STUDIES ON INTER-RELATIONSHIP OF SEED MICROFLORA OF GROUNDNUT *(Arachis hypogea* L.) AND RHIZOSPHERE MICROFLORA WITH SPECIAL REFERENCE TO COLLAR ROT PATHOGEN *(Aspergillus niger* VAN TIEGHEM)

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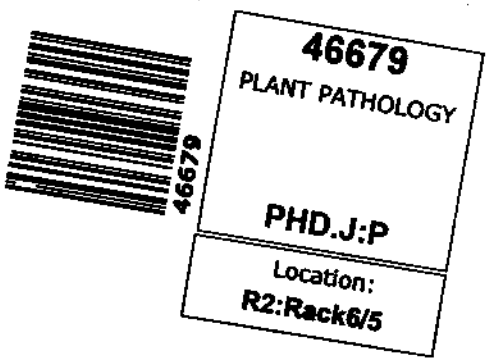

STUDIES ON INTER-RELATIONSHIP OF SEED MICROFLORA OF GROUNDNUT (*Arachis hypogea* L.) AND RHIZOSPHERE MICROFLORA WITH SPECIAL REFERENCE TO COLLAR ROT PATHOGEN (*Aspergillus niger* van Tieghem)

A THESIS
PRESENTED TO
THE FACULTY OF AGRICULTURE
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FOR THE DEGREE
DOCTOR OF PHILOSOPHY

MAHABIR PRASAD SHARMA
OCTOBER, 1988.

RAJASTHAN AGRICULTURAL UNIVERSITY
BIKANER

OCTOBER 5, 1988.

CERTIFICATE I

THIS IS TO CERTIFY THAT THIS THESIS ENTITLED "STUDIES ON INTER-RELATIONSHIP OF SEED MICROFLORA OF GROUNDNUT (Arachis hypogea L.) AND RHIZOSPHERE MICROFLORA WITH SPECIAL REFERENCE TO COLLAR ROT PATHOGEN (Aspergillus niger van Tieghem)" SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN AGRICULTURE IN THE SUBJECT OF PLANT PATHOLOGY OF THE RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER, IS A BONAFIDE RESEARCH WORK CARRIED OUT BY MR. MAHABIR PRASAD SHARMA UNDER MY SUPERVISION AND THAT NO PART OF THIS THESIS HAS BEEN SUBMITTED FOR ANY OTHER DEGREE. THE ASSISTANCE AND HELP RECEIVED DURING THE COURSE OF INVESTIGATION HAVE BEEN FULLY ACKNOWLEDGED.

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CERTIFICATE II

THIS IS TO CERTIFY THAT THIS THESIS ENTITLED "STUDIES ON INTER-RELATIONSHIP OF SEED MICROFLORA OF GROUNDNUT (Arachis hypogea L.) AND RHIZOSPHERE MICROFLORA WITH SPECIAL REFERENCE TO COLLAR ROT PATHOGEN (Aspergillus niger van Tieghem)" SUBMITTED BY MR. MAHABIR PRASAD SHARMA TO THE RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER IN FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN AGRICULTURE IN THE SUBJECT OF PLANT PATHOLOGY HAS BEEN APPROVED BY THE COMMITTEE AFTER AN ORAL EXAMINATION AND EVALUATION BY THE EXTERNAL EXAMINERS.

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(MAHABIR PRASAD SHARMA)

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मूंगफली में कालर गलन का प्रकोप सन् 1983 के सर्वेक्षण में राजस्थान राज्य के अजमेर, भीलवाड़ा, चितौडगढ़, कोटा व उदयपुर जिलों में 5.5 से 42.7 प्रतिशत पाया गया। रोगी पौधों के मूलक्षेत्र के सूक्ष्म वनस्पतिजात में एस्परजिलस नाइजर की संख्या 56.3 से 64.7 प्रतिशत अभिज्ञात हुई। मृदा से ए. नाइजर के पृथक्करण और गणन हेतु एक सापेक्ष वरणात्मक माध्यम विकसित किया गया।

ए. नाइजर द्वारा प्राकृतिक रूप से संक्रमित बीजों से विकसित पौधों पर रोगाणु के प्रभाव का अध्ययन किया गया। ऐसी अवस्था में रोगाणु ने बीज गलन, पूर्व निर्गमन व पौधों में बीज पत्र से दीर्घित बीज पत्राधर पर विकास करके कालर गलन उत्पन्न किया।

मूंगफली के बीज पर ए. नाइजर के निवेश द्रव्य उदभारण के लघुगणकीय बढ़ाव के साथ बीज गलन व पूर्व निर्गमन बढ़ा तथा मूलक्षेत्र में कवक, जीवाणु और एक्टिनोमाईसिटीज की संख्या में कमी हुई।

दैहिक कवकनाशी थाइराम व डाइफालेटान तथा दैहिक कवकनाशी बाविस्टिन व बेलेटान दवा से संक्रमित बीज उपचारित करने पर कालर गलन का नियन्त्रण हुआ और बाविस्टिन के द्वारा मूलक्षेत्र में एक्टिनोमाईसिटीज की संख्या में वृद्धि हुई।

ए. नाइजर से संक्रमित मृदा में नीम व महुआ की खली मिलाने से कालर गलन में 72.9 प्रतिशत कमी हुई। नत्रजन और फास्फोरस की अधिक मात्रा अलग-अलग तथा संयोग में देने पर रोग में कमी हुई तथा मूलक्षेत्र में ए. नाइजर की संख्या में कमी और एक्टिनोमाइसिटीज की संख्या में बढ़ोतरी हुई। गंधक 10.0

व 12·5 ग्राम प्रति किलो मृदा में मिलाने पर रोग आपतन तथा मूलक्षेत्र में ए· नाइजर की संख्या में अत्यधिक कमी हुई।

इस अध्ययन काल में ट्राइकोडर्मा की दो (टी· विरिडि एवं टी· हरजीआनम) एवं एक्टिनोमाइसिटीज तीन ए· नाइजर विरोधी जातियां प्राप्त हुई। ए· नाइजर से संक्रमित बीज ट्राइकोडर्मा से उपचारित करने पर पूर्ण अंकुरित हुए तथा साथ ही रोगाणु के संक्रमण से बचे रहे।

कनकमूल (ग्लोमस फेसीकुलेटस) निवेशन से मूंगफली के पौधों में शुष्क द्रव्य व फास्फोरस अंश की बढ़ोतरी हुई। कक्कमूल निवेशन ने ए· नाइजर के बीजोड़ व मृदोड़ निवेशन द्वारा उत्पन्न कालर गलन को प्रभावित किया व इससे रोग में 66·17 से 67·36 प्रतिशत तक कमी अंकित की गई। कक मूली पौधों की जड़ों में संपूर्ण और आर्थो-डाइहाइड्रोक्सी फिनोल की मात्रा अधिक पाई गई। फिनोल की अधिकतम मात्रा पौधों की 30 दिन की अवस्था में पाई गई।

INTRODUCTION

Groundnut (*Arachis hypogea* Linn.), Papillionaceae (Hedysarae), is a cultivated, annual or weakly perennial herb grown in many tropical and subtropical countries and in the continental parts of temperate countries, in a wide variety of soil, for its seeds, which contain upto 50 per cent of a non-drying edible oil and about 35 per cent protein and are used in the oil and feed industries or for confectionery.

Groundnut is attacked by a large number of soil inhabiting micro-organisms which infect damaged seeds and germinating seedlings. Among them, *Aspergillus niger* is one of the serious pathogens which is world-wide in distribution and affects seed germination and seedling stand and causes collar rot. This pathogen is both seed and soil borne but the severity of the disease in field is governed by several factors including rhizosphere microflora, that affect the survival of *A. niger* in the soil.

The microflora associated with seed and their interaction with chemical seed protectants and rhizosphere microflora may be important and may affect the development

of collar rot in the field. Interaction of A. niger with several species of fungi, bacteria and actinomycetes found in soil around root zones or associated with seed may possibly take place affecting diseases. Also it has been found possible to alter the rhizosphere microflora through soil amendments, fertilizers, organic matter and microbial cultures, seed treatments with chemicals, foliar sprays with such plant nutrients like urea, phosphate and potash. In some cases the changes have been found unfavourable for plant pathogens.

Enough information regarding interrelationship of seed microflora and rhizosphere microflora with respect to collar rot pathogen is not known. Work on seed microflora and rhizosphere microflora of groundnut, seed and rhizosphere microflora as related to A. niger and collar rot development, and effect of various soil amendments and seed treating chemicals on the rhizosphere microflora has been done and results of which are presented herein.

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REVIEW OF LITERATURE

Aspergillus collar rot was first reported on groundnut in 1926 in Java by Jochems (1926). Norwood (1946, 1953) later reported the disease from Australia and studied methods of control of the disease. Gibson (1953) in East Africa contributed to the knowledge of the etiology of this disease. In India, Jain and Nema (1952) observed the disease in Madhya Pradesh and studied methods for its control. In the United States, Jackson (1962) reported that A. niger was responsible for stand depletion in Georgia in 1961-62. Aspergillus niger has been reported from all groundnut growing areas of the world and it is likely that Aspergillus collar rot is found in all these areas.

A. niger is found abundantly in nature in soils and plant debris. The relative abundance of A. niger in cultivated soils varies greatly, but factors influencing its presence or apparent absence are conjectural. Jackson (1962) reported that abundance of A. niger propagules in soil was directly related to high disease incidence in plants growing in the soil. Ashworth et al. (1964) also found a positive correlation with disease and the numbers of particles of a given soil from which A. niger could be

isolated. By sieving soils from different locations, they determined that over 80 per cent of the infested particles in the soils were retained on 10 and 18 mesh sieves.

The pathogen, A. niger, is carried on the groundnut seed surface and in or under the tissues of the testae (Jackson, 1964; Sharma, 1968). Ashworth et al. (1964) found, in their study of spanish peanut,that superficial contamination of seed with A. niger was more common than deep-seated infection. Jackson (1963) surveyed seed stocks in Georgia in 1962 and found that A. niger was not abundant in seed although in a few lots over 80 per cent of the seed cultured yielded A. niger. He concluded that soil-borne inoculum was of more importance in initiating Aspergillus collar rot than seed-borne inoculum. Sharma (1968) found 29-37 per cent of kernels infected with A. niger from pods collected in four districts viz., Ajmer, Deoli (Tonk), Jaipur and Bundi of Rajasthan.

Jackson (1962) suggested that cotyledon infection, that commonly results in growth of the fungus down into the hypocotyl, was important in the initiation of the disease. Gibson (1953) found that the peack death rate occurred about the 17th day after planting and that the period of dying of emerged plants extended from about 10 to 30 days.

High soil and air temperatures may predispose plants to infection. Gibson (1953) found that greater infection occurred among plants raised at 30 to 35 C than in plants raised at 20 C even though the post-inoculation temperature was the same.

Chemical control of Aspergillus collar rot has been achieved through use of seed treatments. The fact that non-systemic seed treatment materials control the disease argues well for the general conclusion that infection of groundnut occurs during or soon after germination. In view of the unusual effect of organic mercury materials when used as seed treatments (Gibson, 1953), non mercurial organic compounds have found great use in areas where collar rot is serious (Gortner and Kruger, 1958). Most commonly used materials include thiram, captan, difolatan, Benlate, Brassicol, ziram, carbendazim (Jackson, 1968; Sidhu and Chohan, 1971; Aulakh and Sunar, 1972; Siddaramaiah et al., 1979; Aulakh and Chohan, 1975). These chemicals, although not noteably good eradicants, are effective in controlling several species of Aspergillus as well as other fungi in soil and on groundnut seeds (Jackson, 1965).

Much work has been done on rhizosphere microflora of several crops by various workers (Ketznelson, 1965; Ketznelson et al., 1948; Garrett, 1956; Rovira, 1965; Starkey, 1958; Lochhead, 1950 and Sadasivan, 1960 and 62).

Rhizosphere has been defined as that portion of soil that is adjacent to root system of a plant, which contains root exudates, sloughed root remains, and large populations of micro-organisms of various nutritional groupings.

The width of the zone of soil influenced by roots varies with the type of plant, age of the plant, soil conditions, environmental conditions, and other factors. Micro-organisms (fungi, bacteria and actinomycetes) in the rhizosphere may exert profound influence upon the plant by decomposition of organic matter, by associative and antagonistic relationships, and by actual parasitism of plant roots.

Soil fungistasis is generally associated with sites of high microbiological activity - the top 15 cm or areas of high moisture, organic matter content or temperature, also has been shown to increase with increasing soil pH (Schuepp and Green, 1968; Schuepp and Frei, 1969). This greater microbiological activity limits the germination of fungus spores, whether by depriving them of nutrients, by liberation of fungitoxins or ethylene or both. Elad and Chet (1987) reported competition for nutrients between germinating oospores of Pythium aphanidermatum and six bacteria isolated from rhizospheres of plants infested from Pythium sp. Seed coating with these bacteria reduced disease incidence in cucumber by 60-75 per cent. Smith(1973)

found that sclerotia of Sclerotium rolfsii remained dormant on soil over which passed an air flow containing 1 ppm ethylene. Spores of Helminthosporium sativum on field soil failed to germinate until the ethylene was removed by passing an air stream over the surface. Soils high in organic matter and nitrogen produced more ethylene than was produced by infertile soils.

Rhizosphere microflora can be altered through soil amendments, fertilizers, organic matter, microbial cultures, seed treatments with chemicals including antibiotics, foliar sprays with plant nutrients like urea, phosphate and potash. In some cases the changes have been found unfavourable for plant pathogens (Rangaswami, 1968).

There are several examples where fungicides controlled a disease, even though the materials or rates used were not considered directly inhibitory to the pathogen. Mc Keen (1949) observed that control of Aphanomyces cochlioides and Pythium aphanidermatum of sugar beets with thiram was partially the result of direct fungicidal action on the pathogen, but he thought that an indirect effect caused by a shift in the microbiological balance was also involved. Domsch (1959) also obtained control of damping-off with captan at rates not considered directly fungicidal to the pathogen, and suggested that biological control initiated by the chemical was involved.

Anderson (1962-64) found that fumigation of soil with chloropicrin controlled pineapple root rot caused by Phytophthora cinnamomi in Hawai for a period of 3 years, although the pathogen could be baited from the soil as early as 2 weeks after treatment. The population of Trichoderma viride was increased by the treatment. In contrast, PCNB increased disease severity over that in non-treated plots and reduced counts of Trichoderma, Penicillium and actinomycetes.

Vaartaja et al. (1964) emphasized that for many soil fungicides, the significant control is probably in part through changes in antagonistic flora, without which the fungicide would be only partially effective. In their work, Dexon alone gave significant control of damping-off of conifers, but stands were poor if Dexon was mixed with anilazine or PCNB. They suggested that anilazine or PCNB interfered with antagonistic flora made possible by Dexon alone.

Application of fertilizers to the soil has been found to affect on the microbial population in soil. Studying the effect of N,P,K fertilization on the microflora of soil and the rhizosphere of ragi, Bagyaraj and Rangaswami (1967) found that by suitably amending the infested soil, the development of a particular fungal disease could often be

partially or significantly checked by the microflora which established in the rhizosphere after the soil treatment. Forms of nitrogen affect propagule survival by affecting multiplication of soil micro-organisms. Henis and Chet (1968) found that only ammonia caused complete loss of germinability of sclerotia of Sclerotium rolfsii in sterile soil, whereas in non-sterile soil, urea, chitin, peptone, calcium nitrate and ammonium acetate caused loss of germinability of the sclerotia when subsequently removed and tested on agar. They suggested that these forms of nitrogen favoured certain soil micro-organisms that could attack the carbonaceous sclerotial wall. They demonstrated high numbers of bacteria, actinomycetes and fungi in crushed sclerotia and in washings of sclerotia for each of the nitrogen treatments that reduced germination.

Leach and Davey (1942) observed in field that nitrogen reduced the sugarbeet disease caused by S. rolfsii. Avizohar-Harshenzon and Shacked (1969) have suggested that this effect may be directly on the fungus, suppressing its ability to compete with other micro-organisms.

Some forms of fertilizer cause much greater pH changes than others. In general, ammonium forms of nitrogen have the greatest acidifying effect on soil, in contrast to nitrate forms, which have a tendency to raise the soil pH,

at least initially. Anhydrous ammonia and the ammonia released by urea initially cause the soil pH to rise markedly, but this is temporary and eventually the pH of soils so fertilized drops well below neutral (Smiley et al., 1970, 1972).

Huber et al. (1965) pointed out that some root diseases are less severe and others more severe when specific forms of nitrogen fertilizer are used. In their examples, diseases caused by Fusarium, Rhizoctonia and Aphanomyces are increased in severity by ammonium nitrogen and decreased by the nitrate form. Verticillium wilt was reduced by ammonium nitrogen (Huber and Watson, 1970). The take-all disease, caused by Gaeumannomyces graminis, was reduced with ammonium nitrogen and made more severe with nitrate nitrogen (Huber et al., 1968). Smiley and Cook (1973) showed that the effect with take-all disease relates to pH, partly of the bulk soil (pH_b) but mostly of the rhizosphere (pH_r), which is related to a shift in the biological balance. Since take-all is severe with ammonium nitrogen at all rhizosphere pH values above 5 if the soil is treated first with methyl bromide to free it of micro-organisms. They concluded that pH_r below 5 controlled G. graminis directly because the fungus can not tolerate these pH values, but between 5.0 and 7.0 the control was indirect, probably through antagonistic rhizosphere micro-organisms.

It is apparent that the pH effect on root diseases operates through shifts in the biological balance. The pH change associated with ammonium or nitrate fertilizer in most cases is not enough to inhibit the pathogen directly. The probable effect is to inhibit the pathogen sufficiently to reduce its growth in competition with others i.e. its inoculum potential and thus its capacity to cause disease and also there might be antagonistic effects.

Sulphur and gypsum amendments in soil have been found to control various plant diseases. Mc Creasy (1967) reported that sufficient sulphur application to give a soil acidity of pH 5.2 controlled potato tuber diseases viz., scab (Streptomyces scabies), powdery scab (Spongospora subterranea), black scurf (Corticium solani) and tuber blight (Phytophthora infestans). He also found that use of lime increased all these diseases. Studying the effect of gypsum and sulphur in soil, Sunar and Chohan (1971) observed increase in number of rhizosphere mycoflora in the uninoculated plants of groundnut. They also found less colonies of Rhizoctonia solani and R. bataticola (causing root rot in groundnut) in rhizosphere mycoflora than in soil not amended. Gypsum and sulphur also supply micro-nutrients essential for healthy growth of the plants and increase the resistance in plants (Kanwar and Chawla, 1963).

The role of oil cakes and crop residues amendments in the control of plant pathogens has been studied and made use of for decades. Gautum and Kolte (1979) found best reduction of Sclerotium rolfsii by soil amendment of castor and Azardirachta indica oil cakes. Lakshmanan and Nair (1984) observed that soils amended with neem and groundnut cakes under dry conditions and ellupa, gingelly and neem cakes under flooded conditions were very effective in reducing the viability of sclerotia of Rhizoctonia solani of the rice pathogen. In nutrient solution growth of Rhizoctonia bataticola, Fusarium oxysporum f. sp. corianderii and F. oxysporum f. sp. cumini were stimulated at lower concentrations and inhibited at higher concentrations (Sankhla et al., 1969). The effect seems to be fungistatic. Singh (1968) got best control of black scurf on potatoes in soil amended with mustard cake.

Zakaria and Lockwood (1980) observed that F. oxysporum and F. solani infested soil amended with linseed, cotton seed and soybean meals reduced chlamydospore population from 10^5/g of soil to 10^2/g in 4-5 weeks. Severity of pea root rot in soil amended with oil seed meal was proportional to the surviving population of F. solani. They also found that total number of fungi, actinomycetes and bacteria were not reduced as greatly by the oil seed meal as the fusaria.

Basically the role of plant residues is one of stimulating micro-organisms antagonistic to plant pathogens. This antagonism may involve rather specific forms of antibiosis, such as that of soybean green manure in the suppression of potato scab (Weinhold and Bowman, 1968). Johnson (1953) found that where sclerotia of Sclerotium rolfsii were destroyed with amendments, bacterial numbers were proportional to the amount of alfalfa meal added and to the rate of destruction of sclerotia. He showed that all sclerotia were destroyed within 6 weeks if 4 per cent of meal was added, 2 months with 2 per cent of meal and 3 months with 1 or 0.4 per cent of meal. The control of Rhizoctonia solani on beans was greatest from fresh-green or dry-plant materials added to soil which stimulated the highest total number of organisms and that decomposed the fastest (Papavizas and Davey, 1960).

Plant residues also control root rots and lower the populations of pathogens by propagule germination-lysis. Mitchell et al. (1941) was first to report germination-lysis, following their observations on the elimination of Phymatotrichum omnivorum (Texas root rot of cotton) sclerotia after organic amendments. It was also described by Chin et al. (1953) to explain the control of Helminthosporium sativum by soybean residues. Menzies and Gilbert (1967) reported the propagule germination and subsequent lysis triggered by volatiles from

decomposing plant residues. The volatile substances included acetaldehyde, isobutyraldehyde, isovaleraldehyde, methanol and ethanol, which stimulated microbial growth at low concentrations but inhibited growth at higher concentrations. Lewis and Papavizas (1971) reported control of aphanomyces root rot of peas by crucifer amendments (cabbage, kale, mustard, turnip), which may result from isothiocyanates and sulfides normally released from crucifer tissues during the decomposition, persisted for no longer than about 15 weeks. During this period, formation, motility and germination of zoospores of Aphanomyces euteiches were inhibited.

A suppressive effect against Fusarium solani f. sp. phaseoli, that lasts for about 28 days results from adding spent coffee grounds to soil (Adams et al., 1968a). The germination-lysis process was completed within the first 48 hrs after amendment, and control was optimal at 7-14 days after amendment, but ultimately populations of the pathogen increased. Control of this same pathogen with materials of high C:N ratios (barley straw, sawdust, cellulose or glucose) is also optimal a few days after the amendment when nitrogen available for pathogen growth is maximally immobilized, but subsides after a few weeks, and disease again may be severe (Snyder et al., 1959; Maurer and Baker, 1965). The period of control varies with the readiness with which the material is decomposed. Control from a single application of glucose may last only

a few days, whereas control with mature barley straw may last a month.

Resistant and susceptible varieties have been found to influence their rhizosphere microflora differently and this has been studied by Atkinson et al. (1974), Larson and Atkinson (1970), Neal (1971), and Neal et al. (1970, 1973), using wheat varieties resistant or susceptible to Cochliobolus sativus root rot. With one set of three wheat varieties, two resistant and one susceptible, kinds of rhizosphere micro-organisms antagonistic to the pathogen on agar plates were studied. Each variety supported a unique rhizosphere flora made up of different percentages of ammonifires, nitrate reducers, starch hydrolyzers, spore formers and organisms requiring various growth factors and amino-acids. The total bacterial counts was about two fold greater for the susceptible than for the two resistant varieties, but about 20 per cent of the bacteria in rhizospheres of two resistant varieties were antibiotic to C. sativus whereas none were in those of susceptible plants.

Studies on the antagonistic behaviour between pathogenic fungi and seed and soil microflora have been made by several workers. Several fungi, bacteria and actinomycetes have been reported to be associated with

groundnut seeds (Garren, 1966; Jackson, 1965, 1968; Joffe, 1969; Sharma, 1968; Barmes, 1971). These investigators have reported the occurrence of different microflora with groundnut seeds but conclusions on ecological implications were not drawn. Joffe (1969) reported antagonism between A. niger and A. flavus, Penicillium funiculosum, P. rubrum and Fusarium solani, but the antagonism with A. flavus was more. Joffe (1972) found A. niger to be predominant in rhizosphere and geocarposphere of groundnut under low moisture conditions and found to reduce infection by F. solani and A. flavus. Chohan (1970b) reported Trichoderma viride and Streptomyces spp., isolated from soils, to be highly antagonistic to A. niger and the addition of T.viride in soil one month before sowing considerably reduced seed and collar rot of groundnut.

Soil contains a variety of fungi and other micro-organisms. Some of these are pathogens and harmful to plant growth whereas the others are beneficial to plant. One such group of beneficial micro-organisms are the mycorrhizal fungi. These fungi form a symbiotic relationship with the roots of the plants in which the host provides carbohydrates for the fungus and the fungus, inturn, supplies nutrients to the host. Smith (1980) mentioned that these fungi invade plant roots virtually forming an extension of

root system and thereby, improves the uptake of nutrients by the plants. Butler and Jones (1955) also narrated that some higher plants have a symbiotic partnership with fungi in which a mutualistic relationship is evident and since the association between the fungus and higher plants is in roots, the term 'mycorrhiza' is applied to it. This fungus root association represents two main types, i.e. endotropic and ectotropic. Maronek et al. (1980) recognised four types of mycorrhizas associated with different groups of plants. They are :

(i) Ecto-mycorrhiza : occur naturally on fine feeder roots as a mantle of fungal tissue of many valuable trees, eg. pine;

(ii) Ericoid-mycorrhizas : associated with plants of Hricaceae family;

(iii) Orchidaceous mycorrhizas : associated with orchids, and

(iv) Vasiculararbuscular mycorrhizas : very widespread group associated with a range of annual and perennial plants, e.g. cereals and grain crops, tomatoes, legumes, citrus and subtropical tree crops, vegetable crops and ornamentals. In this association, the cortex of the root is invaded, usually in limited areas behind the apical

meristamatic zone, by very small spores of a single fungus characterised by special organs known as 'vesicles' and arbuscules'. The fungi concerned are member of Zygomycotina, family Endogenaceae.

Several workers have reported the beneficial effect of mycorrhizal associations on the growth and establishment of various plant species, e.g., Pinus (Bratislayzak, 1964), Legumes (Powell, 1971; Bagyaraj et al., 1978; Islam and Ayanaba, 1981; Bhargava et al., 1986), Gossypium hirsutum (Rich and Bird, 1974), orchids (Warcup, 1975; Bagyaraj and Powell, 1983), peanut (Daft and El-Giahmi, 1976) Zeitschrift Fur Acker, 1977), citrus (Menge, 1977), Horticultural crops (Crews et al., 1978; Maronek, 1981; Cooper, 1982; Bagyaraj and Powell, 1985).

Increase in yield of fruits, plant size and chemical content of shoots, roots and seeds have been reported higher in groundnut infected with Glomus mosseae. Infection with Glomus and Rhizobium stimulated nodulation and acetylene reduction rates. Together these two endophytes contributed greatly to the vigour of the host (Daft and El.Giahmi, 1976). Though the attempts to assess the VA mycorrhizae benefits to crop growth in field are meagre, yet a few field trials indicate that VAM do help in increasing

the yield (Hayman, 1982). Bagyaraj and Sreeramulu (1982), Hayman et al. (1981), and Powell and Bagyaraj (1982) have reported improved plant growth and nutrition of crop plants due to VAM inoculation even under unsterile field condition.

Most physiological studies on VAM have been concerned with benefits conferred by them on their host plants. These benefits are observed mainly as growth responses of mycorrhizal plants and explained primarily in terms of improved uptake of phosphate. Extra phosphate reaches the roots through fungal hyphae, which tap the soluble phosphate in soil beyond the phosphate depletion-zone near the root surface. Such a zone develops around the root surface in soil of moderate or low phosphate status because of poor mobility of phosphate ions in soil (Hayman, 1978; Tinker, 1978). Phosphorus is one of the most important inputs in growing agricultural crops. It is a major plant nutrient and its deficiency can greatly limit plant growth. Many workers have reported that VAM fungi improve plant growth in low to moderate phosphate soils (Gerdeman, 1964 and 1975; Daft and Nicolson, 1966).

In addition to consistent involvement of phosphorus in mycorrhiza assisted nutrition, mycorrhizal fungi are also reported to increase selective uptake of minor elements with poor mobility in soil, e.g. zinc, sulphur and copper

(Manorek et al., 1981; Menge et al., 1978; Munns and Mosse, 1980). Swaminatan and Verma (1979) reported improved zinc uptake by wheat, maize and potatoes in zinc deficient soils in India.

Recently, it has been suggested that mycorrhizal infection may change the biochemical composition of the host plant. Dehne and Schonbeck (1972), working on tomato plants, found higher amount of phenol in mycorrhizal plants compared to uninoculated plants. Higher amount of phenol was recorded in mycorrhizal roots of groundnut compared to non-mycorrhizal roots (Krishna and Bagyaraj, 1984).

Mycorrhizal fungi have the ability to colonize host roots and soil and thus, may have a role in biological control of soil-borne pathogens. Safir (1968) was first to make an attempt to study the interaction of a plant pathogen and a species of VAM fungus. Schenck and Kallam (1978) indicated that presence of VAM could increase, decrease or not affect the disease severity. However, most reports indicate that mycorrhizal fungi decrease the disease severity (Schonbeck and Dehne, 1979).

Schenck (1981), conducted many pot house and microplot studies and indicated that mycorrhizal fungi can deter or significantly reduce the effects of some pathogens on the host. Marx (1973), and Schenck and Kallam (1978)

found that mycorrhizal fungi have a decreasing effect on root infecting fungi and these attributes of mycorrhizae are being recognised as important in modern agriculture. Schenck and Kallam (1978) observed that VAM fungi reduced the effect of several pathogens in their hosts, e.g., Thielaviopsis basicola on tobacco and alfalfa, F.oxysporum f. sp. lycopersici on tomato, Phytophthora megasperma var sojae on soybean, Pyrenochaeta terrestris on onion, and Rhizoctonia solani and Pythium ultimum on poinsettia. All of them were reduced by using mycorrhizal fungi, viz., Glomus fasciculatus, Glomus mosseae and Gigaspora margarita. Schonbeck and Dehne (1977) found that mycorrhizal cotton plants with Glomus mosseae could withstand the stress of infection by Thielaviopsis basicola better than non-mycorrhizal plants. Baltruschat and Schonbeck (1975) reported that chlamydospore production was negatively correlated with mycorrhizal colonization of roots of tobacco and alfalfa and correlated this reduction to higher levels of arginine in mycorrhizal roots. More work with different diseases is needed in this aspect.

Thus, it is seen that survival of soil-borne plant pathogens in soil and development of root diseases by them is influenced by a number of factors including rhizosphere microflora. It is unlikely that the list ends here, many

other factors that have gone unnoticed alongwith cited ones may be playing a major role in the survival of Aspergillus niger in soil. The discovery of such factors and their effective manoeuvre wil help in controlling collar rot disease by hindering the survival of A. niger in soil.

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MATERIALS AND METHODS

Glassware:

Glassware used was of 'Corning make'. Thoroughly washed and cleaned glassware was soaked overnight in chromic acid solution ($K_2Cr_2O_7$ 80 g, distilled water 300 ml, cooled to room temperature and added with constant stirring concentrated H_2SO_4 400 ml). These were then washed in tap water and rinsed with distilled water and dried before use.

Chemicals:

All chemicals used in laboratory work were either of 'Analar' quality or Guaranteed Reagents (GR) were used for all experimental purpose.

Sterilization:

All media, water and other solutions were sterilized at 15 psi (pound per square inch) in an autoclave for 20 minutes. Petriplates were sterilized in an oven at 160 C for two hours.

Media:

1. Martin's rose-Bengal-streptomycin agar medium: (Martin, 1950)

   Used for isolation of rhizosphere fungi -

Agar	-	20.0 g
Dextrose	-	10.0 g
Peptone	-	5.0 g
KH_2PO_4	-	1.0 g
$MgSO_4$, $7H_2O$	-	0.5 g
distilled water	-	1000 ml

Rose Bengal (one part in 30,000 parts of medium) or 30 mg/l

One gram of streptomycin sulphate was dissolved in 100 ml of sterile distilled water, and 3 ml of streptomycin solution was added to the above medium after it was cooled to 40 C.

2. Soil extract agar (Allen, 1957)

Used for the isolation of soil bacteria:

Agar	-	20.g g
Glucose	-	1.0 g
K_2HPO_4	-	0.5 g
Soil extract	-	100 ml
Tap water	-	900 ml
pH adjusted to	-	7.0

Soil extract was prepared by heating 1000 g of garden soil with 1000 ml of tap water in an autoclave for 30 minutes. A small amount of calcium carbonate was added and the soil suspension was filtered through double layer of filter paper.

3. Starch ammonium agar (Kuznetsov and Arjun Rao, 1972)

Used for isolation of soil actinomycetes:

Agar	-	20.0 g
Starch	-	10.0 g
$(NH_4)_2SO_4$	-	1.0 g
K_2HPO_4	-	1.0 g
$MgSO_4 . 7H_2O$	-	1.0 g
NaCl	-	1.0 g
$CaCO_3$	-	3.0 g
Tap water	-	1000 ml
pH adjusted to	-	7.00

4. Czapek's medium with glucose

Used for culturing of actinomycetes:

Agar	-	20.0 g
$NaNO_3$	-	3.0 g
K_2HPO_4	-	1.0 g
KCl	-	0.5 g
$MgSO_4$	-	0.5 g
$FeSO_4$	-	Trace
Glucose	-	30.0 g
Distilled water	-	1000 ml

5. Beef peptone agar (Nutrient agar)

Used for culturing of bacteria

Beef extract	-	3.0 g
Peptone	-	5.0 g
Agar	-	15.0 g
distilled water	-	1000 ml

6. Potato Dextrose Agar (PDA)

Used for the isolation of fungi from seeds and for the maintenance of fungus cultures:

Peeled potatoes	-	200.0 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1000 ml

7. Rice medium

Used for raising A. niger inoculum:

Rice grain	-	50.0 g
Distilled water	-	100 ml

For all media pH was maintained by using 0.1N NaOH and 0.1N HCl with the help of 'Toshniwal pH meter.

Trypan blue-lactophenol stain for mycorrhiza staining (Phillip and Hayman, 1970):

Trypan blue	-	50 mg
Phenol (crystals)	-	20.0 g

Lactic acid	-	20.0 g
Glycerol	-	40.0 g
Water	-	20 ml

(Melted phenol in water until it was dissolved and then added lactic acid and glycerol. Added 50 mg of trypan blue per 100 ml).

Isolation of fungi, bacteria and actinomycetes from grounnut rhizosphere:

For soil dilution and plate count, the method of Timonin (1940) was followed. Groundnut plants were carefully uprooted and gently shaken to remove excess soil adhering to the roots. The roots were later cut with the help of a sterilized blade and were put in 100 ml of sterile distilled water in 250 ml conical flask and were shaken vigorously. This suspension served as the stock solution and from this serial dilutions were made in 9 ml sterile water blanks with the help of one ml pipettes sterilized previously. Martin's rose-Bengal streptomycin agar medium was used for the isolation of soil fungi from dilutions 10^3 and 10^4, starch ammonium agar medium was used for the isolation of rhizosphere actinomycetes from dilutions 10^5 and 10^6. Rhizosphere bacteria were isolated from dilutions 10^6 and 10^7 on soil extract agar medium. For maximum population isolation of A. niger from rhizosphere Martin's rose-Bengal streptomycin agar medium with 5.5 per cent NaCl was used.

One ml of rhizosphere soil suspension of required dilution was placed in each petridish. Fifteen ml of the medium cooled to 40 C was poured to each petridish. The plates were incubated for 3 to 7 days at 27 C. Colonies of fungi, bacteria and actinomycetes were counted on the Quebec colony counter. Fungi after isolation were maintained on PDA, actinomycetes of Czapek's medium with glucose and bacteria on nutrient agar.

The undiluted rhizosphere soil suspension after washing and removing the roots was dried at 105 C for 24 hours and the weight of the oven-dry soil was noted.

Isolation of A. niger from diseased plants collected from different places, their pathogenicity and comparative virulence:

Isolation: Aspergillus collar rotted groundnut plants were collected from five districts, viz., Ajmer, Bhilwara, Chittorgarh, Kota and Udaipur, of Rajasthan, during July, 1983. Diseased collar portion was cut into small bits, immersed in 0.1 per cent mercuric chloride solution for two minutes and finally washed giving four changes in sterile distilled water aseptically. The bits were plated in petriplates containing PDA (4 bits per plate at equal distance). The cultures were purified by transferring the single colony

raised on PDA from diluted spore suspension.

Pathogenicity: For pathogenicity tests, 100 seeds were inoculated separately with each isolate raised on rice medium. These seeds were sown in 30 cm pots filled with steam sterilized sandy-loam soil, at the rate of 10 seeds per pot. All the pots were kept under cage house and watered regularly. Observations on the number of collar rotted plants were taken. Collar rot symptoms appeared within a period of 2 to 20 days after germination. The disease incidence was calculated by the following formula :

$$\text{Disease incidence \%} = \frac{\text{Number of infected plant units}}{\text{Total number (healthy and infected of units assessed)}} \times 100$$

Seed inoculation :

A. niger culture raised on rice medium for 8 days was brought into pastey form by crushing it with waring blender. Groundnut seeds, surface sterilized with 0.1 % $HgCl_2$, were rolled in the culture paste and then air dried for 2 hours by spreading the inoculated seeds on blotting sheet in the laboratory. These inoculated seeds were kept in polythene bags over night at room temperature, before sowing. Inoculum load 5×10^3 per seed was maintained unless specified else where. Inoculum consisted of spore and mycelial bits of A. niger.

Estimation of soil pH and organic matter content :

Soil pH : To the 100 ml of distilled water in a beaker 50 g of dry representative soil sample was added and stirred continuously for half an hour and the beaker set aside for one hour for the sedimentation of soil particles. pH of the soil suspension was determined by dipping the glass electrodes of Systronics pH meter 325 in the above supernatant of soil solution.

Organic matter content : Soil organic matter content was estimated by following the method of Walkley and Black(1947).

One gram of dry soil from representative soil sample was taken in a 500 ml conical flask and added 10 ml of 1N $K_2Cr_2O_7$ solution and 20 ml of concentrated sulphuric acid. The contents of the flask were shaken and kept aside on asbestos sheet for exactly 30 minutes, then added 200 ml of distilled water, 10 ml of phosphoric acid, a pinch of NaF and 1.5 ml of diphenyl amine indicator. The contents of the flask attained a bluish purple colour and titrated against Ferrous ammonium sulphate solution rapidly till the contents became greyish green in colour. A blank was run simultaneously without the soil sample. Organic matter content of the soil sample was calculated from the following formula :

$$\text{Organic carbon \%} = \frac{\frac{10}{5} \times (S-T) \times 0.003 \times 100}{\text{weight of soil sample}}$$

where, S = ml of Ferrous ammonium sulphate used in determination with sample

T = ml of Ferrous ammonium sulphate used in blank titration

Organic matter % = organic carbon % x 1.724

Soil infestation :

For infestation of soil, A. niger was grown for 15 days on sterilized sorghum grains in 500 ml conical flasks at room temperature. Sorghum grains were soaked in tap water for one day, washed thoroughly with running tap water and then all the water was drained off by holding the grains in muslin cloth for two hours. Conical flasks 1/3 filled with soaked grains, plugged with cotton were autoclaved at 20 p.s.i. for 1 hour. Each flask was inoculated by pouring one ml of A. niger spore suspension prepared from the culture actively growing on PDA. The flasks were shaken daily. On 15th day, infested grains were spreaded on blotting sheet for 4-5 hours and then crushed fine in waring blender. This inoculum was mixed in the experimental pots at the rate of 20 g inoculum per kg soil.

Isolation of fungi from groundnut seeds :

Groundnut seeds, surface sterilized with 2 % sodium hypochlorite and unsterilized were plated on PDA at the rate of five seeds per plate. The plates were incubated for 3 to 7 days at 27 C. The fungi developing on the seeds were isolated on PDA slants and maintained for further studies.

Development of collar rot from seeds infected naturally with A. niger :

Development of the pathogen, A. niger from seed to seedling was studied on 1 % water agar in petriplates by the method described by Khare et al. (1977). Five seeds per petriplate were plated on 15-20 ml water agar and incubated at 27 ± 3 C under 12 hours of alternate cycles of artificial day light and darkness. The light was provided by 4 tubes of Philips TLF 40 w/34 hanging horizontally 80 cm above the medium. After two days the lids were removed and the dishes were put in polythene bags for 12 days. Observations were recorded on the appearance of disease and development of A. niger.

In another test, seeds showing growth of A. niger on its surface plated on 1 % water agar were carefully transferred to steam sterilized sandy-loam soil in pots,

kept in laboratory at room temperature 27 ± 3 C under 12 hours of artificial day light and 12 hours darkness. The pots were covered with polythene bags and watered frequently. Daily observations on the appearance of symptoms and development of the pathogen on diseased tissues were recorded.

Antagonistic effect of actinomycetes, fungi and bacteria isolated from groundnut rhizosphere :

Antagonistic effect was studied by two methods:

Pure cultures of actinomycete species from groundnut rhizosphere grown on Czapek's medium with glucose were inoculated to 100 ml flasks containing 25 ml of Chapek's broth with glucose and incubated at room temperaturefor 15 days. The culture filtrate of each actinomycete species was tested for its antagonistic effect by placing 10 mm Whatman No.1 filter paper discs dipped in the culture filtrate on Martin's rose-Bengal agar medium seeded with A. niger. Plates were incubated at room temperature and observed for inhibition zones after 48 hours. The diameter of the inhibition zone was measured in millimeters.

In the second method, each of actinomycetes, fungi, bacteria species was streaked in straight line on Czapek's medium with glucose in petriplates. When the growth became

apparent A. niger was streaked at right angle against each of the actinomycetes, fungal and bacterial species. The plates were incubated for 48 hours and later observed for inhibition zones.

Microbial population changes in groundnut rhizosphere during different stages of crop growth and collar rot development as influenced by different fungicides :

Seeds of groundnut, surfaced sterilized with 0.1 % $HgCl_2$ were inoculated with A. niger grown on rice and treated with different fungicides on 2nd day of inoculation. Such seeds were sown in 30 cm pots filled with sandy-loam soil (15 seeds per pot) kept in cage house. Two controls, one of seeds inoculated with A. niger and another of seeds without A. niger inoculation were maintained. Uniform amount of water was added to each pot throughout the experimental period. The non-systemic and systemic fungicides and their concentration used were as given below :

Fungicide		Conc.(%)
Captan	N-trichloromethyl-thio-4-cyclohexene-1,2-dicarboximide	0.2
Thiram	(Tetramethylthiuram disulphide	0.3
Dithane M-45	Zinc and Manganese salt of ethylene bis dithiocarbamate	0.2
Fytolan	Copper oxychloride	0.3

Agallol	Methoxyethyl mercury chloride	0.25
Seedtox	Phenyl mercuric acetate(1% Hg)	0.25
Agrosan GN	Phenyl mercuric acetate and ethyl mercury chloride mixture	0.15
Difolatan	N-(1,1,2,2-Tetrachloroethyl) sulphenyl-cis-4-cyclohexene-1, 2-dicarboximide,	0.2
Bayleton	1-(4-chlorophenoxy)-3,3-dimethyl-1-1H-(1,2,4-triazole-1-yl) butan-2-one	0.1
Bavistin	methyl-2-benzimidazole carbamate	0.2
Baycor	p-((1,1-biphenyl)-4-yloxyl)-a-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	0.2
Panoram	2-methyl-furan-3-carboxylic acid anilide	0.15
Ridomil	methyl-DL-N-(2,6-dimethylphenyl)-N-(2 methoxyacetyl)-alaninate	0.2

There were two sets with 15 treatments and four replications for each. One set was used to observe development of collar rot disease. Number of diseased plants in each treatment was recorded at 10 and 30 day of sowing. In another set, plant samples were drawn on 10 and 30 day of sowing for isolation of rhizosphere microflora. At particular interval three plants from each pot were dug out carefully at random under each treatment. By serial dilution method from rhizosphere soil suspension fungal, bacterial and actinomycete counts were taken on Martin's rose-Bengal agar, soil extract agar and starch ammonium

agar media, respectively. The plates were incubated for 3 to 5 days at 27 C.

Microbial population changes in groundnut rhizosphere at two stages of crop growth and collar rot development as influenced by organic and inorganic amendments :

(i) Oil cakes :

The experiment was conducted in 15 cm pots filled with 2 kg of sandy-loam soil. The soil was sieved (5 mm meshes) to remove gravel and pieces of plant residues. The inoculum was prepared by culturing A. niger on sorghum grains for 15 days. The grains crushed into a fine powder were mixed in pots so as to 2 % (w/w) of inoculum to soil in each pot. Soil was then amended with oil cakes viz., Kusum, karanj, neem, castor, sal and Mahwa at three doses i.e. 5.0, 10.0 and 15.0 g/kg (w/w) of soil separately in each pot. The pots were placed in the cage house for ten days and equal amount of water was added in each pot on alternate days for the stabilization of the pathogen and the decomposition of oil cakes added to soil.

Eight seeds of groundnut var. RS-1, surface sterilized with 0.1 % $HgCl_2$, were sown in each pot and four replication were used for each treatment. Two controls without oil cake were maintained. One of which consisted of inoculum and another one was without inoculum.

(ii) Gypsum and sulphur amendments :

Groundnut, var. RS-1 was grown in 15 cm pots filled with sandy-loam soil inoculated with A. niger and amended with different doses of sulphur and gypsum. The inoculum raised on sorghum grains was added at the rate of 2 % (w/w) of inoculum to soil in each pot. Sulphur and gypsum at 2.5, 5.0, 7.5, 10.0 and 12.5 g per kg (w/w) of soil in each pot was thoroughly mixed. The pots were then placed in cage house for 7 days and equally watered on alternate days for stabilization of the pathogen. Eight seeds, surface sterilized with 0.1 % $HgCl_2$ were sown in each pot on 8th day of inoculation. Two controls without amendment were maintained. One of which was having inoculum whereas in another no inoculum. Each treatment was replicated four times.

(iii) Nitrogen and phosphorus amendments :

The experiment was conducted by growing groundnut in 15 cm pots filled with 2 kg sandy-loam soil inoculated with A. niger and amended with different amounts of N and P and their combinations. The soil was inoculated with A. niger developed on sorghum seeds for 15 days. The pots were placed in cage housefor 7 days and watered frequently for stabilization of the pathogen.

Nutrient elements were added and thoroughly mixed with soil before sowing. Nitrogen was supplied through urea (46.6 % N) at 25, 50 and 100 ppm and phosphorus phosphoric acid (45 % P_2O_5) at 50, 100 and 200 ppm. A stock solution of these nutrient elements was prepared. The different amounts of the solution were made upto a volume of 50 ml to keep the moisture content in each pot having 2 kg soil constant. All possible combinations between different levels of N and P were tested. Two controls were maintained with and without inoculum of A. niger and both not ammended with N or P.

Eight surface sterilized groundnut seeds were sown in each pot. Four replications were maintained for each treatment.

For each of the amendments experiments, there were two sets with all the treatments and four replications. In one set of each experiment, observations on the appearance of collar rotted plants were recorded. In another set, counts of fungi, bacteria and actinomycetes from rhizosphere were taken on 10 and 30 day of sowing by serial dilution method. Two plants from each pot were dug out at random under each treatment at particular interval for isolation of rhizosphere microflora. Martin's rose-Bengal agar was used to isolate fungi, soil extract agar for bacteria and starch ammonium agar for actinomycete. The plates were incubated for 3 to 5 days at 27 C.

Screening of groundnut germplasm :

Thirty seeds each of groundnut varieties/lines received from NRCG, Junagarh (202 lines), Vanaspati Manufacturers Association of India, Bombay (14 varieties) and ICRISAT (17 varieties), were sown in two rows, one meter long each, in field, containing sandy-loam soil, at a distance, plant to plant 10 cm and row to row 45 cm. The seeds were artificially inoculated with *A. niger*, raised on rice and soil in furrows was also artificially infested by mixing 100 g of inoculum. The plants in each variety affected from collar rot were counted at 10 day interval, within a period of 50 days after germination and the per cent incidence in each variety was calculated. The following scale was used for assessment of reaction :

Scale	Reaction		Disease incidence
0	Immune	(I)	No symptoms of disease
1	Resistant	(R)	1% or less mortality
3	Moderately resistant	(MR)	1-10 % mortality
5	Moderately susceptible	(MS)	11-20 % mortality
7	Susceptible	(S)	21-50 % mortality
9	Highly susceptible	(HS)	51 % or more mortality

Procedure for multiplication of Glomus fasciculatus inoculum:

Glomus fasciculatus mycorrhizal inoculum was received from Dr. D.J. Bagyaraj, Department of Agricultural Microbiology, UAS, GKVK Campus, Bangalore. The culture of mycorrhizal fungus was raised and maintained on sudan grass (Sorghum bicolor (L.) Moench var. sudanense) in 30 cm earthen pots which were filled with a mixture of field soil and sand (1:1 by wt.) and sterilized in autoclave at 20 lb p.s.i. for two hours for two consecutive days. Mycorrhizae inoculum was spreaded on the surface of the sterilized soil and sudan grass seeds were sown @ 10 seeds/pot and watered regularly. After 70 days of sowing, shoot portion of the plants were cut at soil level and the soil in the pots was left to dry to friable condition. The soil was then crumbled and root-lets were cut into small pieces. This mixture of soil and root pieces was used as inoculum which contained hyphae, vesicles, chlamydospores and arbuscules of G. fasciculatus. Inoculum containing 400-500 spores per 50 ml of inoculum was used for inoculations in the experiments.

Estimation of G. fasciculatus spores in soil:

G. fasciculatus spores were obtained by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Approximately 50 ml of soil was suspended in 200 ml of water.

Heavier particles were allowed to settle for 10 minutes and the liquid was decanted through a 60 mesh sieve, fine enough to remove larger particles of organic matter but coarse enough to allow the desired spores to pass through. The sieved suspension was saved and stirred to resuspend all the particles. The heavier particles were allowed to settle down for 10 minutes and the liquid decanted through a 100 mesh sieve, fine enough to retain the desired spores which were examined to determine the presence of spores. The sieved suspension was again allowed to settle down for one hour and the remaining debris parts were removed. Later, the spore suspension was taken with the help of pipette in petriplate, examined under sterioscopic microscope with strong direct light and spores count were taken.

Estimation of VA mycorrhizal fungi for assessment of infection and per cent colonization :

The procedure of Phillips and Haymann (1970) was followed for rapid assessment of mycorrhizae. Roots of groundnut plants inoculated with G. fasciculatus were cut into small pieces and heated at 90 C in 10 per cent KOH solution for 2 hours, washed with fresh KOH and immershed in alkaline solution of hydrogen peroxide (approx. 10 vol.) at 20 C until bleached (for 30 minutes). They are then

rinsed thoroughly in water to remove all the hydrogen peroxide, acidfied with dilute HCl. Finally, the root bits were stained by simmering for 5 minutes in 0.05 per cent trypan blue in lactophenol and the extra stain was removed by putting the stained root pieces in clear lactophenol and then mounted on a clean glass slide. The fungal structures were observed under a compound microscope.

Colonization of root infection was determined by the following formula :

$$\frac{\text{Number of VAM positive segments}}{\text{Total number of segments secured}} \times 100$$

= % colonization

Effect of G. fasciculatus on groundnut plants and Aspergillus collar rot disease development :

Groundnut was grown in 15 cm earthen pots filled with steam sterilized sandy-loam soil with and without mycorrhizal inoculum. The mycorrhizal inoculum was placed 2 cm below the soil surface @ 5 ml per seed before sowing to produce mycorrhizal plants. At the time of sowing Aspergillus niger inoculum was added separately in each of the treatments in different forms, viz., seed inoculation, soil inoculation, seed and soil both inoculated, to produce

collar rot disease. Control was maintained in both the treatments (i.e. with and without mycorrhiza) without A. niger inoculum. Six seeds were sown in each pot and watered regularly.

Observations were recorded on number of collar rot diseased plants in each treatment. After 50 days of sowing, plants were harvested carefully with the roots and, thereafter, dry weight of mycorrhizal and non-mycorrhizal root and shoot and spore population in the soil were recorded for each of mycorrhizal and non-mycorrhizal plants. Per cent colonization and estimation of phosphorus in the plant tissue were also made. Five replications were taken for each treatment and each experiment was repeated.

Estimation of phosphorus in plant tissues :

The method described by Jackson (1958) was used for the estimation of phosporus. Fine powder (0.1 g) of each sample was mixed with 2 ml of triacid mixture (100 ml HNO_3, 10 ml H_2SO_4 and 40 ml of 60 % $KClO_4$) and digested on hot plate at 180-200 C until dense white fumes came out and clear solution remained in the flask. Then the volume of the solution was raised to 100 ml by adding glass distilled water.

For estimation of phosphorus, following three reagents were used :

1. One g ammonium molybdate $\{(NH_4)_6\ MO\ O_2\ 4H_2O)$ in 400 ml distilled water.
2. 1.25 g of ammonium meta-vanadate ($NH_4\ VO_3$) was dissolved in 300 ml of boiling glass distilled water. After cooling 250 ml conc. HNO_3 was added.
3. Finally, solution-1 was poured into solution-2 and the mixture was diluted to one litre.

Five ml each from the extracts were taken into 50 ml volumetric flasks and 5 ml of reagent-3 was added in each flask for the development of colour. The total volume of each solution was made to 50 ml with glass distilled water. The readings were taken at 420 n u on "Klett and Summerson colorimeter" using blue filter. Blank prepared from acid mixture was used simultaneously. The standard solution was prepared by dissolving 0.2195 g of KH_2PO_4 in 500 ml water and 5 ml of HCl and then making the final volume to one litre by adding glass distilled water.

Phenols in mycorrhizal roots of groundnut :

Groundnut was grown in 15 cm pots filled with 3 kg of steam sterilized sandy-loam soil with and without mycorrhizae

(G. fasciculatus) inoculum. The inoculum was placed 2 cm below the soil surface before sowing to produce mycorrhizal plants. Nitrogen, at the rate of 30 mg per kg of soil, was applied to all the pots as urea. Groundnut plants (5 replicates) were harvested after 10, 20, 30 40 and 50 days of growth from mycorrhizal and non-mycorrhizal plants. Shoot portion from roots were cut down at cotyledonary joint and remaining root portion were used for total and ortho-dihydroxy (O-D) phenols.

Preparation of root extract in alcohol :

3 g of tissue from each sample was boiled in 15 ml of absolute alcohol for 2 minutes, then cooled and ground to fine paste with pastle and mortar. The resultant slurry was centrifused at 2000 rpm for 10 minutes and filtered through Whatman No. 42 filter paper. The residue was washed thrice with 10 ml of 95 % alcohol. The filtrate was adjusted to 50 ml with 70 % alcohol. 25 ml of this extract was scrubbed with CCl_4 until free from chlorophyll and other pigments.

Estimation of total phenols :

The method of Bray and Thrope (1954) was used for the estimation of total phenols in mycorrhizal and non-mycorrhizal roots of groundnut.

One ml of alcohol extract was taken into a 25 ml volumetric flask. To this 1 ml of Folin-ciocalteu reagent and 2 ml of 20 % Na_2CO_3 solution was added. After, shaking, the flask was placed in a boiling water bath for exactly 1 min., cooled under a running tap water and then the blue solution was diluted to 25 ml with water. The absorbance at 650 nm was measured in a colorimeter. Standard curve was prepared using catechol and ug total phenol per g tissue was calculated. A blank containing all the reagents minus plant extract was used to adjust the absorbance to zero.

Estimation of O-D phenols :

Estimation of O-D phenols in plant material was determined by colorimetric method (Arnow, 1937).

One ml of the alcohol extract was taken into a 25 ml volumetric flask. To this, 1 ml of 0.05 N HCl, 1 ml of Arnow's reagent (10 g $NaNO_2$ and 10 g Na_2MoO_4 dissolved in 100 ml distilled water), 10 ml of distilled water and 2 ml of 1N NaOH were added. Soon after the addition of alkali, pink colour appeared which was diluted to 25 ml with distilled water. The absorbance at 515 nm was measured in a colorimeter. Standard curve was prepared using catechol and ug O-D phenol/ g tissue was calculated. A reagent blank without extract was maintained to adjust the absorbance to zero.

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EXPERIMENTAL RESULTS

Development of selective medium for isolation of *A. niger* from soil :

Isolation of specific plant pathogenic fungi from infested soil is difficult unless suitable selective medium is available. In many cases saprophytic soil fungi and bacteria over grow the pathogen. Griffin *et al.* (1974) developed a selective medium for isolation of *A. flavus* and *A. niger* group from groundnut fields. Joffe (1969) found pronounced antagonism between *A. flavus* and *A. niger* while studying relationships in plate culture with material from fresh and stored kernels. In 1972, he found reduction in *A. flavus* population in groundnut soils under low moisture conditions and *A. niger* increased and constituted 30-60 % of total mycoflora.

Therefore, in present studies an attempt was made to formulate a relatively selective medium suitable for isolation of *A. niger* from soil. Martin's rose-Bengal agar with streptomycin medium was amended with sodium chloride, which provides a high osmotic concentration in the medium and thus lowers relative humidity. The various doses of NaCl,

viz., 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0 and 12.0 per cent were added in the medium and autoclaved at 15 p.s.i. for 20 minutes. The pH 6.5 was maintained in the medium.

One ml of rhizosphere soil suspension of dilution 10^3 and 10^4 was placed in each petridish. Fifteen ml of the medium cooled to 40 C was poured to each petridish. The plates were incubated for 3 to 7 days at 27 C.

Martin's rose-Bengal streptomycin agar medium with 5.5 per cent NaCl gave maximum number of colonies of A.niger and eliminated most of the fungi which appeared on the medium without NaCl except some of the species of Penicillium and Aspergillus and hence this medium was used to isolate A. niger from soil in all the experiments. There was no growth of A. niger in plates containing 12 per cent NaCl in the medium.

The following characteristics of the medium were helpful in isolation and enumeration of groundnut collar rot pathogen, A. niger :

(a) Initially the individual colonies develop into yellow colour.

(b) Later, as the conidial heads develop, the colonies become black and outer periphery seems yellow (Plate 1).

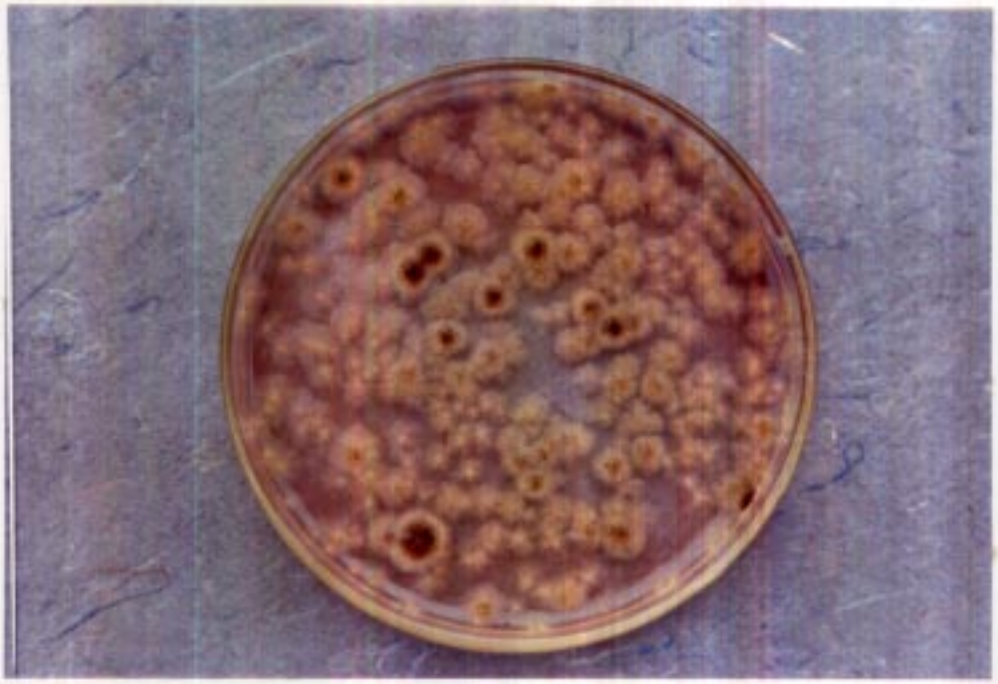


Plate 1. Colonies of A. niger in the specific medium

Survey for Aspergillus collar rot incidence, collection and isolation of rhizosphere microflora from diseased and healthy plants :

During July-August, 1983, survey was done in Ajmer, Bhilwara, Chittorgarh, Kota and Udaipur districts of Rajasthan, for collection of groundnut collar rotted plants and to record incidence of the disease. Samples of diseased and healthy plants were collected separately in polythene bags from each place and were brought in the laboratory for isolation of rhizosphere microflora of healthy and diseased plants, and collar rot pathogen, A. niger.

Disease incidence was recorded by counting healthy and diseased plants at random in one square meter area in groundnut fields. Rhizosphere microflora was isolated by dilution method in laboratory. Results are given in table 1 (A and B).

Maximum disease incidence was recorded in groundnut fields at Ajmer and minimum in fields at Udaipur. A. niger in the rhizosphere of diseased groundnut plants ranged between 56.37 to 64.76 per cent of total mycoflora at different places whereas it ranged from 9.9 to 25.2 per cent in the rhizosphere of healthy plants. The minimum population of actinomycetes and bacteria and maximum population of A. niger

Table 1 A. Collar rot incidence in groundnut and rhizosphere microflora of diseased and healthy plants at different places in Rajasthan

S. No.	District	Disease incidence (%)	Rhizosphere microflora per g of oven dry soil									
			A. niger ($\times 10^3$)		*A. niger* of total mycoflora (%)		Other fungi ($\times 10^3$)		Actinomycetes ($\times 10^6$)		Bacteria ($\times 10^7$)	
			D	H	D	H	D	H	D	H	D	H
1.	Ajmer	25.2-42.7	12.46	2.58	64.7	25.3	6.78	7.62	1.22	1.67	0.81	0.89
2.	Bhilwara	18.0-37.4	15.48	1.98	61.6	14.6	9.62	11.53	2.35	2.76	1.40	1.87
3.	Chittorgarh	17.5-21.4	11.36	1.42	56.3	9.9	8.79	12.79	4.13	4.92	1.79	2.10
4.	Kota	10.0-22.2	12.22	1.67	61.7	15.3	7.58	9.21	2.75	3.22	2.05	2.20
5.	Udaipur	5.5-17.9	10.87	0.85	62.8	11.0	5.43	6.82	3.32	4.43	1.88	2.21

D = diseased plants

H = healthy plants

Table 1 B. Soil characteristics in groundnut fields at different places in Rajasthan

S. No.	District	Texture	Soil type	Organic matter(%)	pH
1.	Ajmer	Sandy	Light	0.57	6.9
2.	Bhilwara	Sandy	Light	0.93	7.1
3.	Chittorgarh	Clayey loam	Heavy	1.62	7.4
4.	Kota	Loamy sand	Medium	1.35	7.2
5.	Udaipur	Sandy loam	Medium	1.65	7.9

was found in rhizosphere of plants collected from Ajmer, where pH and organic matter content of soil was minimum.

Isolation and pathogenicity of A. niger and performance of virulence of different isolates :

Isolation of A. niger from each samole was made on PDA from diseased plants collected from different places. The isolates after purification were maintained on PDA for further studies. For pathogenicity tests, 100 seeds were inoculated separately with each isolate raised on rice medium. These seeds were sown in 30 cm pots filled with steam sterilized sandy-loam soil, at the rate of 10 seeds per pot. All the pots were kept under cage house and watered regularly. Observations on the number of collar rotted plants were taken. Collar rot symptoms appeared within a period of 2 to 20 days after germination. Results are given in table 2.

All the isolates were pathogenic and virulent (Plate 2). Maximum number of diseased plants were found with Ajmer isolate. Reisolations were made on PDA from diseased plants and A. niger appeared consistently in each. Ajmer isolate was used throughout the experiments.

Symptomatology : A. niger causes rotting of seed, pre-emergence soft rot of the hypocotyl and post-emergence collar rot of groundnut seedlings. Infection of the hypocotyl

Table 2. Pathogenicity and comparative disease incidence of five isolates of *A. niger* collected from different places in Rajasthan

S.No.	Isolate number	Place	Per cent collar rot (after 25 days of artificial inoculation of groundnut seeds
1.	AG 1	Ajmer	59.13
2.	AG 2	Bhilwara	48.38
3.	AG 3	Chittorgarh	41.93
4.	AG 4	Kota	51.61
5.	AG 5	Udaipur	32.25

Plate 2

I. Diseased plants from seeds inoculated with A. niger, Ajmer isolate

II. Healthy plants

and the cotyledons of groundnut seedlings occur from the seed-borne and/or soil-borne inoculum of the fungus. From the infected cotyledons the fungus grows into the collar region at the cotyledonary node of the seedling. The rot caused by fungi may be wet or dry and shredded. The former type of rot occurs during the early post-emergence phase while the later type manifests itself during. the late post emergence phase of the seedlings. The rotting proceeds rapidly throughout the tissues of the hypocotyl or the collar region of the affected seedlings, resulting in their eventual collapse and death.

In fields, collar rot affected plants can be easily recognised from the symptoms on the above ground parts. If girdling of the collar region of the affected seedling due to the fungus is complete, the lower-most leaves are chlorotic (Plate 3). If, however, the girdling is partial, the leaves of the branches emerging only from the proximity of the affected region manifest such chlorosis. Drooping, wilting and death of the affected branches of the diseased plants follows in the next few days. The affected collar region becomes shredded with lapse of time and there appears a profuse growth of conidiophores with black conidial heads characteristic of the causal fungus (Plate 4)

Plate 3. Collar rotted plants in groundnut field

Plate 4.A. Rotting of seed and pre-emerged seedling from A. niger in the field

Plate 4.B. Profuse growth of A. niger on collar region of diseased plant

Effect of A. niger inoculum density on collar rot development and rhizosphere microflora of groundnut :

Groundnut seeds, surface sterilized with 0.1 % $HgCl_2$, were inoculated with different levels of A. niger inoculum which consisted of spores and mycelial bits. Thus, 2.5, 5.0, 7.5, 10.0 and 12.5 of log 10^3 levels of inoculum load per seed were used. Hundred seeds from each inoculation were sown in 30 cm pots (10 seeds per pot) filled with sandy-loam soil. Water was given frequently. Observations on number of pre and post-emergence of collar rotted plants were recorded. Rhizosphere microflora counts were made from each treatment on 20 day old plants by dilution method. Results are given in table 3.

No pre-emergence rot of groundnut plants was observed with inoculum load 2.5 x 10^3, but with the increase of inoculum density the pre-emergence rotting increased. Post-emergence rotting was maximum at 5 x 10^3 inoculum load per seed.

A. niger population in rhizosphere increased with the increase in inoculum load on the seed. Population of other fungi, actinomycetes and bacteria in the rhizosphere of inoculated plants decreased with the increase in A. niger population in rhizosphere of groundnut plants. The diseased plants in inoculum load 12.5 x 10^3 per seed consisted of

Table 3. Effect of *A. niger* inoculum density on collar rot development and rhizosphere microflora of groundnut (20 day old plants)

S.No.	Inoculum load per seed ($\times 10^3$)	Collar rot (%)		Rhizosphere microflora per g of oven dry soil			
		Pre-emergence	Post-emergence	*A. niger* ($\times 10^4$)	Other fungi ($\times 10^3$)	Actinomycetes ($\times 10^5$)	Bacteria ($\times 10^6$)
1.	0.0	0.0	0.0	0.99	3.85	6.79	3.63
2.	2.5	0.0	47.91	8.68	2.27	5.88	3.26
3.	5.0	4.1	65.6	11.95	2.07	5.20	2.27
4.	7.5	8.3	62.2	12.18	2.00	4.69	1.73
5.	10.0	29.16	56.2	17.88	1.86	3.80	1.25
6.	12.5	69.79	26.0	19.09	1.15	3.02	1.18
	SEm. ±			0.5251	0.2136	0.2128	0.3016
	CD 5 %			1.32	0.54	0.53	0.76

maximum (77.17 %) A. niger and minimum other fungi (4.9 %), actinomycetes (12.88 %) and bacteria (5.03 %) of the total rhizosphere microflora per g oven dry soil at dilutions 10^4, 10^3, 10^5 and 10^6, respectively.

Inoculum load in relation to collar rot development does not give a true picture, as many of the seedlings do not emerge out of soil because of pre-emergence rotting and also rotting of seeds.

Microbial population changes in groundnut rhizosphere and collar rot development as influenced by different fungicides:

Effect of different systemic and non-systemic seed dressers on collar rot development and rhizosphere microflora was studied by treating A. niger inoculated seeds with the fungicides- Dithane M-45, captan, thiram, aggalol, seed- tox, fytolan, difolatan, panoram, agrosan, baycor, ridomil, bavistin and bayleton at 2.0, 2.0, 3.0, 2.5, 2.5, 3.0, 2.0, 1.5, 1.5, 2.0, 2.0, 2.0 and 1.0 g/kg seed doses, respectively. Seeds were inoculated with A. niger inoculum raised on rice medium. The seeds from each treatment were sown in pots filled with sandy-loam soil. Observations in each treatment on number of collar rotted plants and microbial changes in rhizosphere by dilution method were taken. The results on disease incidence and rhizosphere microflora are presented in table 4 and 5, respectively.

Table 4. Effect of different seed dressing fungicides on collar rot development in groundnut

S. No.	Fungicides	Dose g/ kg seed	Disease incidence (%)	Trans-formed value	Per cent decrease of disease over control
	Non-systemic				
1.	Dithane M-45	2.0	22.5	28.11	67.43
2.	Captan	2.0	13.4	18.66	80.60
3.	Thiram	3.0	8.3	12.02	87.98
4.	Agallol	2.5	17.4	24.67	74.81
5.	Seedtox	2.5	18.3	25.30	73.51
6.	Fytolan	3.0	40.9	39.72	40.81
7.	Difolatan	2.0	9.1	12.65	86.83
8.	Agrosan GN	1.5	13.4	18.66	80.60
	Systemic				
9.	Panoram	1.5	14.1	19.29	79.59
10.	Baycor	2.0	21.6	27.45	68.74
11.	Ridomil	2.0	31.6	34.06	54.26
12.	Bavistin	2.0	4.1	6.01	94.40
13.	Bayleton	1.0	9.2	12.68	86.68
	Control				
14.	With inoculum	-	69.1	56.51	-
15.	Without inoculum	-	0.0	0.0	-
	SEm. ±			4.9804	
	CD 5 %			14.21	

There was minimum 4.1 per cent collar rot when seeds were treated with bavistin. Among other different fungicides used as seed treatment collar rot percentages were 8.3 in thiram, 9.1 in difolatan and 9.2 in bayleton treatments. There was negligible reduction of collar rot in case of fytolan among non-systemic fungicides and ridomil among systemic fungicides (Table 4).

Table 5 indicate that in the rhizosphere of 10 day old plants all the fungicides except fytolan reduced A. niger population significantly as compared to inoculated untreated control. Maximum reduction was found in bavistin, panoram, thiram and bayleton treated seeds. Counts of other fungi were minimum in difolatan. Actinomycetes population was comparatively less in all the fungicidal treatments. Counts of bacteria were less only in ridomil and maximum in bayleton and bavistin.

In rhizosphere of 30 day old plants A. niger population increased in all the treatments as compared to 10 day old plants, but this population was significantly less in comparison to inoculated control. The minimum population of A. niger was found in bavistin, bayleton, difolatan, panoram and agallol, that was 15 to 7 times less compared to inoculated control. In all the fungicidal treated plants, other fungi increased in population and

Table 5. Effect of different systemic and non-systemic fungicides on rhizosphere microflora and collar rot pathogen (*A. niger*) of groundnut

S. No.	Fungicides	Dose g/kg seed	Rhizosphere microflora per g of oven dry soil after 10 and 30 days							
			A. niger ($\times 10^3$)		Other fungi ($\times 10^3$)		Actinomycetes ($\times 10^5$)		Bacteria ($\times 10^7$)	
			10	30	10	30	10	30	10	30
	Systemic									
1.	Panoram	1.5	1.44	3.44	2.70	5.70	2.86	4.79	1.62	1.92
2.	Baycor	2.0	8.40	18.72	2.80	3.96	7.08	8.47	2.12	3.64
3.	Ridomil	2.0	13.58	18.39	2.44	2.71	5.38	5.36	1.55	1.43
4.	Bavistin	2.0	1.13	2.15	3.31	2.74	7.39	9.72	1.23	2.06
5.	Bayleton	1.0	2.89	2.13	3.07	5.24	3.42	4.31	1.21	1.41
	Non-systemic									
6.	Dithane M-45	2.0	8.11	10.39	3.18	2.68	4.24	6.40	2.86	1.62
7.	Captan	2.0	5.77	6.19	2.46	3.64	5.00	6.74	2.53	2.45
8.	Thiram	3.0	2.45	4.50	3.42	5.78	4.04	11.69	2.38	2.30
9.	Agallol	2.5	4.19	4.16	4.64	5.34	3.84	12.67	2.50	2.35
10.	Seedtox	2.5	6.19	7.32	4.17	4.75	5.40	10.49	2.80	2.70
11.	Fytolan	3.0	11.46	20.02	4.23	2.50	4.38	8.71	2.73	3.65
12.	Difolatan	2.0	3.84	3.97	1.89	2.90	5.34	8.66	3.21	4.63
13.	Agrosan GN	1.5	4.78	6.38	4.74	5.53	5.78	9.31	3.62	4.09
	Control									
14.	With inoculum	-	10.38	21.39	2.52	1.26	5.47	5.82	1.77	1.94
15.	Without inoculum	-	0.69	1.03	4.53	5.66	5.93	6.00	2.08	2.32
	SEm. ±		0.4992	0.5231	0.2154	0.2880	0.2647	0.4855	0.3243	0.2567
	CD 5 %		1.15	1.25	0.51	0.69	0.63	1.16	0.77	0.62

as compared to inoculated control and in thiram the increase was maximum but the population was nearly same as found in uninoculated untreated control. Actinomycetes counts were more in all the fungicidal treated plants whereas bacteria increased in fytolan, difolatan, agrosan, baycor treatments as compared to 10 day old plants. However, population of bacteria was more in all the treatments except bayleton, ridomil, panoram and dithane M-45.

Microbial population changes in groundnut rhizosphere and collar rot incidence as influenced by oil cake amendments at different levels :

Effect of different oil cake amendments was studied by growing groundnut in pots filled with sandy-loam soil amended with different dosages (5, 10 and 15 g/kg of soil) of oil cakes viz., Karanj, Kusum, Neem, Sal, Mahwa and Undi (castor) and inoculated with A. niger raised on sorghum grains. Observations on number of collar rotted plants and microflora population in rhizosphere at 10 and 30 day of germination were taken in each treatment. The results are presented in tables 6, 7, 8 and 9.

The results (Table 6) indicate that at the low dosages, disease intensity was more in each oil cake amendment except Karanj where the disease increased with increasing

Table 6. Effect of different levels of oil cakes on collar development in groundnut

S. No.	Oil cakes	Dose g/kg soil	Disease incidence (%)	Trans-formed value	Per cent decrease or increase of disease over control
1.	Karanj	5.0	26.6	30.9	(-)45.8
2.	Kusum	"	77.4	61.8	(+)57.6
3.	Neem	"	26.6	30.9	(-)45.8
4.	Sal	"	68.3	55.9	(+)39.1
5.	Mahwa	"	41.6	42.6	(-)15.2
6.	Castor	"	45.8	42.7	(-) 6.7
7.	Karanj	10.0	35.8	36.5	(-)27.0
8.	Kusum	"	45.8	42.8	(-) 6.7
9.	Neem	"	17.4	24.6	(-)64.5
10.	Sal	"	50.0	45.1	(+) 1.8
11.	Mahwa	"	36.6	37.5	(-)25.4
12.	Castor	"	31.6	34.2	(-)35.6
13.	Karanj	15.0	41.6	36.9	(-)15.2
14.	Kusum	"	26.6	30.9	(-)45.8
15.	Neem	"	13.3	18.6	(-)72.9
16.	Sal	"	32.4	34.9	(-)34.0
17.	Mahwa	"	13.3	18.6	(-)72.9
18.	Castor	"	22.4	28.1	(-)54.3
19.	Control with inoculum	-	49.1	44.8	-
20.	Control without inoculum	-	0.0	0.0	-
	SEm. ±			3.7073	
	CD 5 %			10.4	

dose. At 5 g dose of Kusum and Sal amendment disease was more as compared to inoculated check. At all the dosages, Neem cake reduced collar rot incidence significantly. Minimum disease incidence was recorded with Neem and Mahwa cakes at 15 g dose, where per cent reduction in collar rot was 72.9 over inoculated control.

It is evident from the results (Table 7) that at 5 g dose Karanj oil cake reduced A. niger population and increased the population of other fungi, actinomycetes and bacteria significantly as compared to inoculated control. Neem cake was next in reducing the population of A. niger in rhizosphere whereas Kusum, Mahwa and Sal amendments increased A. niger population

At 10 g dose Neem cake reduced A. niger significantly, followed by castor and Karanj (Table 8). All the amendments except Mahwa and castor increased the other fungi whereas actinomycetes and bacteria counts were more in all the treatments in rhizosphere of 30 day old plants in comparison to both the controls.

Results in table 9 indicate that at 15 g dose A. niger population was less in all the oil cake amendments as compared to inoculated control. Maximum reduction was in Neem cake amendment where the population of A. niger was even less than uninoculated control. Actinomycetes population was significantly more in Mahwa, castor, Kusum and Neem

Table 7. Effect of different oil cakes, at 5 g/kg soil dose, on rhizosphere microflora and collar rot pathogen (*A. niger*) of groundnut

S. No.	Oil cakes	Rhizosphere microflora per g of oven dry soil after 10 and 30 days							
		A. niger ($\times 10^4$)		Other fungi ($\times 10^4$)		Actinomycetes ($\times 10^6$)		Bacteria ($\times 10^7$)	
		10	30	10	30	10	30	10	30
1.	Karanj	2.00	6.89	9.07	13.74	13.57	22.89	2.06	5.16
2.	Kusum	5.51	26.23	7.25	16.04	8.04	17.16	4.08	3.62
3.	Neem	9.11	6.69	6.52	13.06	5.02	8.63	1.24	3.64
4.	Sal	12.54	13.67	1.92	10.67	8.26	11.28	4.27	4.74
5.	Mahwa	7.47	17.33	6.57	8.22	4.24	8.63	1.28	3.05
6.	Castor	7.98	10.37	1.37	7.72	3.97	8.78	5.53	6.99
7.	Control with inoculum	5.74	11.12	5.00	11.74	5.46	8.62	1.62	1.69
8.	Control without inoculum	0.81	1.96	5.09	13.46	5.57	10.20	1.92	2.39
	SEm. ±	0.536	0.770	0.416	0.714	0.388	1.07	0.263	0.36
	CD 5 %	1.28	1.84	0.99	1.70	0.92	2.55	0.63	0.87

Table 8. Effect of different oil cakes, at 10 g/kg soil dose, on rhizosphere microflora and collar rot pathogen (*A. niger*) of groundnut

S.No.	Oil cakes	Rhizosphere microflora per g of oven dry soil after 10 and 30 days							
		A. niger ($\times 10^4$)		Other fungi ($\times 10^4$)		Actinomycetes ($\times 10^6$)		Bacteria ($\times 10^7$)	
		10	30	10	30	10	30	10	30
1.	Karanj	4.34	8.93	3.47	11.77	7.47	9.26	1.91	4.74
2.	Kusum	3.32	19.77	5.53	13.09	9.17	18.37	5.24	3.82
3.	Neem	5.03	4.71	6.42	12.79	5.96	11.28	1.22	3.09
4.	Sal	11.56	13.49	4.28	12.56	11.64	17.34	2.81	3.03
5.	Mahwa	6.32	15.77	6.82	9.03	5.28	14.56	1.69	3.07
6.	Castor	2.19	7.45	4.38	11.65	7.52	11.58	4.68	5.93
7.	Control with inoculum	5.74	11.12	5.00	11.74	5.46	8.62	1.62	1.69
8.	Control without inoculum	0.81	1.96	5.09	13.46	5.57	10.20	1.92	2.30
	SEm. $\pm$	0.536	0.770	0.416	0.714	0.388	1.07	0.263	0.365
	CD 5 %	1.28	1.84	0.99	1.70	0.92	2.55	0.63	0.87

Table 9. Effect of different oil cakes, at 15 g/kg soil dose, on rhizosphere microflora and collar rot pathogen (*A. niger*) of groundnut

S. No.	Oil cakes	Rhizosphere microflora per g of oven dry soil after 10 and 30 days							
		A. niger ($\times 10^4$)		Other fungi ($\times 10^4$)		Actinomycetes ($\times 10^6$)		Bacteria ($\times 10^7$)	
		10	30	10	30	10	30	10	30
1.	Karanj	5.43	9.43	3.35	10.34	5.65	7.55	1.81	3.64
2.	Kusum	1.73	7.00	3.84	11.65	7.49	15.43	5.84	4.54
3.	Neem	1.72	1.53	4.26	8.13	7.26	11.58	0.99	2.64
4.	Sal	6.06	11.52	7.97	13.97	6.68	7.18	2.22	2.96
5.	Mahwa	2.78	4.76	7.90	13.02	10.59	38.62	3.59	5.24
6.	Castor	1.84	5.53	4.55	10.93	7.82	31.28	4.08	5.88
7.	Control with inoculum	5.74	11.12	5.00	11.74	5.46	8.62	1.62	1.69
8.	Control without inoculum	0.81	1.96	5.09	13.46	5.57	10.20	1.92	2.30
	SEm. ±	0.536	0.770	0.416	0.714	0.388	1.07	0.263	0.365
	CD 5 %	1.28	1.84	0.99	1.70	0.92	2.55	0.63	0.87

amendments in comparison to untreated inoculated control. All the oil cakes amendments increased bacterial counts in rhizosphere of 30 day old groundnut plants.

Microbial changes in rhizosphere and collar rot development as influenced by nitrogen and phosphorus :

To find out the effect of nutrients on rhizosphere microflora and collar rot incidence, groundnut, was grown in pots filled with sandy-loam soil infested with A. niger inoculum. Nitrogen and phosphorus (through urea and phosphoric acid) were added, to the infested soil with A. niger, singly or in combinations. Observations were recorded on number of diseased plants and rhizosphere microflora at 10 and 30 day of plant growth.

With increasing dosages of N and P, collar rot incidence decreased significantly. At N_{100} P_{200} dose reduction in disease incidence was maximum (80.84 %), followed by P_{200} alone where per cent reduction in collar rot was 73.89 (Table 10).

Data presented in table 11 indicate that at 10 day of plant growth A. niger population increased in all the treatments compared to check, except N_{50} P_{200} and N_{100} P_{200} dosages. All the levels of N and P reduced the population of A. niger significantly in the rhizosphere

Table 10. Effect of different levels of nitrogen and phosphorus, singly or in combination, on collar rot development in groundnut

S.No.	Nutrients (ppm)	Disease incidence (%)	Transformed value	Per cent decrease or increase of disease over control
1.	N_{25}	51.6	45.98	(+) 8.63
2.	N_{50}	34.1	35.51	(-)28.21
3.	N_{100}	17.4	24.67	(-)63.36
4.	P_{50}	39.1	38.67	(-)17.68
5.	P_{100}	25.6	30.27	(-)46.10
6.	P_{200}	12.4	18.03	(-)73.89
7.	$N_{25} P_{50}$	40.8	39.67	(-)14.10
8.	$N_{50} P_{50}$	31.6	34.06	(-)33.47
9.	$N_{100} P_{50}$	25.8	30.27	(-)45.68
10.	$N_{25} P_{100}$	43.3	41.11	(-) 8.84
11.	$N_{50} P_{100}$	21.6	27.47	(-)54.52
12.	$N_{100} P_{100}$	21.6	24.67	(-)54.52
13.	$N_{25} P_{200}$	31.6	34.06	(-)33.47
14.	$N_{50} P_{200}$	17.4	24.67	(-)63.36
15.	$N_{100} P_{200}$	9.1	12.65	(-)80.84
16.	$N_0 P_0$ (control)	47.5	43.56	-
	SEm. ±		3.2802	
	CD 5 %		9.32	

N = Nitrogen

P = Phosphorus

Table 11. Effect of different levels of nitrogen and phosphorus, singly or in combination on rhizosphere microflora and collar rot pathogen (*A. niger*) of groundnut

S.No.	Nutrients (ppm)	Rhizosphere microflora per g of oven dry soil after 10 and 30 days							
		A. niger ($\times 10^3$)		Other fungi ($\times 10^3$)		Actinomycetes ($\times 10^5$)		Bacteria ($\times 10^7$)	
		10	30	10	30	10	30	10	30
1.	N_{25}	15.32	23.37	1.51	13.60	5.87	15.38	1.37	1.98
2.	N_{50}	13.13	21.47	1.84	22.08	6.31	19.60	1.01	1.61
3.	N_{100}	10.73	19.18	2.27	25.15	7.54	25.14	0.96	1.46
4.	P_{50}	9.99	16.54	2.52	13.78	6.01	31.39	1.23	2.30
5.	P_{100}	9.43	15.28	4.15	16.64	6.30	34.50	2.14	2.98
6.	P_{200}	7.89	11.00	3.90	12.51	8.04	39.48	2.54	3.08
7.	$N_{25} P_{50}$	10.79	13.33	1.86	19.40	3.97	18.20	1.04	2.03
8.	$N_{50} P_{50}$	9.86	10.85	2.17	19.26	4.86	20.36	1.17	2.10
9.	$N_{100} P_{50}$	8.99	8.92	2.50	12.49	5.06	20.31	2.63	2.69
10.	$N_{25} P_{100}$	11.53	19.60	2.51	12.57	6.17	19.04	1.39	1.10
11.	$N_{50} P_{100}$	9.79	15.54	2.45	14.08	6.83	25.52	1.45	1.50
12.	$N_{100} P_{100}$	7.88	9.14	2.11	17.85	8.18	25.90	1.91	1.69
13.	$N_{25} P_{200}$	11.28	17.15	2.11	15.01	6.45	11.21	1.85	1.99
14.	$N_{50} P_{200}$	6.33	14.18	2.42	15.68	6.78	14.98	1.18	1.82
15.	$N_{100} P_{200}$	6.21	9.67	2.90	21.54	6.97	17.34	0.93	1.50
16.	$N_0 P_0$	9.10	27.52	3.24	11.33	5.14	11.21	1.56	1.24
	SEm. ±	0.8742	1.4952	0.2307	1.5847	0.3914	1.4684	0.1467	0.1760
	CD 5 %	2.51	4.30	0.66	4.56	1.12	4.23	0.42	0.50

of 30 day old plants. The counts of other fungi decreased at 10 day, except P_{100} and P_{200} levels, but on 30 day plant growth there was increase in population as compared to control and maximum population was found in N_{100} and N_{100} P_{200} doses. Actinomycetes population increased at both dates of observation except in N_{25}, N_{50} and N_{100} each combined with P_{50} at 10th day and N_{25} P_{200} and N_{50} P_{200} at 30th day. There was not much change in population of bacteria in rhizosphere of 10 day plants. On 30th day bacterial population was found to increase in all the treatments compared to control and maximum population was found in case of phosphorus alone at all the levels.

Microbial changes in groundnut rhizosphere and collar rot development as influenced by different levels of sulphur gypsum :

Influence of sulphur and gypsum on rhizosphere microflora and collar rot development was studied by amending the soil with sulphur and gypsum at 2.5, 5.0, 7.5, 10.0 and 12.5 g/kg of soil. Groundnut was grown in pots filled with sandy-loam soil amended and inoculated with A. niger raised on sorghum grains. Observations on collar rot incidence and rhizosphere microflora of 20 day old plants were taken.

It is evident from table 12 that increase or decrease in collar rot incidence was directly related to

Table 12. Effect of different levels of sulphur and gypsum amendments on collar rot development in groundnut

S.No.	Amendments	Dose g/kg soil	Disease incidence (%)	Trans-formed value	Per cent decrease or increase of disease over control
1.	Sulphur	2.5	56.6	48.8	(+)30.71
2.	"	5.0	51.6	45.9	(+)19.16
3.	"	7.5	33.3	35.0	(-)23.09
4.	"	10.0	17.4	24.6	(-)59.81
5.	"	12.5	12.4	18.0	(-)71.36
6.	Gypsum	2.5	60.8	51.2	(+)40.41
7.	"	5.0	51.6	45.9	(+)19.16
8.	"	7.5	50.0	45.0	(+)15.47
9.	"	10.0	40.8	39.6	(-) 5.77
10.	"	12.5	31.6	34.0	(-)27.02
11.	Control with inoculum	-	43.3	42.1	-
12.	Control without inoculum	-	0.0	0.0	-
	SEm. ±			2.9605	
	CD 5 %			8.52	

the amount of sulphur and gypsum amended in the soil. Collar rot disease was more in 2.5 and 5.0 g doses of sulphur and in 2.5, 5.0 and 7.5 g doses of gypsum amended soil as compared to inoculated check. At higher doses of sulphur amendments disease was significantly less and only at 12.5 g dose of gypsum the disease was less as compared to control. Minimum disease was found in soil amended with sulphur at 12.5 and 10 g/kg of soil and the per cent reduction in collar rot was 71.36 and 59.81, respectively.

Aspergillus niger population in groundnut rhizosphere decreased significantly with the increase in the amount of sulphur amendments from 5.0 g dose whereas in gypsum A.niger population decreased only in 10.0 and 12.5 doses (Table 13). The response of other fungi with sulphur and gypsum amendment was more or less in the same trend as found with A.niger. All doses of sulphur amendment increased actinomycetes in rhizosphere and maximum counts were secured with 7.5 g dose. In gypsum amendments actinomycetes population was maximum with 5.0 g dose and decreased with the increasing amount of gypsum. Bacterial population was significantly more at 2.5 and 5.0 g doses of sulphur whereas gypsum at all doses increased population of bacteria as compared to inoculated control.

Table 13. Effect of different levels of sulphur and gypsum on rhizosphere microflora and collar rot pathogen (A. niger) of groundnut

S.No.	Amendments	Dose g/kg seed	Rhizosphere microflora per g of oven dry seed after 20 days			
			A. niger ($\times 10^3$)	Other fungi ($\times 10^3$)	Actinomycetes ($\times 10^5$)	Bacteria ($\times 10^6$)
1.	Sulphur	2.5	12.21	3.12	8.94	1.86
2.	"	5.0	11.18	3.11	12.61	1.82
3.	"	7.5	7.62	2.94	13.57	1.08
4.	"	10.0	5.70	2.87	11.88	0.99
5.	"	12.5	5.04	1.70	11.74	0.97
6.	Gypsum	2.5	17.52	1.33	8.40	2.33
7.	"	5.0	12.90	2.21	11.06	2.18
8.	"	7.5	12.22	2.91	8.98	1.81
9.	"	10.0	10.71	3.06	8.75	1.47
10.	"	12.5	9.62	3.50	8.54	1.32
11.	Control with inoculum	-	11.05	2.53	7.37	1.28
12	Control without inoculum	-	1.0	3.90	8.36	1.67
	SEm. ±		0.5835	0.2193	0.4411	0.1609
	CD 5 %		1.70	0.64	1.29	0.46

Isolation of fungi, bacteria and actinomycetes species from grounnut rhizosphere and seeds :

By following suitable techniques, 23 actinomycetes, different species of eight fungi (Penicillium, Aspergillus, Rhizopus, Fusarium, Rhizoctonia, Sclerotium) and two species of Trichoderma (T. viride and T. harzianum), seven species of bacteria were isolated from seed and rhizosphere of groundnut. Cultures of all species of fungi were maintained on PDA, of bacteria on nutrient agar and actinomycetes on Czapeck's agar with glucose.

Studies on antagonistic effect of different actinomycetes, bacteria and fungal species against A. niger :

(i) The culture filtrate of each species of actinomycetes isolated from groundnut rhizosphere was tested for inhibition zone of A. niger growth. The culture filtrates of two species induced zones of inhibition when tested against an isolate of A. niger by paper disc method (Plate 5).

(ii) In petridishes by cross streak method three actinomycete species showed strong antagonistic reaction against A. niger. These actinomycete species appear to belong to the genus streptomyces. However, more work is needed for their identification.

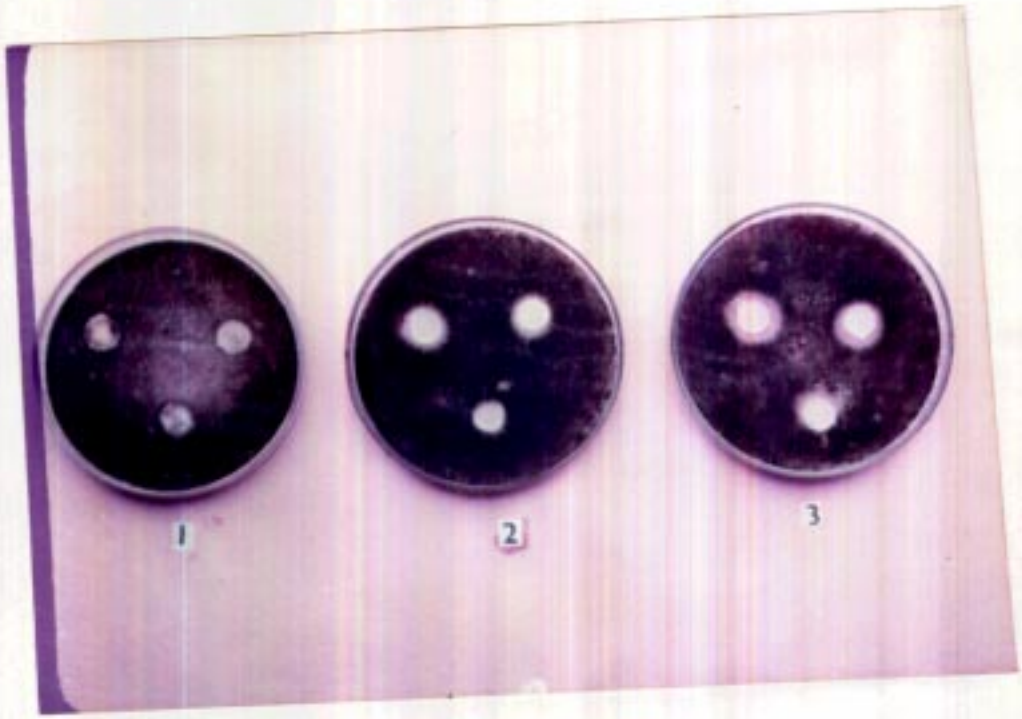


Plate 5.

I. Control showing growth of A. niger on paper disc

II, III. Inhibition zones induced by culture filtrate of two actinomycetes

In case of *T. viride* growth suppressed while *T. harzianum* not only growth of *A. niger* but even grew thereon (Plate 6).

(iii) The effect of seed inoculation of *Trichoderma* and three species of a studied by two methods :

A. Seeds first inoculated with raised on rice medium and or agricultural fungi and acting applied respectively on Maiti and Chakraborty's agar with fungus

B. Seeds, surface sterilized with inoculated with antagonistic tests and them plated on the with *A. niger*.

Seeds inoculated with two sp plated on medium seeded with *A. niger* no growth of *A. niger* and germinated seeds inoculated with *A. niger* and on *Trichoderma* did not germinate and were growth of *A. niger*. In case of actino developed on the seeds in both the ex

Plate 6. Cross streak assay of Trichoderma species and actinomycete against A. niger

I. T. harzianum, II. T. viride, III. Actinomycete

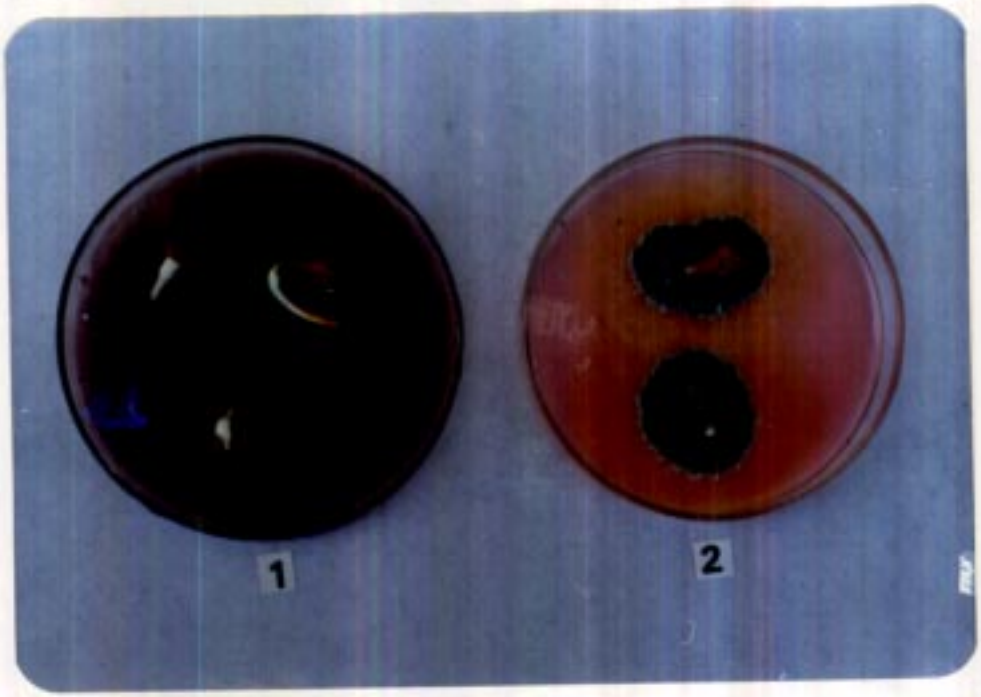


Plate 7 B.

I. Healthy germinated seed with inhibition zone from seed inoculated with T. viride and plated on medium seeded with A. niger

II. A. niger growth on seeds inoculated with A. niger and T. viride

Development of collar rot in seedlings from seeds infected naturally with A. niger :

Transmission of A. niger in seedlings from infected seeds was studied on 1 per cent water agar in petridishes by the method described by Khare et al. (1977) and in pots filled with steam-sterilized sandy-loam soil by the method followed by Chaudary and Mathur (1979). Observations on development of A. niger on seedlings were recorded.

In water agar seedling symptom test 12 % seeds were covered with the growth of A. niger which did not germinate (Plate 8) and 17 per cent of the seeds germinated having growth of A. niger on the cotyledons (Plate 9). As the elongation of hypocotyl took place, A. niger growth developed on it from cotyledons.

Out of 100 seeds transferred to soil, only 78 emerged which showed A. niger growth on cotyledons (Plate 10). Later it passed on cotyledonary nodes and then developed on the hypocotyl and collar region (Plate 11).

Screening of germplasm against A. niger for resistance :

The results on the reaction of different varieties/ lines against A. niger and their respective collar rot incidence are presented in table 14. Out of 233 varieties/

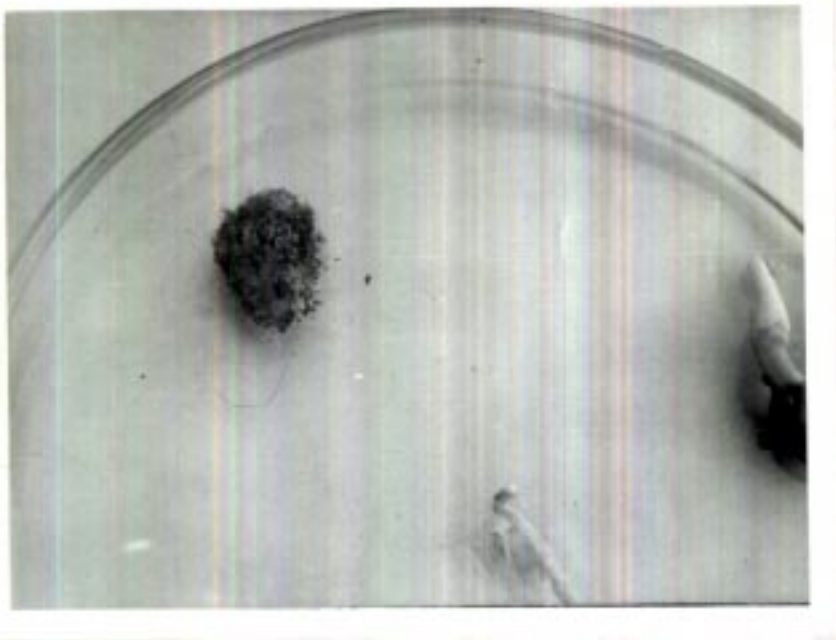


Plate 8. Naturally infected seed of groundnut showing growth of A. niger on 1 % water agar

Plate 9. A. niger growth on cotyledons and hypocotyl on 1 % water agar

Plate 10. A. niger growth on cotyledons emerged from soil

Plate 11. Profuse growth of A. niger on cotyledonary node and hypocotyl/collar region

Table 14. Reaction of groundnut varieties/lines to *A.niger* under artificial inoculation (average data of two years)

S. No.	Source	Variety/line	Origin (country)	Disease incidence	Reaction
	1	2	3	4	5
	NRCG No.				
1.	2965	Ah-30	-	73.3	HS
2.	2967	Ah-44	India	40.0	S
3.	2977	Ah-288	India	33.3	S
4.	2964	Ah-29	-	60.0	HS
5.	2978	Ah-334	India	46.6	S
6.	2980	Ah-447-1	India	53.3	HS
7.	2975	Ah-262	U.S.A.	37.5	S
8.	2970	Ah-84	India	50.0	S
9.	2973	Ah-6140	-	20.0	MS
10.	2957	Ah-7-6	India	35.7	S
11.	2961	Ah-15	Japan	60.0	HS
12.	2918	2878	-	64.2	HS
13.	2906	2794	-	40.0	S
14.	2914	2844	-	60.0	HS
15.	2908	2800	-	71.4	HS
16.	2911	2825	-	80.0	HS
17.	2909	2804	-	42.8	S
18.	2949	Exotic	-	46.6	S
19.	2950	2592	-	60.0	HS
20.	2951	D-5	India	53.3	HS
21.	2944	3069	-	20.0	MS
22.	2946	4079	-	40.0	S
23.	2932	2968	-	13.3	MS
24.	2900	2687	-	53.3	HS
25.	2899	2685	-	57.1	HS
26.	2890	2332	-	40.0	S
27.	2885	638	India	26.6	S

Contd.....

1		2	3	4	5
28.	2888	1011	-	46.6	S
29.	2892	2360	-	42.8	S
30.	2905	2750	-	50.0	S
31.	2893	3506	-	26.6	S
32.	2887	898	-	40.0	S
33.	2866	5-8	-	60.0	HS
34.	2863	VF-780-14	-	46.6	S
35.	2868	19-7	-	40.0	S
36.	2867	14-7-12	-	64.2	HS
37.	2880	69-102	-	6.6	MR
38.	2878	59-127	-	50.0	S
39.	2879	69-101	-	53.3	HS
40.	2873	46-6	-	60.0	HS
41.	2876	53-68	-	40.0	S
42.	2819	Punjab-648-4	India	42.8	S
43.	2824	289-RS-37	-	66.6	HS
44.	2820	Punjab Gold	India	46.6	S
45.	2829	Selection No.12	-	57.1	HS
46.	2807	MPI-2	India	53.3	HS
47.	2803	MF-46-5-143	-	6.6	MR
48.	3362	34-3	-	40.0	S
49.	1957	J-2-5	India	60.0	HS
50.	1936	EC-37485	-	42.8	S
51.	1968	JH-343	-	33.3	S
52.	1937	EC-38279	-	46.6	S
53.	1948	Florunner(106969)	U.S.A.	40.0	S
54.	1955	HG-11	India	57.1	HS
55.	1947	Florigiant(106968)	U.S.A.	46.6	S
56.	1950	8-221-31	India	6.6	MR
57.	1939	EC-39480	Uganda	33.3	S
58.	1940	EC-76444 (B-1)	-	40.0	S
59.	1970	JH-345	-	73.3	HS
60.	1945	F-16	E.Africa	10.0	MR

Cont.....

1	2	3	4	5	
61.	1929	EC-20973	Sudan	53.3	HS
62.	1930	EC-21025	Sudan	40.0	S
63.	1927	EC-20968	Sudan	26.6	S
64.	1917	EC-20920	Sudan	10.0	MR
65.	1919	EC-20927	Sudan	26.6	MS
66.	1922	EC-20949	Sudan	46.6	S
67.	1924	EC-20961	Sudan	13.3	MS
68.	1932	EC-21095	Sudan	33.3	S
69.	1935	EC-37482	-	73.3	HS
70.	1901	EC4078	Fiji	53.3	HS
71.	1911	EC-16672	China	66.6	HS
72.	1895	EC-1690	Tanzania	0.0	I
73.	1893	EC-1541	Nigeria	13.3	MR
74.	1898	EC-1703	Tanzania	100.0	HS
75.	1905	EC-6117	E.Africa	40.0	S
76.	1909	EC-16668	China	42.8	S
77.	1894	EC-1688	Tanzania	26.6	S
78.	1907	EC-6120	E.Africa	46.6	S
79.	1904	EC-6116	E.Africa	93.3	HS
80.	1897	EC-1702	Tanzania	53.3	HS
81.	1884	Dongi(Bhavangadh)	India	20.0	MS
82.	1874	C-1031	India	6.6	MR
83.	1892	EC-1539	Nigeria	53.3	HS
84.	1891	Early Runner	India	80.0	HS
85.	1867	C-176	India	13.3	MS
86.	1865	C-174	India	46.6	S
87.	1871	C-180	India	40.0	S
88.	1870	C-179	India	50.0	S
89.	1861	C-168	India	66.6	HS
90.	1872	C-501	India	20.0	MS
91.	472	VRR-270	India	46.6	S
92.	473	VRR-271	India	40.0	S

Contd.....

	1	2	3	4	5
93.	474	VRR-275	India	10.0	MR
94.	477	VRR-279	India	46.6	S
95.	481	VRR-307	India	20.0	MS
96.	487	TMV-10	India	73.3	HS
97.	517	AH-7326	China	100.0	HS
98.	675	Hua-27	China	73.3	HS
99.	703	NCAC-17014	Bolivia	78.5	HS
100.	820	NCAC-2682	Paraguay	20.0	MS
101.	815	NCAC-1308	Ghana	33.3	S
102.	4875	PI-268858	Brazil	53.3	HS
103.	720	NCAC-16047	Paraguay	20.0	MS
104.	725	NCAC-7238	India	57.1	HS
105.	732	NCAC-15926	U.S.A.	46.6	S
106.	750	NCAC-6755	U.S.A.	66.6	HS
107.	765	NCAC-17340	U.S.A.	10.0	MR
108.	822	NCAC-2719	India	40.0	S
109.	3427	Ah-831	India	6.6	MR
110.	1102	NCAC-769	Uraguay	13.3	MS
111.	1101	NCAC-767	Uraguay	46.6	S
112.	1100	NCAC-766	Uraguay	20.0	MS
113.	1093	NCAC-714	Brazil	35.7	S
114.	1095	NCAC-723	Brazil	10.0	MR
115.	1097	NCAC-738	Brazil	46.6	S
116.	1098	NCAC-761	Uraguay	60.0	HS
117.	1194	NCAC-16071	Paraguay	33.3	S
118.	1189	NCAC-16001	Argentina	13.3	MS
119.	1146	NCAC-17143	Brazil	53.3	HS
120.	305	B-717	India	73.3	HS
121.	303	B-700	India	71.4	HS
122.	301	B-353	Keniya	26.6	S
123.	300	BS-2	India	33.3	S
124.	298	Hong Kong	China	46.6	S

Contd.....

	1	2	3	4	5
125.	297	B-737	India	13.3	MS
126.	296	B-733	India	13.3	MS
127.	306	B-724	India	26.6	S
128.	307	B-725	India	20.0	MS
129.	461	VRR-246	India	46.6	S
130.	460	VRR-242	India	46.6	S
131.	466	VRR-251	India	53.3	HS
132.	1429	RS-16	India	40.0	S
133.	1430	Russian Improved-1	USSR	40.0	S
134.	1431	Short-1	India	66.6	HS
135.	1432	E-22	India	50.0	S
136.	1433	Var-27	Australia	66.6	HS
137.	1434	K-35	India	26.6	S
138.	1435	Kg-6199	India	46.6	S
139.	1437	Taeduk	Taiwan	20.0	MS
140.	1438	Taiwan-No 1	Taiwan	33.3	S
141.	1439	Taiwan No.2	Taiwan	53.3	HS
142.	1440	U4-7-5	Sudan	33.3	S
143.	1441	USA-53	U.S.A.	26.6	S
144.	1442	Ah-8053	Chile	53.3	HS
145.	1448	S7-1-10	Sudan	86.6	HS
146.	1449	U2-1-17	Sudan	66.6	HS
147.	1451	Ah-6661	Nairobi	13.3	MS
148.	1452	Ah-7234	Malaysea	26.6	S
149.	1453	Ah-7770	Kaniuna	80.0	HS
150.	1454	Ah-7865	India	60.0	HS
151.	1455	Ah-7810	India	33.3	S
152.	1456	Ah-7820	India	6.6	MR
153.	1460	Ah-7857	India	20.0	MS
154.	1470	NCAC-17515	India	73.3	HS
155.	341	S-720	India	86.6	HS
156.	302	B-688	India	46.6	S

Contd.....

	1	2	3	4	5
157.	327	S2-11	India	13.3	MS
158.	326	D2/60/1524	India	33.3	S
159.	321	MJ-374	India	66.6	HS
160.	315	Virginia Bunch-46-2	USA	60.0	HS
161.	161	Selection-15	India	20.0	MS
162.	Ak-12-24	Ak-12-24	India	31.5	S
163.		Co.1	India	10.0	MR
164.		CGC-3	India	42.1	S
165.		TG-17	India	31.5	S
166.		TG-16	India	57.8	HS
167.		GAUG-1	India	42.1	S
168.		MK-374	India	100.0	HS
169.		Tyoti	India	15.7	MS
170.		TMV-2	India	68.4	HS
171.		TG-3	India	100.0	HS
172.		J-11	India	36.8	S
173.		SB-XI	India	31.5	S
174.		TG-5	India	30.0	S
175.		JL-24	India	47.3	S
176.		MH-1	India	30.0	S
177.		TMV-12	India	52.6	HS
178.		Chico	U.S.A.	36.8	S
179.		Kadri-3	India	35.0	S
180.		TG-11	India	30.0	S
181.		Gangapuri	India	35.0	S
182.		Selection 13	India	30.0	S
183.		TMV-7	India	21.0	S
184.		BP-1	India	47.3	S
185.		BP-2	India	36.8	S
186.		Kisan	India	31.5	S
187.		GAUG-10	India	25.0	S
188.		RAU-31-3	India	42.1	S

Contd....

1	2	3	4	5
189.	Latur-33	India	40.0	S
190.	C-501	"	15.0	MS
191.	M-13	"	2.0	S
192.	Achali white	"	31.5	S
193.	Karad-4-11	"	10.0	MR
194.	RG-15	"	30.0	S
195.	RS-114	"	13.3	MS
196.	Punjab-1	"	52.6	HS
197.	TMV-10	"	30.0	S
198.	Kapergaon-1	"	36.8	S
199.	GPM	"	35.0	S
200.	M-145	"	26.3	S
201.	M-37	"	45.0	S
202.	S-230	"	42.1	S
VMA, Bombay				
203.	ICGS-11	"	20.0	MS
204.	ICGS-12	"	45.0	S
205.	ICGS-13	"	42.0	S
206.	ICGS-14	"	47.3	S
207.	ICGS-15	"	40.0	S
208.	ICGS-16	"	33.3	S
209.	ICGS-17	"	53.3	HS
210.	ICGS-19	"	40.0	S
211.	ICGS-20	"	36.8	S
212.	ICGS-21	"	17.6	MS
213.	ICGS-22	"	12.5	MS
214.	ICGS-23	"	40.0	S
215.	ICGS-24	"	46.6	S
216.	Robot-33-1	"	40.0	S
ICRISAT				
217.	EPT-1	"	60.0	HS
218.	EPT-2	"	20.0	MS

Contd.....

1	2	3	4	5
219.	EPT-3	India	46.6	S
220.	EPT-4	India	66.6	HS
221.	EPT-5	"	73.3	HS
222.	EPT-6	"	40.0	S
223.	EPT-7	"	60.0	HS
224.	EPT-8	"	73.3	HS
225.	EPT-9	"	53.3	HS
226.	EPT-10	"	86.6	HS
227.	EPT-11	"	53.3	HS
228.	EPT-12	"	33.3	S
229.	EPT-13	"	46.6	S
230.	EPT-14	"	53.3	HS
231.	ICG-1326	"	36.8	S
232.	ICG-1455	"	20.0	MS
233.	KG-1393	"	13.3	MS

Immune	(I)	No symptoms of disease
Resistant	(R)	1 % or less mortality
Moderately resistant	(MR)	1-10 % mortality
Moderately susceptible	(MS)	11-20 % mortality
Susceptible	(S)	21-50 % mortality
Highly susceptible	(HS)	51 % or more mortality

lines tested, one line EC-1690 (NRCG No.1895) was found immune 15 moderately resistant, 29 moderately susceptible, 115 susceptible and 73 were highly susceptible.

Influence of VA mycorrhiza, Glomus fasciculatus Gerdemann Trappe., on groundnut plants :

Several workers have reported the beneficial effect of VA mycorrhiza assocations on the growth of plants in nursery and field soils, particularly in low or medium phosphote soils. Recently work with mycorrhizal fungi has indicated that because of their ability to colonize both roots and soil, they may have a role in the biological control of soil-borne fungal root diseases (Schenck, 1981). The nature of effect exerted by the VA mycorrhizal fungus on the pathogen is not known in most instances. However, in a few studies the differences in chemical constituents between mycorrhizal and non-mycorrhizal plants were observed.

In these studies effect of VA mycorrhiza, G. fasciculatus, on plant growth, total and ortho-dihydroxy phenols in general and on collar rot development in groundnut, in particular was studied.

(i) Response of groundnut plants to G. fasciculatus :

Groundnut, variety RS-1, was grown in pots filled

Table 15. Shoot and root dry weight, phosphorus content in 50 day old groundnut plants as influenced by the inoculation of G. fasciculatus (based on mean of 20 plants)

Treatments	Dry matter (g/plant)		DM shoot / DM root	Phosphorus				Spores/ 50 ml soil	Per cent colonization
				Content(%)		Uptake(mg/plant)			
	Shoot	Root		Shoot	Root	Shoot	Root		
Mycorrhiza inoculation	1.907	1.345	1.41	0.186	0.154	3.547	2.071	187	67
Mycorrhiza not inoculated	0.957	0.719	1.33	0.160	0.082	1.531	0.587	-	-

Plate 12. Stained root of groundnut showing external mycelium, mycelium inside cortex and vesicles of G. fasciculatus (x 400)

with steam sterilized sandy-loam soil with and without mycorrhizal inoculum. Mycorrhizal inoculum contained extramatrical chlamydospores and infected root bits of sudan grass which was infected with G. fasciculatus. Observations on dry matter and phosphorus content of root and shoot, spore population and per cent mycorrhizal root infection were recorded on 50 day old plants.

Results in table 15 indicate that mycorrhizal inoculations induced an increase in shoot and root dry matter as compared to non-inoculated plants. The proportion of dry matter between shoot and root of mycorrhizal plants was more compared to non-mycorrhizal plants. The per cent content and uptake of phosphorus in roots and shoots of mycorrhizae inoculated plants were comparatively higher than uninoculated plants.

(ii) Effect of G. fasciculatus inoculations on collar rot incidence in groundnut :

The influence of G. fasciculatus in collar rot development in groundnut was evaluated by growing groundnut in pots filled with steam sterilized sandy-loam soil with and without mycorrhiza inoculum. A. niger was applied in both the treatments separately in the form of seed inoculation, soil infestation and seed and soil both inoculated. Observations were recorded for appearance of number of diseased plants in all the treatments.

Table 16. Effect of VA mycorrhiza, *G. fasciculatus*, on collar rot development in groundnut with different methods of *A. niger* inoculation

S. No.	Treatments	Disease incidence (%)	Transformed value	Per cent decrease of disease over control
1.	Seed inoculated with *A. niger* + mycorrhiza	16.0	23.38	66.17
2.	Seed inoculated with *A. niger*	47.3	43.42	-
3.	Soil infested with *A. niger* + mycorrhiza	9.8	15.88	75.37
4.	Soil infested with *A. niger*	39.8	33.07	-
5.	Both seed and soil inoculated with *A. niger* + mycorrhiza	19.1	25.71	76.36
6.	Both seed and soil inoculated with *A. niger*	80.8	64.27	-
	SEm. ±		2.731	
	CD 5 %		8.23	

Plate 13. Collar rot development in groundnut with and without mycorrhizal inoculations:

I. Seed and soil both inoculated with A.niger

II. Seed inoculated with A. niger

III. Seed and soil both inoculated with A. niger + mycorrhiza

The data presented in table 16 indicate that mycorrhiza significantly controlled collar rot in groundnut plants developed from seed, soil and seed and soil both. Maximum reduction in collar rot was obtained in the treatment having both seed and soil inoculum. Recent efficiency of disease control by mycorrhiza over their respective checks ranged from 76.36 to 66.17.

(iii) Effect of G. fasciculatus on total and O-D phenols content in root of groundnut plants :

Groundnut was grown in pots filled with steam sterilized sandy-loam soil with and without mycorrhiza. Groundnut plants were harvested at 10, 20, 30, 40 and 50 day of plant growth. Fresh root samples were grounded well, extracted with ethanol. From this extract total and O-D phenols were estimated by the methods of Bray and Thrappe (1954) and Arnow (1937), respectively. Mycorrhizal infection in the roots was determined after staining with trypan blue.

Total and O-D phenol content of mycorrhizal and non-mycorrhizal groundnut roots and percentage mycorrhizal infection are given in the table 17. It is evident from results that total phenol content was higher in mycorrhizal roots. Increase in phenol content was observed from 10th day onward upto 50 day stage, upto which the observations

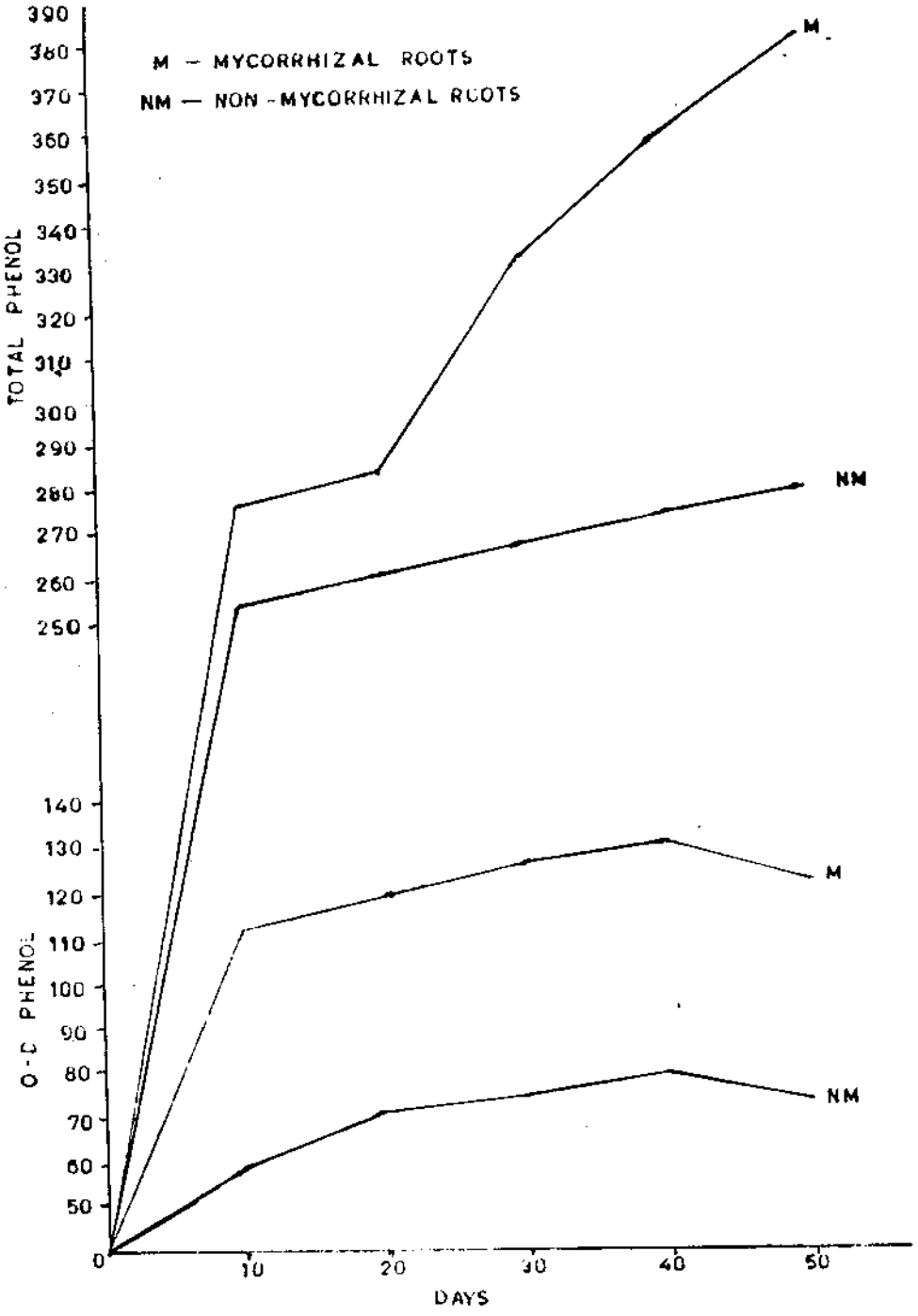
Table 17. Phenol content and per cent colonization of mycorrhizal and non-mycorrhizal roots of groundnut plants

Age of plants (days)	Total phenol (ug/g fresh wt. of sample)		O-D phenol (ug/g fresh wt. of sample)		Per cent colonization	
	M	NM	M	NM	M	NM
10	277.7	254.1	112.4	59.1	0	0
20	285.4	261.6	219.7	71.5	17	0
30	332.2	268.7	127.5	75.1	53	0
40	359.7	274.9	131.6	79.9	59	0
50	381.3	281.2	123.3	74.2	67	0

M = mycorrhizal roots

NM = non-mycorrhizal roots

FIG 1 :- TOTAL AND O-D PHENOL CONTENT IN MYCORRHIZAL AND NON-MYCORRHIZAL GROUNDNUT ROOTS AT DIFFERENT CROP STAGES



were recorded. Maximum increase in phenol content was recorded on 30 day stage. A similar trend was found with respect to O-D phenol content in mycorrhizal and non-mycorrhizal roots, except that it dropped on day 50.

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DISCUSSION

Rhizosphere - site of high micro-biological activity - exerts profound influence upon the plant by decomposition of organic matter, by associatic and antagonistic relationships and by actual parasitism of plant roots. This rhizosphere microflora can be altered through organic and inorganic amendments, microbial cultures, fertilizers, and seed treatment with chemicals including antibiotics. In some cases the changes have been found unfavourable for plant pathogens. Most of the soil invading fungal plant pathogens are incapable to increase sufficiently in growth in competition with others for food and nutrition, and on account of the presence of antagonistic micro-organisms such as fungi, bacteria and actinomycetes in soil. However, in case of permanent soil dwelling plant pathogens, such effects do not affect much in natural conditions for their survival and build up of inoculum.

There are several phytopathogens which can with stand adverse environmental conditions better than others and thus their survival ability is better. In case of collar rot pathogen of groundnut, A. niger was found in the rhizosphere of diseased and healthy plants ranging from 56.3 to 64.7 per cent and 9.9 to 25.3 per cent of total mycoflora, respectively,

in different agroclimatic conditions prevailing in several districts of Rajasthan. Soil type varied from light to heavy with acidic to alkaline in reaction and containing varying amount of organic matters.

Several fungal plant pathogens are known to survive in the rhizosphere of host plants. A. niger is capable to increase sufficiently in population in rhizosphere of groundnut owing to its inherent survival ability (Joffe, 1962 and 1972). In present investigations studying the influence of inoculum density on disease development it was found that with the increase in inoculum load on seed, pre-emergence rot of groundnut increased and there was relative decrease in rhizosphere microflora. Similar results of inoculum density of F. culmorum and its correlation with pre-emergence death of wheat seedlings has been reported by Malalasekera and Colhoun (1969).

Currently emphasis is being placed by various workers on the application of fungicides to infested soil or seed to control collar rot of groundnut by reducing or eliminating population of A. niger (Jackson, 1968; Sidhu and Chohan, 1971; Aulakh and Sunar, 1972; Agnihotri and Sharma, 1972; Aulakh and Chohan, 1975; and Siddaramaiah et. al., 1979). A desirable fungicide is one which selectively kills or inhibits pathogenic fungi in soil when used at

concentrations non-toxic to other soil micro-organisms. The remaining micro-organisms could then serve as a biological buffer to the reestablishment of plant pathogens and also continue activities that are essential to soil fertility (Malcom and Young, 1965). Vaartaja et al. (1964) emphasized that many soil fungicides significantly controlled the disease probably through changes in antagonistic flora and without which the fungicide would be only partially effective. In the present experiments it was found that non systemic fungicides, thiram and difolatan significantly controlled collar rot and reduced A. niger population in rhizosphere and initially decreased actinomycetes but later actinomycetes population increased approximately two times as compared to untreated inoculated control. Systemic fungicides, bavistin and bayleton could control collar rot and decreased A. niger population nearly 10 times compared to untreated control. Leroux and Gredt (1976) showed that bayleton is toxic in vitro to Benicillium species. Bayleton reduced actinomycetes, fungi and bacteria rapidly and subsequently increased but did not return to the level present in untreated and uninoculated control whereas bavistin increased actinomycetes significantly and reduced fungi and bacteria considerably. These experiments were repeated and results were similar.

In subsequent experiments it was observed that fungi, Trichoderma viride and T. harzianum, and three actinomycetes isolated from rhizosphere were antagonistic to the growth of A. niger in vitro. T. harzianum not only inhibited the growth of A. niger but over grew the growth area also. Inhibition of A. niger by metabolites of T. harzianum has been reported by Hutchinson and Cowman (1972). Seeds inoculated with T. viride and T. harzianum germinated and remained free from infection of A. niger growing in petri-plates. It is thus possible to treat seeds with Trichoderma species for elimination of infection from soil-borne A.niger inoculum.

In the present work seeds inoculated with actinomycetes was found that A. niger developed on the seeds. This might be due to lack of sufficient population of actinomycetes required for the inhibition of A. niger. More work on this aspects is needed.

The addition of inorganic and organic amendments, fertilizers to soil have been demonstrated by various workers to reduce the infectivity of several soil-borne plant pathogens. In present investigations efforts were made to control A.niger by providing suitable conditions for the existing microflora of the soil for its increased antibiotic activity as found by many workers in other soil-borne diseases(Bagyaraj and

Rangaswami, 1967; Schuepp and Green, 1968; Schuepp and Frei, 1969).

In most of the cases addition of organic amendments to soil has been found to reduce root rot diseases caused by soil-borne plant pathogens. In the present study, the amendment with lower dosages of most of the oil cakes to soil stimulated A. niger growth in the rhizosphere of groundnut plants whereas at higher dosages population of A. niger and collar rot development was reduced. Karanj cake at lower dose reduced A. niger population possibly due to increase in other fungi, actinomycetes and bacteria which may have suppressive effect due to nutritional competition or increase in number of antagonists in soil. Neem cake may be itself toxic to A. niger as well as other microflora present in the soil at higher concentration. Sankhla et al. (1969) found in vitro that oil cakes at lower concentrations stimulated plant pathogens and inhibited their growth at higher concentrations. The results of disease control, in general, agree with those obtained by other workers for various diseases (Gautum and Kolte, 1979; Lakshmanan and Nair, 1984; and Singh, 1968; Thakore et al., 1987).

Application of fertilizers to the soil has a direct influence on the microbial population and the influence varies with type of fertilizer added (Katznelson et al.,1984;

Reddy and Rao, 1965; Henis and Chet, 1968). Several soil borne pathogens have been controlled by application of fertilizers. In present investigations higher concentration of nitrogen and phosphorus singly or in combination reduced collar rot and A. niger population. The reduction in disease development seems to be more, possibly, due to structural or biochemical changes as Millar and Ohlrogge (1958) reported that higher concentration of phosphate fertilizers results in root development, and increases in root development, in turn, affect the extent to which the soil fertilizer system is brough into contact with the root system. Duncan and Ohlrogge (1958) further found some more beneficial effect of phosphorus on root development when fairly large supplies of nitrogen present in the soil. The reduction of A. niger growth in soil may possibly be due to competition for nutrition with increasing large number of micro-organisms or antagonism of microflora with the pathogen.

In the experiment, soil amendments with different levels of sulphur and gypsum, A. niger population was reduced with the increase in actinomycetes and bacteria in the rhizosphere of groundnut and collar rot disease was reduced. Mc Creasy (1967) reported that sufficient sulphur application to give a soil acidity of pH 5.2 controlled potato tuber diseases (Scab, powdery scab, black surf and

tuber blight). With the application of sulphur and gypsum rhizosphere mycoflora in the uninoculated plants of groundnut increased and in inoculated plants with R. solani and R. bataticola population decreased (Sunar and Chohan, 1971). Gypsum and sulphur also supply micro-nutrients essential for the healthy growth of plants and increase the resistance in plants (Kanwar and Chauwla, 1963). Amendment of soil with sulphur and gypsum before sowing groundnut is therefore beneficial not only to meet the requirement of Ca and S but also to control the collar rot disease of groundnut.

In present investigations on screening of germplasm against collar rot development, it was observed that most of the promising lines for yield parameters are in moderately susceptible to highly susceptible groups. Besides this, some of the varieties/lines are found resistant at other places are susceptible in present studies. This may be due to variation of strain of A. niger and the amount of inoculum used for screening at different places. Dange and Saradava (1985) found J-11 resistant whereas in present experiments it was susceptible. If promising susceptible varieties are grown in soil amended with suitable amendment there is possibility to get more yield and this needs more detailed studies.

Vesicular-arbuscular mycorrhizae are known to enhance the growth and yield, in many crop plants, particularly in soil with low or moderate phosphate status. There are some reports on the control of plant diseases also with VA mycorrhizae inoculations.

Inoculations with VA mycorrhizae, Glomus fasciculatus Gerdemann and Trappe resulted in extensive colonization and spread of the fungus in groundnut roots, enhanced growth of root and shoot. Phosphorus content and uptake was found more in the root and shoot of mycorrhiza inoculated plants. Similar results with mycorrhiza, G. mosseae, inoculations on groundnut have been reported by Daft and El.Giahmi,(1976).

Mycorrhizal fungi can deter or significantly reduce the effects of some pathogens on the host and are considered to have a role in biological control of soil-borne diseases (Schenck, 1981). During present study, the evaluation on the effect of G. fasciculatus inoculations against three types of A. niger inoculations in groundnut have shown that mycorrhizae exhibited significant control of collar rot disease. Mycorrhizal colonization has been found to reduce the effect of several pathogens in their hosts(Schenck & Kallam, 1978). However, the nature of the effect exerted by VA mycorrhizal fungi on the pathogens is not known in most cases. In a few studies the differences in chemical

constituents between mycorrhizal and non-mycorrhizal plants were observed. In present studies the total phenol and O-D phenol content in mycorrhizal groundnut roots was much higher than non-mycorrhizal roots and hence, higher amount of phenols and phosphorus in roots of groundnut mycorrhizal plants might be the factors responsible for increased resistance. Presence of phenolics in plants have been correlated with resistance to pathogens by many workers (Bhatia et al., 1972). Krishna and Bagyaraj (1983) reported that the concentrations of O-D phenol present in mycorrhizal roots inhibited in vitro growth of root pathogen, Sclerotium rolfsii. In the experiment with fertilizers, phosphorus was found to reduce collar rot, thus hitherto, it may be responsible for resistance in roots. However, more detailed experimentation are needed.

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SUMMARY

1. During survey in July-Aug., 1983, in the districts: Ajmer, Bhilwara, Chittorgarh, Kota and Udaipur, of Rajasthan, incidence of collar rot of groundnut ranged from 5.5 to 42.7 per cent. Maximum disease was found in Ajmer, the disease incidence was 25.2 - 42.7 per cent, and minimum in Udaipur where it was 5.5 to 17.9 per cent. In these areas soil type varied from light to heavy and acidic to alkaline in reaction with varying amount of organic matter. A. niger was detected, 56.3 to 64.7 per cent of total mycoflora, from the rhizosphere of diseased plants collected from all these places.

2. A relatively selective medium for isolation and enumeration of A. niger, collar rot pathogen of groundnut, from soil was developed. The medium was composed of: Dextrose, 20.0 g; Peptone, 5.0 g; Potassium dihydrogen orthophosphate, 1.0 g; Magnesium sulphate, 0.5 g; Sodium chloride, 55.0 g; Rose-Bengal, 30 mg; Streptomycin sulphate 30 mg; Agar, 20.0 g, Distilled water, 1000 ml.

3. Pre-emergence rotting and rotting of seeds increased with logarithmically increasing A. niger

inoculum load per seed. Population of other fungi, actinomycetes and bacteria in the rhizosphere of groundnut plants, raised from inoculated seeds, decreased with the increase in A. niger population in the rhizosphere and inoculum load on the seed.

4. Development of A. niger from naturally infected seed to seedling was studied and observed that the pathogen caused rotting of seeds, pre-emergence rot and collar rot by developing first on the cotyledons and then passed to elongated hypocotyl and to collar region of the seedlings.

5. In seed treatment experiments, among non-systemic fungicides, thiram and difolatan controlled collar rot significantly and the disease incidence was 8.3 and 9.1 per cent, respectively, whereas in control it was 69.1 per cent. These fungicides initially decreased actinomycetes in the rhizosphere but in the rhizosphere of 30 day old plants actinomycetes population increased nearly two times as compared to control i.e. seeds inoculated with A. niger.

Systemic fungicides, Bavistin and Bayleton gave disease incidence 4.1 and 9.2 per cent, respectively and also decreased A. niger growth in rhizosphere approximately ten times as compared to inoculated control. Bavistin increased actinomycetes significantly and decreased other fungi and bacteria in the rhizosphere of 10 and 30 day old plants

whereas Bayleton reduced actinomycetes, fungi and bacteria initially and later increased but did not reach the level present in the rhizosphere of 30 day old inoculated plants.

6. Lower dosages (5 g/kg of soil) of different oil cakes increased collar rot incidence except in case of Karang and Neem cakes the disease was reduced significantly. In higher dosage, 15 g/kg of soil, Neem cake and Mahwa cake reduced the disease by 72.9 per cent.

At 15 g/kg of soil dosage of oil cakes, the population of other microflora in rhizosphere increased with the reduction of A. niger population, except in Neem cake where at 10 and 15 g dose the population of all micro-organisms was less. At 5 g dose, Karanj cake increased other fungi, actinomycetes and bacteria and reduced A.niger population significantly in the rhizosphere of 10 and 30 day old plants.

7. In case of nitrogen and phosphorus at N_{100} P_{200} dosage the disease incidence was 9.1 per cent whereas in N_0 P_0 it was 47.5 per cent; in P_{200} dose disease was 12.4 per cent. Higher dosages of N and P and their combinations reduced A. niger population in the rhizosphere of 30 day plants. All the dosages of N and P and their combinations, except N_{25} P_{200} and N_{50} P_{200}, increased actinomycetes

population in the rhizosphere of 30 day plants. Maximum population of other fungi was found in N_{50}, N_{100} and N_{100} P_{200} dosages.

8. Sulphur at 12.5 and 10.0 g/kg of soil dosages reduced collar rot incidence by 71.36 and 59.81 per cent, respectively. A. niger population in rhizosphere was significantly reduced by 12.5, 10.0 and 7.5 g dosages of sulphur. Maximum population of actinomycetes and other fungi were found with sulphur at 7.5 g dose.

9. Two species of Trichoderma, viz., T. viride and T. harzianum and three actinomycetes were found antagonistic to A. niger by cross streak method. Seeds inoculated with Trichoderma species germinated and remained free from A.niger infection when plated in the petridishes containing medium seeded with the pathogen.

10. Out of 233 varieties/lines, one line EC-1690 (NRCG No. 1895) was found immune to A. niger and fifteen lines were moderately resistant having disease incidence of 1-10 per cent. Remaining 217 varieties/lines were either moderately susceptible or susceptible or highly susceptible.

11. Vesicular-arbuscular mycorrhiza, Glomus fasciculatus, inoculations in groundnut plants induced an increase in dry

matter and phosphorus content in root and shoot as compared to non-mycorrhizal plants. The proportion of dry matter between root and shoot in mycorrhizal plants was more as compared to non-mycorrhizal ones.

Mycorrhizal inoculations significantly controlled collar rot development in groundnut from seed and soil-borne inoculum and their combination and the reduction in disease was 66.17, 75.37 and 76.36 per cent, respectively.

Total and ortho-dihydroxy (O-D) phenols were higher in mycorrhizal roots and increase in phenol content was observed from the 10th day onwards, but O-D phenol dropped on 50th day. Maximum increase in phenol content was recorded on 30 day stage of the plant.

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