Discussion

In nature plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognized that 'nature does not work with pure cultures' and that many plant diseases are influenced by associated organisms. Root grow in soil containing a great number of microorganisms (nematode, bacteria, fungi and viruses etc.), whose action is often combined to induce damage. Plant-parasitic nematodes often play a major role in disease interactions. For centuries the economically important grain legume crops have been attacked by plant parasitic nematodes throughout the world. They severely damage a wide range of agricultural crops, causing serious yield losses, especially in the tropical and subtropical regions, where environmental factors favour their survival and dispersal (Sikora and Fernandez, 2005). Most of the diseases caused by nematodes are debilitating, however, in association with other pathogens, the disease picture is often drastically altered. Several workers have reported the interrelationship and interaction between nematodes and fungi on different economically important plants (Powell, 1963, 1971a, 1971b, 1979; Pitcher, 1965; Bergeson, 1972; Taylor, 1979; Sidhu and Webster, 1981b; Rebeiro and Ferraz, 1983; Kumar and Sivakumar, 1981; Uma-Maheshwari *et al.*, 1997). These investigations have led to a change from monopathogenic to multipathogenic disease concept. Though all the plants are being parasitized by one or more pathogens at a given time in nature, the economic losses become evident only when their population or the quality exceed to noxious levels.

In case of chickpea, the major obstacle in the way of increasing production are various diseases which are responsible for yield reduction.

The present study embodies three broad aspects related to the pathogens, root-knot nematode, *Meloidogyne incognita*; wilt fungus, *Fusarium oxysporum* f.sp. *ciceri* and root-rot fungus, *Rhizoctonia solani* in chickpea. Pathogenicity test, interaction of all the three pathogens and strategies for their control by *Trichoderma harzianum*, and oil-seed cakes (neem, castor, piludi and sunflower).

The pathogenicity test confirmed the susceptibility of chickpea (*Cicer arietinum*) var. Avrodhi in terms of damage in plant length, fresh as well as dry weight, pod number, chlorophyll contents, root nodulation, wilt and root-rot development by using different inoculum levels of root-knot nematode, *Meloidogyne incognita*, wilt fungus, *Fusarium oxysporum* f.sp. *ciceri* and root-rot fungus, *Rhizoctonia solani*. Similar studies have also been conducted by other workers to evaluate the susceptibility of pulses to root-knot nematode, *Meloidogyne incognita*, wilt fungus, *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia solani* (Tiyagi and Alam, 1986; Sahoo *et al.*, 1986; Tiyagi and Alam, 1987; Varshney *et al.*, 1987; Mohanty *et al.*, 1989; Khan and Hussaini-Nejed, 1991; Kalita and Phukan, 1993; Samathanam and Seth, 1996; Anwar and Alam, 1997; Siddiqui and Mahmood, 1996; Mahapatra *et al.*, 1999; Haider *et al.*, 2003).

In above studies the pathogenicity was assessed only on the basis of disease development (e.g. root-knot development, wilt and root-rot index, nematode population), whereas, in the present study large number of plant growth parameters have been taken into account to obtain more accurate assessment of plant to the test pathogens (Tables 1 to 6).

Plant damage due to the test pathogens increased with increasing inoculum levels. More damage was caused by *Fusarium oxysporum* f.sp. *ciceri* followed by *Rhizoctonia solani* and *Meloidogyne incognita*. Growth parameters (length, fresh weight and dry weight) of the test plant was reduced at all the inoculum levels of all the three pathogens to a varying degree. The reduction in plant growth increased with an increase in inoculum level. Present findings are in agreement with Tiyagi and

Alam (1988), Azam (1975), Varshney (1982), Fazal *et al.* (1994), Zakiuddin (1984) and Khan (1984).

Significant reduction was also observed in the photosynthetic pigment, chlorophyll contents. In infected plants reduction in growth adversely affects the photosynthesis, which in tern impede development of plant weight, number of flowers and delayed flowering ultimately resulting in reduced yield (Melakeberhan *et al.*, 1985). Nematodes also decreased water uptake by destroying plant roots (Meon *et al.*, 1978). The physiological effects of decreased water availability include decreased nutrient uptake and translocation of solutes, decreased chloroplast activity, decomposition of protein and nucleic acids and increased hydrolytic enzymes (Schoeneweisis, 1978).

The decrease in pod number may also be due to deficiency of food supply to the fertile branch (Tiyagi and Alam, 1989, 1990) and to the deficiency of mineral nutrition (Melekeberhan *et al.*, 1985; Wallace, 1974). Number of nodules were reduced in the test plant due to nutritional interference particularly carbohydrate or physiological changes (Bopaiah *et al.*, 1976a, Taha and Raski, 1969) or anatomical changes (Balasubramanian, 1971) brought about by nematode infection of plants rather than the secretion of hydrolytic and oxidative enzymes (Barker *et al.*, 1972) or competitive phenomenon between rhizobia and nematode (Epps and Chambers, 1962, Malek and Jenkins, 1964).

The rate of nematode multiplication was adversely affected by increasing inoculum levels. Such observations have been given out by several workers (Nath *et al.*, 1979; Walia and Seshadri, 1985; Fazal *et al.*, 1994; Ahmad and Alam, 1997). The decrease in the rate of nematode multiplication with increasing inoculum levels may be due to competition for food and space (Davide and Triantaphillou, 1967; Chapman, 1959; Gupta and Yadav, 1979; Salem and Eissa, 1981).

Interactive effect of root-knot nematode, *Meloidogyne incognita*, wilt fungus, *Fusarium oxysporum* f.sp. *ciceri* and root-rot fungus, *Rhizoctonia solani* on chickpea:

Chickpea is frequently attacked by wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (Woltz and Jones, 1981). Like wilt fungi, root-rot fungi are capable of causing root diseases on their own and have their own inherent mechanism of root penetration. The role of nematode in root-rot diseases in general is related to assisting the fungal pathogen in its pathogenesis and increasing host susceptibility. Nematodes, by wounding provide the fungal pathogen access to root tissue (Inagaki and Powell, 1969). Various species of *Fusarium* and *Rhizoctonia* are prominent fungi that are known to interact with different plant-parasitic nematodes.

The results (Tables 7 to 10) suggested that the nematode and two fungi individually caused significant reduction in total plant length, fresh weight and dry weight as compared to uninoculated control. When both pathogens were inoculated concomitantly, the decline in plant growth parameters (viz. plant length, fresh weight and dry weight) were greater than with these pathogens alone. However, the maximum reduction in all growth parameters was found in the treatment, where the nematode and fungus were inoculated simultaneously (Tables 7 to 10). Maximum plant growth reduction in the simultaneous inoculation of both the pathogens can be assigned to the fact that when a plant is jointly infected by more than one pathogen, it seem reasonable to expect that activities of the other changed its susceptibility due to physiological changes occurred in host plant tissues (Powell, 1979).

Greatest reduction in number of pods and chlorophyll contents was in concomitant inoculation of both the pathogens, could be explained by the fact that both pathogens got equal opportunity to parasitize the root for longer time and alter the host physiology and biochemistry (Khan and Saxena, 1969; Yang *et al.*, 1976; Mani, 1983; Azam *et al.*, 1984).

All the three pathogens affected the nodulation either singly or in combinations when compared to uninoculated control (Tables 7 to 10). In case of combined inoculations the greatest reduction in nodulation was caused by in simultaneous inoculation of both the pathogens. Reduction in number of nodules might have been due to adverse effect of toxic substances of nematode and/or fungus origin on *Rhizobium* or due to the interruption in translocation and/or utilization of host plant material by the nematode and/or fungus. The results are in agreement with Masefield (1958) and Khan & Husain (1988). The rate of nematode multiplication was adversely affected in the presence of *F. oxysporum* f.sp. *ciceri* and/or *R. solani*. Reduced multiplication of nematode in the presence of fungus can be ascribed to the destruction of root tissues by the fungus before the completion of nematode life cycle (Nath *et al.*, 1979; Fazal *et al.*, 1998) or due to possible toxic effect of fungal exudates on nematodes. Some of the biochemical changes occurring during the development of syncytia/giant cells induced by *M. incognita* remain in state of high metabolic activity through continuous stimulation by the nematode (Webster, 1975). They contain maximum DNA, RNA, sugars, hemicellulose, lipids, organic acids, protein and amino acid (Bird, 1972). The enriched medium benefits the fungal pathogens in their interaction with root-knot nematodes. Further physiological alterations by the nematode improve the nutrient status of the host for the fungal pathogens. It is also likely that some toxic fungal secretions might have reduced larval population around the roots (Khan *et al.*, 1984; Shah and Azam, 1992). Therefore, it appears that as a result of fungal parasitism root-knot nematode has failed to establish itself.

Effect of *Rhizobium* on fungi and nematode:

Most leguminous plants are symbiotic with nodule forming *Rhizobium*. Rhizobia fixed nitrogen which benefits plant growth. The inoculation of seeds with *Rhziobium* is known to increase nodulation, nitrogen uptake, growth and yield response of crop plants (Dorosinsky and Kadyrov, 1975, Patil and Shinde, 1980; Herandez and Hill, 1983; Zaidi *et al.*, 2003). In the recent past, rhizobia have gained reputation of controlling soil-borne root-infecting fungi belonging to the genera *Fusarium, Rhizoctonia* and *Macrophomina* (Ehteshamul Haque and Ghaffar, 1993; Sharif *et al.*, 2003; Ozkoc and Deliveli, 2001; Arfaoui *et al.*, 2006; Khalequzzaman and Hossain, 2007) and the root-knot nematode (Siddiqui *et al.*, 1998; Omar and Abd-Alla, 1998; Siddiqui *et al.*, 2001), besides fixation of the atmospheric nitrogen. Rhizobia are reported to produce bacteriocin(Roslycky, 1967), Rhizobiotoxine (Chakraborty and Purkayastha, 1984) which have inhibitory effect on soil-borne plant pathogens. Rhizobia are also reported to produce acid which have antifungal effect (Khokhar *et al.*, 2001). The rhizobia present in the rhizosphere of plants presumably prevent the contact of pathogenic fungi by covering hyphal tip of the fungus and by parasitizing it (Tu, 1978). Besides parasitizing the hyphae rhizobia also produce antibiotics (Malajezuk, 1983) which resulted in the lysis of fungal hyphae (Malajezuk *et al.*, 1984). Rhizobia are gram-negative and may have lectin binding structure in the lipopolysaccharide layer of cell wall membrane (Lotan *et al.*, 1975). Therefore, the mechanism responsible for reduction in nematode penetration may have related to the ability of the bacteria to envelop or bind to root surface lectins, thereby interfering with the normal host recognition (Oostendorp and Sikora, 1990). *Rhizobium leguminosarum* is reported to produce increased levels of phytoalexin (4-hydroxy-2,3,9-trimethoxy pterocarpan) in pea (Chakraborty and Chakraborty, 1989). Rhizobia have several mechanisms of action that allow them to control pathogens. These mechanisms include competition for nutrients (Essalmani and Lahloú, 2002), production of antibiotics (Chakrborty and Purkayastha, 1984; Ehteshamul Haque and Ghaffar, 1993), promotion of plant growth, in terms of better shoot height, root length, dry weight and root nodulation (Siddiqui *et al.*, 2000; Siddiqui and Mahmood, 2001) and induction of plant defence mechanisms (Abdelaziz *et al.*, 1996). Since the rhizosphere provide frontline defence for roots against attack by pathogens, the rhizobia present in the rhizosphere are ideal for use as biocontrol agents.

Effect of antagonistic fungus, *Trichoderma harzianum* on fungi and nematode:

The infection of wilt fungus, *Fusarium oxysporum* f.sp. *ciceri* and root-rot fungus, *Rhizoctonia solani* caused greater reduction in plant growth characters viz. plant length, plant weight (fresh and dry), number of pods and nodulation than caused by the infection of root-knot nematode, *Meloidogyne incognita*. Effect of antagonistic fungus (*Trichoderma harzianum*) was evaluated against the test pathogens (*M. incognita, F. oxysporum* f.sp. *ciceri* and *Rhizoctonia solani*) singly or in their combinations on chickpea plant. It was cleared from the results given in (Tables 7 to 10) that the test pathogens individually caused significant damage to the host. Further, the different combination of *M. incognita, F. oxysporum* f.sp. *ciceri* and *R. solani* brought about more reduction in plant growth parameters. It was also observed that different treatments with antagonistic fungi have effectively contained the pathogenic effect. Different inoculum levels of the antagonistic fungus was tested for their potency. Inoculum level (1.0 g/pot) of antagonistic fungi provided the best result for reducing the plant damage caused by pathogens singly or in different combinations (Tables 11 and 12). The antagonistic fungus not only reduced the pathogenic effects but also improved the plant growth (length, fresh and dry weight, pod number, chlorophyll content and nodulation) of chickpea. These results support many earlier findings where antagonistic fungus have been reported to be highly deleterious to nematodes and fungi (Windham *et al.*, 1989; Saifullah, 1996a,b; Saifullah and Thomas, 1996; Ahmad and Alam, 1997; Perveen *et al.*, 1998; Dubey, 1998; Kubicek and Harman 1998; Duponnois *et al.*, 1998; Rao *et al.*, 1998; Fatemy, 1999; Elad, 2000; Srivastava and Singh, 2000; Dubey, 2000; Viaene and Abawi, 2000; McBeath *et al.*, 2001; Bunker and Mathur, 2001; Kumar and Dubey, 2001; Dubey, 2003; Batta, 2004; Podder *et al.*, 2004; Harman, 2004; Brewer and Larkin, 2005; Santamarina and Rosello, 2006; Rojo *et al.*, 2007) on various crops. The improvement in root nodulation as well as in photosynthetic pigment could be due to direct effect on different treatments or indirectly through control of the pathogens. In any way, improved nodulation as well as chlorophyll content might have, in tern helped the plants for their luxuriant growth.

Use of *Trichoderma spp.* in the biological control of soil-borne fungi like *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kuhn, *Fusarium* spp. and *Macrphomina phaseolina* has also been reported (Elad *et al.*, 1971; Wells *et al.*, 1972; Chet *et al.*, 1981; Bell *et al.*, 1982; Elad, 1986; Chet, 1987; Ghaffar, 1992; Bunker *et al.*, 2001; Devi and Reddy, 2002; Rini and Sulochna, 2006). Besides control of root infecting fungi, *T. harzianum* has also been found to antagonize plant-parasitic nematodes (Saifullah, 1996a; Dos Santos *et al.*, 1992; Siddiqui *et al.*, 2001). *Trichoderma* spp. significantly reduced egg hatching, caused mortality of *M. javanica* juveniles and suppressed nematode populations in soil and root and subsequently reduced root-knot development in both okra and mungbean (Siddiqui *et al.*, 2001).

Trichoderma spp. are active rhizosphere colonizers (Tronsmo and Harman, 1992; de Meyer, 1998; Yedidiya *et al.*, 1999, 2000; Howell *et al.*, 2000) that produce glucanases (Lorito *et al.*, 1996; Witkowska and Maj, 2002), protease (Elad, 2000; Geremia *et al.*, 1993), chitinases (De La Cruz *et al.*, 1992), bioactive metabolites (Antal *et al.*, 2000; Manczinger *et al.*, 2002) and a range of non-volatile and volatile antibiotics (Ghisalberti and Sivasithamparam, 1991; Lida *et al.*, 1993; Padmodaya and Reddy, 1996; Sivasithamparam and Ghisalberti, 1998; Kumar and Dubey, 2001; Dubey and Patel, 2001) and some cell wall degrading enzymes (Bello *et al.*, 1997). Arora *et al.* (1992) reported that root colonization by *Trichoderma* frequently enhances root growth and development. *Trichoderma harzianum* increased root development in other crops under greenhouse or field conditions (Harman, 2000) and in chickpea (Dubey *et al.*, 2007). *T. harzianum* also solubilises phosphorus (Altomare *et al.*, 1999; Sharma, 2003) and stimulate plant growth even in the absence of the pathogen (Windham *et al.*, 1986). Present findings are in agreement with the results reported by above workers.

In the present findings antagonistic fungi not only reduced the intensity of nematode infection but also showed antagonistic effect against *F. oxysporum* and *R. solani*. Antagonistic fungi released some toxic metabolites or enzymes that might have inhibited the growth of fungi.

Identical experiments were also conducted by using unbacterized seeds. All the growth characters (plant length, fresh and dry weight, pod number, chlorophyll content) of the test plant were less than those raised from bacterized seeds. The growth parameters of chickpea plant was further reduced in plant inoculated with pathogens as compared to pathogen inoculated plants in the absence of *Rhizobium*. Rate of nematode multiplication and disease incidence (wilt and root-rot index) was increased due to reduced resistance against invading pathogens. The present findings are in agreement with Bopaiah *et al.* (1976a); Orellana *et al.* (1976), and Tu (1980).

Effect of oilseed cakes and *Trichoderma harzianum* on the development of root-knot nematode, *Meloidogyne incognita*; wilt fungus, *Fusarium oxysporum* f. sp. *ciceri* and root-rot fungus, *Rhizoctonia solani* and on the growth of chickpea.

Organic amendment of soil has been used by farmers since the beginning of the agriculture. It benefits physical and chemical soil properties which are inimical to nematodes (Ahmad *et al.*, 1972), increases soil fertility and aids in pest control (Garcia Alvarez *et al.*, 2004). Organic soil amendments used for nematode control are extremely heterogeneous, including green manuring, animal manuring, soil urban residues, and a variety of agro-indsutrial by-products (Cook and Baker, 1983; Hoitink, 1988; D'Addabbo, 1995). It was found that when crop by-products are used as biofumigant materials the compounds released are mainly aldehydes and isothiocyanates, which have biocidal activity and have been related to control of plant-parasitic nematodes (Sayre *et al.*, 1965; Abawi and Widmer, 2000; Garcia Alvarez *et al.*, 2004). Organic additives are highly effective in suppressing many plant diseases including those caused by nematodes.

In present studies it was observed that when soil was amended with oil-seed cakes (neem, castor, piludi and sunflower) caused significant reduction in the population of phytoparasitic nematode, *Meloidogyne incongita* and the frequency of the pathogenic fungi, *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia solani* and the chickpea growth parameters were increased, the frequency of saprophytic fungi might have increased. The results are in agreement with those of Gour and Prasad (1970), Alam and Khan (1974) Siddiqui (1986) and Tyagi and Alam (1995) on different crops. It is observed that oil –seed cakes in different treatments have effectively controlled the pathogenic effects of both fungi and nematodes. Maximum inhibitory effect against pathogens was found with neem cake followed by castor, piludi and sunflower.

There are numerous theories which explain the mode of action of organic amendments leading to the control of plant parasitic nematodes. These includes :

Organic amendments cause changes in the physical and chemical properties of soil which are inimical to nematodes (Ahmad *et al.*, 1972). Nematicidal/nematostatic substances such as nimbidin, thionimon and azadirachtin from neem, present in the amendments, are released after dissolution in water, or in other words the organic *matter itself is toxic to nematodes (Alam et al., 1978; Siddiqui and Alam, 1990).* Predacious and parasitic activity of soil microorganisms is enhanced by incorporating the organic matter to the soil (Linford, 1937; Mankau, 1962). Several toxic substances eg. ammonia, H_2S, formaldehyde, fatty acid, phenols, amino acids, and carbohydrates etc. released during decomposition of organic matter are toxic to many plant parasitic nematodes (Rodriguez-Kabana *et al.*, 1965; Walker *et al.*, 1967; Alam *et al.*, 1978, 1979). Metabolites of microbes, which are activated by organic amendments, are toxic to plant-parasitic nematodes (Alam *et al.*, 1973; Pandey and Singh, 1990).

Vander Laan (1956) had postulated that organic additives induce some sort of resistance in plants against plant parasitic nematodes. Oil-seed cake amended soil acquire some resistance against plant parasitic nematodes (Sitaramaiah and Singh, 1978; Alam *et al.*, 1980). They correlate this phenomenon with the increase in the phenolic levels in plant roots and proposed that this increase might have caused due to the absorption of phenolics released during amendment decomposition. As a result of the application of organic amendments, plant nutrients are released which accelerate root development and overall plant growth and thus helping the plants to escape nematode attack.

Soil population of pathogenic fungi e.g. *Fusarium* spp., *Rhizoctonia solani* were reduced by incorporating the organic matter to the soil on egg plant, okra and tomato (Khan *et al.*, 1973, 1974a). *Fusarium oxysporum* f.sp. *ciceri* on gram

(Chauhan, 1960), *F. udum* on pigeonpea (Singh and Singh, 1982), fatty acids, aldehydes, ketones, amino acids are released during the decomposition of organic matter have been reported to be toxic to pathogenic fungi (Khan *et al.*, 1974b; Sayre, 1980).

Thus, it appears that improvement in plant growth of chickpea in amended soil was due to reduction in population of plant parasitic nematodes and frequency of pathogenic fungi (Tables 11, 12, 13 and 14) and also due to their manurial effect (oil-seed cake contain about 5-6% N). Increased microbial activity in amended soil is known to bring about unceased conversion of nitrogen to nitrate form (Gunner, 1963) and accelerated enzyme nitrate reductase activity, which results ultimately in increased metabolic activity of plants and plant growth. The frequency of pathogenic fungi like *F. oxysporum* f.sp. *ciceri* and *R. solani* reduced in oil-seed cakes amended soil (Tables 11, 12, 13 and 14). The results has been supported by many workers (Saikia *et al.*, 1999; Walia *et al.*, 1999; Chen *et al.*, 2000).

In present studies it was observed that *Trichoderma* in association with *Rhizobium* and different oil cakes (neem, castor, piludi and sunflower) proved very effective in enhancing all the growth parameters of chickpea plant and reducing the population of root- knot nematode and frequency of both the fungi. (Tables 13 to 16) as compared to individual inoculations of either *T. harzianum* or oil-seed cake. Neem cake is proved to be highly effective against *F. oxysporum* f. sp. *ciceri*, *R. solani* and *M. incognita* followed by castor cake, piludi cake and sunflower cake. *T. harzianum* in combination with neem, karanj and castor oil cakes significantly enhanced the plant growth, nodules and reduction in the nematode population (Pandy, 2005; Dubey et al., 2006) and *Fusarium* wilt (Afrozi *et al.*, 2008).

The results obtained on the integrated management of chickpea are in conformity with findings of Pandey (2005), Dubey (2006), Nikam (2007) and Asfrozi *et al.* (2008).

Therefore, the results of the present findings strongly suggest that oilseed cakes, antagonistic fungi (*Trichoderma spp.*) combination may be beneficial for effective control of diseases 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 organic amendments (oil seed cakes) and antagonistic fungi in mixture rather than using them alone in a disease complex situation which present special problems for the disease management and this will be eco-friendly.