Virus-fungus interactions: Viruses also have interacting effects on fungal pathogens or on fungi other than pathogens. Earlier known viral infection on fungi has been with oomycetous fungi, particularly Chytridiales and Plasmodiophorales, and reviewed by Grogan and Campbell (1967) and Hollings and Stone (1969). Mushrooms have been reported to be prone to viral infection and during 1950s virus infection on mushrooms was observed by mushroom growers in several countries and loss due to it varied slight to 95% (Hollings *et al.*, 1963; Rasmussen *et al.*, 1968). Some pathogenic fungi of plants decline in pathogenesis nature due to virus infection viz., *Endothia*, *Helminthosporium*, *Alternaria* and *Stemphyllium*. Culture collections of fungi in fungal herbaria contain many fungi that degenerate and die, and the reason for some of these is due to virus interactions; some of the virus-infected fungi show even changes in their morphology. "Satellite viurs" first described by Kassanis (1962) needs interaction with tobacco necrosis virus (TNV) for multiplication in plants. Since then

several "satellite" have been discovered and the term is being used to a virus or nucleic acid which is unable to multiply in cells without help of a specific "helper" virus. Tobacco necrosis virus particles are ~ 30 nm in diameter and each particle contains one ssRNA molecule of mol. wt $1.3-1.6 \times 10^6$ (Kaper and Waterworth, 1981) Additional particles of diameter ~ 17 nm were first observed in 1938 in preparations of plant infection with TNV. In further studies with these small particles, it appeared that these small particles do not occur in all T.N.V. - isolates and depend on T.N.V. for their multiplication. Such small particles were called "Satellite virus" (Murant and Mayo, 1982). The first report of fungal virus was published by Gandy (1960a b) who showed that in pure culture mycelium from diseased basidiocarps was weak and tended to grow less, compared with the mycelia that were healthy which preceded profused mycelial growth with anastomosis and rhizomorphs. The occurrence of virus interactions was confirmed by Hollings (1962) and Gandy and Hollings (1962). The virus disease can spread by basidiospores from the diseased basidiocarps (Van Zaayen, 1979). Later virus particles were reported in "killer" strains of yeasts (Bevan and Makower, 1963 - in Bevan and Mitchell, 1974). Still later viruses have been reported to interact with many fungi and fungal parasites (Day and Dodds, 1974; Lemke and Nash, 1974).

Interactions among microorganisms on leaf surface: Leaf surface harbours both saprophytic and parasitic microbes, and nutrient supply to these organisms is from the lechates of the leaf from inside. The moisture for their germination is supplied by transpiration and atmospheric moisture content. The major bacteria on leaf surface are Gram negative ones viz., *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Flavobacterium* etc.; Whereas Gram positive ones are *Lactobacillus*, *Bacillus*, *Corynebacterium* etc. Pathogenic bacteria - *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *glycineae*, *P. syringae* pv. *morsprunorum*, *Erwinia amylovora*, *E. carotovora* etc. can lead their life as non-pathogenic in epiphytic phase on leaf surface. Yeasts are dominant on leaf surface on certain growth stages of plants. *Aureobasidium pullulans*, a dark colored fungus is generally dominant on leaf surface. The interactions between saprophytic and pathogenic micro organism result in the natural imbalance among the microbes and those with antagonistic tolerance can colonize the leaf surface to cause diseases. Some of the

interacting bacteria with fungi are *Erwinia herbicola* and the pathogen of fire-blight due to production of a toxic substance - bacteriocin - like substances which inhibited growth of *E. amylovora* in natural conditions (Beer and Rundle, 1980). Similarly species of *Erwinia* and *Pseudomonas* were able to interact with *Xanthomonas translucens* spp. *oryzicola* causing leaf streak on rice with the result to prevent development of disease symptoms when former were applied 24 hrs earlier than the pathogen, Bacterial interactions with fungi have been reported with several pathogenic fungal forms. A leaf surface bacterium, *Pseudomonas fluorescence* on *Lolium perenne* has been reported to be antagonistic to *Drechslera dictyoides* (Austin et al., 1977). Higher fungus of ascomycetes, *Nectria galligena* infects the plant tissues via wound caused by leaf scar but *Bacillus subtilis* isolated from the same tissues interact antagonistically with it to prevent the disease named Canker (Swinburne, 1973). The bacterium produces two antifungal antibiotics which cause germ tubes of the pathogens to swell and burst (Swinburne et al., 1975). The bacterium interacts with the germ tubes of the fungus and inhibits the growth. Blackman and Fraser (1971) reported that epiphytic bacteria in leaves gave protection from *Botrytis cinerea* by inhibiting spore germination of the fungus. Cucumber mildew caused by *Sphaerotheca fuliginea* can be controlled by spraying with conidia of *Ampelomyces quisqualis*. The interactions here result by checking growth of the pathogen (Jarvis and Slingsby, 1977). Several bacteria and *P. fluorescence* have been discovered to inhibit germination of smut spores due to production of antibiotics.

Interactions between mycophagous soil amoebae and soil-inhabiting fungi: Soil amoebae about whom I have mentioned on the third paragraph of this address are now known to interact with many fungal pathogens, and about six different amoebae have been reported to be mycophagous (Old, 1967, 1977; and Darbyshire, 1978; anderson and patrick, 1980; Esser et al., 1975; Pussare et al., 1979). They range from smaller *Thecamoeba granifera* sp. *minos* to giant *Arachnula* or *Vampyrella* sp. The modes of interaction is by feeding on fungal propagules and modes vary according to the ability of the trophozoites to completely surrounding the hyphae when feeding substrates are in the form of fragmented hyphae, conidia or chlamydospores. Amoebae encircle the conidia, chlamydospores or hyphae and later engulf and digest

them. Secondly if amoebae attack Larger forms and are unable to engulf the whole mass of substrate, perforations in the walls are caused due to interactions and protoplasm of the host propagule is ingested and digested (Dwivedi, 1981, 1986; Chakraborty and Old, 1982; Chakraborty *et al.*, 1983). Amoebae are active to interact and infect many fungal pathogens viz., *Cochliobolus sativus*, *Alternaria alternata*, *Aureobasidium pullulans*, *Botrytis cinerea*, *B. tulipa*, *Cladosporium sp.*, *Helminthosporium*, *Penicillium italicum*, *Rhizopus stolonifer*, *Gaeumannomyces graminis tritici*, *Stemphyllium dendriticum*, *Thielavia basicola* etc.

Due to presence of inocula of soil amoebae along with pathogens, the result of interaction is the amelioration of diseases. Such similar account of decline of 'take-all' disease of wheat has been reported for the first time in pot culture experiments with 'take all' fungus pathogen and amoebae (Dwivedi, 1986). It is a befitting example of interaction between fungal pathogen and soil amoebae which is of applied nature in field condition to control the disease. Destruction of hyphae of the 'take-all' by causing perforations and lysis, due to interaction of soil amoebae, has been demonstrated by Dwivedi (1986, 1993) through the electron microscope studies presenting proof of destruction of hyphae thereby declining the disease. The interaction of amoebae with 'take-all' fungus in pot condition led to the discovery of decline of the disease extendable to field condition by dressing seeds of wheat with cysts of soil amoebae before sowing in soil.

Fungal hyphae interact one another and it is presumed that during adverse conditions in soil environment when nutrient supply diminishes, hyphae penetrate inside hyphae of other fungi for survival and absorb nutrients from the host causing sometimes death of the host hyphae. In some cases coiling of hyphae of the pathogen around the hyphae of the host has been reported and they interact each other, and in competition for nutrient supply, host hyphae become moribund or become dead later. In almost all cases such interactions occurred between pathogens and saprophytic microbes viz., *Rhizopus oryzae* vs. *Bipolaris spicifera*, *Cephalosporium roseogriseum* vs. *Aspergillus flavus*, *F. oxysporum* vs. *A. luchuensis*, *Drechslera australiensis* vs. *R. oryzae* etc. (Dwivedi, 1993).

In conclusion I have to add that I have tried to highlight the subject by plucking flowers from vase of

other persons and of my own but holding them with my own thread to prepare the garlands for offering to this learned gathering.

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