DISCUSSION AND CONCLUSIONS

The economic threshold levels of Meloidogyne spp. on chickpea were earlier determined by several workers (Srivastava et al., 1974; Nath et al., 1979; Ram & Gupta, 1982; Mani & Sethi, 1984) but their findings have been at variance. Damaging threshold level as low as 200 juveniles of M. incognita and M. javanica per Kg soil was reported by Nath et al. (1979) and Srivastava et al. (1974) respectively but Ram & Gupta (1982) and Mani & Sethi (1984) found 1000 and 2000 juveniles of M. javanica and M. incognita as damaging threshold levels respectively. My findings are in conformity with those of Mani & Sethi (1984). The differences observed in damaging threshold levels by different workers can be attributed to the differences in experimental conditions, the cultivars used, or the species and races of the nematode involved.

Effect of nematode parasitism on nodulation has been reported stimulatory (Hussey & Barker, 1976), inhibitory (Miller, 1951; Masefield, 1958; High, 1966; Balasubramanian, 1971; Baldwin et al., 1975; Hussaini & Seshadri, 1975; Sharma & Sethi, 1976a, Bopaiah et al., 1976a; Singh et al., 1977; Srivastava et al., 1979; Raut, 1980; Chahal & Singh, 1984; Singh, 1984) or neutral (Taha & Raski, 1969). I, in my experiments, found it to be
inhibitory (Appendix - I, Table - 1). Reduction in
nodulation has earlier been explained as due to nutritional
interference, particularly carbohydrates, or physiological
changes brought about by nematode infestation than due to
competition for root invasion.

There was increase in the number of galls and final
nematode population with the increase of inoculum level as
earlier reported by Kaul & Sethi (1982) and Mani & Sethi
(1984) on maize and chickpea respectively. Nematode
population was found density dependent (Appendix - I,
Table - 1, Fig. 1) as also reported earlier by (Chapman,
1959; Seinhorst, 1960; Oostenbrink, 1966; Dhawan & Sethi,
1976; Gupta & Yadav, 1979; Dhruj & Vaishnav, 1981; Salem &
The maximum multiplication at low inoculum level might have
been due to less competition for food and space than at high
inoculum level.

Increasing inoculums of M. phaseolina caused
increased root-rotting and wilting and the resultant decrease
in plant growth parameters (Plate - I). Similar effects
caused by other fungal pathogens have also been reported
(Azam, 1975; Varshney, 1982; Zakiuddin, 1984 and Khan,
1986). Nodulation reduction due to fungal parasitism has
also been reported (Gupta, 1974; Orellana et al., 1976;
Zambolim & Schenk, 1984).
Protein content, both in the shoot and root, was found to increase with the increasing inoculum of *M. incognita* or *M. phaseolina* (Appendix - IA, Fig. 1A). Although the amount of buffer soluble protein in shoot was more than in the root but the percentage increase was more in root than in the shoot. Inoculum level dependent increase in protein content of root and shoot as observed in my experiment, is in conformity with the findings of Upadhyay & Banerjee (1986) but contrary to them the percentage increase was more in root than in shoot. Chattorjee & Sukul (1981) had used increase in protein content of root as an index for evaluating root-knot infection in lady's finger. The increase in total protein due to nematode infection has been reported by others also (Hanks & Feldman, 1966; Daney et al. 1971; Sinh et al., 1978).

The peroxidase activity increased up to the inoculum of 2000 juveniles of root-knot nematode and 1.0 gm fungus (Appendix - IB, Fig. 1A). There was no further increase in peroxidase activity at higher inoculums. Increase in peroxidase activity is considered related to the defence mechanism of plants.

On the basis of my findings, it has, therefore, been concluded that the damaging threshold levels of *M. incognita* and *M. phaseolina* were respectively 2000 juveniles of the former and 1.0 gm culture of the latter.
Various combinations of variable inoculums of test pathogens caused significant decrease in plant growth parameters. Reduction in plant growth was directly proportional to the increase in inoculum of test pathogens (Appendix - II, Table - 2, Fig. 2). The effect of interaction of test pathogens on plant growth was synergistic (Plate - 2A&B). Synergistic effects of nematode and fungus interactions have been reported earlier also (Cauquil & Sheperd, 1970; Whitney, 1974; Vaishnav & Sethi, 1978; Sharma et al., 1980; Khan et al., 1980; Singh et al., 1981; Nauza & Webster, 1982; Tchatchoua & Sikora, 1983; Chahal & Chhabra, 1984). Nematode and fungus together affected nodulation more significantly than any one of them singly (Malek & Jenkins, 1954; Nigh, 1966; Hendrick & Southards, 1976; Mani & Sethi, 1986). Adverse effect of fungus on nematode multiplication, as observed in the present findings, has also been observed by others (Sakhuja & Sethi, 1986; Al-Hazni, 1985). According to Powell (1971) the populations of migratory nematodes in general appear to increase as a result of interactions with fungi while those of sedentary nematodes are suppressed under similar conditions due to adverse effect on nematode penetration and direct fungus invasion of giant cells disrupting nematode feeding and subsequent reproduction within the host roots. Contrary to this Tu & Cheng (1971) observed favourable effect of M. phaseolina on reproduction of M. javanica in
kenaf roots when both pathogens were inoculated simultaneously to 5, 10 and 15 days old seedlings. This difference might be due to different host, nematode species or inoculation treatment involved.

Bacterized plants attained better growth and suffered less damage than unbacterized ones in the presence of one or both the pathogens (Appendix - III, Table - 3). It appears that legumes drive possible disease protection from their association with rhizobium due to increased nitrogen status which results in better plant growth. Reduced nematode damage of bacterized plants has also been reported by others (Bopaiah et al., 1976a,b; Sharma & Sethi, 1976a; Upadhyay & Kumar, 1983). However, the findings contrary to this are not uncommon (Ali et al., 1981; Varshney, 1982). Reduced damage of bacterized plants due to fungal pathogens has also been reported (Drapeau et al., 1973; Chou & Schmitthner, 1974; Tu, 1978, 1980). Least plant damage occurred when rhizobium was added 10 days prior to one or both the pathogens but the maximum when pathogen inoculation was followed by rhizobium (Appendix - III, Table - 3, Fig. 3). In case of simultaneous inoculations the plant damage was of intermediate order (Plate - 3A&B). It was possibly because the prior establishment of rhizobium resulted in improved plant growth enabling the plants to restrict the pathogen activity. In case of prior inoculation of pathogens, when nematode was inoculated first and rhizobium and fungus 10 days later, the damage was very high probably because of
physiological and biochemical changes that generally occur in the host tissues as well as in the rhizosphere due to prior nematode establishment resulting in predisposition of plants to fungal attack (Reynolds & Hanson, 1959; Batten & Powell, 1971; Tu & Cheng, 1971; Hutton et al., 1973; Pitcher, 1974; Goswami et al., 1975; Malekaberhan & Evans, 1981; Negron et al., 1982; Al-Hazmi, 1985; Goel & Gupta, 1986 and Mani & Sethi, 1987). Michell & Powell (1972), Chhabra et al. (1977), Reddy et al. (1979) and Patel et al. (1987) on the other hand, reported higher damage when plants were simultaneously inoculated with nematode and fungus. This apparently different relationship might be attributed to the involvement of different species of nematode and fungus, different crops and sets of experimental conditions etc. In treatments where fungus was inoculated first and rhizobium and nematode 10 days later the damage was less than in case of simultaneous inoculation probably because the fungus parasitized the plants less vigorously when present alone than in the presence of nematode. Moreover, prior establishment of fungus reduced nematode multiplication more significantly. When rhizobium and nematode were inoculated first followed by fungus the damage was less compared to prior nematode inoculation followed by rhizobium and fungus 10 days later because of some protection provided by prior establishment of rhizobium. The same pattern was observed when rhizobium and fungus were inoculated prior to nematode (Appendix-III, Table-3, Fig. 3).
Multiplication of nematode was adversely affected by the presence of fungus as discussed earlier. Rhizobium also adversely affected nematode multiplication. Nodulation was also adversely affected by the parasitism of test pathogens when rhizobium was inoculated simultaneously or after the pathogen as discussed earlier but the inoculation of rhizobium prior to pathogens caused no significant reduction in nodulation because of its favourable effect on the plant growth which restricted pathogen's entry to some extent.

Sixty five chickpea vars. were screened separately for their resistance and susceptibility against *M. incognita* and *M. phaseolina* on the basis of modified Husain's (1986) resistance ratings. When evaluated on the basis of percentage reduction in dry shoot weight, nematode multiplication and root-knot and root-rot indices, no variety was found resistant or moderately resistant against either pathogen. One var. gave tolerant response against *M. incognita* and 7 against *M. phaseolina*. Thirty three vars. showed susceptible response against *M. phaseolina* and 37 against *M. incognita*. Twenty seven vars. were found highly susceptible against *M. incognita* and 25 against *M. phaseolina* (Appendix - IV, Table - 4). In most nematological studies varietal screening has been done on the basis of reduction in dry weight and nematode multiplication (Sandhu et al., 1981; Mani & Sethi, 1985; Khan & Khan, 1987; Sasser et al., 1987; Thakar et al., 1987;
Mishra & Gaur, 1989) but in the present study I employed two more parameters namely buffer soluble protein content and peroxidase activity.

Peroxidase is known as a key enzyme required for lignin synthesis and lignin is one of the compounds judged to be phytoalexin which plays a decisive role in disease resistance. Peroxidase catalyses several reactions including those involved in metabolism of phenols and indoles. Protein content of galled roots has earlier been used as an index of root-knot nematode infestation in lady's finger (Chatterjee & Sukul, 1981). It is well known that qualitative protein changes occur in infected plants and the proteins may be of plant and/or pathogen origin. Therefore, the final resistance ratings were done on the basis of similarity of at least two parameters.

When ratings were done on the basis of increase in protein content, no var. gave resistant reaction against nematode but one was evaluated resistant against M. phaseolina. Ten vars. were found tolerant against M. phaseolina and 9 against M. incognita whereas 30 were rated susceptible against M. incognita and 35 against M. phaseolina. Twenty six vars. were highly susceptible against M. incognita and 19 against M. phaseolina (Table - 4A). In this case increase in protein content was more in highly susceptible vars. followed by in susceptible, tolerent, moderately resistant and resistant vars. Uritani
Stahmann (1961) reported increase in protein content of fungus infected tissues while Sharma et al. (1980), Basu & Sukul (1983) and Simte & Dasgupta (1987a) reported increase in protein content of nematode infected plants. Upadhyay & Banerjee (1986) reported inoculum level dependent increase in protein content of both shoot and root of *M. javanica* infected plants over uninoculated check which agrees with my findings of more significant increase in the highly susceptible vars. (highly infected plants) than in susceptible, tolerant, moderately resistant and resistant plants. However, my finding differs with those of Masood & Husain (1975) and Arya & Tiyagi (1982) who reported more protein in resistant than in susceptible and highly susceptible plants.

One var. each was found tolerant against *M. incognita* and *M. phaseolina* when evaluated on the basis of increase in peroxidase activity. Twenty six vars. were found susceptible against *M. incognita* and 37 against *M. phaseolina* while 27 were rated highly-susceptible against *M. phaseolina* and 38 against *M. incognita* (Table - 4A). Peroxidase activity was found high in tolerant vars. followed by susceptible and highly susceptible vars. Noel & McClure (1978) also observed greater peroxidase activity in resistant cotton cultivar clewewilt 6-3-5 than in susceptible M8 cultivar when infected by *M. incognita*. Similarly, Fehrman & Diamond (1967) observed a positive
correlation between peroxidase activity in different organs of potato plants and resistance against *Phytophthora infestans*. Veech & Endo (1970) also reported increase in activity of cytochrome oxidase and peroxidase in soybean infected with *M. incognita*. Increase in the activity of peroxidase after nematode infection was also reported by Hussey & Krusberg (1970), Acedo & Rohde (1971), Huang *et al.* (1971), Mote & Dasgupta (1979), Ganguly & Dasgupta (1981), Mohanty *et al.* (1986), Simte & Dasgupta (1987b). It appears that increase in peroxidase activity after infection with pathogen is in response to resistance activity of the plant. More is the increase in peroxidase activity more is the resistant response of the cultivar.

When all the three parameters were collectively considered for the final rating, only 2 vars. were found tolerant against *M. incognita* and 4 against *M. phaseolina*. Thirty four vars. gave susceptible reaction against *M. incognita* and 38 against *M. phaseolina* while 29 were found highly susceptible against *M. incognita* and 23 against *M. phaseolina* (Table - 4B).

Efficacy of ascorbic acid solution (0.1%), three leaf extracts, four biocontrol agents and six culture filtrates were tested for the management of root-knot nematode and root-rot fungus when present alone or concomitantly and compared with that of *P. lilacinus*. 
Paecilomyces lilacinus significantly improved growth of nematode and nematode plus fungus infected plants in all my treatments but in case of M. phaseolina infected plants only high dose of P. lilacinus was significantly effective (Appendix - V, Table - 5, Fig. 4). Jatala et al. (1979, 1980, 1981) and Morgan-Jones et al. (1984) have also earlier demonstrated that P. lilacinus parasitized eggs, females and larvae of M. incognita and Globodera pallida leading to their eventual death. This results in less development of disease on the host plants thereby improving crop yield. Similar beneficial effects of P. lilacinus have been reported by many other workers (Godoy et al., 1983; Noe & Sasser, 1984; Vellanueva & Davide, 1984; Dickson & Mitchell, 1985; Davide & Zorilla, 1986; Shahzad & Ghaffar, 1987; Cabanillas et al., 1988; Khan & Husain, 1988b; Sharma & Trivedi, 1989). I also observed antifungal effect of P. lilacinus that led to improved growth of infected plants. The antagonistic effect of P. lilacinus on Rhizoctonia solani has earlier been reported by Khan & Husain (1988b). Antagonistic effect of P. lilacinus to M. phaseolina may be attributed to some toxic metabolites or enzymes released by P. lilacinus which inhibited growth of M. phaseolina. Paecilomyces lilacinus is known to produce β (1-3) gluconase (Domsch et al., 1980) and chitinase (Okafor, 1967) extracellularly which are key enzymes in the lysis of fungal cell wall (Mitchell & Allexander, 1963), while Arai et al.

(1973) isolated leucostatin and lilacin, two water soluble peptide antibiotics from Penicillium lilacinus (Paecilomyces lilacinus). Leucostatin is known to be active against gram positive bacteria and many fungi.

It has now been convincingly demonstrated by several workers that P. lilacinus is highly efficaceous biocontrol agent for nematode management and that its antifungal and antibacterial activity has also come to light. In this study too it was found best when compared with other management materials.

Various treatments of ascorbic acid caused variable degree of growth improvement of nematode infected plants (Appendix - V, Table - 5, Fig. 4). Ascorbic acid is known to inhibit lipid oxidation in roots which is so necessary for root-knot larvae to be pathogenic because when lipid oxidation is inhibited the nematode has to use its own lipid reserves resulting in decreased nematode activity and infectivity. The nematodes start aging and eventually die and the damage caused by nematodes is consequently reduced. Higher soil doses and seed treatments were more effective because of high inhibition of lipid oxidation at the site of infection. Foliar applications and 5 ml soil doses were not significantly effective against M. phaseolina. Arrigoni et al. (1975, 1977), on the other hand, visualized the role of ascorbic acid in disease resistance.
Several species of *Cymbopogon* have been reported to possess nematicidal properties (Prem Kumar & Nair, 1976; Sangwan *et al.*, 1985 and Tiyagi *et al.*, 1986). In the present study nematicidal and antifungal efficacy of *C. citratus* has been confirmed (Appendix - VI, Table - 6, Fig. 5). The nematicidal activity of *C. citratus* can be attributed to its major constituents such as citral (about 54-87%) citronellol, geraniol and myicine. Efficacy of leaf extracts of *I. carnea* and *E. crassipes* against the two test pathogens can also be attributed to their major chemical constituents such as polysaccharide ipomose, an anthracene glucoside and one water soluble toxic principle in *I. carnea* and high potash and chlorine contents in *E. crassipes* (Anonymous, 1952, 1954). There appears potential scope for the use of herbal materials in nematode control as these materials are cheap, readily available, generally non-toxic to mammals and easily applicable as dry crop residues, green manuring or as extracts etc. *Eichhornia crassipes* (Water-Hyacinth) and *I. carnea* are common, abundantly available, noxious plants which may prove to be of ample significance for management of nematode problems in India. Moreover, their large scale application in any form would also reduce their own menace as undesirable fast growing plants and their application as organic amendment, would also improve soil fertility. Similarly, other cheap, easily available, nematotoxic herbal materials may also be safely used for this purpose.
Application of medium and high doses of *Bacillus licheniformis* and *Acrophialophora fusispora* improved growth of plants infected with nematode alone and nematode plus fungus, *Bacillus licheniformis* appears to have improved growth of nematode infected plants by their nematode parasitism which adversely affected nematode multiplication and survival (Plate - 4A&B). Reduction in nematode multiplication by the parasitism of *Bacillus penetrans/Pasteuria penetrans* has been reported by several workers (Mankau & Imbriani, 1975; Mankau & Prasad, 1977; Brown & Nardmeyer, 1985; Bird & Brisbane, 1988; Jay Raj & Mani, 1988) *Bacillus licheniformis* was not found effective against *M. phaseolina* although *B. subtilis* A13 was earlier reported to improve growth of *Sclerotium rolfsii* infected plants (Broadbent et al., 1975, 1977). *Acrophialophora fusispora* also improved growth of nematode infected plants and plants infected with nematode and fungus together. *Acrophialophora fusispora* parasitized females and eggs resulting in blackening and rotting of females, thus reducing their multiplication. It was not able to improve growth of plants infected with fungus alone. *Alkaligenes faecalis* though reduced nematode multiplication (Plate - 4A&B) but was not able to improve growth of nematode or fungus or nematode plus fungus infected plants. This was probably due to its mild pathogenic effect on chickpea.
Husain (1988b) reported *A. fusispora*, *P. mindocina* and *Bacillus* sp. as three new biocontrol agents of root-knot and cyst nematodes. The identity of *Bacillus* sp. has now been finally established as *B. licheniformis* and that of *P. mindocina* as *Alkaligenes faecalis*. These three biocontrol agents also show sufficient promise for nematode population management.

Since nematodes and fungi are the common inhabitants of soil, their secretions and excretions might naturally affect each other in various ways. Studies were, therefore, conducted to study the effect of culture filtrates of six fungi. Out of six culture filtrates used, culture filtrates of *A. niger* and *P. lilacinus* in both concentrations were effective for nematode population management when present alone or concomitantly with the fungus (Appendix - VII, Table - 7, Fig. 6). My results concerning efficacy of *A. niger* are in agreement with those of Mankau (1969a,b), Desai et al. (1972), Alam et al. (1973), Gupta et al. (1975), Khan et al. (1984a,b) and Vaishnav et al. (1985). Mankau (1969a) reported that *A. niger* filtrates showed strong positive test for oxalic acid and the toxic principle was thermostable. *Aspersillus niger* filtrate, in my study, also showed antifungal activity. *Paecilomyces lilacinus* culture filtrate was also found nematicidal and antifungal. Nematicidal and antifungal activity of *P. lilacinus* can be attributed to its
toxic metabolites or enzymes released by it such as β(1-3) gluconase, chitinase, leucostatin and lilacin (Domsch et al., 1980; Okafor, 1967; Mitchell & Alexander, 1963; Arai et al., 1973). The 'S' concentration of A. flavus was found toxic against nematodes. Nematotoxicity of A. flavus was also reported by Khan et al. (1984) and Vaishnav et al. (1985) but it was found less nematicidal than A. niger in the present studies. Culture filtrates of A. triticina, A. brassicicola and F. solani were less effective both against nematode and fungus individually or when present together.

When compared with all other test materials, P. lilacinus was found best for nematode population management. However, extracts of C. citratus and E. crassipes, cultures of B. licheniformis and A. fusispora, ascorbic acid and filtrate of A. niger can also be used either alone or in combination for management of root-knot nematode and root-rot fungus.