STUDIES ON THE INTERRELATIONSHIP BETWEEN RHIZOCTONIA SOLANI AND ROOT-KNOT NEMATODE, MELOIDOGYNE INCognita ON MUNGBEAN

ABSTRACT

THESIS
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ABSTRACT

Leguminous pulses occupy an important position in human dietary as a source of protein (17-43%) and supplement to cereal based diet. Pulses offer the most practical means of eradicating widespread protein malnutrition in this country. Importance of grain legumes in the world farming system has been emphasized in recent years and in national nutritional upliftment programme more attention has been given to balanced cereal-pulse diet than that of either component alone. Of all the food crops in India, production of pulses has remained stagnant, fluctuating for more than three decades. Out of the important factors considered to be responsible for the low inputs of grain legumes, pests and pathogens have been recognised as major obstacle. Amongst the various pulse crops grown in India, greengram is the third important widely grown crop of the country. Like other pulse crops its production is also subject to several constraints including those of pests and diseases which play a leading role in the yield reduction. A casual survey of greengram fields in and around the Aligarh district (U.P. State) revealed the frequent and concomitant occurrence of root-knot nematode, *Meloidogyne incognita* and root-rot fungus, *Rhizoctonia solani* associated with the unthrifty growth of this crop. As we know that in nature plants are exposed to multipathogenic conditions and these pathogens as well as other micro-organisms are continuously interacting with each other resulting in greater damage. Therefore, it was
considered desirable to study the pathogenicity and interactive
effects of *Meloidogyne incognita* and *Rhizoctonia solani* on the
physic-morphological characters of greengram cv. T-44 in
presence and absence of *Rhizobium*. Efforts were also made to
minimize the damage caused by these pathogens and in this regard
the efficacy of certain chemicals and organic additives (oil-
seed cakes) were tested against the disease complex. Since
inoculum threshold levels of pathogens may vary under different
sets of experimental conditions, the pathogenicity tests were
conducted using five inoculum levels, each of *M. incognita* and
*R. solani* separately on greengram.

Investigations on the pathogenicity of *M. incognita* and
*R. solani* confirmed the destructive effect of these two
pathogens on the greengram (mungbean) cv. T-44. Lowest inoculum
level of both the test pathogens caused no significant damage or
plant growth reduction. Significant damage to plant growth
occurred at or above 1000 Jg of *M. incognita* and/or 1.0g or
above of *R. solani*/kg soil both in bacterized and unbacterized
plants. The test pathogens also reduced the number of
nodules/plant. However, a significant reduction in nodulation
was recorded at 500 Jg of nematode and/or 0.5g of fungus. *R.
solani* was more damaging than *M. incognita*. A significant
linear relationship between initial and final nematode
population was observed but, the rate of nematode multiplication
decreased with the increase in the inoculum level. Root galling
was, however, directly proportional to inoculum level showing an
increase with the increasing inocula of nematode. Similar trend
was observed with respect to increase in root rotting in the increasing inoculum level of fungus.

The interaction between *M. incognita* and *R. solani* was studied using variable inoculum levels and their combinations. In the individual inoculation of test pathogens, the reduction in plant growth and nodulation was inoculum dependent. The reduction in plant growth and nodulation was directly proportional to the increase in the inoculum level of test pathogens. Initial inoculum level did not cause any significant reduction of plant growth, however, in the increasing inocula and all concomitant inoculations of test pathogens the reduction was statistically significant over uninoculated control. In bacterized plants, the reduction in plant growth and nodulation in simultaneous inoculation of each combination was comparatively less than the sum total of reductions caused by each pathogen alone, thereby showing negative interaction. Whereas, in unbacterized plants, the combination of variable inoculum levels showed a synergistic effect on plant growth reduction (Positive interaction). Nematode multiplied to a varying degree when inoculated alone. The rate of population increase declined at the higher inoculum levels of nematode both in bacterized and unbacterized plants. On the other hand, root galling increased in the increasing inocula of nematode. The fungus, *R. solani* showed an antagonistic effect on the rate of nematode multiplication and root galling. The effect was higher when highest fungal inoculum levels were used with lowest nematode inocula. Root-rot showed considerable enhancement with
the increase in the inoculum levels of fungus alone and in its various combinations with nematode, the highest being in the concomitance of higher inoculum levels of test pathogens.

Studies on the effect of simultaneous and sequential inoculations of test pathogens showed that the reduction in plant growth and nodulation was maximum in simultaneous inoculation, followed by sequential inoculations of nematode, 15 days prior to fungus and least in the treatments where fungus preceded nematode inoculation. In all the concomitant inoculations, the effect of interaction on plant growth and nodulation was less than additive (antagonistic) except, in the simultaneous inoculation of unbacterized plants, where the resultant effect on plant growth was found to be synergistic. The fungus, *R. solani*, whether inoculated simultaneously or sequentially reduced the rate of nematode multiplication and root galling significantly as compared to when nematode was present alone. The reduction was significantly high when fungus preceded the nematode inoculation. On the other hand, the root rotting due to fungus increased markedly in all combinations with nematode, the highest being in simultaneous inoculation and least in the sequential inoculation of nematode prior to fungus.

In general unbacterized plant showed lesser growth and greater damage than the bacterized ones, when inoculated singly or in various combinations of pre-, post- and simultaneous inoculation. Moreover, in absence of *Rhizobium*, the Rf value and gall number was highest than in its presence, both in singly and concomitantly inoculated plants. Similar trend was observed with
respect to root-rot index. Plant health was improved as a result of bacterization and therefore, the bacterized plants suffered lesser damage than the unbacterized ones in different pathogenic treatments.

For determining the effect of test pathogens on biochemical constituents, the studies were conducted to investigate the impact of *M. incognita* and *R. solani* singly or concomitantly on the nitrogen, phosphorus, potassium and chlorophyll contents of greengram cv. T-44 in presence and absence of *Rhizobium* and also their influence on the leghaemoglobin concentration of root nodules. Pathogenic infections caused a considerable variation in the nutrient status (NPK contents) of host plants. Results showed a significant decrease in the NPK contents of leaf, stem and root of plants inoculated with either of the pathogen singly (except in *M. incognita* inoculated roots) or in combination, both in bacterized and unbacterized plants. On the other hand, there was an increased accumulation of NPK contents in the roots of plants inoculated with nematode alone, being significantly higher than control except, the nitrogen content of bacterized plants which, however, did not show any marked variation from that of uninoculated control. Individually *R. solani* caused greater reduction than *M. incognita*. However, the reduction was more pronounced when both these pathogens were inoculated concomitantly.

Similarly, the leaf chlorophyll content also showed marked reduction both in individual and combined pathogenesis of
test pathogens. The loss was significantly high in concomitantly inoculated plants than in those inoculated singly with either of the pathogen alone. Chlorophyll a was reduced to a greater extent as compared to chlorophyll b and total chlorophyll.

Unbacterized plants, in general, recorded a lower concentration of nutrient elements (NPK) and leaf chlorophyll contents as compared to bacterized plants both in presence and absence of test pathogens. Moreover, the plants in absence of Rhizobium also showed a greater decrease in the NPK and chlorophyll contents than in its presence, in different pathogenic treatments.

The observations with respect to the effect of pathogens on leghaemoglobin (lb) concentration revealed that lb content was significantly reduced as a result of nematode and/or fungus infections. Maximum reduction was, however, recorded in the combined pathogenesis of both the test pathogens.

Experiments were also conducted to study the efficacy of two chemical products (carbofuran and bavistin) separately and in mixture and two different doses of four oilseed cakes (neem, castor, mustard, mahua) against root-knot nematode, M. incognita and root-rot fungus, R. solani attacking greengram cv.T-44, singly or concomitantly.

Soil application of chemicals, carbofuran and bavistin (both separately or in mixture) significantly reduced the population of M. incognita and suppressed the root-knot and root-rot development. Consequently, the plant growth and nodulation was also improved significantly as compared to
untreated controls. In all the cases the combined use of chemicals showed a better performance than their individual usage. Nematode and fungus inoculated plants showed a varied response to carbofuran and bavistin treatments. Carbofuran treatment improved the plant growth and nodulation of nematode inoculated plants more significantly than bavistin whereas, the bavistin treatment did so in the fungus inoculated plants. However, in the absence of pathogens or concomitantly inoculated plants both the treatments were at par to each other. Chemical treatments also stimulated the growth and nodulation of uninoculated plants markedly over untreated ones.

Incorporation of oil seed cakes of neem, castor, mustard and mahua proved to be highly effective against the disease caused by *M. incognita* and *R. solani*, alone and in combination on mungbean. Plant growth showed a substantial improvement in both the doses of all the oil cakes over untreated control except in mahua cake treatments. Similarly the nodulation was also enhanced significantly in all oil cake amendments over untreated control. Higher doses (20g/pot) of neem cake was significantly superior to other treatments with respect to the improvement in plant growth and nodulation. On the other hand, the higher dosage of mahua followed by neem cake suppressed the average nematode population, root-knot and root-rot development more significantly, than any other oil cake amendment. In the absence of either of the pathogen, the plant also showed improved growth and nodulation in comparison to untreated controls, when subjected to the oil cake amendments.
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DEDICATED

TO

MY HOMELAND

In the Loving memory of

my
Beloved Uncle Late Gh. Mustafa

and
Dearmost friend Late Showkat Hussain
CERTIFICATE

This is to certify that the thesis entitled "Studies on the interrelationship between Rhizoctonia solani and root-knot nematode, Meloidogyne incognita on mungbean", embodies a faithful record of original and bonafide research work carried out by Mr. Naseer Hussain Shah under my supervision. No part of this thesis has been submitted for any other degree or diploma. It may be submitted to the Aligarh Muslim University, Aligarh for the consideration of the award of the degree of Doctor of Philosophy in Botany.

Dated: 22.11.94

(M. Farooq Azam)
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INTRODUCTION

Pulse crops, also called grain legumes, have remained as mainstay of Indian agriculture for centuries. They occupy an important position in human dietry and constitute the major source of protein to the overwhelming large number of vegetarian population in this country. Besides being important constituents of human diet, pulses also serve as suitable green manure crops and an excellent forage and grain concentrates for cattle feed (Kaul and Sekhon, 1974). Pulse crops play an important role in agricultural economy of India by virtue of their ability to fix atmospheric nitrogen in symbiotic association with Rhizobium. Every pulse plant is in itself a mini-fertilizer factory by contributing substantially to the enrichment of the soil. Together they add many times more nitrogen to our soils per unit area than is added in the form of chemical fertilizers. With the help of their deep penetrating and well spread root system the pulses utilize the limited available moisture more efficiently than many other crops including cereals, and also contribute substantially to the loosening of the soil. Because of this, farmers have chosen to grow pulse crops under highly diversified conditions.

India is the major pulse-growing country of the world, accounting roughly for one third of the total world area under pulses and one fourth of the total world production. Even with an area of about 23 million hectares of land under these crops, the production of food grains has been stagnating between 10-12
million tonnes over the last three decades. This has become a matter of great concern as the country has not been able to achieve self sufficiency in pulse requirements because of large human population which is increasing at an alarming rate. The stagnant production of pulses has adversely affected their availability and the per capita availability of pulses per day has progressively declined from a peak of 64 grams achieved in mid-fifties to less than 40 grams at present as against the FAO/WHO recommendation of minimum pulse requirement of 80 grams per capita (Fazal, 1993).

Over a dozen pulse crops are grown in India, the chickpea (Cicer arietinum L.) and pigeonpea [Cajanus cajan (L.) Millsp.] are the most important ones followed by greengram [Vigna radiata (L.) Wilczek], blackgram [V. mungo (L.) Hepper], pea (Pisum sativum L.), cowpea [Vigna unguiculata (L.) Walp.], lentil (Lens culinaris Medic.), mothbean [V. acontifolia (Jacq.) Marechal], horsegram [Macrotyloma uniflorum (Lam.) Verdc.] and lathyrus (Lathyrus sativus L.). The major pulse growing states are Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh, Bihar, Maharashtra, Orissa, Haryana, Tamil Nadu, West Bengal, Punjab and Gujrat. These are predominantly grown under rainfed and marginal lands with low inputs and without plant protection cover.

Pulses are highly susceptible to pests and diseases which are responsible for reduction and uncertainty in their yields (Grewal, 1988). The crops are heavily attacked by fungi, viruses, bacteria and nematodes. The major diseases caused by
these pathogens include wilts, root-rots, blights, rusts, powdery mildew, mosaics, and stunted growth resulting from the attack by root-knot and cyst nematodes (Nene, 1988).

Greengram is the third important pulse crop of the country next to chickpea and pigeonpea covering an area of 2.86 million hectares representing 12% of the total pulse acreage, but its contribution to total pulse production is hardly 8%. India and central Asia is considered to be the primary centre of origin of greengram (Vavilov, 1926). However, in the recent past it has been introduced to eastern and central parts of Africa, West Indies and USA (Jain and Mehra, 1980). Greengram is grown in almost all the states of India and is cultivated mainly as kharif crop. It is also grown as rabi season crop in southern India, where winter is mild. The crop usually grows on wide range of soils ranging from sandy loam to alluvial, red laterite and black cotton soils. However, it has been observed that crop thrives best on lighter soil with good drainage. In India it is grown from a sea level upto an altitude of 2000 m largely as a dryland crop (Jeswani and Baldev, 1990).

Greengram has shown a significant increase in area as well as in production over time. Comparing the quinquennium average during 1981-82 to that of 1986-87, the area increased from 2.65 to 2.97 million hectares and production from 0.90 to 1.17 million tonnes. During the corresponding period, the productivity increased from 338 kg to 394 kg per hectare. However, the production of greengram after touching a level of 1.18 million tonnes in 1986-87 declined to 1.09 million tonnes
in the following year. This happened despite expansion in area under the crop as the yield declined from 392 to 348 Kg per hectare (Khanna and Gupta, 1988).

Greengram is rich in proteins and vitamin B and its seeds are eaten as dal after boiling. The dried and green stalk leaves of greengram are also used as fodder and green pods are consumed as vegetable.

Unfortunately, like other pulse crops the production of greengram is subject to several constraints including those of pests and diseases which play a leading role in the yield reduction. Greengram, in general, is susceptible to large number of diseases and pests which cause heavy losses to the crop every year. Amongst the various diseases, the diseases caused by fungi and nematodes are major ones and cause considerable losses. The important fungal diseases that take a heavy toll every year in India are anthrocnose (*Colletotrichum Corda spp.*), rusts (*Uromyces* (Link.) Unger. spp.), dry root-rots (*Rhizoctonia De. ex. Fr. spp.*), wilts (*Fusarium Link. ex. Fr. spp.*). The nematodes found associated with this crop includes *Meloidogyne Goeldi* spp., *Heterodera Schmidt* spp., *Rotylenchulus reniformis Linford & Oliver*, *Tylenchorhynchus brassicae Siddiqi*, *Helicotylenchus Steiner* spp. and *Hoplolaimus Daday* spp.

Under natural conditions a plant is a potential host to various micro-organisms and they can influence each other by occupying the same habitat. These micro-organisms often develop symbiotic, synergistic or antagonistic relationship amongst themselves which could primarily be because of nutritional or
spatial competition. It is inherent in nature of all living organisms to co-operate or compete with the other especially if they have similar and overlapping food resources. This is all the more true for the soil which is a complex ecosystem having a wide variety of life forms (including plants and animals). The plants in one or the other form are the direct or indirect sources of food for the consumers of all tropic levels, including plant pathogenic organisms.

In nature plants are rarely exposed to the influence of a single pathogen. Some 63 years ago Fawcett (1931) recognised that nature does not work with pure cultures and that many plant diseases are influenced by associated micro-organisms whose action is often combined to induce damage. It is well known that most of the pathogens like fungi, bacteria, viruses and nematodes are quite capable of causing serious diseases without being influenced by other biotic agents, but the economic damage often becomes more destructive and high when they interact with each other (Powell and Nusbaum, 1960; Powell, 1968; Johnson and Powell, 1969; Husain et al., 1985; Weischer, 1993; Evans and Haydock, 1993; Sitaramiah and Pathak, 1993).

It is unrealistic to assume that a plant, although infected with one pathogen will not be affected by other and it seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Taylor, 1990). The alterations greatly influence the disease development within the particular host and the epidemiology of the pathogen. Therefore, the study of the disease complex is as
important as the study of monopathogenic situation because, under field conditions no soil-borne plant disease can be said to be of monopathogenic origin. Different parasites on the same plant interact which result in the disease complexes and these interactions may lead to susceptibility by predisposition or resistance through preinduction of resistance against a particular parasite (Sidhu and Webster, 1981a).

Plant parasitic nematodes often play a major role in disease interactions. Interactions involving nematodes is more important because they contribute substantially to the variability in crop growth (Zadoks and Schein, 1979). It is possible that some dramatic crop losses involving nematodes are due to the interactions between several determinants that exacerbate the effects (Wallace, 1983). Nematodes interact with different groups of plant pathogens and root symbionts. The association between nematodes and other pathogens or symbionts in plant disease encompasses a wide array of species and results in biological balance that may even lead to an understanding of few interactions. All interactions of plant-parasitic nematodes with other plant pathogens have three components: nematode, host and other pathogen. The plant pathogens known to interact with nematodes are mainly viruses, bacteria and fungi. Disease complexes involving nematodes and other microorganisms are well known in nature and the roles that nematodes play in disease complexes have been the subject of several reviews, including those of Powell (1963, 1971a,b, 1979), Pitcher (1965, 1978), Bergeson (1972), Taylor (1979, 1990) and Khan (1993). Much
Experimental evidence indicates a biological interaction between nematodes and certain soil-borne fungi. The nematodes usually assist and enhance the pathogenicity mechanism of the fungus towards modification in the host plant. They contribute to the disease complex by modifying the physiology of the host plant and occasionally by the mechanical affects they exert on the host. Since the publication of Atkinson's report (1892) that

Fusarium wilt of cotton was more severe in the presence of root-knot nematode (Meloidogyne spp.) than in its absence, a large volume of data has been accumulated which firmly establishes the involvement and the role of plant parasitic nematodes in interactions with fungal pathogens on various crop plants (Powell, 1971a, 1979; Pitcher, 1978; Hussey and McGuire, 1987; Riedel, 1988; Taylor, 1990; Evans and Haydock; 1993, Francal and Wheeler, 1993; Prot, 1993). It is not surprising that many more such complexes may come to light in future

Among the various pulses the greengram is grown fairly in the larger areas in this part (Uttar Pradesh state) of the country. During the course of survey of greengram fields in and around Aligarh district of the U.P. state, which falls between the great Gangetic plains of northern India, the single and concomitant occurrence of plant parasitic nematodes and some pathogenic fungi were observed from the root and soil samples of the crop. Among the plant parasitic nematodes and pathogenic fungi the root-knot nematode, Meloidogyne incognita (Kofoi and White) Chitwood, and root-rot fungus, Rhizoctonia solani Kuhn happen to be dominant species associated with the unthirsty
growth of this crop. Keeping in view the importance of the crop and the nematode-fungus associations it was considered desirable to study the pathogenicity and the interactive effects of *M. incognita* and *R. solani* on the physio-morphological characters of greengram cv. T-44 as there is a paucity of information regarding the interrelationship of nematode-fungal pathogens on this crop. Further the efforts were also made to work out the measures in minimizing the losses caused by these pathogens and in this regard the efficacy of organic amendments (oil seed cakes) and chemicals (pesticides) were tested against the disease complex. With this aim in view, the following aspects have been studied experimentally.

1. Effect of different inoculum levels of *M. incognita* and *R. solani* separately on plant growth, nematode multiplication, gall formation, and root-rot development in presence and absence of *Rhizobium*.

2. Effect of individual and combined inoculation of variable inoculum levels of *M. incognita* and *R. solani* on plant growth, nematode multiplication, gall formation, and root-rot development in presence and absence of *Rhizobium*.

3. Effect of individual, simultaneous, and sequential (pre- or post-) inoculation of *M. incognita* and *R. solani* on plant growth, nematode multiplication, gall formation, and root-rot development in presence and absence of *Rhizobium*.

4. Effect of *M. incognita* and *R. solani* alone and in combination on nitrogen, phosphorus and potassium contents of leaf, stem, and root in presence and absence of *Rhizobium*.
5. Effect of *M. incognita* and *R. solani* alone and in combination on chlorophyll contents of foliage in presence and absence of *Rhizobium*.

6. Effect of *M. incognita* and *R. solani* alone and in combination on leghaemoglobin concentration of root nodules.

7. Efficacy of two different chemical products, carbofuran (a nematicide) and bavistin (a fungicide) separately and in mixture on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence of *M. incognita* and *R. solani* alone and in combination.

8. Efficacy of two different doses of oil seed cakes (neem, castor, mustard, and mahua) on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence of *M. incognita* and *R. solani* alone and in combination.
Disease complexes involving nematodes and other microorganisms are common in nature. In the complex biotic environment of soil, the pathogens are always influenced by associated micro-organisms. Root system irrespective of the nature of plant species, is constantly exposed to abiotic and biotic factors including soil inhibiting micro-organisms which develop several kinds of interrelationships among themselves. Such associations may be beneficial or deleterious to plants. Disease syndromes resulting from root diseases are often caused by microbial interactions. Plant parasitic nematodes have principal role in many interactions, often making plant roots more susceptible to invasion and parasitism by other soil inhibiting microorganisms (Hussey and McGuire, 1987). Several possibilities have been suggested by different workers by which plant parasitic nematodes interact with other micro-organisms. According to Pitcher (1965), nematodes may act as (i) vectors of pathogens capable of self establishment once in contact with the host (ii) vectors of pathogens incapable of self establishment unless introduced below the host epidermis (iii) mechanical wound agents (iv) providers of necrotic infection courts (v) host modifiers (vi) resistance breakers and (vii) deterrents of plant disease. The literature on this subject has been extensively reviewed by several authors (Bergeson, 1972; Hirano, 1975; Pitcher, 1978; Powell, 1979; Taylor, 1990; Khan, 1993) which firmly establish the involvement and role of plant
parasitic nematodes in interactions with other micro-organisms.

2.1 NEMATODE-FUNGUS INTERACTIONS

Interactions between nematodes and fungi have been recognised since 1892, when Atkinson reported that Fusarium Wilt (Fusarium oxysporum f.sp. Vasinfectum (Atk.)Synder & Hansen) was more severe in presence of root-knot nematodes (Meloidogyne spp.) than in its absence. Since this first report, several workers have reviewed the work on interaction of plant parasitic nematodes with fungi on various crop plants (Pitcher, 1978; Powell, 1971a, 1979; Bergeson, 1972; Riedel, 1988; Hasan, 1993; Francl and Wheeler, 1993; Evans and Haydock, 1993). Nematode-fungus interactions have been classified in number of ways and the roles played by nematodes in such interactions have been examined thoroughly. Powell (1971a) categorised the nematode-fungus interactions on the basis of symptomatology of the disease caused by fungi into following three types.

1. Nematode-fungus wilt disease interactions
2. Nematode-fungus root-rot disease interactions
3. Nematode-fungus seedling disease interactions

Although the involvement of nematode-fungus disease complex situation is widespread and the literature is quite extensive, but in the present study the review of literature is confined to the interactions involving nematodes and root-rot fungi, which is further categorised on the basis of nematode parasitisms (i.e. with endo-, semi endo-, and ecto- parasitic nematodes).
The root-rot fungi constitute a category of pathogens where considerable work has been carried out with respect to their interactions with nematodes though not to the extent of wilt-fungi-nematode complexes. Prominent amongst the root-rot fungi are species of Rhizoctonia (R. solani, R. bataticola (Taub.) Butler). Pythium (P. aphanidermatum (Eds.) Fitz., P. ultimum Throw.), Fusarium (F. solani (Mart.) App. Wollenw), Phytophthora parasitica Dastur, Sclerotium rolfsii Sacc., and Colletotrichum coccodes (Wallr.) Hughes that are known to interact with different plant parasitic nematodes. The role of nematodes in root-rot diseases, in general, is related to assisting the fungal pathogen in its pathogenesis and increasing host susceptibility. Much of the literature dealing with the interactions of nematodes with root-rot fungi is contradictory, as noted by Sikora and Carter (1987) and Evans and Haydock (1993) or deals only with selected aspects of relationship.

2.1.1 Interaction with Sedentary Endoparasites

2.1.1.1 Root-Knot nematodes (Meloidogyne incognita)

The frequency of involvement of nematodes and fungi in disease complexes is reflected in number of crops on which such complexes are recorded and amongst the different plant parasitic nematodes of economic importance the root-knot nematodes (Meloidogyne spp.) have been thoroughly studied and commonly found involved in synergistic interactions with root-rot fungi. Steiner (1942) for the first time realised and discussed the importance of root-knot nematode with respect to plant necrosis.
in presence of root-rot fungi. Since then large number of workers have studied the association between root-knot nematode and root-rot fungi on different crops.

A number of such studies underline the significance of nematode infected tissue from which the fungal pathogen derives aggressiveness and becomes pronounced. The root-knot nematode, *M. incognita* have been found to predispose the plant roots for secondary infection (fungal attack) resulting in the greater damage of plants by way of root decay and the disease caused by fungal pathogens become more pronounced and may appear earlier when plants are infected with nematodes. *M. incognita* maximally predisposed tobacco plants to root decay caused by *P. ultimum* (Melendez and Powell, 1970) when the nematode was inoculated four weeks prior to fungus inoculation. Similarly, Nava (1970) found decay in tomato roots only when *M. incognita* was added several weeks ahead to either of fungus *R. solani* or *P. ultimum*. In the roots, where both *R. solani* and *P. ultimum* were present alongwith *M. incognita*, the *R. solani* appeared to be more aggressive than *P. ultimum* and gradually become dominant in the galled tissue. When *M. incognita* inoculation preceded *R. solani* inoculation by at least ten days, the *M. incognita* susceptible tobacco cultivars Dixie Bright 101 and Coker 316 exhibited more severe root-rot than when nematode and fungus were inoculated simultaneously (Batten and Powell, 1971). Powell et al., (1971) showed that when tobacco roots had been previously infected by *M. incognita*, nonpathogenic soil inhibiting fungi became pathogenic and galled roots were extensively decayed following
invasion by these fungi. Golden and Van Gundy (1972) studied the interaction of *M. incognita* with *R. solani* and *Thielaviopsis basicola* (Berk & Br.) Ferraris and reported that *M. incognita* induced changes in the permeability of infected roots resulting in the increased leakage of electrolytes and organic compounds which consequently increased the fungal growth.

Few studies have attempted to elucidate the physiological basis of nematode-fungus interactions. It has been shown, however, that exudates from nematode infected roots accounted for an interaction between *M. incognita* and *R. solani* which produced a disease complex on tomato and okra (Golden and Van Gundy, 1975). Galled roots of field grown tomato and okra infected with *M. incognita* were more susceptible to infection by *R. solani* than non-galled roots on the same plant. Fungal sclerotia developed only on the surface of the galls and the first symptoms of root decay appeared four weeks after initial gall formation. Roots became decayed only when the fungus and nematode were present together. Histological investigations revealed that fungus either penetrated galled roots directly or entered through ruptured tissue caused by swollen female nematode. The fungus initially colonised the giant cells which were destroyed in two or three days before invading other root tissue. In the same study, Golden and Van Gundy (1975) reported that *R. solani* responded to stimuli which originated from nematode infected roots and passed through semipermeable cellophane membrane, by producing sclerotia on the membranes just opposite the galls. The leakage of nutrients from the roots...
attracted the fungus to the galls and initiated sclerotial development. The role of exudates emanating from galls in this interactions was confirmed when it was shown that no decay occurred if roots inoculated with \textit{R. solani} were continuously leached (Van Gundy et al., 1977). However, applying leachates collected from roots infected with \textit{M. incognita} to roots of tomato inoculated with \textit{R. solani} resulted in severe necrosis. Exudates increased between 3 to 14 days after nematode infection with carbohydrates being the principal constituents, whereas after 28 days, nitrogenous compounds increased in the exudates of infected plants. The change in the carbon/nitrogen ratio of gall exudates after 28 days favoured parasitic rather than saprophytic development of the fungus. This work illustrated the role of primary nematode pathogen in altering root exudates to favour the parasitic development of the fungus.

There are certain studies where the reduction in plant growth was found maximum when both nematode and fungus were inoculated simultaneously. The simultaneous associations of \textit{M. incognita} and \textit{R. solani} caused maximum reduction of plant growth in okra (Chhabra et al., 1977), french bean (Reddy et al., 1979), potato (Sharma and Gill, 1979) and egg plant (Azam et al., 1984). On the other hand, highest decrease in plant growth was reported by some other workers when the nematode preceded the fungal inoculation. Raut (1983) reported that prior establishment of \textit{M. incognita} to \textit{R. bataticola} had a synergistic effect on the top growth. However, adverse effect of the nematode was mitigated to a greater extent by the establishment
of fungus prior to nematode. Inoculation of *M. incognita* 3 weeks prior to *R. solani* significantly reduced the plant growth of tomato in comparison to its reverse condition (Chahal and Chabra, 1984). This was attributed to the predisposition of seedling roots by nematode for subsequent damage caused by *R. solani*. Similarly, in sugarbeet, *M. incognita, P. ultimum* and *R. solani* were found to reduce the growth of seedlings but maximum reduction was seen where plants were inoculated with *M. incognita* followed by *P. ultimum* or *R. solani* or *P. ultimum*+*R. solani* (Pandey, 1984). Husain et al., (1985) observed an increased plant growth reduction of pea, in presence of *M. incognita* and *R. solani*, whereas, Abu-El-Amayem et al., (1985) showed similar type of interaction between *M. incognita* and *R. solani* on soyabean. Hasan (1985) reported loss of resistance to *M. incognita* in two chilli cultivars, Jowala (resistant) and Longthin Faizabadi (moderately resistant) infected with *R. solani* or *P. aphanidermatum*. Similarly, in other greenhouse study it was found that tomato cultivars could lose resistance to *M. incognita* in presence of *R. solani* and *Sclerotium rolfsii* (Hasan and Khan, 1985).

Lanjewar and Shukla (1985) reported that rotting of ginger roots by *P. myriotylum* Drechsler was equally severe in presence or absence of *M. incognita* but the presence of fungus decreased nematode reproduction, whilst, Al-Hazmi (1985) found increased severity of root-rot in two french bean cultivars when *M. incognita* was introduced prior to *Macrophomina phaseolina* (Maubl.) Ashby, but nematode reproduction was adversely affected.
when fungus was introduced first. Cultivar Harvester showed more
tolerance to both the pathogens than Romano Italian. Dianese et
al., (1986) suggested that *M. incognita* may interact with
*Cylindrocladium clavatum* Hodges & May to cause root disease of
soyabean. Doshi and Mathur (1987) reported that prior
inoculation with *M. incognita* appeared to prevent root-rotting
of ginger caused by *P. aphanidermatum*, whereas rotting caused by
*F. solani* became most severe.

On cowpea, *M. incognita* interacted with *R. solani* to
reduce plant weight, with greater damage occurring if the
nematode infection was established before inoculation of the
fungus (Varshney et al., 1987), an identical effect was also
noted with *F. solani* on chickpea (Mani and Sethi, 1987).
Similarly, on white jute plants combined inoculation of *M.
incognita* and *R. bataticola* markedly increased the incidence of
root-rot over that found with either organism alone (Mishra et
al., 1988).

Khan and Husain (1988a) reported that individually *R.
solani* was the most aggressive pathogen of cowpea followed by *M.
incognita*, but, the association of *R. solani* caused greater
plant growth reductions to unbacterized plants than the
bacterized ones. There was no significant difference in the
plant growth reduction of bacterised plants whether inoculated
simultaneously with nematode and fungus or when the nematode
inoculation preceded fungus. On the other hand, there was
significantly less reduction when fungus preceded nematode
inoculation. *R. solani* reduced the rate of nematode
multiplication in all combinations as compared to when nematode occurred alone.

Inoculation of cowpea with *M. incognita* and *R. solani* led to the breakdown of resistance to both organisms (Khan and Husain, 1989a) and the greatest decrease in plant dry weight occurred when the lower inoculum level of *M. incognita* were used with higher inoculum levels of *R. solani*. However, *R. solani* irrespective of inoculum level inhibited the multiplication of nematode (Khan and Husain, 1990a) and restricted its penetration on roots (Khan et al., 1992).

Recently, tomato (Srivastava and Singh, 1991) and chilli (Kumar et al., 1992) have also been found to suffer by the effects of disease complexes involving *M. incognita* and *R. solani*, with the greatest damage occurring when both the pathogens were inoculated simultaneously.

### 2.1.1.2 Other species of Meloidogyne

Association of *Meloidogyne javanica* (Treub) Chitwood or *Meloidogyne hapla* Chitwood with *R. solani* resulted in more seedling mortality of soyabean than caused by either pathogen alone (Taylor and Wyllie, 1959). Miller (1968) found that tobacco NC 95 variety *nicotianae* resistant to *M. javanica* and *Phytophthora parasitica* lost its resistance when both the pathogens were inoculated concomitantly. Tu and Cheng (1971) observed an increased root lesion in the roots of kenaf, when *M. javanica* and *Macrophomina phaseoli* were inoculated simultaneously, whereas in *Ligustrum japonicum* L., Alfieri and Stokes (1971) noted leaf chlorosis, abscission, twig die back,
stunting, reduction, and necrosis of roots in the combined inoculation of same two organisms.

Rotting of root system in sugarcane was increased when both Curvularia lunata (Wakker) Boedijn and M. javanica were present with greatest damage occurring when they were inoculated together but with significant damage even when the inoculations were ten days apart (Khurana and Singh, 1971). Similarly, Goswami et al., (1975) observed maximum wilting of tomato plants, when M. javanica was inoculated 3 weeks prior to R. bataticola. However, wilting was comparatively less in simultaneous inoculation and in the sequence of fungus preceding nematode by 3 weeks.

Ibrahim and El-Saedy (1977) found fewer galls on the roots of groundnut cultivar Giza-4, when infected with M. javanica and either F. solani or R. solani than in presence of nematode alone. In other study, simultaneous inoculation of M. javanica with F. solani, F. moniliforme Sheldon, R. solani or Sclerotium bataticola Taub., decreased plant growth of hybrid 17A to a greater extent than the single inoculation by nematode or either of the fungi. M. javanica inoculated at sowing time along with either of the three test fungi increased the percentage of damping-off of tomato seedlings, especially with R. solani; P. debaryanum Hesse was the serious pathogen in its own right (Nath et al., 1984).

Goel and Gupta (1986a) studied the effect of M. javanica and R. bataticola alone and in combination on chickpea seedlings and noted that combined inoculation irrespective of
time, reduced the various growth parameters, compared with when inoculated alone. An identical result was also found with *M. javanica* and *F. oxysporum f. ciceri* (Padwick) Synd. & Hans., on chickpea (Goel and Gupta, 1986b; Upadhyay and Dwivedi, 1987). In a similar study Goel and Gupta (1986a) also found a significant reduction in the number of root galls when nematode was present a week before fungus inoculation. However, when the fungus was introduced 30 days after nematode inoculation the plant growth as well as number of root galls were significantly less, compared to when inoculated after 10, 20, 40 or 50 days of nematode inoculation. The adverse effect of *R. bataticola* and *F. solani* on the multiplication of *M. javanica* was also observed on groundnut, but the reduction in rate of nematode multiplication was maximum, when one or both fungi were inoculated simultaneously with the nematode and the introduction of *F. solani* one week after the nematode did not reduce the galling and nematode population significantly in soil and roots. *R. bataticola* proved more antagonistic to *M. javanica* than *F. solani* (Sakhuja and Sethi, 1986).

Kanwar et al., (1987) found a considerable reduction in the root and shoot length, and dry weight of cowpea in the combined inoculation of *M. javanica* and *R. solani* as compared to uninoculated control. The growth of plant was found to be better in loamy sand which supported higher number of nodules than in sand and sandy loam. Fewer galls were observed in the simultaneous inoculation of both the pathogens than on those infested with nematode alone but, reduction in nodulation was
non significant in all the three soil types. While in another study, a significant reduction in plant growth of cowpea cv. HFC 42-1 was observed, when *M. javanica* was inoculated 3 weeks prior to *R. solani* in pot experiments. Nodules were also significantly reduced in presence of both the pathogens with greatest reduction in nematode treatment only (Kanwar et al., 1988).

*Macrophomina phaseolina* interacted with *M. javanica* on lentil to cause more damage when two organisms were inoculated simultaneously, but the fungus inhibited nematode reproduction especially when it was inoculated earlier (Tiyagi et al., 1988).

*M. javanica* also interacted with fungi not usually thought of as pathogenic, such that *Rhizopus nigricans* Ehrenb and *Penicillium digitatum* Sacc. In the presence of nematode, each fungus caused significant root necrosis (Nath and Kamalwanshi, 1989).

Gupta and Mehta (1989) while studying the interactive effects of different levels of *M. javanica* and *Rhizoctonia* spp., (*R. solani* and *R. bataticola*), observed an increase in various growth parameters of mungbean, when fungus grown on oat meal was added as compared to that grown on PDA broth. However, the number of galls were reduced when nematode and fungus were added simultaneously. The reduction being more pronounced with *R. bataticola* as compared to *R. solani*. On the other hand Anwar and Verma (1993) found a significant reduction in various growth parameters of chickpea, when *M. javanica* and *R. solani* were inoculated concomitantly, the maximum reduction being in simultaneous inoculation than the sequential inoculation. A similar trend was observed with respect to reduction in nematode.
multiplication, galling and root nodulation.

*Cylindrocladium crotalariae* (Loos.) Bell & Sobers causes black rot of peanut roots and severity of this disease was much enhanced by the presence of *M. hapla*, even on *Cylindrocladium* black rot-resistant roots (Diomande and Beute, 1981). Khan and Muller (1982) used gnotobiotic culture to investigate interaction between *R. solani* and *M. hapla* on radish and showed that prior infection by the nematode helped the fungus to colonize the roots. *M. hapla* appeared to exacerbate the loss of alfalfa seedlings in soil contaminated with *Pythium ultimum* (Townshend, 1984) and to be essential for the infection of alfalfa by *Fusarium oxysporum* although this fungus also appeared to suppress the nematode reproduction (Griffin and Thyr, 1988). Another species *Meloidogyne arenaria* (Neal) Chitwood interacted synergistically with *Pythium myriotylum* on peanut causing severity in pod rot (Garcia and Mitchell, 1975) and also interacted with *Aspergillus flaus* Link to cause increased root disease which was accompanied by reduced nematode multiplication (Patel et al., 1986). Recently increased root-rot of Taro plants (*Colocasia esculenta* Lam. long) have been observed in presence of *M. arenaria* and *R. solani* (Ko et al., 1993).

2.1.1.3 Cyst forming nematodes

Despite the similarity of life cycle of cyst and root-knot nematodes, there are many fewer records of interaction of cyst nematodes with fungal pathogens. Grainger and Clark (1963) reported the reduction in the yield of potatoes inoculated with
Globodera rostochiensis (Wollenweber) Behrens and Rhizoctonia solani. Dunn and Hughes (1964) found more reduction in tomato growth when *G. rostochiensis* entered the roots prior to *R. solani* and *Colletotrichum atramentarium* (Berk & Br.) Taub than when fungus preceded the nematode or when the two pathogens entered simultaneously whereas, James (1968) failed to observe any influence of *G. rostochiensis* on disease caused by *C. atramentarium*.

The infected tomato roots with *G. rostochiensis* and gray sterile fungus showed deformation of giant cells and the larvae failed to develop into adult. However, the typical giant cells were produced on plants inoculated with nematode only (Roy, 1968). Dunn (1968, 1970) observed a significant reduction in the growth parameters of tomato when *G. rostochiensis* was added prior to *R. solani* and *C. atramentarium*. Roy (1977) studied the interrelationship between *G. rostochiensis* and *R. solani* and *Colletotrichum coccodes* (Waltr.) Hughes (the causal organism of brown-rot) on tomato cultivar Ailisa Briag and observed greater growth reduction when nematode inoculum preceded fungal inoculation as compared to the situation when fungus preceded nematode inoculation. This complex appeared to be complicated by the possible interrelationship between the two fungi.

*Heterodera schachtii* Schmidt and *R. solani* produced synergistic effect on sugarbeet seedlings (Price and Schneider, 1965; Polychronopoulos et al., 1969 and Polychronopoulos, 1970). The nematode induced wounds facilitated subsequent penetration
and colonization of the fungus. Seedling symptoms characterized by softening and decay of the tissue, developed rapidly after infection by both the pathogens. Polychronopoulos et al. (1969) also studied histology of this interaction and found that giant cells resulting from the infection of *H. schachtii* as well as the adjacent areas, provide highly suitable substrate for the fungal growth. Whitney and Doney (1973) reported synergistic interaction between the cyst nematode *H. schachtii* and fungi *R. solani*, *C. atramentarium* and *Aphanomyces cochlioides* de Bary in root-rot of sugarbeet, whereas Whitney (1974) found similar type of interaction with *H. schachtii* and *Pythium ultimum* on the same plant.

Dave (1975) studied the relationship of *Heterodera glycines* Ichinohe with *R. solani* on soyabean cv. Clark 63 and found that damage caused by *R. solani* in presence of *H. glycines* was no more than additive. The nematode population and development suppressed more in presence of fungus than in its absence. Gupta et al. (1975) observed a significant reduction in the infection of *Heterodera virgini* Edward & Mishra on cowpea, with some soil borne fungi like *Penicillium citrinum* Thom, *Curvularia lunata*, *Rhizoctonia bataticola*, *Rhizopus nigricans* and *Aspergillus terreus* Thom. The severity of interaction between *Heterodera daverti* Wouts & Sturhan and *Fusarium oxysporum* Schlecht and subterranean clover depended greatly on the inoculation sequence of the two organisms (Nordmeyer and Sikora, 1983). If the nematode was added before the fungus, the yield loss was less than the additive effect of the individual
pathogens; simultaneous inoculation and prior inoculation of the fungus gave a greater than additive (i.e. synergistic) effect. Seedling blight of rice caused by *Sclerotium rolfsii* was more severe when the inoculation of *Heterodera oryzicola* Rao & Jayaprakash preceded fungal inoculation. Addition of the fungus followed by the nematode also caused more plant mortality than the fungus alone, but considerably fewer nematodes invaded the roots in this case (Jayaprakash and Rao, 1984).

Walia and Gupta (1986) found that *R. solani* adversely affected *H. cajani* Koshy population when inoculated one week prior to nematode on cowpea. Number of cysts and larvae were significantly reduced over nematode alone. On the otherhand, the fungus when inoculated 2 weeks after the nematode reduced the top growth of plants significantly as compared to check or any other treatment. *H. glycines* has been also reported to interact with *Fusarium solani* (Roy et al., 1989; Hershman et al., 1990) and *Calonectria crotalariae* (Loos.) Bell & Sobers (Overstreet et al., 1990) on soyabean.

### 2.1.2 Interaction with Miragatory Endoparasites

The migratory endoparasitic nematodes particularly the species of *Pratylenchus* Filipjev have also been found involved in certain root-rotting disease complexes. In fact some of the earliest reports were those of Mountain and Benedict (1956) and Benedict and Mountain (1956) on root-rot of winter wheat in Canada, in which *Pratylenchus minyus* Sher & Allen had an interaction with *Rhizoctonia solani* causing significant
reduction in wheat growth. Although, Hendrix et al., (1965) found no interaction between *Pythium* and *Pratylenchus* associated with large percentage of peach trees suffering from peach decline, but the presence of *Criconemoides* Taylor spp. and *Tylenchorhynchus* Cobb spp. was needed for this condition. An interesting work by Edmund and Mai (1966) have shown that *P. penetrans* (Cobb) Filipjev & Sch. Stek., and *Trichoderma viride* Pers. ex Gray caused more reduction in root and shoot growth in both alfalfa and cereals than either pathogen alone, even though *T. viride* was not recognised as an important pathogen. At certain temperatures *Pratylenchus scribneri* Steiner and *Fusarium moniliforme* caused greater reduction of corn fresh weight than either of them alone (Palmer et al., 1967). Olthof (1968) did not found any alteration of barley and tobacco to *P. penetrans*, but the black root-rot caused by *Thielaviopsis basicola* seems to exercise some effects on nematode penetration.

The development of blank shank in tobacco, following invasion of the roots by *Phytophthora parasitica*, was enhanced by inoculation with *Pratylenchus brachyurus* (Godfrey) Filipjev & Sch. Stek., up to one week before fungus inoculation. Addition of nematodes 3 weeks prior the fungal inoculation delayed appearance of the symptom whereas, simple mechanical wounding of roots enhanced symptom development (Inagaki and Powell, 1969). Root-rot severity of canning pea caused by *Aphanomyces euteiches* Drechsler was increased in presence of *P. penetrans* (Oyekan and Mitchell, 1972) and more kidney bean plants were affected by root-rot when subjected to low levels of inocula of *F. solani*
and *E. phaseoli* (Brukh.) Synder & Hans., in presence of *P. penetrans* than the nematode free plants (Hutton et al., 1973). Dave (1975) noted that the damage caused by *R. solani* in presence of nematode *P. scribneri* on soyabeans cv. Clark 63 was more than additive, whilst, Bee-Rodriguez and Ayala (1977) observed increased root necrosis and reduced top and root growth of *Sorghum bicolor* (Linn.) Moench in the combination of *P. zeae* Graham with *Curvularia* Boedign. spp. Miller and Anagnostakis (1977) found a synergistic reduction in the population of *P. penetrans* and *Tylenchorhynchus dubius* (Butschli) Filipjev by a weak pathogen *Trichoderma viride* due to antagonistic behaviour to these nematodes, whereas McIntyre and Miller (1978) reported similar antagonistic interaction between *Phytophthora parasitica* f. *nicotianae* (Dastur) Tucker and *P. penetrans* on tobacco. 

The minor pathogen, Thielaviopsis basicola was assisted by *Pratylenchus crenatus* Loof in penetrating pea roots (Green et al., 1983). On the other hand, *P. penetrans* increased the infection level of *Colletotrichum coccodes* and *R. solani* on Russet Burrank potato roots but the interaction had no effect on yields, even in the presence of wilt fungus *Verticillium dahliae* Kleb (Kotcon et al., 1985). A combination of *Pratylenchus brachyurus* and *P. zeae* interacted with root rot fungus *Fusarium moniliforme* on maize to cause more severe effects on plant growth than from nematodes or fungus alone (Jordaan et al., 1987). The amounts of rotting caused in *Chrysanthemum* roots by *Pythium aphanidermatum* and *R. solani* were increased in the presence of *Pratylenchus coffeae* (Zimmerman) Filipjev & Sch.
Stek., and were further increased when plants were attacked by all three organisms (Hasan, 1988). Similarly Black root-rot of strawberries, caused by *Rhizoctonia* spp. was also exacerbated by *P. penetrans* more so at higher nematode inoculum levels (Lamondia and Martin, 1989).

Another endoparasitic nematode also involved in disease complex with root-rot fungi is the burrowing nematode, *Rodopholus similis* (Cobb) Thorne widely infecting banana (*Musa paradisica* L.) plant. Stover (1966) suggested that *Fusarium solani* and *Rhizoctonia* spp. may contribute to the extension of lesions in banana roots caused by *R. similis* and even to the death of roots, whilst, Pinochet and Stover (1980) found *F. solani*, *F. moniliforme* and *Cylindrocarpon musae* Booth & Stover to be commonly associated with banana root lesions. Coconut seedlings were also severely damaged by *R. similis*, and although the nematode seemed to be necessary for substantial invasion by *Cylindrocarpon effusum* Bugn., to occur, but the damage to plants from the two organisms was not significantly greater than from the nematode alone (Koshy & Sosamma, 1987). On the other hand, the root lesion of arecanut was more extensive when *Cylindrocarpon obtusisporum* (Cook & Harkness) Wollenw., was introduced three weeks after the introduction of *R. similis* (Sundararaju and Koshy, 1987). However, the fungus inhibited multiplication of the nematode, so root lesions and effects on plant growth were actually less except when the nematode was introduced three weeks prior to the fungus.
2.1.3 Interaction with Semi-Endoparasites

The semi-endoparasitic nematodes also took part in the disease complex involving root-rot fungi. The growth of citrus seedlings was retarded more severely with the combination of Tylenchulus semipenetrans Cobb and Fusarium solani than either pathogen alone (Van Gundy and Tsao, 1963). Similarly, O'Bannon, et al., (1967) observed increased root decay caused by Fusarium spp. in the presence of citrus nematode T. semipenetrans but there were differences in decay depending upon the species (F. oxysporum or F. solani). Kumar and Sivakumar (1981) studied the interaction between Rotylenchulus reniformis and Rhizoctonia solani on okra, and found the maximum reduction in plant growth when nematode inoculation preceded the fungus inoculation.

2.1.4 Interaction with Migratory Ectoparasites

Labruyere et al., (1959) noticed "Early Yellowing" disease and root-rot of pea in presence of Hoplolaimus uniformis Thorne and Fusarium oxysporum f. pisi (van Hall) Synder & Hansen. The earlier root-rot was also developed in Chrysanthemum roots when inoculated with Belanolaimus longicaudatus Rau and Pythium aphanidermatum as compared to the inoculation with fungus alone (Littrell and Johnson, 1969). Kisiel et al., (1969) found that Tylenchus agricola de Man promoted the corn root-rot caused by Fusarium roseum Link but not the one caused by Pythium ultimum. On the other hand, Liu and Ayola (1970) observed positive interaction between Trichodorus christiei Allen and Fusarium moniliforme on sugarcane but apparently the infection
by *E. rooseum* was not influenced by *I. christiei*.

### 2.2 INTERRELATIONSHIP BETWEEN NEMATODES, FUNGI AND ROOT-NODULE BACTERIA

*Rhizobium* Frank, induced root nodules are a prime example of symbiosis between legume host and bacteria (Vance, 1983). For an effective nitrogen fixation legume roots must become associated with *Rhizobium* bacteria. The plant (the macrosymbiont) provides an energy source and an ecological niche for the bacteria (the microsymbiont), and the bacteria provide a source of fixed nitrogen for the plant (Vance and Johnson, 1981).

Rhizosphere is a dynamic environment, where the relationship among different micro-organisms, plant and environment are of chemical nature. The environment greatly influences not only the growth and longevity of nodule bacteria in the soil, but also the production and behaviour of the nodules and the development of host plant, and for all these reasons it also influences uptake of nitrogen from the soil and from the air (Van Schreven 1958).

One of the biological factors affecting nodule formation or dysfunction of existing nodules is the presence of nematodes in the rhizosphere. Dysfunction of symbiotic process in legumes also occurs with viral and fungal infections (Tu et al., 1970; Orellana et al., 1976, 1978; Bowen, 1978; Kush, 1982; Tiyagi, 1990; Siddiqui, 1990).

The associations of rhizobia with plant nematodes and fungi in rhizosphere and the beneficial effect of rhizobial
symbiosis on plant nutrition and growth led to investigations into the potential role of nematode and fungal parasitism on nodulation and consequently on symbiotic nitrogen fixation. This chapter primarily presents information on the effect of nematodes and fungi on the symbiotic relationship between rhizobia and legumes.

2.2.1 Nematode - Rhizobia Interactions

The available literature on interactions of nematodes with rhizobia have been recently reviewed by Huang (1987) and Taha (1993). Plant parasitic nematodes and symbiotic organisms commonly occur together in the roots and rhizosphere of the same plant, each having a characteristic but often opposite effect on plant vigor. Several plant parasitic nematodes with different modes of parasitism have been found to cause reduced nodulation on leguminous plants (Romaniko, 1958; Masefield, 1958; Epps and Chambers, 1962; Malek and Jenkins, 1964; Hussaini and Seshadri, 1975; Taha and Kassab, 1980). However, the influence of nematodes on the nitrogen fixing potential is not always adverse but in some cases nematode infestation has been even reported to stimulate nodulation and nitrogen fixation (Baldwin et al., 1975, Hussey and Barker, 1976; Baldwin et al., 1979; Huang, 1987; Verdejo et al., 1988). Other reports indicate that nematode infection had no significant effect on number and size of nodules (Taha and Raski, 1969; Hussey and Barker, 1976; Caroppo and Pelagatti, 1988).
2.2.1.1 Effect of Sedentary Endoparasites

Miller (1951) was the first to observe inhibition of nodulation on plant roots in the presence of root-knot nematode infection. Later, several workers have also reported that root-knot nematode cause reduction in the nodulation of leguminous plants. Balasubramanian (1971) reported that root-knot nematodes namely, Meloidogyne arenaria, M. hapla and M. incognita caused reduction in the bacterial nodulation of soyabean, the maximum being in M. javanica infection. He suggested that reduced nodulation may be due to the direct interference of root-knot larvae with the establishment of nitrogen fixing bacteria or due to the overall reduction of root system. Hussaini and Seshadari (1975) observed hampered nitrogen fixation and reduced nitrogen content of mungbean in presence of M. incognita. The reduction in nitrogen content was due to the overall reduction in nodulation, anatomical changes in nodules and altered host physiology. In another study mungbean plants showed the reduced nodulation, and nitrogen content of root and shoot, when M. javanica was inoculated prior to rhizobia. However, in plants, where Rhizobium inoculation preceded nematode inoculation or in Rhizobium alone inoculation the nodulation was normal (Bopiah et al., 1976a). Barker and Hussey (1976) studied the histopathology of nodular tissue of legumes infected with M. incognita (M. hapla on peanut). M. incognita was found inside the vascular bundles of soyabean nodules and it did not alter the structural integrity of nodules but bacteroids failed to develop adjacent to nematodes. Infected nodules deteriorated
earlier than non-infected nodules. Similarly, nodule development was suppressed on Wando pea and many deteriorated prematurely but only few M. hapla were observed in nodules of peanut which caused a little damage to nematode invaded tissue adjacent to nodules. While in the soyabean, nodule formation was stimulated by M. hapla but nitrogen fixation capacity was inhibited. Different light sources used in phytotron experiments also influenced growth and nodulation of soyabean. A fluorescent plus incandescent light regime resulted in plants with greatest shoot weight, pod number and nodule per gram of root (Hussey and Barker, 1976). Ogbugi (1977) reported that individual inoculation of either of root-knot nematode or Rhizobium on cowpea plants gave high content of galls and nodules respectively, whereas, the simultaneous inoculation of the pathogens produced few or no galls and nodules. Similarly, there was a corresponding decrease in nodulation with the increase in the inoculum level of M. incognita on mungbean (Singh et al., 1977), an identical effect also noted with M. javanica by Srivastava et al., (1979) and M. incognita by Raut and Sethi, (1980a) on soyabean.

Taha and Kassab (1980) reported that inoculation of M. javanica with Rhizoctonia spp. did not affect nodulation on Vigna sinensis Endl., and nodules were also formed on galls caused by M. javanica. On the other hand, M. incognita reduced nodulation and inhibited the nitrogen fixation (about 63%) in the nodular tissue of cowpea. Infected nodules contained different developmental stages of nematodes and the nodules
infected by nematode deteriorated much earlier than uninfected ones. The nematode inoculation prior to rhizobia resulted in maximum reduction in nodulation (Ali et al., 1981). Sharma (1984) observed similar results on pea and suggested that reduced nodulation was due to the nutritional interference by the nematode infestation and overall reduction of root system.

Increasing inoculum levels of root-knot nematode caused gradual reduction in the nodulation of different pulse crops but the significant reduction was observed with 100 juveniles per plant of *M. javanica* on mungbean (Gupta and Mehta, 1989); 500 juveniles of *M. incognita* on chickpea (Siddiqui, 1990) and blackgram (Fazal, 1993) and 1000 juveniles of *M. incognita* on greengram (Fazal et al., 1994), respectively. However, in all the cases, the reduction was comparatively high at the higher inoculum levels.

Cyst forming nematodes (*Heterodera* spp.) has been also found to cause reduction in nodulation and hamper nitrogen fixation in plants. Oostenbrink (1955) noticed that pea plants infected with *Heterodera geottingiana* Liebscher possessed few nodules and exhibited poor growth. However, application of nitrogen fertilizers compensated for the reduced nodulation in nematode infected plants. Ross (1959) observed that sparse nodulation on non fertilized soybean was probably due to the cyst nematode activity, and thereby, substantiating the earlier conclusion of Ichinohe and Asai (1956) and Jones and Moriarty (1956).
Wardojo et al., (1963) reported that *H. trifolii* Goffart plays a competitive role in reducing the number of nodules on white clover roots. Ross (1969) reported that at different nitrogen levels, *H. glycines* besides reducing nodulation and nitrogen fixation caused reduction in the yield of soyabean by inciting deleterious host responses that increased with the nitrogen deficiency.

Barker and Huisingsh (1970) found 93-100% inhibition of nodule development with simultaneous inoculation of *H. glycines* and *Rhizobium japonicum* (Kirchner) Buchanan. The nodular tissue was unsuitable for nematode development as syncytia failed to develop in nodular tissue although a few mature cysts developed on nodules.

Races of *H. glycines* on soyabean differed in their influence on nodulation. Race I significantly reduced nodulation, especially when added simultaneously with rhizobia, while race 2 and 4 did not (Lehman et al., 1971 and Barker et al., 1972). Race 3 did not reduce nodule mass on resistant and susceptible soyabean cultivars as compared to nematode-free plants, except for one instance where nodule mass was also decreased on resistant variety (McGinnity et al., 1980). Results with race 4 were variable, i.e. reducing nodule mass in one instance but increasing it in 3 instances. Differential response of race 3 and 4 on nodule mass compared to race I was attributed to differences in the reproduction rates of *H. glycines* race. When soyabean plants were inoculated with a high density soyabean cyst nematode race I (12500 juveniles per plant) and
rhizobia, complete inhibition of nodule development occurred (Huang and Barker, 1983). Ko et al., (1983) made light microscopic studies to determine the step at which nodulation of soyabean was disrupted by *H. glycines*. They reported that root hairs responded to *R. japonicum* with tips curling, twisting or swelling despite extensive root infection of *H. glycines*. *Rhizobium japonicum* resulted in the formation of only few nodules. Degeneration of bacteroid tissue appeared at 4 weeks accompanied by the disappearance of starch in uninvaded cortical cells. *H. glycines* most likely disrupted nodulation prior to the step of nodule initiation.

In another study Ko et al., (1984) applied a split-root technique to characterize the nature of nodulation supression by race-1 of the soyabean cyst nematode, *H. glycines* on cv. Lee 68. Root halves of each split-root plant were inoculated with *R. japonicum* and other root-halves with different number of soyabean cyst nematode eggs only. They suggested that nodulation and nitrogen fixing capacity were systemically and variously suppressed on both root-halves of the split-root plants 5 weeks after half-root inoculation with 12,500 nematode eggs. Inoculation with 500 eggs caused this suppression only on nematode infected root-half and nodulation on the companion uninfected root-half was stimulated slightly. The nematode infected root half inoculated with 5,000 eggs were excised at two weeks interval, nodulation on remaining uninfected root-halves was not different from that of the non-inoculated control when measured 6 weeks after the nematode inoculation. Thus,
Systemic suppression of nodulation was reversible upon the removal of nematodes. Similarly, application of various levels of KNO₃ to the uninfected root-halves of the split-root plant did not alleviate the suppressed nodulation on the companion nematode infected root-halves, even though plant growth was much improved at certain levels of nitrogen (125μg N/g soil). This indicated that the localized suppression of nodulation by nematodes was caused by other factors in addition to the poor plant growth.

Interference of the nematode with soyabean lectin metabolism was determined as a factor that reduces binding of rhizobia to H. glycines infected soyabean roots and thus suppresses nodule formation (Huang et al., 1984). Soyabean nodules emerging from plants infected with race 1 of H. glycines were poorly organised with less distinct zones of nodular tissue and early appearance of vascular elements and sclerenchyma layers. However, the most conspicuous features in the infected plants were the massive accumulation of starch granules and crystalline arrays of phytoferritin in the plastids of cells in nodular tissue. Their accumulation in nodule of nematode infected soyabean suggests that the metabolism of carbohydrate and iron containing compounds is affected by the presence of cyst nematode (Ko et al., 1985). The presence of syncytia extending into the vascular bundles and by hypertrophoid cells in bacterial tissue was observed in infection by Heterodera daverti in Egyptian clover (Massoud and Ghorab, 1988). Walia et al., (1989) observed a considerable reduction in the root
nodulation of guar at the higher inoculum levels of *H. cajani* (500 and above/pot), however, they suggested that the actual functioning of nodules is hampered even at the low inocula. The reduction in acetylene reduction activity (ARA) increased with increasing inoculum level. On the other hand, a marked reduction in the root nodulation of mungbean was achieved at 100 second stage larvae of *H. cajani* per kg of soil and on clusterbean at 1000 larvae/kg of soil (Dalal and Bhatti, 1989).

### 2.2.1.2 Effect of Semi-Endoparasitic Nematodes

Ayala (1962) reported that mature specimens of reniform nematode, *Rotylenchulus reniformis* were found attached to the bacterial nodules. Gupta and Yadav (1979) studied the pathogenicity of *R. reniformis* on urad (*Vigna mungo*) and found that there was corresponding decrease in the number of reniform nematodes. On the other hand, no deleterious effect on nodule growth was detected on cowpea when *Rhizobium* and *R. reniformis* were simultaneously inoculated, unless *R. reniformis* was inoculated prior to *Rhizobium* (Taha and Kassab, 1980). Meredith et al., (1983) studied the parasitism of *R. reniformis* on *Rhizobium* nodules of soybean root and suggested that *R. reniformis* parasitized the nodular tissues. The swollen posterior portion of the females and egg masses were found protruded from the root nodule surface. Section of nematode infected nodule showed that *R. reniformis* penetrated into the epidermis, nodule cortex and established permanent feeding site in the nodule endodermis but infected soybean root nodules did...
not differ in size and shape from healthy ones. The necrosis induced by the nematode in the endodermal layers and also in nodule and root cortex predisposed the nodular tissues to the infections of other pathogen and their consequent premature breakdown took place.

2.2.1.3 Effect of Migratory Endoparasitic Nematodes

Romaniko (1958) observed that Pratylenchus pratensis (de Man) Filipjev parasitized the nodules of pea, bean, vetch, pea vine, alfalfa and red clover. Germani et al., (1984) investigated that infestation of soybean by P. safaensis Fortunier, reduced nodulation and nitrogen content. They suggested that harmful effect of P. safaensis is similar to that of cyst or root-knot type nematodes as reported by Epps and Chambers (1962). Green (1984) studied that in vitro conditions cyst nematodes, P. thornei Sher & Allen and Ditylenchus dipsaci (Kuhn) Filipjev inactivated the nodules so that fully formed nodules lacked haemoglobin on pea plants. The plants compensated by developing extra nodules giving apparent increases in nodulation.

2.2.1.4 Effect of Ectoparasitic Nematodes

Germani et al., (1981) reported that Scutellonema cavenessi Sher, significantly affected growth and nitrogen fixation of infected soybean plants as compared to uninoculated plants.
2.2.1.5 Effect of Sedentary Endoparasitic and Ectoparasitic Nematodes

Malek and Jenkins (1964) reported that M. hapla, M. javanica, Trichodorus christiei and Criconemboides curvatus Raski apparently interfered directly with the establishment of nodule forming bacteria either by mechanically destroying the root hair, the infection sites, or changing the physiology of roots and thus rendered the roots incompatible to rhizobial infection.

2.2.2 Fungus-Rhizobia Associations

Not much literature is available on the inter-relationships between fungi and root nodule bacteria.

Twng-Wah and Howard (1969) investigated the role of nodule forming Rhizobium japonicum as potential antagonists to Fusarium oxysporum on soyabean. Since growth of Rhizobium is sensitive to acidity, hydrogen-ion-concentration (pH) was chosen as a major environmental variable. Substrates buffered at pH 5.2 permitted severe root cortex necrosis by Fusarium, sparse rhizobial nodulation and no nodulation when Rhizobium and Fusarium inocula were added simultaneously. At pH 7.0-7.6 rhizobial nodulation was good, root-rot was severe with Fusarium alone, but reduced to a trace or none in seedlings exposed currently to both organisms. F. oxysporum with R. japonicum slightly reduced nodulation when compared with Rhizobium alone.

Drapeau et al., (1973) studied antifungal activity of three Rhizobium isolates against eight different fungi. They observed that six fungi viz., Fusarium melanochlorum (Casp.) Sacc., F. culmorum (W.G.Sm.) Sacc., Pyrenocheata terrestris (Hansen)
Gorenz, Walker & Larson, *Colletotrichum destructivum* D'Cara, *Phytophthora cactorum* (Leb & Cohn) Schroet and *Coniothyrium* Corda sp., were inhibited by *Rhizobium* while the growth of remaining two fungi viz., *Rhizoctonia solani* and *Pythium ultimum* was not affected. Some of these fungi are potential phytopathogens on legumes. Gupta (1974) studied the effect of rhizosphere fungi on nodulation and found that all the test fungi (*Alternaria tenuis* Nees, *Aspergillus luchuensis* Inui, *A. nidulans* (Eidam) Winter, *A. niger* Van Tieghem, *Cunninghamella echinulata* Thaxter, *Curvularia lunata*, *Cladosporium herbarum* (Persoon) Link, *Chaetomium* Kunze & Schmidt sp., *Helminthosporium sativum* Pammel, *King & Bakke, Mucor luteus* Linnemann, *Paecilomyces fuscisporus* Saksena, *Penicillium javanicum* Van Beyma, *P. citrinum*, *Rhizopus nigricans*, *Syncephalastrum racemosum* (Cohn) Schroeter, *Thielaviopsis* Went sp. and *Trichoderma lignorum* (Tod) Harz either individually or concomitantly, when mixed with sterilized soil, reduced the number of nodules in comparison to control. However, *M. luteus*, *A. niger*, *A. nidulans*, and *Thielavia terricola* (Gilman & Abbott) Emmons showed maximum inhibitory effect on nodulation. He, further, reported that inhibition may be due to the secretion of toxic substances in the soil which indirectly reduced number of nodules. Chou and Schmitthenner (1974) suggested that more soyabean plants were killed by *Phytophthora megasperma* var. *sojae* Hildebrand, in sterile soil than in combination with *Erdoene mosseae* and *Rhizobium japonicum*. Therefore, they concluded that these two organisms may have a suppressive effect on root-rot severity.
Orellana and Worley (1976) observed that inter and intracellular penetration by *Rhizoctonia solani* hyphae of young functional root-nodules of soybean inoculated with *R. japonicum* was restricted to the outer cortex, penetration of the central tissue may have been impeded by a layer of sclerenchyma. Infected nodules also contained low concentration of leghaemoglobin. Dysfunction in young nodules grown in the presence of *R. solani* may be due to toxic metabolites diffusing throughout the nodules. Such dysfunction interfered with nitrogenase and symbiotic nitrogen fixation activities. Orellana et al., (1976) investigated that *R. solani* significantly reduced the nodule weight of cv. Lee and Kentwen soybean inoculated with *R. japonicum* and grown in a N-free sand nutrient substrate as compared to plants grown with *Rhizobium* alone.

Gray and Hine (1976) observed that in pasteurized field soil, artificially inoculated with *Phytophthora megasperma* Drechs., death of alfalfa seedlings was 24% higher when seeds were bacterized with *Rhizobium meliloti* Dangeard than of seedlings obtained from unbacterized seeds. Increased seedlings death were not observed when treated or untreated seeds were planted in field soil naturally infested with *P. megasperma* and *R. meliloti*. The nodules might have affected the host defence mechanism in such a way that normal mechanisms for limiting fungal activities were altered.

In a glass house experiment the severity of root-rot caused by *P. megasperma* was lessened when rhizobia were applied immediately after planting of soyabean plants (Tu, 1978). At a
given concentration of *P. megasperma*, root-rot severity decreased when the concentration of *Rhizobium* in the soil increased. Thin sections of hyphae, contaminated with rhizobia, showed the consistent bacterial presence inside the hyphae. These observations indicated that rhizobia living saprophytically in soil may reduce the root-rot by parasitizing hyphae of the fungus. Again, Tu (1980) investigated that at a given concentration of *Rhizobium* the severity of root-rot increased with the increase in concentration of root-rot fungus. However, at a given fungal concentration, increasing concentrations of rhizobia decreased the degree of root-rot. Therefore, he concluded that rhizobia protected alfalfa from winter kill by reducing the severity of root-rot and increasing total nitrogen content in alfalfa. Rhizobial protection was accomplished by early inoculation.

Kush (1982) conducted pot experiments using cowpea *Rhizobium* and one per cent inoculum of *Rhizoctonia bataticola* on greengram to study the interaction between symbiosis and root-rot in terms of plant growth and nitrogen fixation. He observed increased nodule fresh weight, nitrogenase activity and root and shoot dry weight in the *Rhizobium* treatment, but in presence of *R. bataticola* nodule fresh weight, nitrogenase activity and plant growth was reduced significantly over *Rhizobium* alone treatment. The microtomy of infected roots showed distortion of outer layers of roots as a possible cause for antagonistic interaction of these two bio-processes. Sawada (1982 and 1983) observed root discoloration and poor rhizobial nodulation of
lucerne seedlings when the soil was naturally infested with rhizobia and \textit{Fusarium oxysporum}. The severity of root-rot was less on nodulated seedlings than on nonnodulated ones when inoculated with \textit{R. meliloti} and \textit{F. oxysporum}. In vitro hyphal growth of \textit{F. oxysporum} was suppressed by multiplication of \textit{R. meliloti} in a mixed culture.

Beagle-Ristaino and Rissler (1982) reported that \textit{Phytophthora} root-rot of soyabean caused by \textit{P. megasperma} f.sp. \textit{glycines}. Kuan & Erwin was more severe on susceptible plants nodulated by \textit{R. japonicum} than on plants not nodulated. However, disease severity of inoculated resistant plants (cv. Harosoy 63) did not differ with or without nodulation. \textit{P. megasperma} f.sp. \textit{glycines} inoculated on cv. Harosoy had fewer nodules than did uninoculated Harosoy and Harosoy-63 plants, the fungus colonised the nodules of Harosoy but not Horosoy-63 soyabean. The lower nodule scores of fungus inoculated Harosoy plants might have been the result of destruction of both roots and root nodules by the fungus. Infected nodules were dark brown and often collapsed. Zamoblim and Schenk (1984) found that the number and weight of nodules were reduced due to infection of \textit{Macrophomina}, \textit{Rhizoctonia} and \textit{Fusarium} spp. in soyabean but increased considerably in presence of \textit{Glomus mosseae} (Nicol. & Gerd.) Gerd. & Trappe. Infection and disease intensity were, however, not significantly affected. There were no differences in growth response to \textit{G. mosseae} and the pathogens in nodulated and non-nodulated plants. Khan (1986) observed a gradual decrease in the number and size of cowpea nodules with the
increase in the inoculum level of R. solani. Similar results were also found with M. phaseolina on the nodulation of chickpea by Siddiqui (1990) and mungbean by Tiyagi (1990). In field trails over two years, the yield of mungbean was increased when Rhizobium symbiont was inoculated on the seeds or drenched at 30 days. However, in presence of M. phaseolina (inoculated by spraying with sclerotial suspension) the nodulation was reduced. Disease severity was higher on plants inoculated with fungus alone than in those with both pathogen and symbiont indicating an effect of the Rhizobium on host metabolism, leading to restraint of the pathogen (Bhattacharyya and Mukherjee, 1990). Infection of mungbean by Macrophomina phaseolina was reduced by the seed treatment with Rhizobium meliloti (Hussain et al., 1990) while in other study Rhizobium meliloti and Bradyrhizobium japonicum Jordan inhibited the growth of M. phaseolina, R. solani and F. solani in vitro and when used as seed dressing or soil drench reduced the infection of these root infecting fungi on both leguminous (soyabean and mungbean) and non-leguminous (sunflower and okra) plants, thereby indicating the biocontrol potential of Rhizobium species against root-rot diseases (Haque and Gaffar, 1993).

2.2.3 Effect of Nematode—Fungus Disease Complexes on root Nodulation

Although, the nematode and fungus infection causes considerable reduction in the root nodulation when they occur individually but in their concomitance the reduction in nodulation has been found to be much more pronounced.
Husain et al. (1985) reported significantly higher reduction in the nodulation of pea when three pathogens *M. incognita*, *R. solani* and Pea mosaic virus were inoculated simultaneously than any one of them alone. Mani and Sethi (1987) achieved similar results on chickpea by inoculating *M. incognita*, *Fusarium oxysporum f. ciceri* and *F. solani* simultaneously. On the other hand, Varshney et al., (1987) observed a considerable reduction in the nodulation of cowpea, when the inoculation of *M. incognita* preceded to that of *R. solani*. Khan and Husain (1988a) found maximum reduction in the nodulation of cowpea when *M. incognita*, *R. reniformis* and *R. solani* were inoculated simultaneously whilst, Gupta and Mehta (1989) observed the same in the concomitance of *M. javanica* and *R. bataticola* on mungbean. Adverse effects on nodulation was also noted by Tiyagi (1990) on mungbean and Siddiqui and Husain (1991, 1992) on chickpea upon the concomitant inoculation of *M. incognita* and *M. phaseolina* and by Anwer and Verma (1993) on chickpea in presence of *M. javanica* and *R. solani*.

2.3 EFFECT ON NUTRIENT ELEMENTS (Nitrogen, Phosphorus and Potassium etc.)

Among the different nutrient elements, the nitrogen phosphorus and potassium also called as primary macroelements are considered to be essential for the normal growth of plant. Nematodes and fungi have been found to cause diminished absorption of nutrient elements, resulting in their inadequate supply to the plants. Studies on the effect of nematodes and fungi on the mineral contents of parasitized plants have
received considerable attention during the past 30 years.

2.3.1 Effect of Nematodes

Vanha, as early as 1893, while studying the mineral composition of sugarbeets infected with *Heterodera schachtii*, reported significant reduction in the calcium, phosphorus, magnesium, nitrogen and potassium contents of the infected plants and concluded that the plants were deprived of these elements as a result of nematode infection. These observations were later confirmed by Wilfrath and Wimmer (1903) and several others. Kruger (1925) and Neuwirth (1930) noted that *H. schachtii* infection did not disturb the absorption ability of sugar beet roots but markedly influenced the uptake of nutrients, particularly that of potassium, which resulted in the appearance of potassium deficiency symptoms. Vosbury and Winston (1921) stated that *Heterodera radicicola* (Greeff.) Muller (=*Meloidogyne*) infection of pine apple seedlings resulted in the destruction of feeder roots leading to the development of deficiency symptoms.

Magistard and Oliveira (1934) found that the total nitrogen absorbed by the root-knot nematode infected pine apple plants was 40-50% less than that absorbed by the healthy plants. Paris and Jehle (1943), later, reported that lima beans, heavily infected with the root-knot nematode, were deficient in phosphorus, although sufficient phosphorus was present in the soil. Tarjan (1950) found low concentrations of essential elements in the *Pratylenchus* infested roots of boxwood plants.
than in the roots of healthy un inoculated plants. Root-knot infected lima beans contained less nitrogen, phosphorus, potassium, calcium, and magnesium than in the healthy plants (Oteifa, 1952). Lowensbery (1956) also reported less potassium content in the leaves of walnut (Juglandis hindsii Jepson) seedlings infected with Pratylenchus vulnus Allen & Jensen than in the leaves of healthy seedlings. Therefore, he concluded that the appearance of potassium deficiency symptoms was either due to the utilization of this element by the nematode or because of impaired absorptive capacity of roots or both. Sher (1957) obtained similar results with rose plants. Dropkin and King (1956) studied the uptake of P$^{32}$ in the healthy and galled tomato roots and observed that the phosphorus contents of nematodes present in the roots remained uniform thereby suggesting that nematodes did not absorb phosphorus from galled tissues during the course of their development. They also observed that in infected plants there was less translocation of phosphorus out of the galled roots to the aerial parts as compared to the healthy plants.

There are, however, some contradictory findings. Hunter (1958) suggested that Meloidogyne incognita acrita Chitwood caused no interference with the absorption or translocation of minerals including labelled phosphorus P$^{32}$. Bodovora (1961) observed that in root-knot infected cucumber plants uptake of P$^{32}$ was slowed down in the beginning, but, later on it levelled up. Oteifa and Elgindi (1962), on the other hand, noticed that both healthy and galled tomato roots infected with M. javanica
were capable of absorbing $^{32}P$, but in diseased plants a major amount of $^{32}P$ was accumulated in the galled tissue and only a limited amount was translocated to aerial parts, whereas in case of healthy roots a major fraction of $^{32}P$ was translocated to the aerial parts. Consequently they stipulated that prolonged parasitism leads to reduced translocation. The infection of both, *Pratylenchus penetrans* and *M. incognita acrita*, greatly reduced fresh and dry weight of shoot and root of pepper (*Capsicum frutescens* Rodsch.) (Shafiee and Jenkins, 1963). Furthermore both the nematodes brought about accumulation of nitrogen, phosphorus, potassium and sodium in the infected roots of plants. However, higher amount of potassium was detected only in the leaves of plants inoculated with *P. penetrans*. This led them to conclude that the imbalance in mineral content of nematode infected plants was not only due to the consumption of elements (nitrogen, phosphorus, potassium and sodium) by the nematodes but also because of the overall disturbed physiology of infected plants. Jenkins and Malek (1966), while investigating the effect of four nematode species, including *Meloidogyne hapla* on vetch (*Vicia villosa* Bro. Fl. Lusit.), concluded that nematodes in some way alter the plant mechanisms of absorption, translocation and accumulation of mineral constituents.

Bergeson (1966), by using split root technique, demonstrated that excess of sodium and potassium in the roots of tomato infected with *M. incognita* was due to the metabolic upsets in which the minerals were mobilised to the infection
site. He also pointed out that the excess of these elements due to the failure of infected roots to translocate them to other parts appeared to be slight. Dasgupta and Deb (1969) concluded that in tomato plants, infected with M. javanica and M. arenaria, the absorption of $^{32}$P was adversely affected and the differences were significantly greater after 14 days. In healthy plants, on the other hand, the absorption of phosphorus, nitrogen, potassium and magnesium remained high at all stages of growth as compared to the diseased plants while, accumulation of nitrogen, phosphorus and potassium in roots per gram of dry weight was higher in diseased plants. Thus, they concluded that root-knot infection adversely affected the absorption and translocation capability of plants.

Haque et al. (1972) reported that roots of infected plants contained more nitrogen, phosphorus and potassium than roots of uninfected plants. On the contrary, there was less nitrogen, phosphorus and potassium in aerial parts of the infected plants and thus showed deficiency symptoms. Meloidogyne incognita besides being pathogenic to mungbean was found to interfere bacterial nodulation and hamper nitrogen fixation. This reduction in nitrogen content may be due to overall reduction in root nodulation, anatomical changes in nodules and altered host physiology (Hussaini and Seshadri, 1975). Jatala and Jensen (1976) stated that sugarbeet plants inoculated with M. hapla had lower quantities of B, K, and P in leaf tissue than non inoculated plants. They also noted that plants inoculated with H. schachtii had lower quantities of B, K, Mg, Mn, Cu, and
In. Sharma and Sethi (1976) came to the conclusion that either M. incognita or H. cajani adversely affected the root nodulation and nitrogen content of cowpea plants. They observed maximum reduction (78.44%) in case of concomitant inoculations and that M. incognita reduced nitrogen content to a greater extent than H. cajani. Bopaiah et al., (1976a) stated that infestation of M. javanica interfered with the nitrogen fixation ability of Vigna radiata plants and reduced the nitrogen content of shoot and root. Ismail and Saxena (1976) reported that inoculation of M. incognita caused greater disturbance in the NPK in susceptible tomato plants than in resistant plants. Of the three elements, nitrogen and potassium were greatly influenced by nematode infestation. A significant reduction in the nitrogen content of shoot of mungbean was also observed with the increase in the inoculum level of M. incognita in presence and absence of Rhizobium (Singh et al., 1977).

Ali et al., (1981) noticed an antagonistic interaction between M. incognita and Rhizobium leguminosarum (Frank) Frank on cowpea. They observed that M. incognita reduced nodulation and inhibited nitrogen fixation by about 63% in the nodular tissues. Sharma (1984) reported that M. incognita infection interfered with the symbiotic nitrogen fixation and reduced the nitrogen content of pea plants. Meloidogyne incognita also reduced the number of nodules and total nitrogen uptake of mungbean cultivar ML-31 and G-65 irrespective of the initial inoculum level (Chahal and Chahal, 1987, 1989a), an identical effect also noted by Sharma and Tiyagi (1990) on pea.
2.3.2 Effect of Fungi

Like nematodes fungi are also known to disrupt the normal uptake of macro-elements. Reddy et al., (1969) observed that the concentrations of total phenols, flavanoids, amino nitrogen and total nitrogen were considerably reduced in rice seedlings infected with *Pyricularia oryzae* Cavara than the healthy seedlings. Reduction in nitrogen, phosphorus and potassium contents in bean pods due to infection of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav., has also been reported (Hegde and Munjal, 1971). Sivaprakasam et al., (1974) observed that infection of brinjal plants with *Verticillium dahliae* reduced the phosphorus and potassium content in the leaves.

Reduction in the contents of phosphorus, potassium, calcium, magnesium and iron in coriander plant parts (leaves and fruits) infected with *Protomyces macrosporus* Unger was also noticed (Gupta, 1975). Similarly, *Coccinia cordifolia* Cong., infected with powdery mildew (*Erysiphe cichoracearum* DC.) showed a marked decrease in nitrogen, phosphorus and potassium contents as compared to healthy leaves (Jamal and Khan, 1976). Orellana et al., (1976) observed 63% decrease in the fixed nitrogen per soyabean plant due to the infection of *Rhizoctonia solani*. *Puccinia helianthi* Schw. infection on sunflower leaves is also reported to cause reduction in the contents of sodium, potassium and phosphorus (Patil and Kulkarni, 1977). A significant decrease in NPK content was observed in infected leaves of date-palm and was indirectly proportional to the advancement of the
disease caused by *Graphiola phoenicis* Poit. (Kapur *et al.*, 1978). Bisen (1983) observed appreciable decrease in the nitrogen content of bean leaves infected with *Curvularia lunata* as against the healthy leaves. Prasad *et al.*, (1976) stated that *Puccinia carthami* Corda infection on safflower (*Carthamus tinctorius* L.) plants reduced the phosphorus content in the leaves. Similar observation was also reported by Siddaramaiah *et al.*, (1979) due to infection of *Puccinia arachidis* Speg. on groundnut.

Metabolic contents of healthy leaf discs of eggplant when compared with that of discs attacked by fungus, *Alternaria tenuissima* (Nees ex. Fr.) Wiltshire showed a decrease in the soluble amino nitrogen, protein nitrogen and total nitrogen (Basalah *et al.*, 1984). Mishra and Rath (1986) observed a decrease in dry matter content as well as in the level of potassium, phosphorus and calcium in rotten tissue (due to Fusarial rot) of brinjal fruits as compared to adjacent healthy tissue. Reduction in nitrogen (total proteinaceous and non-proteinaceous was also recorded in the leaves of coriander infected with *Protomyces macrosorum* (Prasad *et al.*, 1989). Khan and Husain (1990b) studied the effect of individual and combined inoculation of *Rotylenchulus reniformis*, *M. incognita* and *R. solani* on the nutrient levels in cowpea and reported that all the pathogens whether inoculated singly or concomitantly caused a significant reduction in nitrogen, phosphorus and potassium content of root and leaf except in the inoculation of *M. incognita* alone or in combination with *R. reniformis* where an
increased accumulation of phosphorus and potassium was observed in roots. Individually *R. solani* caused greater reduction than caused either by *M. incognita* or *R. reniformis*. However, phosphorus content of leaf was not significantly reduced in plants inoculated with *R. reniformis*.

There are, however, some contradictory findings of different workers regarding the mineral content of plants infected with nematode and/or fungus. Chitwood et al., (1951) stated that higher inoculum level of either *M. incognita* or *M. javanica* resulted in an increase in the magnesium and iron and a decrease in potassium and calcium content in the roots of susceptible peach seedlings, whereas there was an increase in the magnesium, calcium and potassium contents of leaves. Healed and Jenkins (1963 & 1964) reported that inoculation of 10,000 specimens of *Pratylenchus penetrans* on *Ilex rotunda* Colebr. ex Wall., and *Berberis juliana*, Schneider caused significant increase in the calcium and potassium content in leaves of both the plants but a decrease in nitrogen content of *B. juliana* leaves. In the roots, on the other hand, they observed more nitrogen and less of phosphorus, potassium and magnesium in *I. rotunda* and excess of calcium in *B. juliana*. Nasr et al., (1980) reported that the NPK contents of both, root and leaves, were significantly greater in bitter almond plants infested with *M. javanica* and *M. incognita* than the uninfested plants. They further reported that there were no significant differences in mineral concentration of roots and leaves between nematode infested nemaguard peach plants and control, except that the Fe
concentration of root was significantly less in infested plants. Price et al., (1982) suggested that infection of oat plants with Heterodera avenae Wollenweber did not impeded uptake or transport of nutrients and roots compensated for reduced and altered root growth by increasing rates of uptake of phosphorus and potassium. Ibrahim et al., (1982) observed that infection of cotton plants with Fusarium oxysporum f. vasinfectum induced a significant reduction in K, Mn, Mg, Zn, and Cu, and a significant increase in N and Ca. Nematode infection, however, produced a significant increase in N and Zn and a decrease in Cu and Fe. Saxena (1984) reported that infection of powdery mildew (Erysiphe polygoni DC.) increased nitrogen, phosphorus and potassium contents in the leaves of fodder guar. Similarly, Taphrina deformans (Berk.) Tul infected leaves of Prunus persica Batsch had higher quantities of nitrogen, phosphorus and calcium (Sharma & Sharma, 1990).

2.4 EFFECT ON PHOTOSYNTHETIC PIGMENTS (Chlorophyll contents)

Chlorophylls, the green pigments of plants are the most important pigments responsible for the conservations of light energy into chemical energy and are thus active in process of photosynthesis. Chlorophyll a and b are the most abundant pigments in the plants. Nematodes and fungi are known to interact with the metabolism of photosynthetic pigments in plants. However, the information about their influences on the photosynthetic pigments and photosynthesis is limited.
2.4.1 Effect of Nematodes

When nematodes infect one part of the host plant, e.g. roots, they rapidly disrupt the host physiological process in one way or the other. Loveys and Bird (1973) reported that *M. javanica* infection caused the reduction in net photosynthetic rate and chlorophyll contents of tomato. Nematode invasion is also known to bring a change in the concentration of nutrient elements such as Fe, Zn, Mn, K etc. which play an important role for the constituents of plants e.g. Fe and Mn in photosynthetic pigments. Change in the concentration of these elements in plants even to a small extent, appear to have a profound impact on host physiology which in turn appear to have a major cause in limiting the growth of host plant and cause imbalance in translocation process. (Bird and Loveys, 1975, McClure, 1977).

Singh *et al.*, (1977) observed a gradual decrease in the chlorophyll content of mungbean with the increase in the inoculum level of *M. incognita* both in presence and absence of *Rhizobium*. The bacterised plants had higher concentration of chlorophyll than the unbacterized ones. The reduction in chlorophyll content was attributed to the alteration of host nutrition and physiology, as reported earlier (Bergeson, 1966; Doney *et al.*, 1970). Melakeberhan *et al.*, (1985a) found a significant decrease in the chlorophyll content of frenchbean, within two weeks after infection with different inoculum levels of *M. incognita*. In another study the chlorophyll content at the end of experiment was markedly lower in the frenchbean plants inoculated with *M. incognita* (single generation) at the bud
stage than at either of earlier stages (BPLE, TRIF). While chlorophyll b content in all plants did not change significantly with increasing inoculum level, the total chlorophyll (a+b) in the TRIF and BDS plants and chlorophyll a content in all plants was significantly lower and become increasingly so with increasing inoculum level (Melakeberhan et al., 1986).

Upadhyay and Banerjee (1986) reported that decrease in the chlorophyll content of chickpea due to M. javanica infection was because of proportional increase in the concentration of pheophytin. The imbalance in chloroplast pigments may be correlated with the general chlorosis and die back caused by the infection of nematodes.

The increased number of nematodes (M. incognita) caused significant reduction in the chlorophyll content (a,b) of mungbean leaves, which ultimately lead to the reduced production and supply of carbohydrates to nodules for carrying out nitrogen fixation (Chahal and Chahal, 1987). Quantitative changes in the chlorophyll content of root-knot infected plants have also been reported on pigeonpea (Anwar and Alam, 1989) and on chickpea (Ahmad and Kumar, 1990; Tiyagi and Alam, 1990).

Sharma and Trivedi (1992) observed a decreased photosynthetic efficiency and reduced chlorophyll content in the brinjal plants 90 days after treatment with M. incognita. However, application of Paecilomyces lilacinus (Thom.) Samson mitigated the ill effect of nematodes and increased the net photosynthetic rate and chlorophyll content to a better extent as compared to Verticillium chlamydosporum Goddard. Chandel et al., (1993)
found a higher reduction in the chlorophyll content of susceptible pigeonpea cultivar when inoculated with *M. incognita*, whereas Vashisth *et al.*, (1994) observed a similar effect of root-knot nematode on the chlorophyll content of some blackgram cultivars.

Similarly, the cyst nematode *Heterodera* spp. has also been reported to cause the considerable reduction in the photosynthetic pigments. Kaushal and Madavi (1992) studied the effect of *H. avenae* on the photosynthesis and chlorophyll content of *Triticale* and observed reduced photosynthetic efficiency and chlorophyll contents at the higher levels of nematode density, similar effect was also noted by Nagesh and Dhawan (1988) on wheat. Siddiqui (1993) found a significant reduction in the chlorophyll content of pigeonpea leaves in *H. cajani* infections.

### 2.4.2 Effect of Fungi

Fungus also had an adverse effect on the photosynthetic pigments. *Taphrina maculans* Butler infected leaves of turmeric (*Curcuma longa* L.) possessed lower contents of chlorophyll than the healthy ones (Agarwal *et al.*, 1982), an effect also noted by Srinivasan (1982) on arecanut infected with yellow leaf spot disease. Parmar *et al.*, (1983) observed 98% reduction in the pigments of *Cichorium intybus* L. leaves infected by *Alternaria cichoriij* Nattrass. Murumkar and Chavan (1985) reported that wilt fungus *F. oxysporum* f.sp. *ciceri* caused a marked reduction in chlorophyll content of chickpea, while as Singh *et al.*, (1986b)
found reduced chlorophyll and carotenoid contents in downey mildew (*Pencospora arborescens* (Berk.) de Bary) infected leaves of opium. Reduction in chlorophyll (a, b and total), carotenes and xanthophyll contents were also recorded in the leaves of coriander infected with *Protomyces macrosporus* (Prasad et al., 1989). Similar marked decrease of chlorophyll content also occurred in *Taphrina deformans* infected peach leaves (Sharma and Sharma, 1990). Buonaurio (1991) determined the chlorophyll content of chloroplasts from faba bean leaves infected with *Uromyces viciae* (Pers.) Schröet and observed decreased chlorophyll contents from the beginning of urediospore differentiation to postule eruption (8-14 days) after inoculation. In addition there was a significant reduction in chlorophyll a/b ratio. Similarly, in onion leaves infected with *Pencospora destructor* (Berk.) Casp., a significant gradual loss in the contents of chlorophyll a, b and total chlorophyll was observed with an increase in the infection of foliage (Sugha et al., 1992).

2.4.3 Effect of Nematode-Fungus Disease Complexes

Only few reports are available on the combined effect of nematode-fungus disease complexes on the chlorophyll contents of plants. Tiagi (1990) while studying the effect of *M. incognita* and *Macrophomina phaseolina* alone and in combination on the chlorophyll content of mungbean observed a significantly higher reduction in chlorophyll content (a, b and total) in concomitant inoculations than in either of them alone. Similar
effect in photosynthetic pigments (chlorophyll and carotenoids) was also noticed in simultaneous inoculation of *M. incognita* and *R. solani* on the potted French bean plants (Shah, 1993). Siddiqui and Mahmood (1994) examined the effect of the *Heterodera cajani*, *Fusarium udum* Butler and *Bradyrhizobium japonicum* on the chlorophyll content in the wilt disease complexes of pigeonpea. Individually each pathogen reduced the chlorophyll contents but simultaneous inoculation had a synergistic effect on it. *B. japonicum* caused increased chlorophyll contents.

### 2.5 EFFECT ON LEGHAEEMOGLOBIN CONCENTRATION

Among the several families of the Plant Kingdom, the family leguminosae contributed a good deal to the nitrogen economy of nature through symbiotic association with the bacteria of the genus, *Rhizobium* in nodules formed on the root system. Based on colour, three types of nodules have been met with in legumes. The pink nodules possess leghaemoglobin, while the green and the brown ones have legcholeglobin and legmethaemoglobin respectively. Out of these, leghaemoglobin is active in nitrogen fixation while the other two are relatively inactive but they are interconvertible (Chopra and Subba Rao, 1967).

Leghaemoglobin (lb) is generally localized in the cytoplasm of cells containing bacteroids and not inside the prebacteroidal membrane (Verma et al., 1978). Leghaemoglobin appears to be a product of the *Rhizobium* legume complex, since the pigment is not present in bacteria when cultured alone.
Leghaemoglobin is a protein which is synthesized exclusively in the nitrogen fixing root nodules and is restricted to infected cells within these nodules where it constitute 25-30% of the total soluble protein of the cell. Leghaemoglobin has myoglobin like properties, which is a muscle protein whose function is thought to be that of assisting diffusion of $O_2$ into tissues and possibly providing a "store" of $O_2$ (Bray, 1983).

Several workers have strongly suggested that leghaemoglobin is involved in nitrogen fixation. The fact that nodules lacking leghaemoglobin are unable to fix nitrogen and that a correlation exists between leghaemoglobin concentration and the rate of nitrogen fixation is apparent from numerous investigations (Virtanen et al., 1947a,b). Leghaemoglobin is similar to mammalian haemoglobin and acts as an $O_2$ carrier in the process of Nitrogen fixation in nodules. It is probably important in the oxidation metabolism that provides the energy for driving nitrogen fixation, but it may also have a role in protecting the oxygen sensitive Nitrogen fixing enzyme from the effects of atmospheric oxygen (Bidwell, 1979).

Very little information is available regarding the effect of pathogens on leghaemoglobin concentration. Orellana and Worley (1976) suggested that, if fungal incited cytopathogenic dysfunction occurs near or at the time of nodule cell differentiation and nodule expansions then leghaemoglobin synthesis, $O_2$ diffusion and nitrogen fixation process might be affected or completely suppressed. Orellana and Fan (1978).
reported that infection by bean yellow mosaic virus in *Phaseolus vulgaris* reduced the leghaemoglobin concentration in nodules. Sharma and Sethi (1975), on the other hand, showed that combined inoculation of *M. incognita*, *Heterodera cajani* and *Rhizobium* on cowpea adversely affected nodulation and leghaemoglobin contents. Both nematode species thrived well and completed their life cycle on nodular tissue but *M. incognita* caused greater reduction in leghaemoglobin content than *H. cajani*. Bopaiah et al. (1976b) noted that *M. javanica* infection in mungbean reduced the leghaemoglobin content in root nodules by 12% over the control. They further reported that maximum leghaemoglobin content was in the plants inoculated with *Rhizobium* alone, moderate in plants treated with carbofuran, aldicarb and fensulfothion but significantly low in plants treated with methomyl oxamyl and DBCP.

McGinnity et al. (1980) indicated that nodulation and nitrogen fixation are influenced by cultivar x *Rhizobium* strain and soyabean cyst nematode race interactions and that nematode is not equally deleterious to all susceptible cultivars. Leghaemoglobin (lb) per gram of nodule was lower in soyabean plants infected with *Heterodera glycines* race I than in nodules of check plants. Since nodules from nematode infected plants had a higher Lbc/Lba ratio, this suggested that nodule development was impaired with nematode infection (Huang and Barker, 1983). Significant reduction in overall lb content, however, suggested that nodules were senescent. Reduction in nitrogen fixing efficiency results therefore, either from reduced number and
size of nodules, or their impaired development or early senescence on soyabean infected with *H. glycines* or all act jointly and the overall effect is deleterious for crop. Green (1984) observed that *in vitro* inoculation with *Pratylenchus thornei*, *Ditylenchus dipsaci* and cyst nematode in-activated the root nodules of pea in such a way so that fully formed nodules lacked by leghaemoglobin.

Chahal and Chahal (1988, 1989a,b) found significant reduction in the leghaemoglobin and bacteroid content and nitrogenase activity in chickpea and mungbean nodules on the roots infected with *M. incognita*. Verdejo et al., (1988), however, did not find any alteration in functioning of nodules on black bean and pea. They found an increase in leghaemoglobin content in nodules on pea, and decrease in nodules on black bean and suggested that pea nodules are in-determinate (apical) and therefore able to grow and form young tissue rich in lb when the plant is short of nitrogen. Black bean nodules are determinate (spherical) and once formed are less able to develop so that young tissue can only form in new nodules.

Khan and Husain (1989b) studied the effect of *Rotylenchulus reniformis*, *Meloidogyne incognita* and *Rhizoctonia solani* alone and in combination on the leghaemoglobin content of cowpea nodules and observed a significant reduction in lb content in all the pathogenic treatments, but the reduction was maximum in the combined inoculation of all the three pathogens. Individually *R. solani* caused greater reduction than either of *R. reniformis* or *M. incognita*. 

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2.6 MANAGEMENT OF NEMATODE AND FUNGAL DISEASES

Much emphasis has been given on the study of interaction of Meloidogyne spp. and root rot infecting fungi in the causation of disease complexes, whereas relatively little information is available on the control of such complexes. During the last few years there has been intensification of interest in the possibilities of influencing the soil environment by physical, chemical and biological means in order to render it relatively unfavourable to disease producing organisms. Except for Brodies (1970) brief review there is hardly any comprehensive effort to high-light the significance of multipathogenic scenario vis-a-vis their management. Recently, Khan and Reddy (1993) have discussed the various methods adapted to deal with the management of different plant pathogens involved in complex diseases. In this chapter the methods involving use of chemicals and organic amendments for the management of nematode-fungus disease complexes has been briefly reviewed.

2.6.1 Management by Chemical Methods

Chemicals have been used to control nematodes and other soil-borne plant pathogens for a long time (Wilhelm, 1966). There is a vast literature reporting the use of nematicides and fungicides against a primary target organism without consideration of their activity against other pathogens present in the soil. A brief review of Brodie (1970) on the imperatives of tackling the fungus -nematode complexes and subsequent
realization of multiferous activities of systemic chemicals, coupled with a better understanding of complex diseases, impressed upon the workers the need to evolve a multiple treatment plan with single or variety of chemicals in varying ratios to achieve optimal minimization of potentially damaging population. The reviews of Katan and Eshel (1973), Altman and Campbell (1977), Rodriguez-Kabana and Curl (1980) can be referred to in exploring the modalities for evolving chemical formulations exhibiting true features of "broad spectrum biocides", which Brodie (1970) has termed "super granule".

Soil fumigants have been used singly or in combination against pathogens involved in complex diseases. Usually combination of fumigants have been found to have chemical compatibility coupled with toxicity against soil biota like insect pests, fungi, and weeds capable of causing substantial damage. Good and Rankin (1964) explored the possibility of working out a common treatment schedule for nematodes and fungi posing a serious complex production problem for cotton and legume fields. Of the 11 soil fumigants representing specific nematode toxicity and a broad spectrum nature, only vapam and mylone individually and mixture of DD and vorlex were found to be effective against the nematode Pratylenchus brachyurus and the fungus Sclerotium rolfsii. Good (1964) found that amongst all fumigants i.e, MBr, SMDC, DMTT, DD, and DBCP, tested at 10 and 20 inches depth against root-knot-Fusarium wilt of okra and soft seeded weed, only MBR significantly reduced the disease complex. DD, on the other hand, could slightly check the
nematode-fungal complex but only when applied at a 10 inch depth, whereas DBCP was much more effective than DD at the same depth.

Meagher and Jenkins (1970) found that MCP (methyl bromide and chloropicrin), Ditrapex and EDB were effective against *Verticillium* wilt of strawberry and MCP and EDB against *M. hapla*. Only EDB exhibited dual efficiency against nematode-fungal complex. The authors concluded that in case of the nematode fungus complex and short duration crops, only low cost nematicides like EDB should be selected. For durable control MCP was found to be effective against *Pratylenchus-Verticillium* wilt complex of tobacco. Chloropicrin was effective only against *Pratylenchus* (Taylor et al., 1970). Methyl bromide, chloropicrin, DD mixture dazomet and mercury salts tested against *Heterodera avenae* and *Gaeumannomyces* (Ophiobolus) *graminis* (Sacc.) Sacc. were effective in the first year against both the organisms. Dazomet was effective against the fungus in the second year also but in the third year the fungus population increased in all the treated plots except those that received two successive applications of DD mixture. Chloropicrin double application was effective against the nematode. On the otherhand, two formalin applications caused a population build up of *H. avenae* which at the end of third year almost trebled (Williams and Salt, 1970).

Farley and Riedel (1971) working on the efficacy of DD and D140 against *Pratylenchus-Verticillium* complex of tomato
showed that DD at preplanting or D_{140} at transplanting time reduced the nematode population but was ineffective against the wilt fungus. In *Verticillium dahliae-Globodera rostochiensis* complex of potato, a mixture of methyl bromide and chloropicrin was found to have comparatively more fungicidal and nematicidal efficacy than either benomyl (fungicide) or aldicarb (nematicide) tested alone. However, its residual effect could not last up to the third crop (Hide and Corbett, 1974). DBCP in addition to nematicidal action possesses fungicidal properties also. Its twin potential in combination with fungicides like sodium azide is synergized. This combination was found to be potentially effective against nematode fungus complex in soyabean. (Kinloch and Schenck, 1978). In *Pratylenchus-Verticillium* complex of strawberry also, a combination of ditrapex and benlate was very effective (Szczygiel and Reabandal, 1982).

The non fumigant nematicides have been also tried alone and in combination with fungicides and herbicides so as to attain a goal of multiple pest control. Brodie and Hauser (1970) used a compatible mixture of aldicarb (a nematicide), pentachloronitrobenzene (a fungicide) and trifluraline (a herbicide) in a ratio of 8:2:1. The mixture was reported to be effective against pest complex of cotton that involved *Helicotrichia longicaudatus*, *Fusarium* wilt and weeds like *Richardia scabra* L. and *Digitaria sanguinalis* Scop. Replacement of aldicarb with fensulfothion was not effective against the nematode. Similarly, the herbicidal efficacy of trifluraline too
seemed to be synergized in combination. Effective wilt control was also related to decline in nematode population rather than merely due to the efficacy of fungicide against wilt fungus. The study clearly indicates the possibility of amalgamation of various chemicals with proven biocidal efficacy against a pest disease complex of a given crop. Jones and Overman (1976) noticed an improvement in the yield of tomato affected by nematode like Belonolaimus longicaudatus, Trichodorus christiei, Meloidogyne acrita, and wilt fungi Fusarium oxysporum Schlecht. ex. Fr. f.sp lycopersici (Sacc.) Synder & Hansen race 2 and Verticillium albo-atrum Reinke & Berth, when carbofuran was applied to higher soil pH (6.5-7.5). They suggested that, although carbofuran is not fungicidal, its negative influence should be seen in the light of nematode-fungus interaction. Abu El-Amayem et al., (1978) used CGA 12223 and benomyl for the control of damping-off of tomato disease and found that the separate use of both the chemicals was ineffective but their combined use provided significant reduction in the disease intensity. Meloidogyne incognita - Phytophthora infestans f.sp nicotianae de Bary complex was managed by a combination of nemacur and dasanit (Fortnum and Curin, 1984) whereas, on tomato Rotylenchulus reniformis-Rhizoctonia disease complex was effectively controlled by the use of bavistin and aldicarb in mixtures (Zakiuddin, 1984). Abu-El-Amayem et al., (1985) also explored a suitable nematicide-fungicide combination against M. incognita - Rhizoctonia solani complex on soyabean. They found a potent action of carbofuran-benomyl combination against the
nematode and of phenamiphos / carbofuran-benomyl against the fungus. Aldicarb and phenamiphos also have been reported to check *Fusarium* wilt of maize effectively although, indirectly through their toxic action against nematodes (Minton et al., 1985). Csinos et al. (1986) reported that blank shank (*P. parasitica* Dastur var. *nicotianae* Tucker) root-knot complex was best managed when metalaxyl and fenamiphos were used together. Kimpinski et al., (1987) recorded a positive correlation between aldicarb application and yield of barley and wheat, however, they did not observe a significant relation between the treatment and decline in nematode population and fungal disease. Hasan (1989) demonstrated that aldicarb, carbofuran and phorate significantly reduced the severity of disease complex involving *Heterodera cajani* and *Fusarium udum* on pigeonpea. Pandey and Singh (1990) reported that application of temik and brassicol as mixture significantly reduced the disease complex caused by *R. bataticola* - *M. incognita* on chickpea. However, the plant growth was comparatively better in temik + brassicol treatments than in any one of them alone.

### 2.6.2 Management by Organic Amendments

Plant residues and their decomposition products are important components of soil. Crop residues, green manure crops and other organic materials are added to arable soil by the farmers just as in nature, the leaves, needles and roots are continuously added to uncultivated soil. The influence of these residues is felt, either directly or indirectly, by the multitude of living components of that soil including soil fungi.
and plant roots. Incorporation of plant residues and organic amendments has been recognised as an effective way of achieving substantial population reduction of plant pathogenic forms like fungi, bacteria, nematodes etc. (Patrick and Toussoun, 1965; Sayre et al., 1964). There are indications that quite often during the decomposition process of one or other kind of organic material an identical mechanism may operate leading to the population reduction of pathogenic organisms.

Amongst organic amendments tested so far highly consistent and satisfactory control has been achieved by amending the soil with oil cakes. Lear (1959) demonstrated that amending the soil with castor pomace reduced the populations of root-knot, *M. javanica* and *H. schachti* and also the incidence of disease by amending the soil with castor pomace. Mankau (1962, 1963) obtained about 100 per cent reduction in the numbers of *Tylenchulus semipenetrans* by using castor pomace, however, the efficacy was more in green house and less in the field (Mankau, 1963). Miller and Taylor (1970) obtained successful control of *Heterodera tabacum* Lownsbery & Lownsbery by addition of castor pomace. In India also considerable work has been done on the control of plant parasitic nematodes by the application of oil seed cakes (Singh, 1965; Khan et al., 1966, 1974a,b; Singh and Sitaramaiah 1966, 1971a,b; Mathur and Prasad, 1973; Desai et al., 1979; Bhattacharya and Goswami, 1988; Darekar et al., 1990a; Rao et al., 1991; Mishra and Gupta, 1992). Singh (1965) pointed out that karanj cake (*Pongamia glabra* Vent.) reduced root-knot development on tomato both in pot and field,
however, for field higher dose was required. Khan et al., (1966) and Singh and Sitaramaiah (1966, 1971a) observed that soil amendments with oil-cakes considerably reduced the incidence of root-knot nematode on tomato and okra. Margosa and peanut cakes gave consistent control of root-knot disease. Oilcakes not only reduced galling by root-knot nematodes but also the population of the nematode in the root tissue and egg laying capacity of females. Khan et al., (1966, 1974b) noticed that water extracts obtained from oil-cakes or from de-oiled cakes brought about marked inhibition in larval emergence of *M. incognita* and suppression in nematode population of soil. Singh and Sitaramaiah (1973) reported that oil cakes of margosa, peanut, castor, mustard, linseed, mahua and coconut, when incorporated into the soil three weeks before planting, significantly reduced the incidence of root-knot nematode, caused by *M. javanica* on okra and tomato.

Trivedi et al., (1978); Desai et al., (1979); Alam et al., (1980); Goswami and Vijayalakshmi (1983); Jagdale et al., 1985a,b and Vijayalakshmi and Goswami (1986) noted reduction in the plant parasitic nematodes on chilli, tobacco, egg plant, tomato, betelvine, and greengram by amendments with different oil cakes. Bhattacharya and Goswami (1988) found that 4 per cent and 1 per cent dosages of neem and groundnut oil cakes, respectively, were the optimum doses for the control of *M. incognita* on tomato. Spot application of neem, karanj, mahua and castor oilseed cakes reduced *M. incognita* population and the root gall index, and increased the tomato yield than in furrow
application more effectively. Spot application of neem cake recorded the highest yield (Darekar et al., 1990a). In furrow application neem, castor, groundnut, and honge cake, significantly reduced the root galling on okra due to M. incognita, where neem gave the least root-knot index (Reddy and Khan, 1991). Dwivedi and Pandey (1992) observed a significant reduction in total number of nematodes, egg sacs, eggs, males and females/plant on greengram with the application of neem, mustard, linseed and mahua oilcakes. The greatest reduction occurred in neem cake application than the other amendments. Oil cakes of mustard, neem, mahua, linseed, castor and sawdust reduced the population of plant parasitic nematodes associated with soyabean in microplots. Soil Rhizobium population increased in all treatments over fallow at 60 days and 120 days after sowing except, with mahua cake application (Mishra and Gupta, 1992). Hossain et al. (1992) observed increased effectiveness of mustard and cotton oilcakes to control root-knot nematode with the increase in the decomposition period. At highest rate maximum reduction in root galling was achieved but at this rate plants suffered from phytotoxicity. However, when the oilcakes were allowed to decompose for 30 days before planting no phytotoxicity appeared. Chopped leaves of neem, castor, mustard and marigold when incorporated in soil, reduced the gall rating and number of juveniles of M. incognita significantly and supported tomato plants with heavier shoot and root systems (Owino et al., 1993a).
Adverse effect of organic amendments on the population of pathogenic fungi has also been obtained by several workers (Mitchell et al., 1941; Toussoun et al., 1963; Khan et al., 1973, 1974b,c; Kannaiyan and Prasad, 1981; Sinha and Prasad, 1986; Goswami and Meshram, 1991). Organic materials substantially suppressed the population of Phymatotrichum Bow. (Mitchell et al., 1941); Helminthosporium Link. ex Fr. (Chinn and Ledingham, 1957); Fusarium oxysporum Schlecht ex Fr. f. cubense (Smith) Wollen and Reink. (Sequeiera, 1962); F. solani, F. phaseoli (Toussoun et al., 1963) and R. solani (Davey and Papavizas, 1959; Papavizas and Davey, 1960). Singh and Pandey (1965) reported that groundnut and castor oil-cakes inhibited the population of Pythium aphanidermatum in soil. Oil cakes have also been found effective in suppressing the population of pathogenic fungi like R. solani, Colletotrichum spp. and Fusarium spp. in the rhizoplane of egg plant, okra and tomato (Khan et al., 1973; 1974c) and F. udum on pigeonpea (Singh and Singh, 1981, 1982).

Soils amended with organic amendments such as rice chaff, mustard cake, neem cake, saw dust and farm yard manure significantly reduced the seedling infection of rice owing to R. solani. Significant increase in shoot growth was noticed in soils amended with organic amendments while reduction in root growth of rice seedlings was also observed owing to the addition of organic amendments such as sunflower cake, mustard cake, coconut cake and farmyard manure (Kannaiyan and Prasad, 1981). Population of Pythium spp. on ginger (Sadavandan and
Iyer, 1986), Ganoderma lucidum (Leyss.) Karst. on coconut (Gunasekaran et al., 1986) and F. oxysporum f. ciceri and Sclerotium sp. on gram (Sinha and Prasad, 1986) were also found to be reduced in presence of different oilcake amendments. Goswami and Meshram (1991) observed a marked change in the frequency and genera of fungal flora three weeks after the decomposition of mustard and karanj seed cakes.

Although, a considerable success has been achieved in the recent years for controlling plant diseases caused by nematodes and fungi yet the effects of organic matters on nematode fungus disease complexes have received little attention. Khan et al., (1973, 1974c) showed that incorporation of oil cakes, viz., margosa, castor, groundnut in natural field soil, resulted in population decline of parasitic fungi and nematodes in the rhizosphere of tomato and egg plant. Azam (1975) tested the efficacy of oil seed cakes (neem, mustard, castor, groundnut and mahua) in nematode-fungal disease complexes involving M. incognita, R. solani, Pythium spp. and Colletotrichum atramentarium on egg plant and noted a significant improvement in the plant growth at the lower doses as compared to higher doses. Optimal reduction in root-knot index and larval population was found in pots treated with 50 g cakes, however, this dose was phytotoxic. Khan and Husain (1988b) observed no significant improvement of cowpea plant growth in the groundnut cake seed treatments, when inoculated with R. reniformis or M. incognita alone and in combination with R. solani. However, seeds treated with neem cake significantly
improved the plant growth and reduced the nematode multiplication of both the nematode species when inoculated either individually or concomitantly with test pathogens. Oil cake amendments of neem, mustard and karanj caused a significant reduction in the severity of complex disease caused by *M. incognita*, *F. oxysporum* on chickpea and *M. incognita-M. phaseolina* complexes on mungbean. Amongst the oilcakes neem cake proved to be most effective in reducing the disease and in improving the growth of the test plants (Tiyagi, 1990). Similarly, *M. incognita-M. phaseolina* disease complex of frenchbean (*Phaseolus vulgaris* L.) was substantially managed by the application of neem and mustard cakes with a most profound effect of mustard (Srivastava and Singh, 1990) whilst, in another study neem cake followed by the saw dust gave the better control of *M. incognita* on mungbean and soil amended with neem cake had recorded a significant increase in *Rhizobium*, *Azotobacter* Beijerinck and fungi and also showed a greatest increase in plant growth (Pandey et al., 1991).
3. MATERIALS AND METHODS

The different materials used and the methods employed during the course of proposed experimental programme are generalised as follows:

3.1. Selection of Test Crop and Pathogens

The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and the root-rot fungus *Rhizoctonia solani* Kühn, were used as the test pathogens and greengram (mungbean) *[Vigna radiata* (L.) Wilczek] cv. T-44 as test plant throughout the course of these investigations.

3.2. Preparation and Sterilization of Soil Mixture.

Sandy loam soil, which is commonly found in Aligarh was collected from fertile field in Aligarh Muslim University Agriculture farm. The soil was then passed through a coarse sieve (1 mm pore size) to remove stone particles and debris etc. Organic manure at the rate of 3:1 (soil:organic manure) was thoroughly mixed with the soil and 15 cm earthen pots were then filled with this soil-organic manure mixture @ 1 kg/pot. A little water was poured in each pot before transferring them to an autoclave for sterilization at 101b pressure for 1 hour. Sterilized pots were allowed to cool down at room temperature before use for experiments.

3.3. Raising and Maintenance of Test plant

Five uniform seeds of greengram cv. T-44 were surface sterilized with 0.1% mercuric chloride for 2 minutes, then
thoroughly washed thrice in distilled water and sown in pots containing sterilised soil. After emergence, the seedlings were thinned and only one seedling was allowed to grow in each pot. When plants attained the age of one week, these were inoculated with test pathogens, as per the schedule of the experiments.

Another experiment was also established on the similar pattern using bacterized seeds. The surface sterilized seeds were treated with greengram (mungbean) strain of *Rhizobium* (*R. phaseoli* Dangeard) obtained from IARI. Sucrose solution 5% used as sticker, was mixed with respective rhizobial culture. Seeds were then mixed with this mixture in such a manner that uniform coating formed on their surface. These bacterized seeds were dried at room temperature and then sown in autoclaved earthen pots containing soil-organic manure (3:1) mixture.

3.4. Preparation of Nematode Inoculum.

Culture of root-knot nematode, *M. incognita* was maintained on brinjal in concrete microplots. Large number of egg masses from heavily infected brinjal roots were handpicked with the help of sterilized forcep from the previously maintained pure cultures of *M. incognita* on brinjal in concrete microplots. Egg masses were washed with distilled water and placed on a small coarse sieve (1 mm pore size) fitted with moist tissue paper. The sieve was placed in 10 cm diameter petridish containing water just touching its lower portion. A series of such assemblies were kept to get required number of second stage juveniles (*J2*) for inoculation. The second stage
juveniles (J2) which were hatched out were collected along with the water from petridishes after 24 hours and transferred to a beaker. Fresh water was added to petridishes after withdrawing the nematode suspension every time. The process was repeated up to 5-7 days. These second stage juveniles served as the inoculum of root-knot nematode. Counting of the juveniles was done from the collected suspension of nematode. An average of five counts were made to determine the density of nematode in the suspension. Each sample was transferred to counting dish and counted under a stereoscopic microscope (Southey, 1986). Calculations were made for the total suspension to be used for inoculation.

3.5. Isolation of Fungus from Infected Roots

Greengram plants showing distinct galls and exhibiting root-rot symptoms were collected in polythene bags from infected fields. Serial washing technique (Harley and Waid, 1955) was employed to isolate fungus from infected roots. Roots were transferred to an sterilized dish containing sterile distilled water and gently freed of soil particles. The process was repeated till all the soil particles were removed. Later the roots were cut into approximately 5 mm pieces and transferred to petridishes containing 0.1% mercuric chloride solution. After one minute root pieces were washed at least thrice in distilled water and dried on filter paper. Five of these root pieces were then placed in each of 10 petriplates containing potato dextrose agar (PDA). (For 1 litre: Potato = 200g, Dextrose=20 g, Agar =20g and Distilled water=1000ml) with the help of sterilized forceps.
under aseptic conditions. Petriplates were incubated at 28±2°C for 10 days. The fungus that developed on root segments were examined and identified. On confirmation of its identity as *Rhizoctonia solani* its pure culture was prepared.

3.5.1. Raising and Maintenance of Fungus Culture

For obtaining sufficient inoculum the fungus was later cultured on 'Richard' liquid medium (Riker and Riker, 1936) having following composition.

- Potassium nitrate = 10.0 g
- Potassium dihydrogen phosphate = 5.0 g
- Magnesium sulphate = 2.5 g
- Ferric chloride = 0.02 g
- Sucrose = 50.0 g
- Distilled water = 1000 ml

The medium was prepared and filtered through muslin cloth, sterilized in an autoclave at 15 lb pressure for 15 minutes in 250 ml Erlenmeyer flasks each containing 100 ml of liquid medium. The fungus was inoculated in each flask with the help of inoculation needle, in an aseptic chamber. Inoculated flasks were then incubated at 28±2°C for about 15 days to allow sufficient growth of the fungus. Pure cultures were continuously maintained on PDA by reinoculation of the fungus after every 15 days.

3.5.2. Preparation of Fungal Inoculum

After incubating the flasks for about 15 days the
required medium was filtered through Whatman filter paper No.1. The mat was washed in distilled water and gently passed between sterile blotting sheets to remove excess amount of liquid. The inoculum was prepared by mixing 10 gm fungal mycelium in 100 ml of sterilized distilled water and blending it for 30 seconds in a mixer (Sternerding, 1964). In this way each 10 ml of this homogenate contained 1 gm of fungal mycelium.

3.6. Inoculation Technique

One week old seedlings of greengram (mungbean) cv. T-44 were inoculated with 2000 second stage juveniles of *M. incognita* and 2g mycelium of *R. solani* throughout the course of these investigations, unless stated otherwise. Feeder roots of both bacterized and/or unbacterized seedlings were exposed just before inoculation by carefully removing the top layer of soil and required quantity of inoculum was poured uniformly all around the exposed roots using sterilised pipette. Exposed roots were immediately covered by leveling the soil properly.

Both individual and combined inoculation of both the test pathogens were done depending upon the experiment. Throughout these studies each treatment was replicated thrice and uninoculated plants were kept as control. Watering was done whenever required. Experiments were terminated after 60 days of inoculation.

3.7. Recording of Observations

3.7.1. Parameters Used

The plants were uprooted after 60 days of inoculation.
and their roots were gently washed of the soil, taking utmost care to avoid losses and injury during the entire operation. For measuring length and weight the plants were cut with sharp knife just above the base of the root emergence zone. The length of the shoots and roots were recorded in centimeters from the cut end to the top of the first leaf and to the longest root respectively. The excess of water was removed by putting the plant parts between the folds of the blotting sheets, for sometime before taking their fresh weights. The weight was recorded in grams. For dry weight, the roots and shoots were kept in bamboo envelops for drying in an oven at 60°C for 2-3 days. Reduction in dry plant weight (root+shoot) was calculated in terms of per cent reduction for the interpretation of the results.

3.7.2. Root Nodule Estimation

Nodulation was estimated by counting the number of nodules per root system and percentage of nodulation reduction over control was calculated.

3.7.3. Root-knot Estimation

The root-knot nematode galls were estimated by counting the number of galls per root system. The degree of root infection caused by root-knot nematode was assessed according to the rating scale of Taylor and Sasser (1978) for the presence of root galls as under 1 = No gall, 2 = 1-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = 101 and above galls.
3.7.4. Determination of Root-rot Index

The relative amount of \textit{R. solani} damage was determined by scoring the extent of disease on the following scale (Batten and Powell, 1971).

1 = less than 10\% root-rot,
2 = 11-25\% root-rot,
3 = 26-50\% root-rot,
4 = 51-75\% root-rot,
5 = 76-100\% root-rot (severe root-rot)

3.7.5. Nematode population Estimation

For extraction of nematodes from the soil, 250 g sub sample of well mixed soil from each treatment was processed through Cobb's sieving and decanting method followed by modified Bearman funnel technique (Southey, 1986). The nematode suspension was collected after 24 hours and number of the nematodes were counted in the counting dish by taking three replicates of 2 ml suspension from each sample. Mean of the three such samples was obtained and the population of nematodes per kg soil was calculated.

For estimation of nematode population in roots, 1.0 g of root, from each replicated treatment was macerated, for 45 seconds in an electrically operated wearing blender, in enough water. Counting was done from the suspension thus obtained as described above. The total final population was obtained by adding the soil as well as root population and the reproduction factor (Rf) was calculated by the formula of Oostenbrink (1966).
as follows: \( R_f = \frac{P_f}{P_i} \)

Where \( P_f \) represents the final population and \( P_i \) represent the initial population of the nematodes.

### 3.7.6. Statistical Analysis

The data obtained were analysed statistically and significance of variance was calculated at \( P < 0.05 \) and \( P < 0.01 \) levels.
4. EXPERIMENTAL-II: PATHOGENICITY AND INTERACTION

4.1 Introduction

Plant-parasitic nematodes, often referred to as "hidden enemies", are among the most widespread and important pathogens causing crop loss. They are major pathogens in their own right and through their interaction with other disease causing agents (Sidhu and Webster, 1977, 1981b). Since plant-parasitic nematodes are in constant association with saprobic, pathogenic and symbiotic micro-organisms present in the rhizosphere, therefore, it is logical to consider interactions between these groups of organisms in terms of their combined effects on plant growth. In the complex biotic environment of soil, the pathogens are always influenced by associated micro-organisms. As they occupy the same environmental niche, such organisms besides influencing the plants are likely to influence each other as well. Interaction and interrelationship between different micro-organisms such as nematode-fungus (Powell 1971a,b, 1979; Pitcher, 1978; Webster, 1985; Hussey and McGuire, 1987; Francl and Wheeler, 1993; Evans and Haydock, 1993), nematode-Rhizobium (Malek and Jenkins, 1964; Taha and Kassab, 1980; Huang, 1987; Taha, 1993; Fazal, 1993) and fungus-Rhizobium (Tu, 1978; Sawada, 1983; Zambolim and Schenk, 1984; Khan, 1986; Siddiqui, 1990) have been reported from time to time. Thus, in place of long held belief of one pathogen-one disease, the new concept of multipathogen acting in concert causing economic injury to an organism has been emerged. Whatever the basis for interaction
between nematodes and other micro-organisms in inducing plant
disease the pathogenic interrelationships often show additive,
synergistic or antagonistic effects on plant disease
development.

In a recent study, a constant and concomitant
occurrence of root-knot nematode, *Meloidogyne incognita* and a
fungus, *Rhizoctonia solani* was observed in several greengram
(mungbean) growing areas of Aligarh district of Uttar Pradesh
State, India. Patches of several damaged mungbean plants were
observed in many fields. Examinations of soil and root samples
from such poor patches revealed the presence of moderate to high
population of *M. incognita* associated with the root-rot fungus,
*R. solani*. These observations evoked an interest to study the
problem to determine whether the damage was casual or because
of interaction of these two pathogens. In the present study an
individual and interactive effects of both the pathogens in
various combinations on different growth parameters of greengram
(mungbean) cv. T44 have been investigated and discussed.

4.2 Materials and Methods

4.2.1 Plant Materials

Seeds of greengram (*Vigna radiata*) cv. T-44 surface
sterilized with 0.1% mercuric chloride and rinsed thrice with
distilled water, were sown in 15 cm diameter earthen pots @ 5
seeds/pot filled with steam sterilized soil and farm yard manure
(3:1 v/v). In another set of experiments the bacterized seeds
were used, treating them with *Rhizobium* (mungbean strain) before
sowing using 5X sucrose \((C_{12}H_{22}O_{11})\) solution as sticker. One 7-day old healthy seedling was retained in each pot. The plants were irrigated whenever required. The procedure is described in Chapter-3 (3.3).

4.2.2 Inoculation of Test Pathogens

Inoculum of both the test pathogens \(M.\ incognita\) and \(R.\ solani\) was obtained as per the procedure described in Chapter-3 (3.4, 3.5, 3.5.1, 3.5.2). Seven days old seedlings of greengram were inoculated by pipetting the required quantity of inoculum of each pathogen around the root zones of seedlings. The details of procedure adapted is described in Chapter-3 (3.6).

4.2.3 Parameters

Two months after inoculation, experiments (4.3.1, 4.3.2, 4.3.3, 4.3.4, 4.3.5) were terminated and following parameters were considered for describing the results.

1. Dry plant weight (root+shoot) (plant growth),
2. Nodulation,
3. Gall number,
4. Rate of nematode multiplication, and
5. Root-rot index.

All these parameters were assessed according to the procedures described in Chapter-3 (3.7.1, 3.7.2, 3.7.3, 3.7.4, 3.7.5).

Per cent decrease in total dry weight of plant
(root+shoot) and nodulation over uninoculated control was calculated for all experiments. Whereas for nematode multiplication, gall number, root-rot index the per cent increase (+)/decrease (-) was calculated against their respective inoculated controls.

4.2.4 Statistical Analysis

The data of all the experiments (4.3.1, 4.3.2, 4.3.3, 4.3.4, 4.3.5) obtained were analysed statistically for critical difference (C.D.) and the significance of variance was calculated at P<0.05 and P<0.01 levels as per procedure described by Panse and Sukhatme (1989).

4.2.5 Experimental Design.

4.2.5.1 Determination of Inoculum Threshold Levels of Meloidogyne incognita and Rhizoctonia solani (Experiment. 4.3.1.)

In order to determine the inoculum threshold levels of M. incognita and R. solani the seedlings raised from bacterized and unbacterized seeds were inoculated with 250, 500, 1000, 2000, and 4000 second stage juveniles (J2) of M. incognita. Similarly, the seedlings were also inoculated with 0.25, 0.5, 1.0, 2.0, and 4.0 g mycelium of R. solani for determining fungal inoculum threshold.

4.2.5.1.1 Experimental

The experiment was designed according to the following treatment scheme:

a) Uninoculated control,
b) Inoculated with different inoculum levels of M. incognita
c) Inoculated with different inoculum levels of *R. solani* (0.25, 0.5, 1.0, 2.0 or 4.0 g fungus/plant).

4.2.5.2 Effect of Individual and Concomitant Inoculation of Variable Inoculum Levels of *M. incognita* and *R. solani* (Experiments 4.3.2, 4.3.3.)

In order to find out the role of *M. incognita* and *R. solani* when present individually or concomitantly in the aggravation of plant damage, disease development and nematode reproduction on greengram cv. T.44, the seedlings were inoculated with different inoculum levels of both the test pathogens either individually or in various combinations.

4.2.5.2.1 Experimental

Greengram seedlings both bacterized and/or unbacterized were inoculated with different inoculum levels of test pathogens according to scheme shown in Table A.

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Nematode inoculum (number of larvae/pot)</th>
<th>Fungus inoculum (g mycelium/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Uninoculated</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Inoculated</td>
<td>500</td>
<td>--</td>
</tr>
<tr>
<td>3. -do-</td>
<td>1000</td>
<td>--</td>
</tr>
<tr>
<td>4. -do-</td>
<td>2000</td>
<td>--</td>
</tr>
<tr>
<td>5. -do-</td>
<td>--</td>
<td>0.5</td>
</tr>
<tr>
<td>6. -do-</td>
<td>--</td>
<td>1.0</td>
</tr>
<tr>
<td>7. -do-</td>
<td>--</td>
<td>2.0</td>
</tr>
<tr>
<td>8. -do-</td>
<td>500</td>
<td>0.5</td>
</tr>
<tr>
<td>9. -do-</td>
<td>1000</td>
<td>0.5</td>
</tr>
<tr>
<td>10. -do-</td>
<td>2000</td>
<td>0.5</td>
</tr>
<tr>
<td>11. -do-</td>
<td>500</td>
<td>1.0</td>
</tr>
<tr>
<td>12. -do-</td>
<td>1000</td>
<td>1.0</td>
</tr>
<tr>
<td>13. -do-</td>
<td>2000</td>
<td>1.0</td>
</tr>
<tr>
<td>14. -do-</td>
<td>500</td>
<td>2.0</td>
</tr>
<tr>
<td>15. -do-</td>
<td>1000</td>
<td>2.0</td>
</tr>
<tr>
<td>16. -do-</td>
<td>2000</td>
<td>2.0</td>
</tr>
</tbody>
</table>
In order to study the effect of early establishment of either of test pathogen, greengram seedlings were inoculated with 2000 infective stages (J2) of nematode, M. incognita and 2.0 g mycelial mat of fungus, R. solani individually or concomitantly, before or after 15 days of one another and/or simultaneously.

4.2.5.3.1 Experimental

Greengram (mungbean) seedlings (both bacterized and unbacterized were inoculated with M. incognita and R. solani singly or in various combinations of pre-, post- (with an interval of 15 days) or simultaneously as per the following schedule:

1. Uninoculated control,
2. M. incognita (Mi) alone,
3. R. solani (Rs) alone,
4. M. incognita + R. solani simultaneously (Mi+Rs),
5. M. incognita 15 days prior to R. solani (Mi --> Rs), and
6. R. solani 15 days prior to M. incognita (Rs --> Mi).

Uninoculated bacterized/unbacterized plants served as control in all experiments. Each treatment was replicated thrice. After inoculation the pots (from each experiment) were placed in a randomised block design on benches of greenhouse. Plants were allowed to grow for two months. After two months of inoculation the experiments were terminated and data on various
parameters mentioned in 4.2.3. were recorded and statistically analysed (4.2.4).

4.3 Experimental results

4.3.1 Effect of different inoculum levels of *M. incognita* and *R. solani* separately on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence and absence of *Rhizobium*

In order to determine the inoculum threshold levels of root-knot nematode, *Meloidogyne incognita* and root-rot fungus, *Rhizoctonia solani* on mungbean, the pathogenecity tests were conducted using 250, 500, 1000, 2000, and 4000 freshly hatched second stage juveniles (J₂) of nematode and 0.25, 0.50, 1.0, 2.0, and 4.0 gram of fungal culture per plant grown in one kg of steam sterilised soil (contained in 15 cm earthen pots) in presence and absence of *Rhizobium*.

It is evident from the data presented in Table-1.1, 1.2, 1.3, 1.4; Fig. 1, 2 and Appendix-IA, IB that irrespective of pathogens, the reduction in plant growth (length, fresh and dry weights of root and shoot) was directly proportional to inoculum level of each pathogen both in bacterized and unbacterized plants. The growth of unbacterized plants was comparatively less than that of bacterized ones both in inoculated and uninoculated plants.

4.3.1.1 Effect on plant dry weight (root+shoot)

The plant growth (total dry weight) was adversely affected by both the pathogens. There was a gradual decrease in plant dry weight with the corresponding increase in the inoculum
Table-1.1. Effect of different inoculum levels of Meloidogyne incognita on plant dry weight, nodulation, nematode multiplication, and gall formation in presence of Rhizobium.

<table>
<thead>
<tr>
<th>Inoculum levels</th>
<th>Plant Dry Weight</th>
<th>Nodules/root system</th>
<th>Nematode multiplication</th>
<th>Number of galls/root system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Root+Shoot)</td>
<td>% reduction over control</td>
<td>Number</td>
<td>% reduction over control</td>
</tr>
<tr>
<td>Control</td>
<td>3.30</td>
<td>-</td>
<td>49</td>
<td>-</td>
</tr>
<tr>
<td>(Uninoculated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 Mi</td>
<td>3.15</td>
<td>4.54</td>
<td>44</td>
<td>10.20</td>
</tr>
<tr>
<td>500 Mi</td>
<td>2.98</td>
<td>9.69</td>
<td>32</td>
<td>34.69</td>
</tr>
<tr>
<td>1000 Mi</td>
<td>2.73</td>
<td>17.27</td>
<td>27</td>
<td>44.89</td>
</tr>
<tr>
<td>2000 Mi</td>
<td>2.46</td>
<td>25.45</td>
<td>21</td>
<td>57.14</td>
</tr>
<tr>
<td>4000 Mi</td>
<td>2.28</td>
<td>30.30</td>
<td>17</td>
<td>65.30</td>
</tr>
</tbody>
</table>

C.D. (P 0.05) 0.43  12.32  2.78  7.69
C.D. (P 0.01) 0.61  17.18  4.05  11.19

Table-1.2. Effect of different inoculum levels of Rhizoctonia solani on plant dry weight, nodulation, and root-rot development in presence of Rhizobium.

<table>
<thead>
<tr>
<th>Inoculum levels</th>
<th>Plant Dry Weight</th>
<th>Nodules/root system</th>
<th>Root-rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Root+Shoot)</td>
<td>% reduction over control</td>
<td>Number</td>
</tr>
<tr>
<td>Control</td>
<td>3.30</td>
<td>-</td>
<td>49</td>
</tr>
<tr>
<td>(Uninoculated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25gfs</td>
<td>3.03</td>
<td>8.18</td>
<td>41</td>
</tr>
<tr>
<td>0.5gfs</td>
<td>2.85</td>
<td>13.63</td>
<td>27</td>
</tr>
<tr>
<td>1.0gfs</td>
<td>2.68</td>
<td>18.78</td>
<td>23</td>
</tr>
<tr>
<td>2.0gfs</td>
<td>2.33</td>
<td>29.39</td>
<td>18</td>
</tr>
<tr>
<td>4.0gfs</td>
<td>1.89</td>
<td>42.72</td>
<td>14</td>
</tr>
</tbody>
</table>

C.D. (P 0.05) 0.56  10.96  0.27
C.D. (P 0.01) 0.79  15.58  0.39

Each value is mean of three replicates,
*M* = Meloidogyne incognita, *Rs* = Rhizoctonia solani,
RF (Nematode reproduction factor) = PF (final population)/Pi (initial population)
FIG. 1—EFFECT OF DIFFERENT INOCULUM LEVELS OF *M. INCognita* AND *R. Solani* ON PLANT DRY WEIGHT MODULATION, NEMATODE MULTIPLICATION, ROOT GALLING AND ROOT-ROT DEVELOPMENT IN PRESENCE OF RHIZOBium.
Table 1.3. Effect of different inoculum levels of *Meloidogyne incognita* on plant dry weight, nematode multiplication and gall formation in absence of Rhizobium.

<table>
<thead>
<tr>
<th>Inoculum levels</th>
<th>Plant Dry Weight</th>
<th>Per cent reduction over control</th>
<th>Nematode multiplication Rf=Pf/Pi</th>
<th>Number of galls / root system</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Root+Shoot) &quot;g&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td>3.03</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>250 Mi</td>
<td>2.81</td>
<td>7.26</td>
<td>32.4</td>
<td>74</td>
</tr>
<tr>
<td>500 Mi</td>
<td>2.63</td>
<td>13.20</td>
<td>26.3</td>
<td>33</td>
</tr>
<tr>
<td>1000 Mi</td>
<td>2.38</td>
<td>21.45</td>
<td>18.2</td>
<td>125</td>
</tr>
<tr>
<td>2000 Mi</td>
<td>2.12</td>
<td>30.03</td>
<td>12.8</td>
<td>158</td>
</tr>
<tr>
<td>4000 Mi</td>
<td>1.73</td>
<td>42.90</td>
<td>10.6</td>
<td>179</td>
</tr>
<tr>
<td>C.D. (P&lt; 0.05)</td>
<td>0.56</td>
<td>2.65</td>
<td>9.58</td>
<td></td>
</tr>
<tr>
<td>C.D. (P&lt; 0.01)</td>
<td>0.79</td>
<td>3.85</td>
<td>13.95</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.4. Effect of different inoculum levels of *Rhizoctonia solani* on plant dry weight and root-rot development in absence of Rhizobium.

<table>
<thead>
<tr>
<th>Inoculum levels</th>
<th>Plant Dry Weight</th>
<th>Per cent reduction over control</th>
<th>Root-rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Root+Shoot) &quot;g&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td>3.03</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0.25g Rs</td>
<td>2.66</td>
<td>12.21</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5g Rs</td>
<td>2.48</td>
<td>18.15</td>
<td>2.0</td>
</tr>
<tr>
<td>1.0g Rs</td>
<td>2.15</td>
<td>29.04</td>
<td>2.5</td>
</tr>
<tr>
<td>2.0g Rs</td>
<td>1.89</td>
<td>37.62</td>
<td>3.0</td>
</tr>
<tr>
<td>4.0g Rs</td>
<td>1.49</td>
<td>50.82</td>
<td>4.3</td>
</tr>
<tr>
<td>C.D. (P&lt; 0.05)</td>
<td>0.64</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>C.D. (P&lt; 0.01)</td>
<td>0.92</td>
<td></td>
<td>0.53</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates.

Mi = *Meloidogyne incognita*, Rs = *Rhizoctonia solani*

Rf (Nematode reproduction factor) = Pf (final population)/ Pi (final population)
FIG 2.-EFFECT OF DIFFERENT INOCULUM LEVELS OF M. INCognita AND R. SOLANi ON PLANT DRY WEIGHT, NEMATODE MULTIPLICATION, ROOT GALLING AND ROOT-ROT DEVELOPMENT IN ABSENCE OF RHIZOBium.
level of each pathogen. However, statistically significant (P<0.05) reduction in plant dry weight over control was found only when 1000 or more infective stages (J2) of *M. incognita* or 1.0 g or more fungal mycelium of *R. solani* was inoculated per plant per kg of soil both in bacterized and unbacterized plants. In presence of *Rhizobium* the inoculation of plants with 250, 500, 1000, 2000, and 4000 J2 of *M. incognita* resulted in 4.54, 9.69, 17.27, 25.45, and 30.90% dry weight reductions, whereas with increasing inoculum levels (0.25, 0.50, 1.0, 2.0, and 4.0 g) of fungus, *R. solani* the plant weight was reduced by 8.18, 13.63, 18.78, 29.39, and 42.72, respectively (Table-1.1, 1.2; Fig. 1).

Unbacterized plants also responded in a similar way to different pathogenic treatments, but they showed greater growth reductions than that observed in bacterized plants. The reduction in plant growth being highest (ranging from 12.21-50.82%) in *R. solani* treatments and lowest (7.26-42.90%) in *M. incognita* treatments (Table-1.3, 1.4; Fig. 2).

4.3.1.2 Effect on nodulation

A considerable reduction in root nodulation was observed due to the parasitism of both the test pathogens but the reductions caused by *R. solani* were greater than *M. incognita*. Reduction in nodulation was also directly proportional to the increase in the inoculum level of each pathogen. However, the significant reduction in nodulation over control occurred only when 500 or more infective stages (J2) of
M. incognita or 0.5g or more fungal mycelium of R. solani were inoculated. Higher inoculum level (4g/pot) of R. solani caused 71.42% reduction whereas, in the higher inocula (4000 Jg/pot) of M. incognita the reduction in nodulation was 65.30% (Table-1.1, 1.2; Fig. 1).

4.3.1.3 Effect on nematode multiplication

The final nematode population was highest in and around the plants inoculated with 4000 juveniles/plant and lowest in those inoculated with 250 juveniles/plant (Appendix-IA, IB). However, the rate of nematode multiplication of both bacterized and unbacterized plants decreased with the increase in the inoculum level from 250 to 4000 suggesting it to be a density dependent phenomenon. But in unbacterized plants the nematode reproduction factor (Rf) was comparatively higher than the bacterized plants at all inoculum levels. Maximum nematode reproduction (Rf=29.3 for bacterized plants and Rf=32.4 for unbacterized plants) occurred at the lowest (250 Jg/plant) inoculum level and minimum (Rf=8.82 for bacterized plants and Rf=10.6 for unbacterized plant) at the highest (4000 Jg/plant) inoculum level (Table-1.1, 1.3; Fig. 1, 2).

4.3.1.4 Effect on root galling

Root galling increased significantly with the increase in the initial inoculum levels of M. incognita both in bacterized and unbacterized plants, the highest being in unbacterized plants. The number of galls ranged between 74-179 in unbacterized (Table-1.3; Fig. 2) and 62-165 in bacterized
plants (Table-1.1; Fig. 1) with the increased inoculum levels from 250-4000 larvae.

4.3.1.5 Effect on root-rot development

With the increase in the initial inoculum levels of *R. solani* (from 0.25g - 4.0g), there was a gradual increase in the root-rot index of both bacterized and unbacterized plants. But in unbacterized plants the root-rot development was maximum as compared to bacterized ones. In the increasing inocula of fungus the root-rot indices ranged between 1.5-3.5 in bacterized plants (Table-1.2; Fig. 1) and 1.5-4.3 in unbacterized plants (Table-1.4; Fig. 2). There was no significant difference of root-rot between the lower two inoculum levels (0.25g and 0.5g) but in other increasing inocula of fungus showed significant variation in the root-rot when compared to initial inoculum level.

4.3.2 Effect of individual and concomitant inoculation of variable inoculum levels of *M. incognita* and *R. solani* on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence of Rhizobium

Results of the data presented in Table-2.1, Fig. 3 and Appendix-IIA, clearly indicate that with the increase in the inoculum levels of each pathogen [*M. incognita* (Mi) or *R. solani* (Rs)] there was a corresponding decrease in plant growth parameters viz., length, fresh and dry weights of root and shoot but, in case of combined inoculation (Mi+Rs) the degree of their individual effects were modified to a greater or lesser extent. Lower inoculum levels of both the test pathogens individually did not cause any marked variation in the plant.
Table 2.1. Effect of individual and concomitant inoculation of variable inoculum levels of *Meloidogyne incognita* and *Rhizoctonia solani* on plant dry weight, nodulation, nematode multiplication, gall formation and root-rot development in presence of *Rhizobia*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry Plant Weight (g)</th>
<th>Modules / Root system</th>
<th>Nematode multiplication</th>
<th>Galls / Root system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root+shoot reduction</td>
<td>Number / reduction</td>
<td>Per cent reduction</td>
<td>Per cent reduction</td>
</tr>
<tr>
<td>Control</td>
<td>3.07</td>
<td>59.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>500 Mi</td>
<td>2.78</td>
<td>9.49</td>
<td>49.30</td>
<td>16.86</td>
</tr>
<tr>
<td>1000 Mi *+</td>
<td>2.56</td>
<td>16.1</td>
<td>37.60</td>
<td>36.59</td>
</tr>
<tr>
<td>2000 Mi</td>
<td>2.30</td>
<td>25.08</td>
<td>29.60</td>
<td>50.08</td>
</tr>
<tr>
<td>0.5g Rs</td>
<td>2.60</td>
<td>15.30</td>
<td>40.60</td>
<td>31.30</td>
</tr>
<tr>
<td>1.0g Rs</td>
<td>2.26</td>
<td>26.38</td>
<td>33.00</td>
<td>44.35</td>
</tr>
<tr>
<td>2.0 Rs</td>
<td>2.02</td>
<td>34.20</td>
<td>25.00</td>
<td>57.84</td>
</tr>
<tr>
<td>0.5g Rs+500 Mi</td>
<td>2.32</td>
<td>24.42</td>
<td>36.00</td>
<td>39.29</td>
</tr>
<tr>
<td>0.5g Rs+1000 Mi</td>
<td>2.18</td>
<td>28.99</td>
<td>28.60</td>
<td>51.77</td>
</tr>
<tr>
<td>0.5g Rs+2000 Mi</td>
<td>1.98</td>
<td>35.50</td>
<td>20.00</td>
<td>66.27</td>
</tr>
<tr>
<td>1g Rs+500 Mi</td>
<td>2.00</td>
<td>34.85</td>
<td>29.60</td>
<td>50.08</td>
</tr>
<tr>
<td>1g Rs+1000 Mi</td>
<td>1.85</td>
<td>39.73</td>
<td>25.00</td>
<td>57.84</td>
</tr>
<tr>
<td>1g Rs+2000 Mi</td>
<td>1.52</td>
<td>50.48</td>
<td>17.00</td>
<td>71.33</td>
</tr>
<tr>
<td>2g Rs+500 Mi</td>
<td>1.81</td>
<td>41.04</td>
<td>20.60</td>
<td>65.26</td>
</tr>
<tr>
<td>2g Rs+1000 Mi</td>
<td>1.59</td>
<td>46.20</td>
<td>16.30</td>
<td>72.51</td>
</tr>
<tr>
<td>2g Rs+2000 Mi</td>
<td>1.28</td>
<td>58.30</td>
<td>10.60</td>
<td>82.12</td>
</tr>
</tbody>
</table>

Each value is a mean of three replicates,

Mi = *M. incognita*, Rs = *R. solani*, (+) = Per cent increase, (-) = Per cent decrease

Rf (Nematode reduction factor) = Pf (final population / Pi (initial population)}
growth but the growth parameters were substantially reduced at higher inoculum levels and in all combined treatments.

4.3.2.1 Effect on plant dry weight (root+shoot)

Both the test pathogens (M. incognita and R. solani) in single species inoculation adversely affected the plant growth (based on total dry weight of plant). With the increase in the inoculum level of each pathogen a gradual decrease in plant growth was observed. However, significant reduction in plant growth over control was found when 1000 or more second stage juveniles (J2) of M. incognita or 1.0g or more fungal mycelium of R. solani was inoculated per plant. Individually higher inoculum levels (1.0g or 2.0g/plant) of R. solani caused a greater reductions (26.38% at P<0.05 and 34.20% at P<0.01) as compared to higher inocula (1000 or 2000 larvae/plant) of M. incognita (16.61% at P<0.05 and 25.08% at P<0.01). Combination of variable inoculum levels of both test pathogens were found to be more damaging than their individual effects. The plant growth (total dry weight) was significantly reduced at each combination in comparison to uninoculated control. The reduction in simultaneous inoculation of both the pathogens at each combination was comparatively less than the total sum of reductions caused by each pathogen alone, thereby showing negative interaction. Amongst the simultaneous inoculations the per cent loss in dry plant weight was higher ranging from 41.04-58.30%, when 2.0g of fungus R. solani was inoculated with three increasing (500, 1000, 2000) inoculum levels of nematode, M.
incognita, followed by 34.85-50.48% and 24.42-35.50% in the
concomitant inoculations of above nematode inocula with 1.0g and
0.5g fungus, respectively (Table-2.1; Fig.3).

4.3.2.2 Effect on root nodulation

Reduction in nodulation was significant over
uninoculated control in all the treatments of test pathogens
whether inoculated alone or in various combinations. R. solani
alone caused greater reductions (ranging from 31.53-57.84%) than
that of M. incognita (16.86-50.08%). In the combined inoculation
of both the test pathogens the magnitude of reduction in
nodulation enhanced more significantly, the highest (82.12%)
being in the concomitance of higher inoculum levels
(2.0gRs+2000Mi) and lowest (39.29%) in the combination of lower
inoculum levels (0.5g Rs+500Mi). At all these combinations the
reduction in nodulation were relatively lower than the sum total
of reductions caused by each pathogen alone (negative
interaction (Table-2.1; Fig.3).

4.3.2.3 Effect on nematode multiplication

When present alone the rate of nematode multiplication
was found to be density dependent being highest (Rf=27.5) when
the initial inoculum level of nematode was low (500
larvae/plant) and lowest (Rf=11.15) when the initial inoculum
level was high (2000 larvae/plant). The fungus R. solani
produced an inhibitory effect on the rate of nematode
multiplication reducing them significantly to 45.63% when
highest fungal inoculum level (2.0 g/plant) was used in
combination with lowest nematode inoculum level (500 larvae/plant). However, the reduction was comparatively less 43.53% and 41.43% in its (2.0 g fungus) concomitance with 1000 and 2000 nematode larvae respectively (Table-2.1; Fig. 4).

4.3.2.4 Effect on root galling

The number of root galls per plant increased from 88-133 with an increase in the inoculum level of *M. incognita* from 500-2000 J2. But, the fungus, *R. solani* irrespective of the level of inoculum affected the root galling significantly to a varying degree. The inhibition in root galling exhibited a trend almost similar to that obtained for nematode multiplication. The reduction in root galling varied between 31.13 and 60.90% in its combination with lower and higher fungal inoculum levels, respectively (Table- 2.1; Fig 4).

4.3.2.5 Effect on root-rot development

The root-rot indices caused by *R. solani* increased substantially with the increase in the inoculum level of fungus alone and in its various combinations with nematode, *M. incognita* (Table-2.1; Fig. 4). However, there was no significant difference in the root-rot index when 0.5g of fungus was inoculated alone or in combination with 500J2 of nematode. In single inoculation of *R. solani* the root-rot indices increased from 1.2-2.8 whereas, in presence of nematode the severity of root-rot increased more significantly ranging from 2.5-3.8.
FIG. 4 — EFFECT OF INDIVIDUAL AND COMBINED INOCULATION OF VARIABLE INOCULUM LEVELS OF M. INCognita AND R. SOLANI ON NEMATODE MULTIPLICATION, ROOT GALLING AND ROOT-ROT DEVELOPMENT IN PRESENCE OF RHIZOBiUM.
4.3.3 Effect of individual and concomitant inoculation of variable inoculum levels of *M. incognita* and *R. solani* on plant growth, nematode multiplication, gall formation, and root-rot development in absence of *Rhizobium*

The experimental results presented in Table-2.2, Fig.5 and Appendix-IIB revealed that in absence of *Rhizobium* the plant growth parameters did not reach to that level as they were noted in presence of *Rhizobium*. Moreover, the unbacterized plants in general suffered more damage in plant growth than the bacterized ones, when inoculated alone or in various combinations with test pathogens. Plant growth reductions due to different pathogenic treatments followed the same trend as was observed in bacterized plants.

4.3.3.1 Effect on plant dry weight (root+shoot)

No significant reduction in plant growth (total dry weight) was observed in the lowest inoculum levels (500Jg of *M. incognita* or 0.5g of *R. solani*) of either pathogen alone. However, with the further increase in the inoculum level of each pathogen the plant dry weight was reduced significantly as compared to uninoculated control. At the highest (2.0g/plant) inoculum level of fungus, *R. solani* the reduction in plant growth was maximum (35.40%) as compared to corresponding (2000Jg/plant) inoculum level of nematode, *M. incognita* (27.37%).

In comparison to uninoculated control, all the combined inoculum levels of test pathogens caused significantly higher reductions than their individual effects. The reduction in plant
Table 2.2 Effect of individual and concomitant inoculation of variable inoculum levels of Meloidogyne incognita and Rhizoctonia solani on plant dry weight, nematode multiplication, gall formation and root-rot development in absence of Rhizobium.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Dry Weight (g)</th>
<th>Nematode multiplication</th>
<th>Galls/root system</th>
<th>Root-rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Root+Shoot)</td>
<td>Per cent reduction</td>
<td>RF = PF/Pi</td>
<td>Per cent reduction</td>
</tr>
<tr>
<td>Control</td>
<td>2.74</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Uninoculated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 M1</td>
<td>2.45</td>
<td>10.58</td>
<td>29.35</td>
<td>-</td>
</tr>
<tr>
<td>1000 M1</td>
<td>2.19</td>
<td>20.07</td>
<td>19.59</td>
<td>33.25</td>
</tr>
<tr>
<td>2000 M1</td>
<td>1.99</td>
<td>27.37</td>
<td>13.69</td>
<td>53.35</td>
</tr>
<tr>
<td>0.5g Rs</td>
<td>2.29</td>
<td>16.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.0g Rs</td>
<td>1.97</td>
<td>28.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.0g Rs</td>
<td>1.77</td>
<td>35.40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5g Rs+500 M1</td>
<td>1.80</td>
<td>34.30</td>
<td>23.65</td>
<td>19.42</td>
</tr>
<tr>
<td>0.5g Rs+1000 M1</td>
<td>1.66</td>
<td>39.41</td>
<td>16.13</td>
<td>17.66</td>
</tr>
<tr>
<td>0.5g Rs+2000 M1</td>
<td>1.45</td>
<td>47.08</td>
<td>11.46</td>
<td>16.28</td>
</tr>
<tr>
<td>1g Rs+500 M1</td>
<td>1.50</td>
<td>45.25</td>
<td>20.55</td>
<td>29.98</td>
</tr>
<tr>
<td>1g Rs+1000 M1</td>
<td>1.39</td>
<td>49.27</td>
<td>13.92</td>
<td>28.94</td>
</tr>
<tr>
<td>1g Rs+2000 M1</td>
<td>1.20</td>
<td>56.20</td>
<td>10.03</td>
<td>26.73</td>
</tr>
<tr>
<td>2g Rs+500 M1</td>
<td>1.35</td>
<td>50.72</td>
<td>17.74</td>
<td>39.55</td>
</tr>
<tr>
<td>2g Rs+1000 M1</td>
<td>1.17</td>
<td>57.29</td>
<td>11.94</td>
<td>39.05</td>
</tr>
<tr>
<td>2g Rs+2000 M1</td>
<td>.995</td>
<td>63.68</td>
<td>8.48</td>
<td>38.05</td>
</tr>
</tbody>
</table>

C.D. (P< 0.05)  .47  2.29  10.45  .28
C.D. (P< 0.01)  .63  3.11 14.20  .39

Each value is a mean of three replicates,
M1 = M. incognita, Rs = R. solani, (+) = Per cent increase, (-) = Per cent decrease,
RF (Nematode reproduction factor) = PF (final population) / Pi (initial population)
FIG. 5—EFFECT OF INDIVIDUAL AND COMBINED INOCULATION OF VARIABLE INOCULUM LEVELS OF M. INCognita AND R. SOLANI ON PLANT DRY WEIGHT, NEMATODE MULTIPLICATION, ROOT GALLING AND ROOT-ROT DEVELOPMENT IN ABSENCE OF RHIZOBIUM
dry weight at each combination was comparatively higher than the sum total of reductions caused by each pathogen alone, thus showing a positive interaction (synergistic effect). For example, the sum total of per cent reduction in plant dry weight caused by 500 *M. incognita* (Mi) and 2.0 g *R. solani* (Rs) in single species inoculations was 45.98% but in concomitant inoculations with 500Mi+2.0g Rs the per cent reduction was higher (50.72%). A similar trend was also observed for other combinations, however, in the treatments involving equal (2000Mi+2.0gRs) inoculum levels of both the test pathogens the reduction was marginally high (slightly synergistic effect) than the sum total of reduction caused by the same inoculum levels of each pathogen alone (Table-2.2; Fig.5).

4.3.3.2 Effect on nematode multiplication

The rate of nematode multiplication at each level of nematode inoculum was comparatively higher in unbacterized plants than that of bacterized ones. With an increase in the initial inoculum level of *M. incognita* (Mi) the rate of nematode multiplication decreased significantly being lowest (Rf=13.69) in highest inoculum level (2000J2/plant) and highest (Rf=29.35) in the lowest inoculum level (500J2/plant). Presence of fungus *R. solani* (Rs) irrespective of combination levels reduced the nematode multiplication rate markedly except, in the concomitance of lower fungal inoculum level with higher nematode level (0.5g Rs+2000Mi). However, the reductions were comparatively less than that observed in bacterized plants. In
presence of fungus, irrespective of inoculum level the reduction in nematode multiplication was maximum at lowest nematode inoculum level (500Jg/plant) and minimum at highest level (2000Jg/plant) (Table-2.2; Fig.5).

4.3.3.3 Effect on root galling

In the absence of Rhizobium, root galling caused by the nematode, M. incognita increased substantially with the increase in the inoculum level from 500 to 2000 Jg/pot. However, in comparison to bacterized plant the corresponding increase in the number of galls was high at the same inoculum levels.

The galling experienced a significant loss when nematode was inoculated simultaneously with different inoculum levels of fungus, R. solani. But the inhibition in root galling was comparatively less than that observed in bacterized plants. In comparison to other concomitant treatments, the reduction in root galling was markedly high (49.65%) when highest fungal inoculum level (2.0gRs) was inoculated with lowest nematode inoculum level (500Jg/Mi). However, in the concomitance of lower fungal inoculum level and higher nematode level (0.5gRs+2000Mi) the reduction in galling was not significant over the same inoculum level of nematode alone (Table-2.2; Fig. 5).

4.3.3.4 Effect on root-rot development

Unbacterized plants in general had higher root-rot indices as compared to bacterized plants in various treatments of fungal inoculation alone and in its combination with nematode. The root-rot indices increased significantly from 1.5-
3.0 When initial inoculum level (0.5 g/plant) of fungus was raised to 2.0 g/plant, however, the severity of root-rot increased more significantly in presence of different inoculum levels of nematode, *M. incognita*. The magnitude of increase ranged between 1.8 and 4.2, respectively (Table-2.2; Fig. 5).

### 4.3.4 Effect of individual, simultaneous and sequential (pre- or post-) inoculation of *M. incognita* and *R. solani* on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence of *Rhizobium*

It is evident from the data presented in Table-3.1, Fig. 6 and Appendix-III A that inoculation of *M. incognita* and *R. solani* alone and in different combinations significantly reduced the different plant growth parameters viz., length, fresh and dry weights of root and shoot, as compared to uninoculated control. Simultaneous inoculation of test pathogens caused higher reductions than the sequential inoculations.

#### 4.3.4.1 Effect on plant dry weight (root+shoot)

Both the test pathogens caused significant reduction in plant dry weight over uninoculated control when inoculated alone. The *R. solani* caused greater reduction (32.15%) than *M. incognita* (26.68%). However, there was no significant difference in between the two treatments. In combined inoculations the reduction in dry weight was significantly higher than their individual inoculation. Maximum reduction (54.01%) was found in treatments where the nematode and fungus were inoculated simultaneously (*Mi+Rs*) followed by 48.87% in sequential inoculation of nematode 15 days prior to fungus (*Mi--->Rs*) and
Table 3.1. Effect of individual, simultaneous, and sequential inoculation of *Meloidogyne incognita* and *Rhizoctonia solani* on plant dry weight, nodulation, nematode multiplication, gall formation, and root-rot development in presence of *Rhizobium*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Dry Weight</th>
<th>Nodules/Root system</th>
<th>Nematode multiplication</th>
<th>Galls/Root system</th>
<th>Root-rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root:Shoot (g)</td>
<td>Per cent reduction</td>
<td>Number</td>
<td>Per cent reduction</td>
<td>Rf = PF/PI</td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td>3.11</td>
<td>-</td>
<td>62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ni</td>
<td>2.28</td>
<td>26.68</td>
<td>26</td>
<td>58.06</td>
<td>12.75</td>
</tr>
<tr>
<td>Rs</td>
<td>2.11</td>
<td>32.15</td>
<td>20</td>
<td>67.74</td>
<td>-</td>
</tr>
<tr>
<td>Ni + Rs</td>
<td>1.43</td>
<td>54.01</td>
<td>10</td>
<td>83.87</td>
<td>5.82</td>
</tr>
<tr>
<td>Ni ——&gt; Rs</td>
<td>1.59</td>
<td>48.87</td>
<td>14</td>
<td>77.41</td>
<td>6.67</td>
</tr>
<tr>
<td>Rs ——&gt; Ni</td>
<td>1.87</td>
<td>39.87</td>
<td>16</td>
<td>74.19</td>
<td>5.10</td>
</tr>
</tbody>
</table>

C.D. (P 0.05) 0.22 5.95 1.21 2.64 0.12
C.D. (P 0.01) 0.31 8.46 1.83 4.00 0.19

Each value is a mean of three replicates,

Ni = *M. incognita* 2000 larvae (J2)/ plant,

Rs = *R. solani* 2 g mycelium / plant,

Ni + Rs = (Both pathogens simultaneously),

Ni ——> Rs = 1st pathogen (Ni) 15 days prior to 2nd (Rs) pathogen,

Rs ——> Ni = 1st pathogen (Rs) 15 days prior to 2nd (Ni) pathogen.

Rf (Nematode reproduction factor) = PF (final population) / PI (initial population)
in its reciprocal treatment (Rs— Mi). In all concomitant treatments the resultant effect on plant growth (based on total dry weight) was less than the sum total of their individual effects (negative interaction). Amongst the concomitant inoculations, prior inoculation of fungus followed by nematode (Rs— Mi) caused significantly lesser reduction than the simultaneous inoculation (Mi+Rs) and prior inoculation of nematode (Mi— Rs), however, there was no significant difference in between the treatments receiving both the pathogens simultaneously or nematode 15 days prior to fungus (Table-3.1; Fig. 6).

4.3.4.2 Effect on nodulation

Reduction in nodulation followed the same trend as was observed in plant growth. Individually *R. solani* caused greater reduction (67.74%) than *M. incognita* (58.06%). However, the reduction was significantly (P<0.01) higher in concomitant inoculations than the individual treatments. In simultaneous inoculation (Mi+Rs) the nodulation was reduced more significantly (83.87%) as compared to the inoculation of fungus prior to nematode (Rs— Mi) but there was no significant difference between the treatment in which nematode preceded fungal inoculation and other two treatments (Mi+Rs or Rs— Mi) (Table-3.1; Fig. 6).

4.3.4.3 Effect on nematode multiplication

The rate of nematode multiplication was maximum (Rf=12.75) when nematode occurred individually but, in different
combinations with fungus the multiplication rate reduced significantly. The highest reduction (60.00%) was observed in the sequence of \textit{R. solani} 15 days prior to \textit{M. incognita} followed by (54.35%) in simultaneous inoculation and least (47.68%) in the treatment where \textit{M. incognita} preceded \textit{R. solani} by 15 days. The treatments receiving nematode prior or after the fungus differed significantly (P<0.05) among themselves but there was no critical difference in RF value between simultaneous inoculation (Mi+Rs) and either of the two sequential treatments (Mi-->Rs/Rs-->Mi) (Table-3.1; Fig. 6).

4.3.4.4 Effect on root galling

The roots bore more galls (125) when \textit{M. incognita} was present singly. However, the presence of fungus irrespective of time of inoculation affected the root galling significantly to a varying degree. Reduction in the galling was high (65.60%) in prior inoculation of \textit{R. solani} (Rs-->Mi), followed by 58.40% in simultaneous inoculation (Mi+Rs) and least (34.40%) in the treatment where nematode preceded the fungus (Mi-->Rs). All the combined treatments differed significantly in between (Table-3.1; Fig. 6).

4.3.4.5 Effect on root-rot development

Root-rotting due to fungus \textit{R. solani} increased substantially in all concomitant inoculations with nematode \textit{M. incognita}. The maximum (3.6) being in simultaneous inoculation and least (3.3) in the sequential inoculation of nematode 15 days prior to fungus.
Table 3.2. Effect of individual, simultaneous, and sequential inoculation of Meloidogyne incognita and Rhizoctonia solani on plant dry weight, nematode multiplication, gall formation, and root-rot development in absence of Rhizobiun.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Dry Weight (g)</th>
<th>Nematode multiplication</th>
<th>Galls/Root system</th>
<th>Root-rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninoculated)</td>
<td>2.82</td>
<td>27.30</td>
<td>14.11</td>
<td>-</td>
</tr>
<tr>
<td>Rs</td>
<td>1.80</td>
<td>36.17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mi + Rs</td>
<td>1.01</td>
<td>64.18</td>
<td>8.28</td>
<td>77</td>
</tr>
<tr>
<td>Mi --&gt; Rs</td>
<td>1.25</td>
<td>55.31</td>
<td>9.60</td>
<td>91</td>
</tr>
<tr>
<td>Rs --&gt; Mi</td>
<td>1.48</td>
<td>47.51</td>
<td>6.78</td>
<td>65</td>
</tr>
</tbody>
</table>

C.D. (P 0.05) .21  1.56  6.36  .35
C.D. (P 0.01) .31  2.36  9.63  .53

Each value is a mean of three replicates,

Mi = M. incognita 2000 larvae (J2) / plant,
Rs = R. solani 2g mycelium / plant,
Mi + Rs = Both pathogens simultaneously,
Mi --> Rs = 1st pathogen (Mi) 15 days prior to 2nd (Rs) pathogen,
Rs --> Mi = 1st pathogen (Rs) 15 days prior to 2nd (Mi) pathogen,
RF (Nematode reproduction factor) = Pf (final population) / Pi (initial population)
Fig. 7—Effect of individual, simultaneous, and sequential inoculation of M. incognita and R. solani on plant dry weight, nematode multiplication, root galling and root-rot development, in absence of Rhizobium.

MI = M. incognita
RS = R. solani
MI+RS = Simultaneous inoculation
MI+RS, first pathogen is RS-MI; 2 days prior to second
sequential inoculations the effect of interaction was less than simple additive (antagonistic). The treatment in which nematode preceded the fungus resulted in the higher growth reduction (55.31%) as compared to its reciprocal treatment (47.51%). Both of these sequential treatments differed significantly in between and from that of simultaneous inoculations (Table-3.2; Fig. 7).

4.3.5.2 Effect on nematode multiplication

Whether present alone or in different combinations with fungus the rate of nematode multiplication was comparatively higher in unbacterized plants than bacterized ones. When nematode occurred alone the nematode reproduction factor was Rf=14.11. In simultaneous or sequential inoculation with the fungus, *R. solani* the Rf value was significantly (*P<0.01*) reduced but to a lesser extent than that observed in bacterized plants. The maximum reduction (51.94) in nematode multiplication was recorded in the sequential inoculation of *R. solani* 15 days prior to *M. incognita* (Rs---Mi) followed by 41.31% in simultaneous inoculation (Mi+Rs) and least (31.96) in the treatment where nematode preceded fungal inoculation (Mi---Rs). Both the treatments in which nematode preceded (Mi---Rs) or succeeded fungal inoculation (Rs---Mi) differed significantly in between, however, they were at par with the simultaneous inoculation (Table-3.2; Fig. 7).

4.3.5.3 Effect on root galling

The unbacterized plants harboured maximum number of galls than bacterized ones when nematode was present alone or in
concomitance (simultaneously or sequentially) with the fungus.

The number of galls were 141, when *M. incognita* was present singly but, the fungal inoculation reduced the galling significantly (*P*<0.01) to 53.90% when it (*R. solani*) preceded *M. incognita* by 15 days, followed by (45.39%) in simultaneous inoculation and least (35.46%) in the sequence of *M. incognita* 15 days prior to *R. solani*. All the concomitant treatments showed significant variations in between (Table-3.2; Fig. 7).

4.3.5.4 Effect on root-rot development

Plants in absence of *Rhizobium* (unbacterized) also showed higher root-rot indices as compared to that observed in presence of *Rhizobium* (bacterized) when fungus was inoculated alone or in various combinations with nematode. The root-rot indices due to fungus, *R. solani* experienced a substantial enhancement in presence of nematode, *M. incognita*, the maximum (4.3) being in simultaneous inoculation and minimum (3.8 and 4.0) in the treatments receiving nematode 15 days prior (*Mi---Rs*) and after the fungus (*Rs---Mi*). There was no significant difference in between the treatments receiving both the test pathogens simultaneously (*Mi+Rs*) and fungus in advance to nematode (*Rs---Mi*), but the prior inoculation of nematode (*Mi---Rs*), differed significantly from that of simultaneous inoculation (Table-3.2; Fig. 7).

4.4 Discussion

For determining the inoculum threshold levels of both the test pathogens, nematode and fungus, the seedlings of
Greengram were separately inoculated with different inoculum levels of nematode, *M. incognita* (250, 500, 1000, 2000 and 4000 juveniles/J2/plant) and the fungus, *R. solani* (0.25, 0.5, 1.0, 2.0 and 4.0 g of mycelium/plant). Lowest inoculum levels of both the test pathogens caused no significant damage or plant growth reductions but, with the increasing inoculum levels there was an increased plant growth reduction, whether inoculated with nematode or fungus. There was a significant reduction in the total dry weight of the plant, when the plants were inoculated with 1000 or more nematodes and 1.0 g or more fungus/plant both in bacterized (Table 1.1, 1.2; Fig. 1) and unbacterized plants (Table 1.3, 1.4; Fig. 2). However, the higher inoculum levels 2000 nematodes/plant and 2.0 g fungus/plant caused significantly higher reductions in plant growth as compared to lower ones and these levels of inocula of the test pathogens were used for other investigations.

The damaging threshold levels of *Meloidogyne incognita* on greengram have been determined by several workers (Raut and Sethi, 1980b; Chahal and Chahal, 1987, 1989a; Tiyagi and Alam, 1988; Fazal *et al.*, 1994) but their findings have been at variance. Damaging threshold levels as low as 250 larvae per 800 g soil have been reported by Chahal and Chahal (1989a). However, Raut and Sethi (1980b) found it at the higher inoculum level of 1000 larvae per 500 g of soil.

The present findings (1000 infective stages of *M. incognita* as the damaging threshold level) are in conformity with those of Tiyagi and Alam (1988), Chahal and Chahal (1987)
and Fazal et al., (1994). The differences observed in the damaging threshold levels by the different workers can be attributed to the differences in the experimental conditions, cultivars used and the races of nematode involved.

Similarly, the increasing inoculum levels of *R. solani* caused increased root rotting and resultant decrease in plant growth and nodulation but the marked reduction in the plant growth was recorded at or above 1.0 g of fungus per plant (Table-1, 2, 4; Fig. 1, 2). Similar damaging results of *R. solani* on the plant growth of different crops have been reported by several workers (Azam, 1975; Varshney, 1982; Zakiuddin, 1984; Khan, 1986) and thus substantiated the present findings. The reduction in plant growth may be due to the structural and physiological aberrations caused by the test pathogens (Tiyagi, 1990).

The number of nodules/plant decreased significantly at all the inoculum levels, except the lowest inoculum level (250 nematodes or 0.25 g fungus/plant) of each test pathogen. Reduced nodulation in different pathogenic treatments may be attributed to the nutritional interference particularly carbohydrates or physiological changes brought about by the nematode infestation (Nutman, 1958; Balasubramanian, 1971; Hussaini and Seshadri, 1975; Sharma and Sethi, 1976; Bopaih et al., 1976a) or to the secretion of toxic metabolites by fungal infection (Orellana et al., 1976; Kush, 1982; Zambolim and Schenk, 1984). Inhibitory effects of nematode and/or fungal parasitism on nodulation have been also reported by several workers (Khan, 1986; Chahal and Chahal, 1987, 1988, 1989a; Siddiqui, 1990; Tiyagi, 1990). On the
otherhand there are some contradictory reports of stimulation of nodulation by nematode parasitism (Hussey and Barker, 1974, 1976).

The number of galls/plant increased gradually with an increase in the inoculum level of nematode (Table-1.1, 1.3; Fig.1,2). These findings are in agreement with those of Raut and Sethi (1980a,b), Mani and Sethi (1984) and Fazal et al., (1994), who also observed increased galling in the increasing inoculum levels of root-knot nematode on soyabean, chickpea and mungbean, respectively.

The rate of nematode multiplication on the otherhand, decreased with the corresponding increase in the inoculum levels (Table-1.1,1.3;Fig.1,2). The reason for reduced multiplication of nematodes with the increasing inoculum levels may be due to competition for the food and space (Triantaphyllou, 1960; Davide and Triantaphyllou, 1967). Since the root surface area for both the lower and higher inoculums remained the same, crowding of nematodes at high inoculum densities created competition among the nematodes which resulted in their natural death and reduced multiplication. The high rate of multiplication at lower levels of inocula, could possibly be due to the positive factors like abundance of food, space, less competition and the ability of the host to support these levels of population.

The progressive decrease in the plant growth and nematode multiplication with the increasing inoculum levels of nematode has been reported by Chapman (1959); Oostenbrink (1966); Mishra and Gaur (1981); Salem and Eissa (1981); Khan
The results of the above experiments clearly demonstrate that plant growth reduction was directly proportional to the increase in the inoculum level of test pathogen both in bacterized and unbacterized plants. Although reduction in plant growth was comparatively higher in unbacterized plants than in bacterized ones but there was no difference in the inoculum threshold levels of either of the test pathogen in presence and absence of *Rhizobium*. The fungus, *R. solani* was more damaging pathogen than that of nematode, *M. incognita*.

Various combinations of variable inoculum levels of test pathogens caused significantly higher reductions in plant growth (total dry weight) as compared to their individual effects (Table-2.1,2.2; Fig.3,5). Reduction in the total dry weight of concomitantly inoculated (Mi+Rs) plants was relatively less than the sum total of reductions caused by each pathogen alone (negative interaction) in bacterized plants (Table-2.1; Fig.3). However, in unbacterized plants the reduction was comparatively greater than the sum total of reductions (positive interactions) caused by same level of pathogen alone (synergistic effect) (Table-2.2; Fig.5). Additive/ synergistic/ antagonistic effects of nematode and fungus interactions on plant growth have been also noticed on different pulse crops by several workers (Abawi and Jacobsen, 1984; Abu-El-Amayem et al., 1986; Gupta et al., 1987; Haseeb et al., 1990; Baheti and Yadav (1991); Fazal et al., (1991) and Fazal et al., (1994).
Root nodulation due to *Rhizobium* decreased significantly at each inoculum level of test pathogens when inoculated individually or concomitantly. However, the reduction was more pronounced in all the combinations of test pathogens being less than the sum total of reductions caused by each pathogen alone (Table 2.1; Fig. 3). Similar effects on the nodulation in the combined inoculation of nematode and fungus have been reported earlier by Mani and Sethi (1987), Tiyagi (1990), Siddiqui and Husain (1991) and Anwar and Verma (1993).

It is presumed that reduction in the number of nodules may be due to the adverse effect of toxic substances released from the nematode and/or fungus infected roots on *Rhizobium* itself. Nutman (1958) reported that nodulation depend upon the supply and translocation of certain materials particularly the carbohydrates from the shoot. Therefore, the reduced nodulation might have been due to the interruption in translocation and/or utilization of the host materials during gall formation or their consumption directly by the nematode and/or fungus. Further, he suggested that rhizobia infection takes place through the root hairs into the cortex, where the sites of nodulation exists. As the nematode or fungus infection depletes the root hairs the rhizobial infection is inhibited. Reduction in nodulation may also be attributed to the changes in the host metabolism due to
nematode and/or fungus infection which makes it unsuitable or less preferred by the Rhizobium.

Maximum plant growth (total dry weight) reductions in the concomitant inoculations of both the test pathogens can be assigned to the fact that when a plant is jointly infected by more than one pathogen, it seems reasonable to expect that activities of one pathogen influence the activities of the other pathogen thereby, changing its susceptibility due to the altered physiological functions (Powell, 1979). It is well known that nematodes besides providing infection courts, which facilitate the entry of the fungus (Smith, 1954) also induce certain physiological/biochemical alterations within the host which favours fungus infection and enhances the disease development. These alterations result in the increased plant growth reductions. There may be some other possible mechanisms which explain the elevated fungal disease intensity in presence of nematodes. There are strong indications that nematodes especially root-knot nematode may induce physiological or biochemical changes in their hosts which enhance the development of fungi and/or predispose their host to fungal pathogens.

Nematodes seems to favour all stages of fungal infection and development. By modifying the composition of root leachates they can promote the growth of fungi in the rhizosphere and favour their pathogenic development. Moreover, these modifications of the rhizosphere environment may limit the development of the organisms antagonistic to pathogenic fungi (Noguera and Smits, 1982). Their feeding sites and cells they
modify especially the giant cells induced by root-knot nematode, may serve as favourable substrate which help the fungus to establish within the plant and promote their development (Van Gundy, et al. 1977). Nematode induced or produced factors appear to be translocated from the nematode feeding sites to other parts of their host, especially in the above ground parts. These factors seem to modify the resistance of the host tissue to the fungi and directly stimulate fungal growth (Nicholson et al., 1985; Hillocks, 1986). Results obtained by Golden and Van Gundy (1975); Van Gundy et al., (1977) and Khan and Muller (1982) are the direct indications that biochemical changes induced by the nematodes in root leachates are responsible for the improvement of rhizosphere colonization of *Rhizoctonia solani* and its attraction by root-knot induced galls respectively.

However, there are other findings which have concluded that there was no interaction between nematodes and fungi in disease complexes or that nematodes did not predispose their host to the fungal pathogens. It has been also reported that nematodes may suppress the infection on their host by pathogenic fungi (Orion and Netzer, 1981; Nordmeyer and Sikora, 1983).

Discrepancies between observations may probably result from differences in experimental conditions, nematode or fungus inoculum levels used and/or species involved and from the differences in the considered nematode-fungus-plant (cultivar) combinations.
The nematode multiplication rate and root galling was found to be adversely affected in all combinations with the fungus, R. solani in contrast to where nematode was present alone. The effect was more when the highest fungal inoculum level was used in combination with the lowest nematode inoculum level (Table-2.1,2.2; Fig.4,5). This inhibition in the root galling and nematode population may be attributed to fungal antagonism. Detrimental effects of various fungi on nematode population has been reported by earlier workers (Al Hazmi, 1985; Sakhuja and Sethi, 1986; Tiyagi, 1990; Siddiqui, 1990; Anwar and Verma, 1993; Shah et al., 1993) and this has been ascribed to the destruction of root tissues before the completion of nematode life cycle (Carter, 1980; Khan and Husain, 1990a).

In combined infection the fungus component of interaction dominate in the complex and suppress the development and reduce the resulting population density of nematodes (Powell, 1971a). Generally, population of sedentary forms such as Meloidogyne, Heterodera and Globodera spp. show reduced population in presence of wilt fungi, (Fusarium spp. and Verticillium spp.) or in few instances in the presence of the root-rot fungi, Rhizoctonia, Pythium and Phytophthora spp. (Powell, 1971a; Salem, 1980; Nordmeyer and Sikora, 1983; Ribeiro and Ferraz, 1983; Hasan, 1984; Al-Hazmi, 1985; Griffin and Thyr, 1986; Starr and Veech, 1986; Griffin et al., 1988; Hasan, 1989; Starr et al., 1989; Gray et al., 1990). The suppression of the sedentary forms has been implicated with the impairment of the nutrient supply to the developing egg laying adult nematodes.
available to them through syncytia or giant cells induced by invading juveniles. This is borne out by the fact that disintegration of syneytia/giant cells occur in fungus infected plants. Moreover, the population of sedentary forms remain sedentary at one place and thus are subjected to the influences of the changes in the host system as a result of fungal infection (Powell, 1971a).

Another possible reason could be the secretion of toxic metabolites by the fungi which suppress the hatching and development of nematode juveniles (Mankau, 1969a,b; Shukla and Swarup, 1971; Desai et al., 1972; Alam et al., 1973; Arai et al., 1973; Azam, 1975; Azam et al., 1979; Khan et al., 1984; Mani et al., 1986; Sakhuja and Sethi, 1986; Singh et al., 1986a; Ciancio et al., 1988; Shah and Azam, 1992; Shah et al., 1993), in addition to certain physiological alterations in the host due to the infection and interaction of nematode and fungus.

Contrary to these reports Prasad et al., (1980) and Varshney (1982) have reported significant increase in nematode population when the plants were inoculated with nematode and fungus simultaneously or sequentially in comparison to nematode alone.

The severity of the root-rot increased when the nematode was inoculated simultaneously with the fungus especially at the higher inoculum levels of both the test pathogens (Table-2.1,2.2; Fig.4,5). This severity of root-rot caused by R. solani may be associated with the nutrient mobilization in root leachates induced by M. incognita (Van
Gundy et al., 1977). They demonstrated that exudates from M. incognita infected tomato roots attracted the hyphae of R. solani, enhanced sclerotial formation and increased the severity of root decay. When root leachates of plants inoculated simultaneously with the nematode were permanently removed no root-rot occurred. Moreover, when root leachates produced by M. incognita infected plants were applied to roots of plants inoculated with R. solani, severe root-rot developed, whereas roots inoculated with R. solani receiving root leachates from control plants were free from decay. Similar results were also obtained by Azam et al., (1977). Increased root-rot in presence of root-knot nematode have been also reported by other workers on different crops (Reddy et al., 1979; Al-Hazmi, 1985; Siddiqui, 1990; Ko et al., 1993).

The results of the data pertaining to the experiments involving individual, simultaneous and sequential inoculation of test pathogens (Table-3.1,3.2; Fig. 6,7) indicate that both the pathogens caused significant reduction in plant dry weight whether inoculated singly or in various combinations (simultaneously or sequentially) as compared to uninoculated control. However, the maximum reduction in the plant growth (total dry weight) was found in the treatments, where the nematode and fungus were inoculated simultaneously followed by the inoculations of nematode 15 days prior to fungus and least in its reciprocal treatment (Table-3.1,3.2;Fig. 6,7). Similar trend was observed with respect to reduction in nodulation (Table-3.1; Fig.6). All the concomitant inoculations of test
pathogen showed the negative interaction, wherein the effect of combined inocula on plant growth (dry weight) was less than additive (antagonistic) except in the simultaneous inoculation of test pathogens in unbacterized plants where the resultant effect was more than simple additive (synergistic) thereby showing positive interaction (Table-3.2; Fig.7).

The mechanism of elevated disease in the disease complexes involving nematode and fungus have been discussed earlier in first chapter. Comparatively higher reductions in the plant growth and nodulation in simultaneous inoculation in contrast to sequential inoculation could be explained by the fact that each organism has got equal opportunity to parasitize the roots and alter the morphology, anatomy, cytology and biochemistry of the host plants (Khan and Sexana, 1969; Yang et al., 1976; Mani, 1983) while in remaining treatments the parasitism of one of the two pathogens is restricted by 15 days. It is likely by that time the seedling have progressed to such a stage of their development that they no longer remain susceptible to one of the two parasites. The results are in agreement with those of Inagaki and Powell (1969); Mitchell and Powell (1972); Chhabra et al., (1977); Reddy et al., (1979); Azam et al., (1984); Khan and Husain (1988a); Mukhtar and Khan (1989); Tiagi (1990); Kumar et al., (1992); Anwar and Verma (1993) and Shah et al., (1993).

In sequential inoculations, the plant damage was maximum when nematode preceded fungal inoculation than that of reverse condition. Similar observations have been recorded by
several workers on other crops (Porter and Powell, 1967; Batten and Powell, 1971; Al Hazmi, 1985; Goel and Gupta, 1986a; Evans, 1987; Mai and Abawi, 1987; Mani and Sethi, 1987; Varshney et al., 1987; Franci et al., 1988; Hasan, 1989; Gray et al., 1990; Siddiqui, 1990).

The lowest growth reduction in the plants infected with fungus and succeeded by nematode may be attributed to the fact that when present alone the fungus parasitized the plant less vigorously than in the presence of nematode and it is likely that by that time the plants were inoculated with nematode the fungus got sufficient time to colonize the cortex and thereby prevented their entry to vascular parenchyma which normally is the feeding site of root-knot nematode (Nemec, 1910; Christie, 1936; Linford, 1937; Semdner, 1963). Moreover, fungus metabolites produced adverse effect on nematode or deteriorated the feeding cells, which limits the deleterious effects of nematode and thereby resulting in less damage to the host plant (Zaidi and Tiyagi, 1989; Tiyagi, 1990) whereas, in prior establishment of nematode to fungus, the nematode predisposed the plants in advance to fungal attack (Reynolds and Hansen, 1957; Goel and Gupta, 1986a; Mani and Sethi, 1987; Siddiqui, 1990). In addition, nematodes might have provided infection courts through which fungal entry might have been facilitated resulting in the aggressive behaviour of the fungus (Smith, 1954; Hasan, 1993). Further physiological and biochemical changes in the various tissues of the host as well as in the rhizosphere area caused by the prior infection of nematode, favouring good growth of fungus.
cannot be discounted in the light of observations made by Goel and Gupta (1986a).

The nematode galling and the multiplication of *M. incognita* was adversely affected in all combinations in the presence of fungus due to its inhibitory effect on nematodes in contrast to where the nematode was present alone as discussed earlier. The reduction was significantly high when fungus was introduced prior to nematode and least in its reciprocal treatment (Table-3.1, 3.2; Fig.6,7). It could be due to extensive colonization of the hypertrophoid and hyperplastic regions of nematode galls by the fungus as also the rapid invasion of areas anterior and posterior to female nematode and egg masses, such fungal infection progressed very rapidly, resulting in the drastic tissue disintegration (Powell and Nusbaum, 1960; Ryder and Crittenden, 1965; Melendez and Powell, 1967; Polychronopoulos *et al.*, 1969; Powell *et al.*, 1971a; JayaPrakash and Rao, 1984; Walia and Gupta, 1986). On the other hand, in the treatments where the fungus succeeded nematode the reduction was comparatively less because by the time the fungus was inoculated, the nematode got sufficient time to multiply and thereby limiting the effects of lately inoculated fungus. It seems to be the reason of highest population of nematodes in this treatment as compared to other combinations.

Root-rot caused by fungus was also found to be increased in all combinations in presence of nematode, the highest being in simultaneous inoculation and least in the sequential inoculation of nematode preceding fungal inoculation.
Increased root-rot in presence of nematode may be due to the nutrient mobilization in the root leachates of *M. incognita* infected roots which in turn benefits the fungal growth and increases the disease incidence (Owens and Specht, 1966; Van Gundy et al., 1977). Similar observations with respect to the increased root-rot in presence of nematode have been reported by several workers on different crops (Reddy et al., 1979; Kumar and SivaKumar, 1981; Tiyagi, 1990; Siddiqui, 1990).

The unbacterized plants in all the experiments (Table-1.3, 1.4, 2.2, 3.2; Fig.2, 5, 7) attained less growth and suffered greater damage due to the different pathogenic treatments as compared to that observed in bacterized plants. The greater reduction in unbacterized plants has a positive correlation with the increased nematode multiplication and gall formation in *M. incognita* infected plants and enhanced root-rot development due to *R. solani* infection. It can be attributed to the reduced resistance against the invading pathogens in less vigorously growing plants in absence of *Rhizobium* (Bopaiah et al., 1976a; Orellana et al., 1976; Tu, 1980; Kush, 1982).

On the other hand, the plant health was improved as a result of bacterization (Table-1.1, 1.2, 2.1, 3.1; Fig.1, 3, 6) and therefore the bacterized plants when inoculated individually or in various combinations suffered comparatively less damage than the unbacterized plants. It appears that legumes derive possible disease protection from their association with *Rhizobium* due to increased nitrogen status, which impart more vigour to the
plants thereby reducing the damage to some extent caused by invading pathogens (Kush, 1982; Khan, 1986; Hussain et al., 1990; Haque and Gaffer, 1993). Bacterization of the seeds or roots with the effective strains of Rhizobium have been found to mitigate the ill effects of nematode and fungus on several pulse crops (Bopaiah et al., 1976a; Orellana et al., 1976; Sharma and Sethi, 1976; Kush, 1982; Dalal and Bhatti, 1989; Hussain et al., 1990; Siddiqui, 1990; Tiyagi, 1990; Fazal, 1993; Haque and Gaffar, 1993). However, the findings are at variance with those of Ali et al., (1981) and Varshney (1982) who observed greater damage to cowpea plants in presence of Rhizobium.

4.5 Summary

Investigations on the pathogenicity of Meloidogyne incognita and Rhizoctonia solani confirmed the destructive effect of these two pathogens on the greengram (mungbean) cv. T-44. Lowest inoculum level of both the test pathogens caused no significant damage or plant growth reduction. Significant damage to plant growth occurred at or above 1000Jg of M. incognita and/or 1.0g or above of R. solani/kg soil both in bacterized and unbacterized plants. The test pathogens also reduced the number of nodules/plant. However, a significant reduction in nodulation was recorded at 500Jg of nematode and/or 0.5g of fungus. R. solani was more damaging than M. incognita. A significant linear relationship between initial (Pi) and final (Pf) nematode population was observed but the rate of nematode
multiplication (Rf) decreased with an increase in the inoculum level. Root galling was, however, directly proportional to inoculum level showing an increase with the increasing inocula of nematode. Similar trend was observed with respect to increase in root rotting in the increasing inoculum level of fungus.

The interaction between *M. incognita* and *R. solani* was studied using variable inoculum levels and their combinations. In the individual inoculation of test pathogens, the reduction in plant growth and nodulation was directly proportional to the increase in the inoculum level of test pathogens. Initial inoculum levels did not cause any significant reduction of plant growth, however, in the increasing inocula and all concomitant inoculations of test pathogens the reduction was statistically significant over uninoculated control. In bacterized plants the reduction in plant growth and nodulation in simultaneous inoculation of each combination was comparatively less than the sum total of reductions caused by each pathogens alone, thereby showing negative interaction. Whereas, in unbacterized plants the combination of variable inoculum levels showed a synergistic effect on plant growth reduction (positive interaction).

Nematode multiplied to a varying degree when inoculated alone but, with the increase in the inoculum level there was a gradual decrease in the rate of population increase of nematode both in bacterized and unbacterized plants. On the other hand, root galling increased in the increasing inocula of nematode. The fungus, *R. solani* showed an antagonistic effect on the rate of nematode multiplication and root galling. The effect was higher.
when highest fungal inoculum levels were used with lowest nematode inocula. Root-rot showed considerable enhancement with the increase in the inoculum levels of fungus alone and in its various combinations with nematode, the highest being in the concomitance of higher inoculum levels of test pathogens.

Studies on the effect of simultaneous and sequential inoculations of test pathogens showed that the reduction in plant growth and nodulation was maximum in simultaneous inoculation, followed by the sequential inoculations of nematode 15 days prior to fungus and least in the treatments where fungus preceded nematode inoculation. In all the concomitant inoculations the effect of interaction on plant growth and nodulation was less than additive (antagonistic) except in the simultaneous inoculation of unbacterized plants, where in the resultant effect on plant growth was found to be synergistic. The fungus, *R. solani* whether inoculated simultaneously or sequentially reduced the rate of nematode multiplication and root galling significantly as compared to when nematode was present alone. The reduction was significantly high when fungus preceded the nematode inoculation. On the other hand the root rotting due to fungus increased markedly in all combinations with nematode, the highest being in simultaneous inoculation and least in the sequential inoculation of nematode prior to fungus.

In general, unbacterized plants showed lesser growth and greater damage than the bacterized ones, when inoculated singly or in various combinations of pre-, post-, and simultaneous inoculation. Moreover in absence of *Rhizobium* the
Rf value and gall number was highest than in its presence, both in singly and concomitantly inoculated plants. Similar trend was observed with respect to root-rot index. Plant health was improved as a result of bacterization and therefore the bacterized plants suffered lesser damage than the unbacterized ones in different pathogenic treatments.
5. EXPERIMENTAL - II: BIOCHEMICAL STUDIES

5.1 Introduction

Normal plant growth requires an optimum balance of nutrient elements together with their normal uptake and distribution within the plant. The general notion has been that nematode and/or fungus infection directly diminish absorption of nutrient elements, resulting in an inadequate supply to the plant (Sivaprakasam, et al., 1974; Gupta, 1975; Jamal and Khan, 1976; Kapur et al., 1978; Elkins et al., 1979; Price et al., 1982; Bisen, 1983; Melakeberhan et al., 1985a, b; Melakeberhan, 1986; Prasad et al., 1989; Khan and Hussain, 1990b). However, the degree to which these pathogens modify the availability, uptakes and translocation of nutrients to plants, and the physiological mechanism that influence the crop yield, are not fully understood. In assessing the effect of pathogens on the crop yield it is important to compare the range of physiological parameters in addition to the morphological parameters (Melakeberhan et al., 1986). The metabolic destruction and altered host physiology result in the change of most nutrients important for normal activities of plant. For example, a decrease in soil nitrogen directly influences plant chlorophyll contents and together with decreased potassium influences photosynthesis. Leguminous crops are likely to suffer additionally as the degree of colonization of nitrogen fixing Rhizobium is reduced (Husain et al., 1985; Chahal and Chahal 1989a). The effective functioning of nodules in the symbiotic

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nitrogen fixation is dependent on bacteroid and leghaemoglobin (Chopra and Subba Rao, 1967). Leghaemoglobin (Lb), a soluble protein delivers oxygen to aerobic endosymbiotic bacteroids at an oxygen tension sufficiently low so as not to inhibit the oxygen sensitive bacteroid nitrogenase (Nadler and Avissar, 1977). The Lb content and the extent of bacteroid tissue in nodules have a direct correlation with the amount of nitrogen fixed by legumes (Chopra and Subba Rao, 1967). Dysfunction of existing nodules and reduction in Lb and bacteroid contents have been found to occur with nematode and fungal infections (Chahal and Chahal, 1989b; Khan and Husain, 1989b). Reduced leghaemoglobin concentration of nodules (Khan and Husain, 1989b) associated with decreased leaf chlorophyll, plant nitrogen (Melakeberhan et al., 1985a; Chahal and Chahal, 1989a) and potassium concentration (Melakeberhan, et al., 1987) with a parallel decrease in photosynthetic rates (Melakeberhan, 1986) are indications of some of the complex interactions involving nutrients and host physiology.

The aim of present study was to investigate the impact of nematode-fungus disease complex on NPK status and chlorophyll contents of greengram (mungbean) cv. T-44 in presence and absence of Rhizobium and also their influence on leghaemoglobin content of root nodules.

5.2 Materials and Methods

5.2.1 Plant Materials and Inoculation of Test Pathogens

One week old seedlings of greengram (mungbean) cv-T44
raised from bacterized and unbacterized seeds were inoculated with 2000 juveniles of *M. incognita* and 2.0 g mycelium of *R. solani* plant alone and in combination (simultaneously) (procedure described in Chapter 3). After inoculation the pots were placed on the benches of glass house (25±2°C). Plants were irrigated whenever required. There were three replicates of each treatment in all the experiments (5.3.1, 5.3.2, 5.3.3, 5.3.4). The experiments were terminated two months after inoculation and the data on the various biochemical parameters (nitrogen, phosphorus, potassium, chlorophyll contents and leghaemoglobin concentration) were recorded as per the procedures described below and then statistically analysed.

### 5.2.2 Analysis of Nitrogen, Phosphorus and Potassium

The plants were uprooted carefully after 60 days of inoculation and shoot and root systems were washed gently with double distilled water. After washing fully mature leaves and some part of stem and root systems were dried in an oven at 78±2°C separately for 24 hours. These oven dried samples of root, stem and leaves were powdered and passed through 72 mesh sieve. The powder was kept in a desiccator so as to ensure the availability of perfectly dry material. The samples were then digested for determining the NPK contents by following the method of Linder (1944) briefly described below.

#### 5.2.2.1 Digestion of Samples

100 mg dry powder of each sample (root, stem or leaf) was taken separately in a 50 ml kjeldahl flask. Two ml of pure
sulphuric acid (H$_2$SO$_4$) was carefully added and the mixture was gently heated for about two hours to dissolve the powder. Dense fumes ceased to come out at this stage and acid turned the contents black. After cooling the flask for about 15 minutes, 0.5 ml of chemically pure 30% hydrogen peroxide (H$_2$O$_2$) was added dropwise. The solution was heated again for about 30 minutes, until it turned light yellow in colour. After heating, the flasks were left for cooling for 10 minutes and then added with 3 or 4 drops of hydrogen peroxide followed by gentle heating for about 15 minutes to get a clear extract. Excess of hydrogen peroxide was avoided which would otherwise oxidise the ammonia in the absence of organic matter. The peroxide digested material was transferred to 100 ml volumetric flask with three or four washings with double distilled water (DDW) and the volume made up to mark. This served as the stock solution for the estimation of N, P and K. Suitable aliquots for determining nitrogen, phosphorus and potassium contents were taken from these sulphuric acid peroxide digested samples. The methods employed for the estimation of these elements are briefly described below.

5.2.2.2 Nitrogen Estimation

The Nitrogen content of the sample was estimated after Nesselerisation as described by Linder (1944).

About 10 ml of the peroxide digested material was transferred to 50 ml volumetric flask and the excess of acid partially neutralized with 2 ml of 2.5 N sodium hydroxide. To
this, 1 ml of 10% sodium silicate was added to prevent turbidity. The volume was made up to 50 ml with double distilled water and 5 ml aliquot of this solution was taken in a 10 ml graduated test tube and 0.5 ml of Nessler's reagent (10 g of Potassium iodide (KI) dissolved into 100 ml of double distilled water to which a solution of mercuric chloride (6.0 g in 100 ml of DD water) was added in small lots with shaking till a slight permanent precipitate was formed. To this was added 80 ml of 9N potassium hydroxide solution and then diluted to 200 ml with double distilled water. The solution was kept overnight and supernatant (clear solution) used next day. This reagent was kept in a dark coloured bottle because of its photosensitivity), was added drop by drop, shaking thoroughly after the addition of each drop. Double distilled water was added to make the volume upto 10 ml and the contents were allowed to stand for 5 minutes to attain the maximum colour development. The solution was then transferred to colorimetric tube and its optical density measured at 525 nm using a "Spectronic 20" colorimeter. A blank (9.5 ml double distilled water + 0.5 ml Nessler's reagent) was run with each set of determinations. The amount of Nitrogen in the aliquot was read from calibration curve, obtained by using known dilutions of a standard ammonium sulphate solution, which followed Bear's Law. Nitrogen in each sample was calculated in terms of percentage on dry weight basis.

5.2.2.3 Phosphorus Estimation

Total phosphorus in the sulphuric acid-peroxide digest
was estimated by the method of Fiske and Subba Rao (1925). A 5 ml aliquot was taken in 10 ml graduated test tube and 1 ml molybdate reagent (6.25g of ammonium molybdate dissolved in 75 ml of 10 N H₂SO₄ and volume made to 250 ml by adding 175 ml of distilled water) was added with care, followed by 0.4 ml of amino-2-napthol-4-sulphonic acid (ANSA) (0.5g of 1,2,4 ANSA dissolved in 195 ml of 15% sodium bisulphite solution and added with 5% of 20% sodium sulphite). The colour turned to blue. Distilled water was then added to the blue solution to make the volume upto 10 ml. The solution was shaken thoroughly, kept to stand for five minutes and then transferred to a colorimetric tube. The optical density was read at 620 nm on a "Spectronic 20" colorimeter. A blank was run for each determination. The standard curve was prepared by using known concentrations of monobasic potassium phosphate solution. Phosphorus was calculated on percentage dry weight basis.

5.2.2.4 Potassium Estimation

Potassium was estimated flame photometrically. One ml of an aliquot (peroxide digested material) was suitably diluted with double distilled water in graduated tube. It was read by using potassium filter. A blank containing only distilled water was run simultaneously. The readings were compared with calibration curve plotted for different dilutions of standard potassium sulphate solution. The potassium was expressed on percentage dry weight basis.
5.2.3 Estimation of Chlorophyll Contents

The fresh leaf samples after being brought to laboratory were removed from the ice bags and their surface gently cleaned with moist cotton to remove any particulate matter deposited over them. The chlorophyll contents were estimated by following the method of Arnon (1949). One gram fresh sample was crushed gently with 80% acetone in a mortar and pestle. To this was added a little pinch of calcium carbonate (CaCO₃). The samples after being ground to a fine pulp were centrifuged (5000 rpm for 5 minutes) and the supernatant transferred to a 100 ml volumetric flask. The residue was repeatedly ground and centrifuged till it turned colourless, thus ensuring the complete extraction of chlorophyll from the tissue. The volume of the extract was made 100 ml by adding 80% acetone. The absorption of the solution was read at 663 and 645 nm on spectrophotometer. The chlorophyll (a and b) contents were analysed by applying the formulae given by Maclachlan and Zalik (1963). The total chlorophyll was estimated by applying the formulae given by Arnon (1949).

\[
\text{mg Chlorophyll a /g tissue} = \frac{12.3 \text{D}663 - 0.86 \text{D}645}{d \times 1000 \times W} \\
\text{mg Chlorophyll b /g tissue} = \frac{19.3 \text{D}645 - 3.60 \text{D}663}{d \times 1000 \times W} \\
\text{mg total Chlorophyll /g tissue} = \frac{20.2 \text{D}645 + 8.02 \text{D}663}{d \times 1000 \times W}
\]

where, D663 and D645 represent the values of optical densities at the respective absorption spectra.
\[ V = \text{final volume of chlorophyll extract in 80\% acetone}, \]
\[ W = \text{fresh weight of tissue extracted}, \]
\[ d = \text{length of the light path}. \]

5.2.4 Estimation of leghaemoglobin from Nodules

For the measurement of leghaemoglobin from the excised root nodules, a method of Appleby and Bergerson's (1980) described by Sadasivam and Manickam (1992) was followed. Fresh or thawed nodules (0.5 g) were crushed in the flat bottomed tube and mixed with equal amount (volumes) of phosphate buffer (0.1 M sodium \ potassium phosphate buffer (pH 7.4)) and macerated in a mixer. The mixture was then filtered through two layers of cheese cloth. Nodule debris was discarded and the turbish reddish brown filtrate thus formed was clarified by centrifuging at 10,000 g for 10-30 minutes and then diluted suitably. To a suitable volume (3 ml) of the extract an equal amount of alkaline pyridine reagent (0.8 g NaOH dissolved in 5 ml water and cooled, followed by the addition of 33.8 ml of pyridine (33.2 g), dissolved and diluted to 100 ml with distilled water. In this way 4.2 M pyridine in 0.2M NaOH was produced), was added and mixed. The solution turned greenish yellow due to the formation of ferric hemochrome. The hemochrome thus formed was divided equally into two tubes. To one portion a few crystals of sodium dithionite was added to reduce hemochrome and it was stirred without aeration. After 2-5 minutes absorbance was red at 556 nm against a reagent blank in a Spectronic 20 spectrophotometer. To the other portion few
crystals of potassium hexacyanoferate were added to oxidise the hemochrome and absorbance was read at 539 nm. The leghaemoglobin (Lb) concentration was estimated by applying the formula given below:

$$\text{Lb concentration (mM)} = \frac{A_{556} - A_{539} \times 2D}{23.4}$$

where, D is the initial dilution (calculation is based upon the equation: $E = 23.4 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$)

The amount of leghaemoglobin was thus calculated and expressed in mg/g nodules.

5.2.5 Statistical Analysis

The results of the data obtained from the experiments were analysed statistically and significance of variance was calculated at $P < 0.05$ and $P < 0.01$ levels.

5.3 Experimental Results

5.3.1 Effect of M. incognita and R. solani alone and in combination on nitrogen, phosphorus and potassium contents of root, stem, and leaf in presence of Rhizobium

The data summarized in Table-4.1 and Fig.8 clearly indicate that both the pathogens [M. incognita(Mi) or R. solani(Rs)] whether inoculated singly or concomitantly (Mi+Rs) caused substantial decrease in the NPK contents of root, stem and leaf except, in the roots of M. incognita inoculated plants where an increased accumulation of all the three elements were observed, as compared to uninoculated control.

In presence of either of the pathogen (Mi or Rs) alone no marked variation in the nitrogen content of roots was noticed in comparison to uninoculated control, however, there was a
Table 4.1. Effect of Meloidogyne incognita and Rhizoctonia solani alone and in combination on Nitrogen, Phosphorus and Potassium contents of root stem and leaf in presence of Rhizobium.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nitrogen per cent dry matter</th>
<th>Phosphorus per cent dry matter</th>
<th>Potassium per cent dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Stem</td>
<td>Leaf</td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>4.03</td>
<td>3.61</td>
<td>5.24</td>
</tr>
<tr>
<td>(+5.45)</td>
<td>(-20.22)</td>
<td>(-8.20)</td>
<td>(+6.80)</td>
</tr>
<tr>
<td>Rss</td>
<td>3.75</td>
<td>2.48</td>
<td>4.50</td>
</tr>
<tr>
<td>(-6.94)</td>
<td>(-31.30)</td>
<td>(-14.12)</td>
<td>(-7.98)</td>
</tr>
<tr>
<td>Ni+Rs</td>
<td>3.27</td>
<td>1.97</td>
<td>3.99</td>
</tr>
<tr>
<td>(-18.85)</td>
<td>(-45.42)</td>
<td>(-23.85)</td>
<td>(-27.21)</td>
</tr>
</tbody>
</table>

C.D. (P= 0.05) .33 .12 .22 .01 .01 .02 .09 .26 .13
C.D. (P= 0.01) .51 .18 .34 .02 .02 .03 .14 .40 .19

Each value is a mean of three replicates,
Ni = M. incognita 2000 1st stage juveniles/plant,
Rss = R. solani 2 g fungus/plant,
Ni + Rss = M. incognita + R. solani simultaneously,
Figures in parentheses indicate per cent decrease (-) and per cent increase (+) over uninoculated control.
Fig. 8—Effect of M. Incognita and R. Solani alone and in combination on NPK contents of root, stem and leaf in presence of Rhizobium.
substantial (P<0.01) loss of 18.85% in nitrogen content when both the pathogens were inoculated concomitantly (Mi+Rs). Moreover, the difference between the nitrogen content of nematode and fungus inoculated plants was statistically significant (P<0.05). The nitrogen content of stem and leaf, on the other hand experienced a severe loss (P<0.01) upon individual (Mi or Rs) as well as combined pathogenesis (Mi+Rs). The magnitude of loss varied from 20.22-45.42% in stem and 8.20-23.85% in leaf, respectively (Table-4.1, Fig.8).

The observations (Table-4.1; Fig.8) on the impact of selected pathogens on the phosphorus contents reveals that plants treated with M. incognita showed marked (P<0.05) increase of 6.8% in the phosphorus content of root. However, in R. solani alone (Rs) and concomitantly inoculated (Mi+Rs) plants the amount of phosphorus was considerably (P<0.01) decreased to 7.98% and 27.21%, respectively.

In case of stem and leaf the M. incognita caused significant (P<0.01) reduction of 9.86% and 9.63% and the severity of loss increased almost two fold (16.14% in stem and 18.79% in leaf) in presence of R. solani alone. The per cent reduction enhanced further upto 35.42% in stem and 33.49% in leaf in the combined parasitism of both the tests pathogens (Mi+Rs).

Similarly, the potassium content of roots increased significantly (P<0.01) to 6.37% in M. incognita treated plants. However, it was substantially (P<0.01) decreased to 7.96% in presence of R. solani alone and 26.29% in its combination with
M. incognita. The potassium content of stem and leaf also exhibited a marked (P<0.01) decrease ranging from 13.39-29.46% in former and 15.80-40.87% in later in the corresponding pathogenic treatments, respectively (Table-4.1; Fig.8).

5.3.2 Effect of M. incognita and R. solani alone and in combination on nitrogen, phosphorus and potassium contents of root, stem, and leaf in absence of Rhizobium

It was observed from the results of data presented in Table-4.2 and Fig.9 that in the absence of Rhizobium the concentration of all the three elements (NPK) was comparatively less both in inoculated and uninoculated plants as compared to their Rhizobium treated counterparts. Further in all pathogenic treatments the unbacterized plants suffered a greater damage in the amount of nutrient elements than the bacterized ones. The change in concentration of NPK followed the same trend as was observed in Rhizobium treated plants in the different pathogenic treatments. However, the degree of variation differed depending upon the plant parts analysed. In case of roots an elevated level of nitrogen 19.14% at P<0.01 was observed upon the individual inoculation of M. incognita as compared to uninoculated control and it was substantially decreased in other pathogenic treatments. R. solani alone did not cause any marked reduction in the nitrogen content of roots in comparison to uninoculated control but the value differed significantly at (P<0.01) in between nematode (Mi) and fungus (Rs) inoculated plants. On the other hand, the per cent loss in nitrogen content was significantly (P<0.01) high (37.23%) in concomitantly
Table 4.2: Effect of Meloidogyne incognita and Rhizoctonia solani alone and in combination on Nitrogen, Phosphorus and Potassium contents of root, stem and leaf in absence of Rhizobium.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root per cent dry matter</th>
<th>Stem per cent dry matter</th>
<th>Leaf per cent dry matter</th>
<th>Root per cent dry matter</th>
<th>Stem per cent dry matter</th>
<th>Leaf per cent dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninoculated)</td>
<td>2.82</td>
<td>2.52</td>
<td>4.93</td>
<td>.305</td>
<td>.199</td>
<td>.398</td>
</tr>
<tr>
<td>M_i</td>
<td>3.36</td>
<td>1.78</td>
<td>4.36</td>
<td>.338</td>
<td>.165</td>
<td>.354</td>
</tr>
<tr>
<td>Rs</td>
<td>2.49</td>
<td>1.41</td>
<td>3.86</td>
<td>.280</td>
<td>.141</td>
<td>.300</td>
</tr>
<tr>
<td>M_i + Rs</td>
<td>1.77</td>
<td>1.05</td>
<td>3.09</td>
<td>.186</td>
<td>.121</td>
<td>.259</td>
</tr>
</tbody>
</table>

Each value is a mean of three replicates,
M_i = M. incognita 2000 1st stage juveniles/plant,
Rs = R. solani 2 g fungus/plant,
M_i + Rs = M. incognita + R. solani simultaneously,
Figures in parentheses indicate per cent decrease (-) and per cent increase (+) over uninoculated control.
FIG. 9—EFFECT OF M. INCognITA AND R. SOLANI ALONE AND IN COMBINATION ON NPK CONTENTS OF ROOT, STEM AND LEAF IN ABSENCE OF RHIZOBium
inoculated (Mi+Rs) plants. The nitrogen content of stem and leaf decreased significantly (P<0.01) in both single and combined treatments of test pathogens and the magnitude of loss ranged from 29.36-58.33% in stem and 11.56-37.32% in leaf (Table-4.2; Fig.9).

The phosphorus content of the roots increased significantly (P<0.01) to 10.81% in M. incognita alone, however, it showed a substantial (P<0.01) decrease of (8.13%) in R. solani alone and (39.01%) in combined inoculation of both the test pathogens (Mi+Rs). Similarly, in stem and leaf the phosphorus content was found to be declined considerably in all pathogenic treatments. While, the M. incognita infected plants showed a decrease of about 17.08% and 11.05% in stem and leaf respectively, the other plants infected with R. solani alone and in its combination with M. incognita (Mi+Rs) experienced a much higher decrease ranging from 29.14-39.19 in stem and 24.62-34.92% in leaf (Table-4.2; Fig.9).

The potassium content of root, stem and leaf fluctuated in a similar way as that of phosphorus in different pathogenic treatments. The roots exhibited a marked (P<0.01) increase (11.57%) in its potassium content from that of uninoculated control when treated with M. incognita alone and a substantial (P<0.01) decrease of 14.35% and 40.74% in R. solani and concomitantly inoculated (Mi+Rs) plants, respectively. The potassium content of stem and leaf also declined similarly in the individual as well as combined parasitism of both the test pathogens and the extent of loss varied from 18.37-46.85% in
5.3.3 Effect of *M. incognita* and *R. solani* alone and in combination on chlorophyll contents of foliage in presence and absence of *Rhizobium*

The data summarized in Table-5.1, 5.2 and Fig.10,11 shows the impact of *M. incognita* and *R. solani* alone and in combination on the chlorophyll contents of bacterized and unbacterized plants. The chlorophyll contents (Chlorophyll a, b and a+b) at the end of experiment was markedly low in all pathogenic treatments as compared to uninoculated control. However, the reduction was significantly high in the concomitance of both the test pathogens. Chlorophyll b declined to a lesser extent as compared to chlorophyll a and total chlorophyll (a+b) both in bacterized and unbacterized plants.

5.3.3.1 Effect on bacterized plants

In bacterized plants, a significant (P<0.01) reduction in the chlorophyll content was noticed both in the single and combined inoculation of test pathogens. *R. solani* alone caused greater reduction than that of *M. incognita*, however, there was no significant difference in between the chlorophyll contents (Chla and total chlorophyll a+b) of nematode and fungus inoculated plants. Chlorophyll b although declined significantly as compared to uninoculated control, did not show any marked variation in between nematode, fungus or concomitantly inoculated plants. Reduction in the chlorophyll a and total chlorophyll (a+b) on the other hand, was significantly high in concomitantly inoculated plants than in the inoculation of
Table 5.1. Effect of *Meloidogyne incognita* and *Rhizoctonia solani* alone and in combination on chlorophyll contents of foliage in presence of *Rhizobium*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chl. a</th>
<th>Per cent reduction over control</th>
<th>Chl. b</th>
<th>Per cent reduction over control</th>
<th>Total Chl. (a+b)</th>
<th>Per cent reduction over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninoculated)</td>
<td>1.328</td>
<td>--</td>
<td>.980</td>
<td>--</td>
<td>2.308</td>
<td>--</td>
</tr>
<tr>
<td>M1</td>
<td>.992</td>
<td>25.30</td>
<td>.821</td>
<td>16.22</td>
<td>1.813</td>
<td>21.44</td>
</tr>
<tr>
<td>Rs</td>
<td>.872</td>
<td>34.33</td>
<td>.762</td>
<td>22.44</td>
<td>1.634</td>
<td>29.20</td>
</tr>
<tr>
<td>Mi + Rs</td>
<td>.726</td>
<td>45.33</td>
<td>.677</td>
<td>30.91</td>
<td>1.403</td>
<td>39.21</td>
</tr>
</tbody>
</table>

C.D. (P<0.05) .14 .10 .19  
C.D. (P<0.01) .21 .15 .29  

Each value is a mean of three replicates,  
Mi = *M. incognita* 2000 IIInd stage juveniles/plant,  
Rs = *R. solani* 2 g fungus/plant
Fig. 10 - Effect of M. incognita and R. solani alone and in combination on the chlorophyll contents in presence of Rhizobium
Table 5.2. Effect of *Meloidogyne incognita* and *Rhizoctonia solani* alone and in combination on chlorophyll contents of foliage in absence of *Rhizobium*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chl.a</th>
<th>Per cent reduction over control</th>
<th>Chl.b</th>
<th>Per cent reduction over control</th>
<th>Total Chl.</th>
<th>Per cent reduction over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninoculated)</td>
<td>1.18</td>
<td>--</td>
<td>.887</td>
<td>--</td>
<td>2.067</td>
<td>--</td>
</tr>
<tr>
<td>Mi</td>
<td>.822</td>
<td>30.33</td>
<td>.730</td>
<td>17.70</td>
<td>1.552</td>
<td>24.91</td>
</tr>
<tr>
<td>Rs</td>
<td>.740</td>
<td>37.28</td>
<td>.640</td>
<td>27.84</td>
<td>1.380</td>
<td>33.23</td>
</tr>
<tr>
<td>Mi + Rs</td>
<td>.586</td>
<td>50.33</td>
<td>.555</td>
<td>37.42</td>
<td>1.141</td>
<td>44.79</td>
</tr>
</tbody>
</table>

C.D. (P < 0.05) = .08
C.D. (P < 0.01) = .12

Each value is a mean of three replicates,
Mi = *M. incognita* 2000 IInd stage juveniles/plant,
Rs = *R. solani* 2 g fungus/plant
FIG. 11—EFFECT OF M. INCognita AND R. SOLani ALONE AND IN COMBINATION ON THE CHLOROPHYLL CONTENTS IN ABSENce OF RHIZOBiUM
either pathogen alone. Chlorophyll a experienced a maximum loss of 25.30\% in *M. incognita* (Mi), 34.33\% in *R. solani* (Rs) and 45.33\% in concomitantly inoculated (Mi+Rs) plants, followed by 21.44\%, 29.20\% and 39.21\% in total chlorophyll *(a+b)* and 16.22\%, 22.24\% and 30.91\% in chlorophyll b, respectively in the corresponding pathogenic treatments (Table-5.1; Fig.10).

5.3.3.2 Effect on unbacterized plants

Unbacterized plants, in general, recorded a lesser concentration of chlorophyll contents in foliage and much pronounced decrease in all pathogenic treatments as compared to that observed in bacterized plants. The reduction in chlorophyll contents followed the same trend as was noticed in bacterized plants, however, the degree of variation differed with the treatments. While, the chlorophyll b declined to a lesser extent varying from 17.70-37.42\%, the chlorophyll a and total chlorophyll *(a+b)* experienced a greater loss ranging from 30.33-50.33 in former and 24.91-44.79\% in later in the different pathogenic treatments of nematode and fungus respectively. Chlorophyll a did not differ significantly in between nematode and fungus inoculated plants but the chlorophyll b and total chlorophyll *(a+b)* showed a marked variation in between nematode, fungus *(P<0.05)* or concomitantly inoculated plants *(P<0.01)* (Table-5.2; Fig.11).

5.3.4 Effect of *M. incognita* and *R. solani* alone and in combination on leghaemoglobin content of root-nodules

Data presented in Table-6 and Fig.12. clearly indicate
Table-6. Effect of *Meloidogyne incognita* and *Rhizoctonia solani* alone and in combination on leghaemoglobin concentration of root-nodules.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leghaemoglobin mg/g of nodules</th>
<th>Per cent reduction over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninoculated)</td>
<td>4.61</td>
<td></td>
</tr>
<tr>
<td>Mi</td>
<td>2.94</td>
<td>36.22</td>
</tr>
<tr>
<td>Rs</td>
<td>2.70</td>
<td>41.43</td>
</tr>
<tr>
<td>Mi + Rs</td>
<td>2.09</td>
<td>54.66</td>
</tr>
</tbody>
</table>

C.D. (P( 0.05)) .16  
C.D. (P( 0.01)) .25  

Each value is a mean of three replicates,  
Mi = *M. incognita* 2000 1Ind stage juveniles/plant,  
Rs = *R. solani* 2g fungus/plant
FIG. 12—EFFECT OF M. INCOCNITA AND R. SOLANI ALONE AND IN COMBINATION ON LEGHAEOMOGLOBIN CONCENTRATION OF ROOT NODULES
that both the test pathogens *M. incognita* and *R. solani* whether present singly or in combination (simultaneously) caused a significant ($P<0.01$) reduction in the leghaemogoblin concentration of root nodules. The fungus, *R. solani* caused a greater reduction (41.43\%) than that of *M. incognita* (36.22\%) when compared to uninoculated control. However, the magnitude of loss enhanced greatly (54.66\%) when both the pathogens were inoculated in combination.

5.4 Discussion

Nitrogen, phosphorus and potassium contents of root, stem and leaf experienced a substantial loss in both individual and combined inoculation of *M. incognita* and *R. solani*. However, in the inoculation of *M. incognita* alone the roots showed an increased accumulation of all the three elements (Table-4.1, 4.2; Fig.8,9). Adverse effect of nematode and/or fungus infections on the nodulation or nitrogen fixation has been already reported by several workers (Taha and Raski, 1969; Bopaiah et al., 1976a; Drellana et al., 1976; Sharma and Sethi, 1976; Ali et al., 1981; Kusk, 1982; Varshney, 1982; Mani, 1983; Sharma, 1984; Chahal and Chahal, 1987, 1989a,b; Khan and Hussain, 1990a). Reduction in the nitrogen content of root, stem and leaf in the inoculated plants might have been due to one or more of the following reasons i) Nematode or fungus might have interfered the establishment and multiplication of rhizobia and/or transformation in bacteroids, ii) In the process of symbiotic nitrogen fixation certain amount of energy in the form
of adenosine triphosphate (ATP) is required to reduce the triple bond of nitrogen molecule. This energy is released as a result of oxidation of photosynthate derived metabolites in the bacteroids (Brill, 1977). Since the infection of plants with nematode or fungus is known to reduce the photosynthetic activity, it is possible that the availability of less photosynthates might have adversely affected nitrogen fixation,

iii) Leghaemoglobin, a soluble protein present in the root nodules helps in the diffusion and proper regulation of oxygen concentration required for the respiration of nitrogenase containing *Rhizobium* bacteroids in the nodules (Cutting and Schulman, 1969; Burns and Hardy, 1975; Nadler and Avissar, 1977). Considerable reduction in the leghaemoglobin contents in the nodular tissue of nematode and fungus infected plants, as observed in the present study in another experiment (Table-6; Fig.12), might have caused an imbalance in the oxygen supply, thereby adversely affecting the nitrogenase activity in bacteroids. On the other hand, less synthesis of nitrogenase in the nematode and/or fungus infected plants (Kush, 1982; Chahal and Chahal, 1987,1988,1989a,b) may be one of the reason of reduced nitrogen fixation as the function of nitrogenase is to reduce dinitrogen into ammonia.

iv) Nodules require healthy cortical tissue for their proper development. The failure or the poor development of nodules on the nematode and/or fungus infected roots might have also imposed nitrogen deficiency in plants.

v) Utilization of free aminoacids, present in the infected tissue may be another reason of reduced nitrogen
The accumulation of nitrogen, phosphorus and potassium contents in the nematode infected roots as compared to uninoculated control and other treatments may be attributed to the i) impaired translocation to the foliage due to mechanical blockage or modification of vascular system (ii) highest metabolic activity of galled roots, which would have mobilised these elements to the infection site (Owens and Novotony, 1960) and/or (iii) increased permeability of infected roots (Bergeson, 1966). Other workers have also reported that root-knot infected plants suffer from the deficiency of nitrogen, phosphorus and potassium contents as a result of their accumulation in the roots (Feldman et al., 1961; Shafiee and Jenkins, 1962; Haque et al., 1974) or due to the absorption of these elements by the roots of infected plants (Tarjan, 1950; Hunter, 1958; Dasgupta and Deb, 1969).

Reduction in phosphorus and potassium contents may be due to the disturbed host physiology as a result of interacting pathogens. Moreover, high rate of carbohydrate metabolism due to the increased respiratory activity of infected tissue might also explain the reduced phosphorus and potassium contents (Shaw and Colotelo, 1961; McCombs and Winstead, 1964).

The result (Table-5.1, 5.2; Fig.10, 11) of present studies also indicate significant decrease of chlorophyll contents (Chl, a, b and total a+b) over uninoculated control when inoculated by either of pathogens (M. incognita or R. solani) alone and/or in combination. The reduction in chlorophyll contents of infected
plants may be due to the metabolic disruption, inhibition of chlorophyll synthesis or both (Singh et al., 1986b; Sharma and Sharma, 1990; Sugha et al., 1992) or the alterations of host nutrition and physiology (Bergeson, 1966; Doney et al., 1970; Singh et al., 1977). Nematode infection generally cause change in the concentration of certain elements such as iron (Fe) and manganese (Mn) which play an important role in the constituents of photosynthetic pigments (Devlin and Witham, 1986). The changed concentration of these elements impaires the chlorophyll synthesis and is believed to have a profound impact on host physiology which inturn appears to be a major cause in limiting the growth of host plant and in impairing translocation process (Bird and Loveys, 1975; McClure, 1977; Melakeberhan et al., 1985a). Increased pheophytin content in nematode parasitism (Upadhyay and Banerjee, 1986) and decrease in soil nitrogen content (Melakeberhan and Webster, 1993) as observed in other experiments of present study (Table-4.1,4.2;Fig.8,9) could also account for decreased chlorophyll contents.

The highest disease index in the combined pathogenesis results in the reduced transpiration pull and impairment of nutrient supply to the branches (Kumar et al., 1992), thereby influencing the chlorophyll synthesis and photosynthesis which inturn impede the development of plant in terms of reduced plant weight and yield (Melakeberhan, et al., 1985a). The results of present study substantiate the findings of Tiyagi (1990), Shah (1993) and Siddiqui and Mahmood (1994) who also reported considerable decrease in the chlorophyll contents in nematode
fungal disease complexes of different pulse crops. There are several other reports in which fungus and/or nematode infection alone has caused reduction in the chlorophyll contents of some plants (Agarwal et al., 1982; Murmunkar and Chavan, 1985; Melakeberhan et al., 1986; Chahal and Chahal, 1987; Ahmad and Kumar, 1990; Tiyagi and Alam, 1990; Sugha et al., 1992).

The plants in absence of *Rhizobium* (unbacterized) generally had lower concentration of nutrient elements (NPK) (Table-4.2; Fig.9) and chlorophyll contents (Table-5.2; Fig.11) as compared to that observed in presence of *Rhizobium*. Moreover, the unbacterized plants suffered a greater loss in amount of nutrient elements (NPK) and leaf pigments (chlorophyll contents) than the bacterized ones when inoculated individually and in combination with the test pathogens. The nematode infected roots of unbacterized also plant showed significantly higher amount of (NPK) in comparison to uninoculated control and other treatments, while in bacterized plants the amount of these elements was slightly higher than uninoculated control. This increased concentration of nutrient elements may be due to higher gall index caused by *M. incognita* in absence of *Rhizobium*.

The increased loss of nutrient elements and photosynthetic pigments in unbacterized plants has a positive correlation with increased disease index due to nematode and fungus infections. In absence of *Rhizobium* plants grew less vigorously and had reduced resistance or less tolerance against
invading pathogens (Kush, 1982) thereby, resulting in the increased damage. Several other workers have also found the reduced concentration of nutrient elements (nitrogen) and photosynthetic pigments (chlorophyll) and their increased loss in different pathogenic treatments in absence of Rhizobium (Singh et al., 1977; Tiyagi, 1990; Siddiqui, 1993; Siddiqui and Mahmood, 1994). The presence of Rhizobium in rhizosphere on the other hand presumably protects the host roots against root disease infections (Haque and Ghaffar, 1993) due to increased nitrogen status and hence resulting in lesser damage (Kush, 1982; Tiyagi, 1990).

Leghaemoglobin (lb) concentration of root nodules was significantly reduced in single and concomitant inoculation of M. incognita and R. solani (Table-6;Fig.12). An adverse effect of nematode and/or fungus infection on the leghaemoglobin concentration of nodules have been reported by earlier workers (Orellana and Worley, 1976; Huang and Barker, 1983; Chahal and Chahal, 1987, 1988, 1989b; Khan and Hussain, 1989b).

This impairment in leghaemoglobin concentration as a result of pathogenic infections may be due to the dysfunction or premature decay of nodules (Taha and Raski, 1969; Orellana et al., 1976; Bowen, 1978; Kush, 1982), suppression of the nodulation (Hussey and Barker, 1974; Hussaini and Seshadri, 1975; Sharma and Sethi, 1976; Kush, 1982; Chahal and Chahal, 1988, 1989a; Siddiqui and Husain, 1991), decreased photosynthetic activity in diseased plants (Melakeberhan, et al., 1984, 1985a, 1986; Hussey, 1985) or interference with the

Results are in accordance with the findings of Khan and Husain (1989b) who also observed a significant reduction in leghaemoglobin content of cowpea root nodules in the *Meloidogyne* and *Rhizoctonia* disease complexes.

5.5 Summary

Pathogenic infections caused a considerable variation in the nutrient status (NPK contents) of host plants. Results of the data showed a significant decrease in the NPK contents of leaf, stem and root of plants inoculated with either of the pathogen singly (except in *M. incognita* inoculated roots) or in combination, both in bacterized and unbacterized plants. There was, on the other hand, an increased accumulation of NPK contents in the roots of plants inoculated with nematode alone being significantly higher than uninoculated control except, the nitrogen content of bacterized plants which, however, did not show any marked variation from that of uninoculated control. Individually, *R. solani* caused greater reduction than *M. incognita*, however, the reduction was more pronounced when both these pathogens were inoculated concomitantly.

Similarly, the leaf chlorophyll content also showed marked reduction both in individual and combined pathogenesis of test pathogens. The loss was significantly high in concomitantly inoculated plants than in those inoculated singly with either of the pathogen alone. Chlorophyll a was reduced to a greater
extent as compared to chlorophyll b and total chlorophyll. Unbacterized plants, in general, recorded a lower concentration of nutrient elements (NPK) and leaf chlorophyll contents as compared to bacterized plants both in presence and absence of test pathogens. Moreover, the plants in absence of *Rhizobium* also showed a greater decrease in the NPK and chlorophyll contents than in its presence, in different pathogenic treatments.

The observations with respect to the effect of pathogens on leghaemogloboin (Lb) concentration revealed that Lb content was significantly reduced as a result of nematode and/or fungus infections. Maximum reduction was, however, recorded in the combined pathogenesis of both the test pathogens.
6. EXPERIMENTAL-III: NEMATODE-FUNGUS DISEASE COMPLEX MANAGEMENT

6.1 Introduction

Protection of crop plants from the disease causing agents have been the focal point of scientists concern in dealing with these organisms. This is particularly true for pulse crops which occupy an indispensible place in our daily diet as a source of protein. The major obstacle in the way of increased pulse production are various diseases and pests which are responsible for reduction and uncertainty in pulse yields (Grewal, 1983, 1988). Efforts directed towards the formulation of management systems by and large have been aimed against the monopathogenic situation although, the occurrence of disease complex involving plant parasitic nematodes is not uncommon in nature (Powell, 1979). Much emphasis has been given on the study of nematode fungal disease complexes which are now recognised to cause significant crop losses (Abu-El-Amayem et al., 1985; Husain et al., 1985; Varshney et al., 1987; Khan and Husain, 1988a; Siddiqui and Husain, 1992). Disease management strategies are being modified specially to reduce the amount of damage caused by nematode-fungal pathogen interactions. In most cases, control of nematode component of an interaction is fundamental in controlling the disease complex. Control measures can be classified as chemical, physical, cultural, biological and regulatory methods. Chemicals have been considered as by far the most effective and efficient means of managing the pathogenic populations of fungi, bacteria, nematodes etc. Chemicals such as nematicides, fungicides and herbicides have been formulated and
are being successfully used against their respective targets singly (Dekker, 1977; Van Gundy and McKenry, 1977; Wright, 1981) and in mixture (Brodie and Hauser, 1970; Abu-El-Amayem, 1985; Roberts et al., 1988).

There are, however, major compulsions for the diversion of research priorities from chemical methods to other alternatives because of the prohibitive costs of chemicals and their adverse ecological impacts. The use of the organic amendments has a greater relevance to present day need of avoiding pollution hazards caused by chemicals. This is more so in the situation like ours where organic wastes are easily available in large quantities. Plant residues/organic amendments serve as triple purpose. They form an antagonist, improve crop growth and act as deterrents for various pathogenic forms. Organic amendments such as oil cakes have been reported to check the population of pathogenic life forms like fungi, bacteria, nematodes etc. through a variety of mechanisms (Sayre et al., 1964; Patrick and Toussoun, 1965; Cook, 1977; Sitaramaiah, 1990).

Organic amendments are usually bulky and need to be applied in large quantities but their lower efficacy as contrasted to chemicals is out weighed by their cheapness and relative availability. The methods involving use of chemicals and organic amendments to deal with management of different plant pathogens have been investigated in the present study. The objective of the study was to evaluate the efficacy of two different chemicals (separately or in mixture) and various oil

6.2 Materials and Methods

6.2.1 Substances used for the management of test pathogens

Two chemicals (carbofuran and bavistin) and four oil seed cakes (neem, castor, mustard and mahua) were used separately (as mentioned in experiment Nos. 6.3.1, 6.3.2) to manage the disease incidence caused by *M. incognita* and *R. solani* whether present singly or concomitantly on greengram (mungbean) cv. T-44.

6.2.1.1 Use of chemicals (Experiment 6.3.1)

Two different chemicals carbofuran (Furadan3G) a nematicide and bavistin (a fungicide) were used separately or in equal mixture as soil drench. Carbofuran (2,3- Dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) a highly active systemic granular carbamate insecticide/nematicide with a broad spectrum antinematode activity and bavistin (a commercial formulation of carbendazim 50% w.p. (methyl benzimidazole-2Yl (carbamate) having broad antifungal spectrum were obtained from local market. The concentration of each chemical was kept at 200 ppm on active ingradient basis. In the treatments where their mixture was used, equal quantity (200 ppm + 200 ppm) of each chemical was taken. 50 ml of the above concentration of each chemical was added to the 15cm earthen pots containing autoclaved soil. Surface sterilized and bacterized seeds of
green gram (mung bean) cv. T-44 (0-4 seeds/pot) were sown in each pot (containing sterilized soil) 24 hours after the chemical treatments.

6.2.1.2 Use of oil cakes (Experiment 6.3.2)

Four oil seed cakes viz., neem (Azadirachta indica A. Juss), castor (Ricinus communis Linn), mustard (Brassica compestris Linn.), and mahua (Madhuca indica Gmel.) were obtained from local market. Each oil cake was mixed uniformly with 1kg sterilized soil (contained in 15 cm earthen pots) at the rate of 10 and 20g/Kg soil. The pots receiving oil seed cakes were watered to ensure proper decomposition. After 14 days (2 weeks) waiting period, surface sterilized and bacterized seeds of green gram (mung bean) cv. T-44 were sown at the rate of four seeds per pot.

6.2.2 Inoculation of test pathogens

One week after germination one healthy bacterized seedling of green gram (mung bean) cv. T-44 was retained per pot. Each set of plants in both the experiments (6.3.1, 6.3.2) was inoculated with 2000 (J2) second stage juveniles of M. incognita and 2.0 g mycelium of R. solani per plant alone and in combination (simultaneously) as per the procedure described in Chapter 3. All the treatments in each experiment (6.3.1, 6.3.2) were replicated thrice and after treatments and inoculations pots were kept in glasshouse (25±2°C).

6.2.3 Parameters

Sixty days after inoculations, both the experiments
were terminated. The following parameters were considered to
describe the results of the experiments (6.3.1 and 6.3.2).
1) Total dry weight of the plants (root+shoot),
2) Number of nodules per root system,
3) Number of galls per root system,
4) Rate of nematode multiplication (Rf),
5) Root-rot development (Root-rot index).

Each of the above parameters were determined by
standard methods (described in Chapter 3) and mean value was
calculated.

Per cent increase or decrease in total dry weight,
nodulation and root-rot indices and decrease in Rf value and
number of galls were calculated over their respective controls.
Per cent increase or decrease in the parameters of uninoculated
treated plants were calculated against uninoculated untreated
plants (control), whereas, those of inoculated and treated
plants over their respective inoculated untreated plants
(control).

6.2.4 Statistical Analysis

The experiments (6.3.1. and 6.3.2.) were laid down in
randomized block design. For analysing the variance, randomized
block design was further extended to split the factors and
analysed by extending the methods of Fischer (1950). While the
chemicals (Expt. 6.3.1.) and oil cakes (Expt. 6.3.2.) were
considered as factor one (F1) and test pathogens (nematode and
fungus) in both the above mentioned experiments (Expt. 6.3.1.

150
and 6.3.2.) were considered as factor two (F2). The significance of variance was calculated at P<0.05 level.

6.3 Experimental Results

6.3.1 Efficacy of two different chemicals (carbofuran and bavistin) separately and in mixture on plant growth, nodulation, nematode multiplication, gall formation, and root-rot development in presence of M. incognita (Mi) and R. solani (Rs) alone and in combination

6.3.1.1 Plant Growth

Both the pathogens, M. incognita and R. solani, when inoculated singly or concomitantly, caused a marked reduction in plant growth parameters viz., length, fresh and dry weights of root and shoot as compared to uninoculated control. In general, the plant growth did not differ significantly in between nematode and fungus alone treatment but in their concomitant inoculations the reduction in plant growth was significantly higher than their individual effects. Soil application of chemicals, carbofuran (Ca) and bavistin (Ba) separately or in mixture (Ca+Ba) reduced the individual and combined adverse effect of both the test pathogens and consequently improved the plant growth parameters substantially as compared to untreated control (Table-7; Fig.13,14 and Appendix-IV).

6.3.1.2 Plant dry weight (root+shoot)

6.3.1.2.1 Effect of pathogens

Significant reduction in plant growth (total dry weight) was noticed in all treatments in comparison to uninoculated control. Individually R. solani caused greater growth reductions (37.65%) than M. incognita (30.69%), but there
Table 7. Efficacy of two different chemicals, Carbofuran and Bavistin separately and in mixture on plant dry weight, nodulation, nematode multiplication, gall formation and root-rot development in presence of Meloidogyne incognita and Rhizoctonia solani alone and in combination.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Dry Weight</th>
<th>Modules / Root system</th>
<th>Nematode multiplication</th>
<th>Galls/root system</th>
<th>Root-rot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root +</td>
<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
<td>RF = Pf/Pi</td>
</tr>
<tr>
<td></td>
<td>Shoot (g)</td>
<td>Variation</td>
<td></td>
<td>Variation</td>
<td>Reduction</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td>3.16</td>
<td>-</td>
<td>59.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. incognita</td>
<td>2.19</td>
<td>-30.69</td>
<td>29.00</td>
<td>-51.34</td>
<td>12.29</td>
</tr>
<tr>
<td>R. solani</td>
<td>1.97</td>
<td>-37.65</td>
<td>22.30</td>
<td>-62.58</td>
<td>-</td>
</tr>
<tr>
<td>M. incognita + R. solani</td>
<td>1.23</td>
<td>-61.07</td>
<td>13.60</td>
<td>-77.18</td>
<td>6.59</td>
</tr>
<tr>
<td>Carbofuran</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
<td>4.05</td>
<td>+28.48</td>
<td>72.00</td>
<td>+20.80</td>
<td>-</td>
</tr>
<tr>
<td>M. incognita</td>
<td>2.99</td>
<td>+36.52</td>
<td>50.30</td>
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C.D. (P< 0.05)

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<td>1.91</td>
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Each value is a mean of three replicates,
(-) = Per cent reduction over untreated uninoculated control.
(+)= Per cent increase over respective untreated controls.
(++)= Per cent increase over fungus alone (untreated),
(-)= Per cent decrease over respective untreated inoculated controls,
RF (Nematode reproduction factor) = PF (Final population) / Pi (Initial population)
FIG. 13 - EFFICACY OF CARBOFURAN AND BAVISTIN SEPARATELY AND MIXTURES ON THE PLANT DRY WEIGHT AND NODULATION

UN = UNTREATED
CA = CARBOFURAN
BA = BAVISTIN
CA + BA = CHEMICALS IN MIXTURES

- CONTROL
- M. INCognita
- R. SOLani
- Simultaneous inoculation
FIG. 14 — EFFICACY OF CARBOFURAN AND BAVISTIN SEPARATELY AND IN MIXTURES ON NEMATODE MULTIPLICATION, ROOT GALLING AND ROOT-ROT DEVELOPMENT.
was no significant difference of the plant weight in between two treatments. In concomitantly inoculated plants (Mi+Rs) the reduction in plant growth was significantly higher (61.07%) than in plants inoculated with either of the pathogen alone. The effect of interaction was found to be antagonistic (Table-7; Fig. 13).

6.3.1.2.2 Effect of Chemicals

In general, soil application of chemicals was beneficial for the overall plant growth. Both the chemicals, when applied separately or in mixture, improved the plant growth significantly over untreated controls. The plant weight did not differ significantly in between carbofuran and bavistin treatments, however, when these chemicals were applied to nematode and fungus inoculated plants separately, the difference in plant weight was statistically significant. In all the cases combined use of chemicals showed a better performance than their individual applications.

In the absence of either of the pathogen, the plant growth (dry plant weight) showed a substantial increase in all the chemical treatments being highest with carbofuran+bavistin (Ca+Ba) (31.32%) and lowest in carbofuran (28.48%) and bavistin (26.26%) alone treatments. Treatments involving combined use of carbofuran+bavistin and bavistin alone differed significantly among themselves while all other treatments were at par to each other.

The growth of nematode, fungus, and concomitantly inoculated plants also improved significantly in all chemical
treatments over untreated controls. Combined application of chemicals resulted in the maximum improvement of plant growth than their individual effects. The plant growth (total dry weight) was increased significantly by 50.68%, 70.05% and 69.10% in M. incognita, R. solani and concomitantly inoculated (Mi+Rs) plants, respectively when chemicals were applied in mixture. On the other hand, the individual application of chemicals showed a varied effect on the nematode and fungus inoculated plants. In M. incognita inoculated plants, the carbofuran treatment increased the plant growth more significantly (36.52%) than that of bavistin (21.0%) whereas, in fungus infected plants the bavistin treatment recorded significantly higher increase (49.74%) in plant weight than the carbofuran (27.91%). However, in concomitantly inoculated plants there was no significant difference of plant dry weight in between individual treatments of carbofuran and bavistin (Table-7; Fig. 13).

6.3.1.3 Nodulation

6.3.1.3.1 Effect of pathogens

The number of nodules were also found to be reduced significantly in all pathogenic treatments. The maximum reduction (77.18%) was found in the combined inoculation of M. incognita and R. solani (Mi+Rs) followed by 62.58% in R. solani and 51.34% in M. incognita alone treatments (Table-7; Fig.13).

6.3.1.3.2 Effect of chemicals

The application of chemicals exerted a beneficial effect on nodulation both in inoculated and uninoculated plants.
Increase in nodule formation was more pronounced in combined use of chemicals (Ca+Ba) than their individual applications. In uninoculated plants the nodulation was increased by 20.80% in carbofuran, 24.16% in bavistin and 29.19% in carbofuran+bavistin (Ca+Ba) treatments. Carbofuran and bavistin alone treatments were at par to each other but both of them differed significantly from that of combined application (Ca+Ba). In M. incognita inoculated plants significantly higher increase in nodulation was observed in carbofuran+bavistin (82.75%) and carbofuran alone (73.44%) treatments with no significant difference in between them, but in bavistin alone treatment the increase in nodulation was significantly less (48.27%) than the other two treatments. On the other hand, in fungus inoculated plants the application of carbofuran caused comparatively lesser increase (52.46%) in nodulation than that of bavistin (79.37%) and carbofuran+bavistin (97.30%) treatments. But all the treatments differed significantly among themselves. Similarly, in concomitantly inoculated (Mi+Rs) plants the combined use of chemicals (Ca+Ba) improved the nodulation more significantly (76.47%) than in either of chemicals alone (47.05% in bavistin and 36.76% in carbofuran). Increase in nodulation did not differ significantly in between carbofuran and bavistin alone treatments (Table-7; Fig. 13).

6.3.1.4 Nematode multiplication

The experimental results on nematode multiplication presented in Table-7 and Fig.14 indicated that nematode
multiplied several folds in untreated inoculated controls. However, in presence of fungus, *R. solani* the multiplication of nematode was significantly suppressed. The Rf value was 12.29 when nematode occurred individually but it was substantially reduced (46.37%) when fungus was inoculated concomitantly with the nematode (Mi+Rs).

### 6.3.1.4.1 Effect of chemicals

Soil application of either of the chemical (carbofuran and bavistin) separately or in mixture suppressed the average Rf value of *M. incognita* both in individual and combined parasitism of test pathogens in comparison to untreated control. Combined usage of chemicals (Ca+Ba) were comparatively superior then their individual usage. In the inoculation of nematode alone the Rf value of *M. incognita* was significantly reduced to 34.09% in bavistin alone, 47.51% in carbofuran alone and 57.20% in carbofuran+bavistin (Ca+Ba) treatments. However, when the chemicals were applied to concomitantly inoculated (Mi+Rs) plants (with the exception of bavistin alone treatment) the decrease in nematode reproduction was almost higher as compared to the plants receiving nematode inoculum and chemicals only. The decrease in Rf value was significantly high (61.76%) in the treatments receiving mixture of chemicals (Ca+Ba) followed by 52.80% in carbofuran alone and 22.61% in bavistin alone treatments. In both, nematode (Mi) and concomitantly inoculated (Mi+Rs) plants the bavistin alone treatment caused significantly lesser reduction in Rf value as compared to that of carbofuran.
alone and mixture of chemicals (Ca+Ba) and Rf value in bavistin alone treatment was at par with that of untreated control in concomitantly inoculated plants. However, there was no significant difference in the Rf value of carbofuran alone and carbofuran+bavistin (Ca+Ba) treated plants both in (Mi) and (Mi+Rs) inoculated plants (Table-7; Fig.14).

6.3.1.5 Galls

Root-knot nematode caused severe galling (143) when it was inoculated individually but, in presence of fungus, R. solani the root-galling was significantly suppressed by 52.02% (Table-7; Fig. 14).

6.3.1.5.1 Effect of chemicals

Application of chemicals, alone and in combination inhibited the root-galling significantly in individually as well as concomitantly inoculated plants. The per cent loss in the galling was significantly high in treatments receiving both the chemicals in mixture (Ca+Ba), whereas in bavistin alone treatment the reduction was comparatively less than other two treatments (carbofuran alone and Ca+Ba). All the chemical treatments differed significantly in between themselves. In M. incognita inoculated plants the reduction in galling ranged between 45.73-73.91% in the single and combined application of carbofuran and/or bavistin while, in concomitantly inoculated (Mi+Rs) plants the corresponding figures ranged between 40.23-68.51% in the respective chemical treatments (Table-7; Fig. 14).
6.3.1.6 Root-rot development

The root-rot due to fungus, *R. solani* increased significantly (34.61%) in presence of nematode, *M. incognita*. However, in different chemical treatments the root-rot showed a noticeable decrease, both in fungus inoculated (Rs) and concomitantly inoculated (Mi+Rs) plants. Carbofuran alone treatment reduced the root-rot to lesser extent than that of bavistin alone and its combination with bavistin (Ca+Ba). The root-rot indices differed significantly in between the chemical treatments. In fungus inoculated plants, the root-rot was markedly decreased to 19.23% in carbofuran, 38.46% in bavistin and 46.15% in carbofuran + bavistin (Ca+Ba) treatments, whilst in concomitantly inoculated (Mi+Rs) plants a reduction of 14.28, 34.28 and 58.28% was observed in the respective chemical treatments (Table-7; Fig. 14).

On the basis of above findings it can be concluded that combined application of chemicals gave better control of disease caused by *M. incognita* and *R. solani*, singly or concomitantly, with respect to the improvement in plant growth and reduction in nematode multiplication and root-rot development the different chemical treatments can be arranged in descending order of efficacy as follows:

Carbofuran + bavistin (Ca+Ba) > Carbofuran > Bavistin.

6.3.2. Efficacy of two different doses of oil seed cakes (neem, castor, mustard, and mahua) on plant growth nodulation, nematode multiplication, gall formation, and root-rot development in presence of *M. incognita* and *R. solani* alone and in combination

Results (Table-8, Fig. 15, 16 and Appendix-V) shows the
Table-6. Efficacy of two different doses of oil seed cakes on plant dry weight, nodulation, nematode multiplication, gall formation, and root-rot development in presence of *Meloidogyne incognita* and *Rhizoctonia solani* alone and in combination.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Dry Weight</th>
<th>Modules / Root system</th>
<th>Nematode multiplication</th>
<th>Gall/root system</th>
<th>Root-rot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Root + Per cent)</td>
<td>Number</td>
<td>Per cent : Rf=Pf/Pi</td>
<td>Per cent</td>
<td>Number</td>
</tr>
<tr>
<td>Untreated</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control (Uninoculated)</td>
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Table continued
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Table Continued
Table 8. Continued

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<th>Treatments</th>
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<th>Balls/root system</th>
<th>Root-rot</th>
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<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
<td>RF=Pf/Pl</td>
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</table>

C.D. (P< 0.05)

| Treatments | .09 | 1.06 | .38 | 3.99 | .06 |
| Cakes | .14 | 1.59 | .81 | 8.46 | .12 |
| Treatments x Cakes | .28 | 3.19 | 1.15 | 11.97 | .18 |

Each value is a mean of three replicates.

(-) = Per cent decrease over untreated uninoculated control,
(+ ) = Per cent increase over respective untreated controls,
(+ )* = Per cent increase over fungus alone (untreated),
(-) = Per cent decrease over respective untreated inoculated controls,
RF (Nematode reproduction factor) = Pf (final population) / Pi (initial population)
Fig. 16—Efficacy of two different doses of oil seed-cakes on the nematode multiplication, root galling, and root-rot development.
impact of two different doses (10g or 20g/pot) of four oil seed cakes viz., Neem (Ne), Castor (Ca), Mustard (Mu) and Mahua (Ma) on plant growth and the disease caused by M. incognita (Mi) and R. solani (Rs) alone and in combination.

6.3.2.1 Plant Growth

In untreated set, the single as well as combined inoculation of M. incognita (Mi) and R. solani (Rs), resulted in significant decrease of different plant growth characters viz., length, fresh and dry weight of root, and shoot and nodulation. Individually R. solani (Rs) caused greater reduction than that of M. incognita (Mi) but in their combined inoculation (Mi+Rs) the reduction in plant growth was significantly higher than their individual effects. In general, application of oil seed cakes irrespective of dosage, with the exception of higher dose of mahua cake (Ma20), brought about a considerable improvement in the plant growth characters of both inoculated as well as un inoculated plants. However, in different pathogenic treatments both the dosage (10g or 20g/pot) of mahua cake (Ma) failed to cause any marked variation in plant growth, though they reduced the nematode multiplication, galling and root-rotting significantly (Table-8; Fig.15, 16; Appendix-V).

6.3.2.2 Plant dry weight (root+shoot)

6.3.2.2.1 Effect of pathogens

Both the pathogens, M. incognita (Mi) and R. solani (Rs) whether present singly or concomitantly (Mi+Rs) caused significant (P(0.05)) reduction in the total dry weight of plant,
in comparison to uninoculated control. Individually, *R. solani* caused greater growth reduction (40.43%) than that of *M. incognita* (29.62%) but, the reduction in the growth of concomitantly inoculated (Mi+Rs) plants was significantly high (61.11%) than the singly inoculated plants, though, it was less than the total sum of reduction caused by each pathogen alone (Table-8; Fig. 15).

**6.3.2.2 Effect of oil cakes**

Plant growth (total dry weight) was significantly enhanced in all oil cake amended soils, except in mahua cake (Ma), in comparison to those grown on unamended soils. Higher dose (20g/pot) of neem cake (Nc) was significantly superior to other oil cake amendments in improving the plant growth whereas, there was no significant difference in between the treatments involving other oil seed cakes both at lower and higher doses.

In uninoculated plants maximum enhancement (44.75%) in the plant growth was recorded in the higher dose of neem cake (Nc20) and minimum (10.81%) at the lower dose of mahua (Ma10). At higher dose of mahua cake (Ma20) plant growth was at par to that of unamended control . Similarly, the soil application of either of the oil cakes [neem (Nc), castor (Ca), mustard (Mu)] significantly increased the growth of *M. incognita* (Mi), *R. solani* (Rs) and concomitantly (Mi+Rs) inoculated plants. Maximum improvement in the growth of inoculated plants was achieved at the higher doses of oil cakes (20 g/pot). In all the cases,
higher dose of neem cake (Na2O) increased plant growth more significantly than the other treatments. However, there was no significant difference of plant growth in between the different doses of other oil cakes [Castor (Ca) and mustard (Mu)] though they improved the growth of plants substantially in comparison to unamended control.

In M. incognita (Mi) inoculated plants the degree of enhancement in plant dry weight ranged between 26.75-43.42% in neem, 16.66-26.31% in castor and 12.28-20.61% in mustard cake at their low (10g) and high (20g) doses, respectively. Similar trend was also observed in R. solani (Rs) and concomitantly inoculated (Mi+Rs) plants. The maximum enhancement of plant growth being in neem cake amendments and least in mustard cake (Table-8; Fig. 15).

On the basis of above findings with respect to improvement in plant growth (total dry weight) the treatments can be arranged in order to efficacy as follows:
Neem 20g > Castor 20g > Neem 10g > Mustard 20g > Castor 10g > Mustard 10g > Mahua 10g.

6.3.2.3 Nodulation
6.3.2.3.1 Effect of pathogens

The root nodulation showed a marked (P<0.05) reduction in all pathogenic treatments. Reduction in nodulation was significantly high (82.76%) in concomitantly inoculated (Mi+Rs) plants, followed by 65.82% in R. solani (Rs) and 56.90% in M. incognita inoculated plants (Table-8; Fig. 15).
6.3.2.3.2 Effect of oil cakes

The application of all oil seed cakes exerted a beneficial effect on the nodulation of both inoculated and uninoculated plants. Significant \((P<0.05)\) increase in nodulation was observed at both dosages \((10\text{g or } 20\text{g/pot})\) of oil cakes but higher dose of mahua cake \((\text{Ma}_{20})\) failed to cause any marked improvement of nodulation in uninoculated plants.

Nodulation of uninoculated control was significantly increased even at the lower doses of oil seed cakes over unamended control, but the highest value \((85.3)\) was noted in the higher dose of neem cake \((\text{Ne}_{20})\), which was also significantly superior to all other treatments. Nodulation in the lower doses of mahua \((\text{Ma}_{10})\), mustard \((\text{Mu}_{10})\) and castor \((\text{Ca}_{10})\) and higher doses of castor \((\text{Ca}_{20})\) and mustard \((\text{Mu}_{20})\) were at par to each other whereas in between the lower and higher doses of each oil cake the nodulation differed significantly. \textit{M. incognita} and \textit{R. solani} inoculated \((\text{Mi}+\text{Rs})\) plants responded in a similar manner to different oil cake amendments. In concomitantly inoculated \((\text{Mi}+\text{Rs})\) plants nodulation was also increased significantly in both the doses of oil seed cakes over untreated control. However, there was no significant difference in between the treatments both at lower and higher dosages \((\text{Table-8}; \text{Fig.15})\). With respect to improvement in nodulation different treatments can be arranged in order of efficacy as follows:

\[
\text{Neem 20g } \text{ Mustard 20g } \text{ Castor 20g } \text{ Neem 10g } \text{ Castor 10g } \text{ Mustard 10 g } \text{ Mahua 10g.}
\]
6.3.2.4 Nematode multiplication

Results presented in Table-8 and Fig. 16 indicated that the *M. incognita* multiplied several folds in the untreated inoculated control. On concomitant inoculation with fungus, *R. solani*, the multiplication of nematode was significantly suppressed in comparison to *M. incognita* inoculation alone. The rate of multiplication of *M. incognita* was 12.79 when inoculated singly but it was suppressed by 45.42% in presence of *R. solani*.

6.3.2.4.1 Effect of oil cakes

Application of oil seed cakes significantly suppressed the average reproduction factor (Rf) of both *M. incognita* (Mi) and concomitantly inoculated (Mi+Rs) plants (Table-8; Fig. 16). Higher dosages (20g/pot) of oil cakes were significantly more effective than the lower dosage (10g/pot). In general, higher doses of mahua (Ma20), neem (Nc20) and castor (Ca20) were found to be most effective and also significantly superior than other treatments whereas, in *M. incognita* alone inoculated plants only mahua (Ma20) and neem cake (Nc20) showed a better performance than other oil cakes. However, there was no significant difference in between these treatments. On the other hand, the Rf value in higher doses of castor (Ca20), mustard (Mu20) and all other lower doses (Ma10, Nc10, Ca10, and Mu10) were at par to each other but they differed significantly from that of unamended control.
The reduction in the Rf value of nematode was more pronounced in concomitantly inoculated plants (Mi+Rs) than that of nematode alone (Mi). Maximum reduction in the Rf value of *M. incognita*, (Mi) whether present singly (45.73%) or concomitantly (Mi+Rs) (55.87%) was observed in higher dose of mahua cake (Ma20) while the least reduction (15.55% for Mi and 15.75% for Mi+Rs) occurred in the lower dose of mustard cake (Mu10). The Rf value in lower doses of castor (Ca 10), and mustard (Ma 10) was at par to unamended control.

6.3.2.5 Galls

*M. incognita* caused severe galling (136) on the roots of greengram when present alone. However, the gall formation was significantly reduced (52.20%) in presence of the fungus, *R. solani* (Table-8; Fig. 16).

6.3.2.5.1 Effect of oil cakes

In general, soil amended with different dosages of oil seed cakes significantly suppressed the average gall formation of both *M. incognita* (Mi) and concomitantly (Mi+Rs) inoculated plants over untreated controls. The reduction in gall number was more pronounced in concomitantly inoculated (Mi+Rs) plants than those inoculated with nematode (Mi) alone. The suppressive effect of each oil cake was dose dependent and higher doses of mahua (Ma20) and neem (Nc20) cake were found to be most efficacious than the other treatments. In *M. incognita* (Mi) inoculated plants, the reduction in the number of galls was significantly superior to other treatments in higher doses of
mahua (Ma20), (51.98%) and neem (Nc20) (48.82%) cakes. The difference in the number of galls between lower (10 g/pot) and higher (20 g/pot) doses of each oil cake was significant. In concomitantly inoculated (Mi+Rs) plants, though the reduction was significantly greater in higher doses of mahua (Ma20) (58.00%) and neem (Nc20) (49.23%) cakes when compared to other treatments but there was no significant difference in between these two treatments and other treatments involving lower and higher doses of other oil cakes. Lower dose of mustard cake (Mu10) failed to cause any marked reduction in the root galling (Table-8; Fig. 16).

6.3.2.6 Root-rot development

In fungus inoculated (Rs) plants, root-rot index was 2.6, but in presence of nematode, M. incognita (Mi) it was significantly increased to 34.61% (Table-8; Fig. 16).

6.3.2.6.1 Effect of oil cakes

Oil cake amendments, in general, suppressed the development of root-rot significantly both in singly or concomitantly inoculated (Mi+Rs) plants in comparison to untreated control. Higher dose of mahua cake (Ma20) declined the root-rot indices more significantly than other treatments. However, there was no significant difference among the different doses of other oil cake treatments. On the otherhand, the root-rot index differed significantly in between lower (10g) and higher (20g) dosages of all the oil cakes both in single (Rs) and concomitant (Mi+Rs) inoculation.

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The reduction in root-rot indices of individually (Rs) and concomitantly inoculated (Mi+Rs) plants ranged from 26.92-42.30% for Rs, 28.57-45.71% for Mi+Rs in mahua cake; 19.23-26.92% for Rs, 17.14-31.42% for Mi+Rs in neem cake; 11.53-23.07% for Rs, 14.28-28.57% for Mi+Rs in mustard cake and 11.53-19.23% for Rs, 17.14-28.57% for Mi+Rs in castor cake at their low (10g) and high (20g) doses (Table-8; Fig.16). Thus, with respect to reduction in nematode multiplication, galling and root-rot indices the different treatments can be arranged in order of efficacy as follows:

Mahua 20g > Neem 20g > Castor 20g > Mahua 10g > Mustard 20g > Neem 10g > Castor 10g > Mustard 10g.

6.4 Discussion

In the present study, efficacy of two chemicals (carbofuran, a nematicide and bavistin, a fungicide) separately or in mixture and certain oil cakes (neem, castor, mustard and mahua) were evaluated against root-knot nematode, Meloidogyne incognita and root-rot fungus, Rhizoctonia solani, when present singly or concomitantly on greengram (mungbean) cv. T-44.

Soil application of both the chemicals (carbofuran and bavistin) separately or in mixture resulted in significant improvement of the plant growth and nodulation over untreated controls (Table-7; Fig.13). Similarly, nematode multiplication, gall formation and root-rot development was also found to be effectively reduced in all chemical treatments (Table-7; Fig.14). Improved plant growth in chemical treatments over
untreated control can be attributed to their persistent antinematode or antifungal effects which inhibit the penetration and development of the pathogens.

In general, application of chemicals in mixture showed a better performance than their individual effects. Carbofuran alone and in combination with bavistin hampered the nematode multiplication more effectively than that of bavistin alone. However, the bavistin application (singly or in mixture with carbofuran) did so in reducing the root-rot development. Reduction in the root-knot development and population of nematodes with the application of carbofuran are in agreement with the earlier findings of Jaiswal et al., (1987); Stephen et al., (1989); Reddy and Khan (1991) and Fazal (1993). Carbofuran is a systemic nematicide belonging to organo-carbamate. The way systemic nematicides work in protecting the plants from nematode infection has been summarized by Bunt (1987). When applied as soil treatment these nematicides influence the penetration, feeding, movement, invasion, hatching, and reproduction of nematodes inside or around the root tissue by two way action i.e. by a direct contact action through soil water and by a systemic action via the plant (Bunt, 1975; Steele, 1976; Evans and Wright, 1982). On the otherhand, the carbofuran reduced the activity of fungus, R. solani and disease caused by it alone and in combination with nematode. It has been reported that toxicity of certain systemic nematicides including carbofuran may manifest itself in reducing the microbial growth, basic activity in metabolism and reproduction of some pathogenic fungi (Bollen
et al., 1954, 1958; Bollen, 1961; Tu, 1975; Tu and Miles, 1976; Bertoldi et al., 1977, 1978; Tiyagi, 1990). Therefore, the adverse effect of carbofuran on the fungus may be attributed to this fact. Bavistin, a systemic fungicide besides controlling the disease of fungus and concomitantly inoculated (Mi+Rs) paints markedly, appears to have a positive effect on suppressing the nematode population and root-knot development. Nematicidal properties of this fungicide has already been reported for some other nematodes including M. incognita (Khan and Husain, 1988b; Jafee and McInnis, 1990). Maximum improvement of plant growth in the combined application of chemicals (Ca+Ba) may be ascribed to their sufficient biological activity or much biocidal effects (Brodie, 1970; Brodie and Hausar, 1970). Several workers (Fortnum and Curin, 1984; Abu-El-Amayem et al., 1978, 1985; Pandey and Singh, 1990) have also attained a multiple pest control by using compatible mixture of nematicides and fungicides on the root-knot and root-rot disease complexes of different crops.

Both the chemicals alone and in mixture significantly enhanced the nodulation and plant growth in presence and absence of pathogens. This may be due to the stimulatory effect of the chemicals (Johnson, 1970; Haq et al., 1987; Khan and Husain, 1988b), more availability of nitrogen in presence of nematicides (Charles and Paul, 1958; Singh et al., 1985) which in turn enhance the biomass of the plants or reduced pathogenic effects of nematode and fungus in chemical treatments (Tiyagi, 1990; RadhaKrishnan and Chatrath, 1991). Secondly cytokinin like
activity of bavistin (carbendazim) (Thomas, 1974) can also be attributed to the increased nodulation as the application of kinetin has resulted in increased number of nodules, fresh/dry weight and total nitrogen content (Nandwal et al., 1981). Results of present study confirms the findings of other workers (Walia and Sheshadari, 1985; Khan and Husain, 1988b; Tiyagi, 1990; Darekar et al., 1990b; RadhaKrishnan and Chatrath, 1991) who also observed a stimulatory effect of different chemicals (nematicides/fungicides) on the plant growth and nodulation on different crops.

Therefore, the results of the present findings strongly suggest that a fungicide-nematicide combination may be beneficial for effective control of disease caused by the root infecting pathogens and their possible amalgamation may show a proven biocidal efficacy against a pest disease complex of a given crop. Hence, a satisfactory control programme could involve the use of systemic chemicals in mixture rather than using them alone in a disease complex situation which present special problems for the disease management.

The farmers have been using organic materials for improving soil fertility since the advent of agriculture. However, it has become known only recently that these organic additives are highly effective in suppressing many plant diseases including those caused by nematodes and fungi. It is well known that whenever some organic material are added to the soil, there occurs an ecological succession of micro-organisms. Thus, successive phases of biochemical degradation and
succession of micro-organisms may guide the control of plant pathogens in the soil.

All the oil seed cakes, viz., neem, castor, mustard and mahua, used in the present study had a beneficial effect on the plant growth of mungbean. Their incorporation into the soil proved to be highly effective in reducing the incidence of root-knot and root-rot disease caused by M. incognita and R. solani, respectively. Besides minimizing the ill effects of nematode and fungus singly or concomitantly the oil cake amendments also improved the plant growth and nodulation substantially as compared to untreated control (Table-8; Fig.15,16). Enhanced plant growth and nodulation and suppression in the population parameters of nematode, root-knot and root-rot development has a positive correlation with the amount of oil cakes applied. Higher doses of the oil cakes resulted in the better performance than the lower doses. Mahua cake even at the lowest concentration though suppressed the nematode population and fungal damage (root-rot indices) more effectively in comparison to other oil cakes yet, did not improved the plant growth to a desired level and was inferior to other treatments in this regard. It appears that mahua cake tended to be phytotoxic at the given dosages. Phytotoxicity of this oilcake at higher dosages has also been reported by Azam (1975) on tomato plants, thus substantiating the present findings. With respect to the improvement in plant growth, the neem cake proved to be highly effective, followed by castor and mustard cake. A variety of mechanisms has been known to involve in the suppression of
nematode and fungal diseases and improvement in plant growth due to the oil cake amendments (Patrick and Toussoun, 1965; Sayre et al., 1964; Cook, 1977; Sitaramaiah, 1990). The nature of soil environment is so intricate that it is very difficult to conceive the activities occurring inside the soil. Control of disease in the amended soil does not seem to be the result of only one specific but, various intriguing factors. These mechanisms alter the disease severity through inseparable interactions of soil, host, and pathogen. Modification in physical, chemical and biotic environment of soil, by the addition of decomposable organic matter, has been found to influence the incidence of many plant diseases.

Interaction between, micro-organisms and other biotic components of the soil, lead to the transformation of organic matter from an unavailable into an available form to the plants. Microbial activities also limit certain soil inhabitant pathogens. The metabolic products of these organisms or decomposition products of the organic matter may induce physiological resistance in the host plants.

Furthermore, amendments of soil with oilcakes have been reported to be highly deleterious to nematodes and fungi and check their population through a diverse mechanisms (Sitaramaiah, 1990; Khan and Reddy, 1993).

Organic amendments quicken degradation resulting into the products more readily consumable and assimilable by the plants for their development. Oil cakes also result in improving the texture and increasing the water holding capacity of soil.
which helps in absorption of minerals by the plants more efficiently and consequently result in their luxurious growth. Increased plant growth in the soil amended with oil seed cakes as compared to those in unamended soil is understandable as these substances besides serving as organic manures, arrests the growth of fungi upon decomposition on one hand when added to culture medium (Khan, 1969) brings about reduction in the population of the fungi in fields and reduces the severity of other pathogenic fungi (Synder et al., 1959; Davey and Papavizas, 1959, 1961, 1963; Lewis and Papavizas, 1971; Saxena et al., 1971; Khan et al., 1973, 1974a; Singh and Singh, 1981, 1982; Kannaiyan and Prasad, 1981; Khan and Husain, 1988b; Tiyagi, 1990) and nematode population on the other hand when incorporated to soil (Singh and Sitaramaiah, 1966, 1971a,b; Khan, 1969, 1970, 1971, 1972; Alam et al., 1977a,b,c, 1979, 1980, 1982; Bhattacharya and Goswami, 1987, 1988; Zaki and Bhatti, 1989; Owino et al., 1993a,b). There may be several reasons attributed for the suppression of parasitic fungi with organic amendments. Khan et al., (1974b) have found water soluble fractions of oil seed cakes viz., neem, mahua, groundnut, castor and some bitter principles of neem like nimbidine and thionimone inhibitory to the growth of various test fungi including Rhizoctonia solani. Several fatty acids (Sayre et al., 1964, 1965; Toussoun et al., 1968), aldehydes and ketones (Khan, 1972), amino acids, carbohydrates, free sulphur (Ahmad et al., 1972) released during the decomposition of organic amendments may be toxic to the pathogenic fungi (Sayre
et al., 1965; Sayre, 1980; Singh and Pandey, 1965; Khan et al., 1974b). Moreover, the suppressive effect of organic amendments on parasitic fungi may also be due to the stimulation of microbial activity and biocontrol agents (Lockwood, 1960; Lloyd and Lockwood, 1966; Singh and Singh, 1981).

Similarly, the nematode control by organic amendments have been attributed to the accumulated toxicity of decomposing products (Patricks et al., 1965; Singh and Sitaramaiah, 1973; Alam et al., 1980; Owino et al., 1993a) or increase in the predacious or parasitic activity of soil biota (Muller and Gooch, 1982; Rodriguez-Kabana, 1991) or an increased host resistance due to the increase in the phenolic contents (Alam et al., 1977c, 1979, 1980). Consequently the organic amendments alter the physio-chemical properties of soils that make the soil environment unfavourable for the development and activities of plant parasitic nematodes (Vander Laan, 1956; Sitaramaiah, 1990).

Organic additives also release nutrients which accelerate rapid root development and improve the soil conditions for a better plant growth and nodulation thus helping the plant to escape nematode/fungus attack. This theory has been substantiated by the results where the oil seed cakes have improved the plant growth and nodulation in absence of either of the pathogen. Present studies provide an ample proof, that oil seed cakes besides controlling the disease caused by nematode and fungus singly or concomitantly also improved the plant growth substantially due to the changes in soil structure,
water holding capacity and changes in soil pH. Moreover additives also served as organic manures.

Thus, the present results corroborated the findings of Azam (1975), Khan and Husain (1988b), Tiyagi (1990) and Srivastava and Singh (1990) who achieved a successful control of nematode fungal disease complexes with oil seed cakes on vegetables, cowpea, mungbean and french bean, respectively.

6.5 Summary

The present chapter of the thesis embodies the results of two different experiments conducted to evaluate the efficacy of two chemical products (separately or in mixture) and four oil seed cakes (at two different doses) against root-knot nematode, M. incognita and root-rot fungus, R. solani attacking greengram (mungbean) cv. T-44 singly or concomitantly.

Soil application of chemicals, carbofuran and bavistin (both separately or in mixture) significantly reduced the population of M. incognita and suppressed the root-knot and root-rot development. Consequently the plant growth and nodulation was also improved significantly as compared to untreated controls. In all the cases the combined use of chemicals showed a better performance than their individual usage. Nematode and fungus inoculated plants showed a varied response to carbofuran and bavistin treatments. Carbofuran treatment improved the plant growth and nodulation of nematode inoculated plants more significantly than bavistin whereas, the bavistin treatment did so in the fungus inoculated plants.
However, in the absence of pathogens or concomitantly inoculated plants both the treatments were at par to each other. Chemical treatments also stimulated the growth and nodulation of uninoculated plants markedly over untreated ones.

Incorporation of oil seed cakes of neem, castor, mustard, and mahua proved to be highly effective against the disease caused by *M. incognita* and *R. solani* alone and in combination on mungbean. Plant growth showed a substantial improvement in both the doses of all oil seed cakes over untreated control except, in mahua cake treatments. Similarly the nodulation was also enhanced significantly in all oil cake amendments over untreated control. Higher dose (20g/pot) of neem cake was significantly superior to other treatments with respect to the improvement in plant growth and nodulation. On the other hand, the higher dosage of mahua followed by neem cake suppressed the average nematode population, root-knot and root-rot development more significantly than any other oil cake amendment. In the absence of either of the pathogen the plant also showed improved growth and nodulation in comparison to untreated controls, when subjected to the oil cake amendments.


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Effect of different inoculum levels of *Meloidogyne incognita* and *Rhizoctonia solani* separately on plant growth, nematode multiplication, and root-knot development in presence of *Rhizobium*.

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<th>Dry weight &quot;g&quot;</th>
<th>Total Nematode Population</th>
<th>Root-Knot index</th>
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<td>C.D. (P 0.01)</td>
<td>1.69</td>
<td>2.96</td>
<td>3.25</td>
<td>1.03</td>
<td>0.713</td>
</tr>
<tr>
<td>R. solani</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 g</td>
<td>25.4</td>
<td>18.0</td>
<td>43.4</td>
<td>5.00</td>
<td>1.69</td>
</tr>
<tr>
<td>0.50 g</td>
<td>24.3</td>
<td>17.2</td>
<td>41.7</td>
<td>4.59</td>
<td>1.38</td>
</tr>
<tr>
<td>1.0 g</td>
<td>21.3</td>
<td>15.5</td>
<td>36.8</td>
<td>4.10</td>
<td>1.12</td>
</tr>
<tr>
<td>2.0 g</td>
<td>19.3</td>
<td>13.3</td>
<td>32.6</td>
<td>3.34</td>
<td>1.04</td>
</tr>
<tr>
<td>4.0 g</td>
<td>18.2</td>
<td>11.1</td>
<td>29.3</td>
<td>3.04</td>
<td>0.930</td>
</tr>
<tr>
<td>C.D. (P 0.05)</td>
<td>2.10</td>
<td>2.14</td>
<td>2.65</td>
<td>0.844</td>
<td>0.480</td>
</tr>
<tr>
<td>C.D. (P 0.01)</td>
<td>2.59</td>
<td>3.04</td>
<td>3.79</td>
<td>1.19</td>
<td>0.681</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates.
Effect of different inoculum levels of *Meloidogyne* *incognita* and *Rhizoctonia solani* separately on plant growth, nematode multiplication, and root-knot development in absence of *Rhizobium*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length “cm”</th>
<th>Fresh weight “g”</th>
<th>Dry weight “g”</th>
<th>Total Nematode : Root-Knot : Population : Root-Knot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total</td>
<td>Shoot</td>
</tr>
<tr>
<td>Control (uninoculated)</td>
<td>25.8</td>
<td>18.6</td>
<td>44.4</td>
<td>5.15</td>
</tr>
<tr>
<td>M. <em>incognita</em></td>
<td>250</td>
<td>24.6</td>
<td>17.7</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>22.8</td>
<td>16.7</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>20.6</td>
<td>13.7</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>20.1</td>
<td>12.0</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>19.3</td>
<td>11.2</td>
<td>30.5</td>
</tr>
<tr>
<td>C. D. (P 0.05)</td>
<td>2.09</td>
<td>1.66</td>
<td>3.81</td>
<td>0.714</td>
</tr>
<tr>
<td>C. D. (P 0.01)</td>
<td>2.98</td>
<td>2.37</td>
<td>5.41</td>
<td>1.01</td>
</tr>
<tr>
<td>R. <em>solani</em></td>
<td>0.25 g</td>
<td>24.2</td>
<td>17.6</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>0.50 g</td>
<td>23.0</td>
<td>15.3</td>
<td>38.3</td>
</tr>
<tr>
<td></td>
<td>1.0 g</td>
<td>19.7</td>
<td>13.3</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>2.0 g</td>
<td>18.5</td>
<td>11.4</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td>4.0 g</td>
<td>17.8</td>
<td>10.6</td>
<td>28.6</td>
</tr>
<tr>
<td>C. D. (P 0.05)</td>
<td>2.27</td>
<td>2.13</td>
<td>4.50</td>
<td>1.04</td>
</tr>
<tr>
<td>C. D. (P 0.01)</td>
<td>3.28</td>
<td>3.03</td>
<td>6.40</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates
**APPENDIX-IIA**

Effect of individual and concomitant inoculation of variable inoculum levels of *Meloidogyne incognita* and *Rhizoctonia solani* on plant growth, nematode multiplication, and root-knot development in presence of *Rhizobium*.

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Length “cm”</th>
<th>Fresh weight “g”</th>
<th>Dry weight “g”</th>
<th>Total nematode population</th>
<th>Root-Knot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control (uninoculated)</td>
<td>27.40</td>
<td>19.50</td>
<td>47.00</td>
<td>6.86</td>
<td>1.91</td>
</tr>
<tr>
<td>Ni-500</td>
<td>26.20</td>
<td>19.00</td>
<td>45.20</td>
<td>6.36</td>
<td>1.71</td>
</tr>
<tr>
<td>Ni-1000</td>
<td>25.80</td>
<td>16.10</td>
<td>41.90</td>
<td>5.83</td>
<td>1.38</td>
</tr>
<tr>
<td>Ni-2000</td>
<td>23.70</td>
<td>15.50</td>
<td>39.20</td>
<td>4.54</td>
<td>1.26</td>
</tr>
<tr>
<td>Rs-0.5g</td>
<td>25.50</td>
<td>19.20</td>
<td>44.20</td>
<td>6.14</td>
<td>1.66</td>
</tr>
<tr>
<td>Rs-1.0g</td>
<td>24.20</td>
<td>15.80</td>
<td>40.00</td>
<td>5.80</td>
<td>1.33</td>
</tr>
<tr>
<td>Rs-2.0g</td>
<td>23.40</td>
<td>14.30</td>
<td>37.70</td>
<td>5.51</td>
<td>1.15</td>
</tr>
<tr>
<td>0.5Rs+500Ni</td>
<td>23.60</td>
<td>16.40</td>
<td>40.00</td>
<td>5.96</td>
<td>1.60</td>
</tr>
<tr>
<td>0.5Rs+1000Ni</td>
<td>22.90</td>
<td>15.10</td>
<td>38.00</td>
<td>5.71</td>
<td>1.26</td>
</tr>
<tr>
<td>0.5Rs+2000Ni</td>
<td>21.00</td>
<td>13.50</td>
<td>34.50</td>
<td>4.25</td>
<td>1.18</td>
</tr>
<tr>
<td>1.0Rs+500Ni</td>
<td>22.20</td>
<td>13.50</td>
<td>35.70</td>
<td>5.48</td>
<td>1.18</td>
</tr>
<tr>
<td>1.0Rs+1000Ni</td>
<td>19.70</td>
<td>13.20</td>
<td>32.90</td>
<td>5.50</td>
<td>1.13</td>
</tr>
<tr>
<td>1.0Rs+2000Ni</td>
<td>18.40</td>
<td>12.90</td>
<td>31.30</td>
<td>4.00</td>
<td>1.10</td>
</tr>
<tr>
<td>2.0Rs+500Ni</td>
<td>20.50</td>
<td>13.00</td>
<td>33.60</td>
<td>4.57</td>
<td>.990</td>
</tr>
<tr>
<td>2.0Rs+1000Ni</td>
<td>17.60</td>
<td>12.40</td>
<td>30.00</td>
<td>4.18</td>
<td>.876</td>
</tr>
<tr>
<td>2.0Rs+2000Ni</td>
<td>15.80</td>
<td>12.10</td>
<td>27.90</td>
<td>3.26</td>
<td>.840</td>
</tr>
</tbody>
</table>

C.B. (χ² 0.05)  1.60  1.33  2.26  .846  .293  1.10  .450  .135  .486  
C.B. (χ² 0.01)  2.16  1.79  3.43  1.14  .395  1.45  .607  .181  .655

Each value is a mean of three replicates,
Ni = *M. incognita*, Rs = *R. solani*
**APPENDIX-IIB**

Effect of individual and concomitant inoculation of variable inoculum levels of *Helicodera insagittata* and *Fusarium solani* on plant growth, nematode multiplication, and root-knot development in absence of *Rhizobium*.

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Length in &quot;cm&quot;</th>
<th>Fresh weight &quot;g&quot;</th>
<th>Dry weight &quot;g&quot;</th>
<th>Total nematode population</th>
<th>Root-knot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>26.30</td>
<td>19.10</td>
<td>45.40</td>
<td>6.13</td>
<td>1.77</td>
</tr>
<tr>
<td>(uninoculated)</td>
<td>24.90</td>
<td>18.80</td>
<td>43.70</td>
<td>5.60</td>
<td>1.54</td>
</tr>
<tr>
<td>Ni-500</td>
<td>23.40</td>
<td>15.20</td>
<td>38.60</td>
<td>5.00</td>
<td>1.13</td>
</tr>
<tr>
<td>Ni-1000</td>
<td>22.00</td>
<td>15.20</td>
<td>37.20</td>
<td>3.83</td>
<td>1.07</td>
</tr>
<tr>
<td>Rs-0.5g</td>
<td>23.50</td>
<td>18.60</td>
<td>42.10</td>
<td>4.93</td>
<td>1.26</td>
</tr>
<tr>
<td>Rs-1.0g</td>
<td>22.70</td>
<td>16.00</td>
<td>38.70</td>
<td>4.50</td>
<td>1.15</td>
</tr>
<tr>
<td>Rs-2.0g</td>
<td>20.70</td>
<td>14.30</td>
<td>35.00</td>
<td>4.00</td>
<td>.940</td>
</tr>
<tr>
<td>0.5Fs+500Ni</td>
<td>20.90</td>
<td>15.00</td>
<td>35.90</td>
<td>4.55</td>
<td>1.25</td>
</tr>
<tr>
<td>0.5Fs+1000Ni</td>
<td>19.00</td>
<td>14.40</td>
<td>33.40</td>
<td>4.20</td>
<td>1.20</td>
</tr>
<tr>
<td>0.5Fs+2000Ni</td>
<td>18.50</td>
<td>13.40</td>
<td>31.90</td>
<td>3.56</td>
<td>1.00</td>
</tr>
<tr>
<td>1.0Fs+500Ni</td>
<td>19.90</td>
<td>13.20</td>
<td>33.10</td>
<td>3.90</td>
<td>1.03</td>
</tr>
<tr>
<td>1.0Fs+1000Ni</td>
<td>18.10</td>
<td>12.70</td>
<td>30.80</td>
<td>3.52</td>
<td>.910</td>
</tr>
<tr>
<td>1.0Fs+2000Ni</td>
<td>17.10</td>
<td>12.00</td>
<td>29.10</td>
<td>3.26</td>
<td>.850</td>
</tr>
<tr>
<td>2.0Fs+500Ni</td>
<td>18.00</td>
<td>12.10</td>
<td>30.10</td>
<td>2.76</td>
<td>.904</td>
</tr>
<tr>
<td>2.0Fs+1000Ni</td>
<td>15.60</td>
<td>11.00</td>
<td>26.60</td>
<td>2.43</td>
<td>.860</td>
</tr>
<tr>
<td>2.0Fs+2000Ni</td>
<td>13.20</td>
<td>9.80</td>
<td>23.00</td>
<td>2.36</td>
<td>.840</td>
</tr>
</tbody>
</table>

C. D. (P = 0.05) | 1.35 | 1.38 | 1.85 | 1.01 | .250 | 1.17 | .426 | .119 | .472 |

C. D. (P = 0.01) | 1.82 | 1.86 | 2.50 | 1.37 | .339 | 1.58 | .574 | .160 | .655 |

Each value is a mean of three replicates,  
Ni = *H. insagittata*  Rs = *F. solani*
Appendix-III A

Effect of individual, simultaneous, and sequential inoculation of *Meloidogyne incognita* and *Rhizoctonia solani* on plant growth, nematode multiplication, and root-knot development in presence of *Rhizobium*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length <strong>cm</strong></th>
<th>Fresh weight <strong>g</strong></th>
<th>Dry weight <strong>g</strong></th>
<th>Total nematode population (Soil+Root)</th>
<th>Root-knot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control (uninoculated)</td>
<td>26.60</td>
<td>18.50</td>
<td>45.10</td>
<td>7.13</td>
<td>1.96</td>
</tr>
<tr>
<td><strong>Mi</strong></td>
<td>22.20</td>
<td>15.10</td>
<td>37.30</td>
<td>4.62</td>
<td>1.36</td>
</tr>
<tr>
<td><strong>Rs</strong></td>
<td>21.70</td>
<td>14.00</td>
<td>35.70</td>
<td>4.20</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Mi + Rs</strong></td>
<td>16.00</td>
<td>11.90</td>
<td>27.90</td>
<td>3.16</td>
<td>.950</td>
</tr>
<tr>
<td><strong>Mi -&gt; Rs</strong></td>
<td>16.10</td>
<td>12.30</td>
<td>28.40</td>
<td>3.47</td>
<td>1.02</td>
</tr>
<tr>
<td><strong>Rs -&gt; Mi</strong></td>
<td>16.40</td>
<td>12.59</td>
<td>28.90</td>
<td>3.80</td>
<td>1.13</td>
</tr>
</tbody>
</table>

C.D. (P 0.05) 1.28 .385 1.46 .683 .471 .548 .190 .072 .220
C.D. (P 0.01) 1.82 .548 2.08 .972 .670 .780 .271 .102 .312

Each value is a mean of three replicates,

**Mi** = *M. incognita* 2000 larvae/plant

**Rs** = *R. solani* 2g mycelium/plant

**Mi + Rs** = Simultaneous inoculation

**Mi -> Rs** = First pathogen (Mi) 15 days prior to 2nd pathogen (Rs)

**Rs -> Mi** = First pathogen (Rs) 15 days prior to 2nd pathogen (Mi)
### Effect of individual, simultaneous and sequential inoculation of Meloidogyne incognita and Rhizoctonia solani on plant growth, nematode multiplication, and root-knot development in absence of Rhizobium.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length cm</th>
<th>Fresh weight g</th>
<th>Dry weight g</th>
<th>Total nematode</th>
<th>Root-knot index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control (uninoculated)</td>
<td>25.30</td>
<td>18.10</td>
<td>43.40</td>
<td>6.43</td>
<td>1.76</td>
</tr>
<tr>
<td>Mi</td>
<td>20.90</td>
<td>14.60</td>
<td>35.50</td>
<td>3.30</td>
<td>1.43</td>
</tr>
<tr>
<td>Rs</td>
<td>19.00</td>
<td>13.20</td>
<td>32.20</td>
<td>3.06</td>
<td>1.05</td>
</tr>
<tr>
<td>Mi + Rs</td>
<td>14.40</td>
<td>10.60</td>
<td>25.00</td>
<td>2.13</td>
<td>.810</td>
</tr>
<tr>
<td>Mi --&gt; Rs</td>
<td>15.00</td>
<td>11.50</td>
<td>26.50</td>
<td>2.31</td>
<td>.896</td>
</tr>
<tr>
<td>Rs --&gt; Mi</td>
<td>15.10</td>
<td>12.40</td>
<td>27.50</td>
<td>2.81</td>
<td>1.08</td>
</tr>
</tbody>
</table>

C.D. (P( 0.05) | .851  | .766  | 2.13 | .418  | .364  | .593  | .284  | .075 | .218 |
C.D. (P( 0.01) | 1.22  | 1.09  | 3.02 | .595  | .518  | .844  | .404  | .107 | .310 |

Each value is a mean of three replicates,

Mi = M. incognita 2000 larvae/plant
Rs = R. solani 2g mycelium/plant
Mi+Rs = Simultaneous inoculation
Mi --> Rs = First pathogen (Mi) 15 days prior to 2nd pathogen (Rs)
Rs --> Mi = First pathogen (Rs) 15 days prior to 2nd pathogen (Mi)
Efficacy of two different chemicals (carbofuran and bavistin) separately and in mixture on plant growth, nodulation nematode multiplication, and disease development in presence of Meloidogyne incognita and Rhizoctonia solani alone and in combination.

### Plant length "cm"

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Root Mean</th>
<th>Shoot Mean</th>
<th>Total Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>F</td>
</tr>
<tr>
<td>Untreated</td>
<td>117.90</td>
<td>114.20</td>
<td>114.60</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>119.80</td>
<td>120.30</td>
<td>119.80</td>
</tr>
<tr>
<td>Bavistin</td>
<td>120.10</td>
<td>119.20</td>
<td>120.90</td>
</tr>
<tr>
<td>Carbofuran + Bavistin</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>119.87</td>
<td>119.07</td>
<td>119.07</td>
</tr>
</tbody>
</table>

C. D. (P 0.05)

| Treatments | .530 | | .578 | | .900 |
| Chemicals  | .530 | | .578 | | .900 |
| Treatments x Chemicals | 1.06 | | 1.15 | | 1.80 |

### Plant fresh weight "g"

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Root Mean</th>
<th>Shoot Mean</th>
<th>Total Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>F</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.05</td>
<td>1.39</td>
<td>1.30</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>3.13</td>
<td>2.51</td>
<td>2.39</td>
</tr>
<tr>
<td>Bavistin</td>
<td>3.21</td>
<td>2.34</td>
<td>2.49</td>
</tr>
<tr>
<td>Carbofuran + Bavistin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.91</td>
<td>2.24</td>
<td>2.20</td>
</tr>
</tbody>
</table>

C. D. (P 0.05)

| Treatments | .099 | | .115 | | .194 |
| Chemicals  | .099 | | .115 | | .194 |
| Treatments x Chemicals | .199 | | .231 | | .389 |

Continued
<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Root</th>
<th>Shoot</th>
<th>Total</th>
<th>Number of nodules /root system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>F</td>
<td>N+F</td>
</tr>
<tr>
<td>Untreated</td>
<td>.830</td>
<td>.572</td>
<td>.458</td>
<td>.322</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>1.15</td>
<td>.806</td>
<td>.655</td>
<td>.510</td>
</tr>
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C.D. (P (0.05)

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C.D. (P (0.05)

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Each value is a mean of three replicates,
C = Uninoculated, N = M. incognita 2000 larvae/plant, F = R. solani 2g fungus/plant, N+F = M. incognita + R. solani simultaneously, Rf = Nematode Reproduction factor, Pi = initial population, Pf = final population
**APPENDIX-V**

Efficacy of two different doses of oil seed cakes on plant growth, nematode multiplication, and disease development in presence of Meloidogyne incognita and Rhizoctonia solani alone and in combination.

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<th>N</th>
<th>F</th>
<th>N+F</th>
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<th>Shoot C</th>
<th>N</th>
<th>F</th>
<th>N+F</th>
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<th>N</th>
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C.D. (P0.05)
- Treatments .325
- Chemicals .404
- Treatments x Chemicals .977

continued
### Plant Fresh Weight "g"

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continued
### Appendix V. continued

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C.D. (P(0.05))

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Each value is a mean of three replicates,

- **C** = Uninoculated (Control)
- **N** = *M. incognita* 2000 larvae/plant,
- **F** = *R. solani* 2g fungus/plant,
- **N+F** = *M. incognita* + *R. solani* simultaneously,
- **Rf** = Nematode Reproduction factor,
- **Pi** = Initial population,
- **Pf** = Final population