REVIEW OF LITERATURE

In natural or crop ecosystem, plants are exposed to various kinds of pathogens, both animate and inanimate and three kinds of relationships are observed viz., neutralism, synergism and antagonism. Among them, synergistic effects of root knot nematodes with other soilborne pathogens play a crucial role in devastating the cultivated crops. Nematodes besides causing direct damage to the plant, are also notorious for causing disease complexes by involving fungi, bacteria, viruses, mycoplasma like organisms (MLOs) and other nematodes (Siddiqui et al., 2005). Nematodes are often involved in the formation of disease complexes in which they act as the primary pathogen and cause wounds in host plants to make easy entry for some fungus/bacterium as the secondary pathogen(s) resulting in injury to the host plants. Keeping in mind the importance of severe damage to the crops during cultivation due to disease complex, various researchers have been studying the effect of the interactions of nematodes with other soilborne plant pathogens and their management. Over the past 20 years worldwide, a large number of research papers have been published on the above aspects and it is difficult to include all the published papers in the present study. Hence, research on interaction between the most devastating nematodes effects with soilborne pathogens and their control measures have been conducted, are given under the following headings.

2.1 Survey on the distribution, prevalence and intensity of nematode-fungal disease complex

2.2 Isolation, pathogenicity and identification of nematode and fungal cultures

2.3 Economic Threshold Level (ETL) of nematode and virulent fungi

2.4 Study the interaction effects of nematode and virulent fungi on disease severity

2.5 Management of nematode-fungal disease complex
2.1 Survey on the distribution, prevalence and intensity of nematode-fungal disease complex

Survey of plant diseases provides basic information on outbreak of a disease, its severity and amount of damage caused by pathogen(s) on a particular crop (Qadri et al., 2005). A number of systematic surveys were conducted in various districts of Karnataka to observe the pattern of distribution, prevalence and intensity of the disease complex caused by the association of nematodes and soilborne pathogens in certain agricultural and horticultural crops. But the literature on surveys of disease complexes on crop plants involving plant parasitic nematodes and soilborne fungal pathogens under field conditions is very much limited (Sharma and McDonald, 1990).

During the survey of root disease of chickpea (Cicer arietinum L.) in Allahabad region, Pandey and Singh (1990) revealed the association of M. incognita, F. oxysporum and R. bataticola. With regard to severity, 83% villages showed less than 25% disease intensity and the remaining villages recorded 26 - 50%. Sharma and McDonald (1990) conducted a survey in many countries and reported that the species of Meloidogyne, Hoplolaimus, Pratylenchus and Xiphinema on cereals were strongly suspected in increasing the severity of diseases caused by fungal pathogens. Tiwagi and Alam (1992) observed an increasing trend in the damage caused by M. incognita and F. oxysporum f. sp. ciceri as a disease complex of chickpea at a younger stage and a decrease in plant growth.

Rao and Krishnappa (1995) assessed the distribution and severity of disease complex in chickpea in 15 districts of Karnataka and reported that M. incognita - Fusarium wilt disease complex was observed in most of the districts except in Mandya and Kolar. They also observed that the distribution of population levels of the two organisms (M. incognita and F. oxysporum) in different districts is not consistent due to various ecological factors and their interaction effects.

Rao and Krishnappa (1996 a) reported that occurrence of Meloidogyne and Fusarium associated with chickpea in Karnataka was influenced by various ecological factors, viz., agro-climatic zones, soil type, types of farming, growth stage of the crop cultivars and previous crop grown. The population of
Meloidogyne was maximum in the eastern dry zone having red soil under irrigated conditions, during flowering stage, in cultivars local and Annigeri-1 with ragi and sunflower as the previous crops. On the other hand minimum population was observed in the North eastern dry zone, under rainfed conditions during vegetative stage and no population in clay, sandy loam and murrum soils, in cultivars Vishwas, Chaffa and Bhima with paddy, wheat, soyabean, castor, potato and cotton as the previous crops. Similarly, maximum population of Fusarium was observed in North eastern dry zone, having black soil under rainfed conditions, during flowering stage in cultivars Chaffa and Annigeri-1 with red gram, cucumber and chick pea as the previous crops, whereas minimum population was recorded in eastern dry zone, under rainfed conditions, during vegetative stage in cultivar Bhima, and no population in clay and murrum soils with paddy, wheat, castor and cotton as the previous crops.

Rao and Krishnappa (1996 c) studied the quantitative relationship between environmental variables and wilt disease complex through simple correlation and multiple linear regression analysis based on field data in Karnataka. Maximum and minimum air temperatures, soil pH, potassium and nitrogen had a positive correlation with wilt disease complex while relative humidity, phosphorus and organic carbon content showed a negative correlation. The joint influence of biotic and abiotic factors was accounted by 82 % variation in the wilt disease complex, while abiotic factors alone explained the variability by 67 %. Maximum air temperature, soil pH and carbon content were found to be important variables in influencing the wilt disease complex.

A preliminary survey was carried out on the occurrence of Meloidogyne - Fusarium disease complex associated with chickpea in Karnataka. The results revealed co-infection of Meloidogyne spp. and Fusarium spp. in 15 districts of Karnataka. In addition to Meloidogyne spp. other plant parasitic nematodes viz., Heterodera, Helicotylenchus, Hoplolaimus, Pratylenchus, and Applenchoideis were also found associated with wilted plants and dominated over other fungi. The other fungi detected from wilted chickpea were Rhizoctonia solani, R. bataticola, Verticillium sp. and Sclerotium rolfsii. The frequency of occurrence of nematode and fungal pathogens was found very high in the soil as compared to the roots (Rao and Krishnappa, 1997).
Survey conducted by Haseeb and Shukla (2005) on disease complex in pigeonpea [Cajanus cajan (L.) Millsp.] in districts of Uttar Pradesh like Agra, Aligarh, Bulandshahr, Hathras and Mathura indicated that wilt disease complex caused by Heterodera cajani, M. incognita and F. udum, is the most severe constraint during its cultivation. Severity of wilting was observed directly proportional to the population of H. cajani and M. incognita. Infection of F. udum was also found in the roots of stunted chlorotic and wilted plants. The highest population of M. incognita larvae was observed at Hathras followed by Aligarh, Agra, Mathura and Bulandshahr. Infection of F. udum in roots was observed in all the districts.

A survey was conducted in five major groundnut (Arachis hypogaea L.) growing districts of Punjab State and severe infection of nematodes like Tylenchorhynchus vulgaris, Aphelenchus avenae and Siddqia citri on the crop was noticed. Other important nematodes were recorded as Pratylenchus thronei, P. zeae, Hemicyclophora punensis, Macroposthonia xenoplax and Xiphinema insigne. Further, soilborne pathogens such as F. solani, Neocosmopora vasinfecta and Rhizoctonia spp. were found to be associated in these areas (Sakhuja and Sethi, 1985).

In mulberry, infection of nematodes is considered to be a serious threat for the sericulture industry. About 42 species belonging to 24 genera were reported from almost all countries, where sericulture is practiced. In India, root knot disease caused by M. incognita was reported for the first time by Swamy and Govindu (1965) from Mysore, Karnataka, in sandy soil under irrigated farming. All the five major species of Meloidogyne viz., M. incognita, M. javanica, M. hapla, M. arenaria and M. mali have been reported to cause root knot disease (Toida, 1984). However, M. incognita and M. javanica have been reported on mulberry from India (Narayanan et al., 1966; Nirula and Kumar, 1966). Besides Meloidogyne species, occurrence of various other nematodes such as Xiphinema basiri, Paratylenchus clanchistus, Longidorus martini and Rotylenchulus reniform have also been reported (Adylov, 1979; Thangavelu et al., 1989). The disease reduces about 12 % leaf production and leaf quality, especially leaf protein, which is the most essential nutrient to the silkworm for the synthesis of silk. The leaf protein is converted into silk protein sericin and fibroin through the biological process. Therefore, the leaf quality is one of the main criteria to obtain good quality of cocoons and silk (Govindaiah et al.,
1991; Paul et al., 1995; Sharma and Gupta, 2005 b). The study on prevalence of *M. incognita* with reference to different farming systems and soil types in Karnataka indicated that the disease incidence and intensity were very high in red sandy soil followed by red loamy soil; while in black cotton soil, the incidence was found to be very poor. Considering different farming systems, high incidence and intensity were found in irrigated gardens while poor or negligible incidence and intensity were noticed in rainfed gardens (Mathur et al., 1969; Thangavelu et al., 1989; Teotia et al., 1992; Govindaiah et al., 1994; Philip et al., 1997; Sharma and Sarkar, 1998). The dynamics of established nematode population (*M. incognita*) at different depths of rhizosphere soil viz., 0 - 15; 15 - 30; 30 - 45; 45 - 60; 60 - 75 and 75 - 90 cm revealed that there is a significant increase in nematode population density upto the depth of 15 - 30 cm, however, it then gradually decreases with the increase in depth upto 75 - 90 cm. Further, significantly higher population is recorded around the root zone of the plant followed by inter-stump and inter-ridge regions (Sharma, 1998 a). As far as the races of *M. incognita* affecting mulberry, out of the 4 races it is reported that the root knot nematode *M. incognita* affecting mulberry in Karnataka is categorized as race-2 (Sharma and Sarkar, 1998) and as race-3, which is prevalent in Tamil Nadu, irrespective of the crops grown (Kumar and Rajendran, 1995).

Root rot disease also poses a major problem for mulberry cultivation and is becoming more alarming due to its epidemic nature and potentiality to completely kill the plant. The disease appears in all types of soil and climate throughout the year (Sharma and Gupta, 2005 b). The first report of root rot on mulberry caused by *Macrophomina phaseolina* (Tassi) Goid, was reported by Butler and Bisby (1960) in India. Survey was taken in eight districts of Tamil Nadu covering 812 mulberry gardens and per cent root rot damage was noticed from 0.6 to 33.3 in different districts. Highest level of 33.3 % damage was recorded in Gundadam village of Erode district. In Coimbatore district, 14.3 % root rot damage was reported (Gangwar and Thangavelu, 1991). Philip et al. (1995; 1997) have conducted a survey on existence of root rot in all Southern States of India. The percentage of infection in individual garden varied from 3 - 30 that reduces more than 14 % of leaf production. Large scale mortality of plants due to disease was observed in Kerala and Tamil Nadu States. *F. solani* and *F. oxysporum* are associated as causal pathogens at all the places. Among
them, the former is more virulent and causes maximum damage to mulberry plantation. There are some villages in Wayanad and Thiruvananthapuram districts of Kerala State where entire gardens were found to be destroyed by the disease. Survey undertaken by Rajendran (1996) in Thiruchirapalli, Coimbatore and Thanjavur districts reported that the mean level of root rot (*M. phaseolina*) incidence was noticed as 30, 26 and 21 %, respectively for the above districts. Sridhar (2000) made root rot survey in Coimbatore and Erode districts of Tamil Nadu. He documented that *F. semitectum*, *F. moniliformae* and *M. phaseolina* were isolated from root rot infected samples. Per cent disease of root rot was recorded ranging from 5.6 to 83.3 % and mean of root rot incidence was registered 16.1 % in Coimbatore as against 45.32 per cent in Erode. Recent survey carried out by Chandramohan (2005) in Coimbatore and Udumalpet districts. *M. phaseolina* was predominant in Coimbatore area with 85.7 % of root rot, while mixed infection of *M. phaseolina* and *Fusarium* spp. was recorded as 90.0 %, causing root rot in Udumalpet area.

A preliminary survey was undertaken in seven major sericultural areas of Karnataka to identify the soilborne fungal pathogen(s) associated with root knot disease to cause disease complex in mulberry. Three fungi were isolated from affected samples and only *F. solani* was found to be pathogenic to mulberry causing disease complex alongwith nematode. Maximum root knot disease complex was observed in Mysore district with higher nematode (*M. incognita*) and *F. solani* populations (Naik et al., 2004).

### 2.2 Isolation, pathogenicity and identification of nematode and fungal cultures

Diagnosis of disease plays an important role in disease management. Accurate diagnosis of disease requires isolation and identification of the pathogen(s) and complete fulfillment of Koch's postulates. Further, it might probably be accepted that an association of more than one pathogen also causes the disease, which may lead to confusion in identification of the actual pathogen involved. For this, the collection of diseased samples, isolation, identification and pathogenicity of the isolated microbe(s) are very much essential (Sharma, 2005).

The nematode is identified mainly on the basis of morphological features, host range and biochemical characters. The morphological characters include
stylet, second stage larvae, male and female (Jairajpuri, 1986). The identification of root knot nematode especially *Meloidogyne* species *viz.*, *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* which are reported on the mulberry can be done on the basis of perineal patterns of female and North Carolina differential host test (Hartman and Sasser, 1985). Further, the perineal pattern of (*Meloidogyne* species) female is also being the most predominant character for routine identification of the species nowadays. Fingerprint like pattern formed by cuticular striae surrounding the vulva and anus of the mature *Meloidogyne* female is called perineal patterns (Eisenback, 1985).

There are various types of tests that may be used for identification of fungal pathogens. First, it must be made sure by the symptoms appearing on the host plants whether these are developed by fungal infection. Later, these can also be confirmed by solid puffy growth on agar medium through culturing. Unknown fungi can be identified on the basis of their morphological and cultural characters. Morphological characters consists of vegetative and reproductive structures, which include the shape, size, colour and arrangements or attachment of spores on the sporophores or the fruiting bodies (Agrios, 2000; Sharma, 2005).

The genus *Botryodiplodia* belongs to the class Deuteromycetes (Sub class: Coelomycetes), order Sphaeropsidales and family Sphaeropsidaceae, is the asexual spore stage of members of the divisions of Mycota, in which conidia formed within pycnidia which may remain entirely closed, open by a pore or a long slit, or finally become disc-like. Generally, *B. theobromae* have pycnidia black, 200 µm in diameter, more or less hairy. Conidiophores are hyaline, 50 µm long, elongate, slightly brownish, one septate, 25 - 34 x 12 - 15 µm in size (Gillman, 1966). *B. theobromae* is seen on a great variety of dead plants, including those which also grow in temperate regions, and is commonly found in the tropics but cannot be observed in cooler zones (Pirozynski, 1968).

The genus *Fusarium* is fusiform, septate/non-septate spores, borne on a stroma. The stroma or sporodochia develop on artificial media rarely. The presence of macroconidia with a foot cell is the most definite character (Booth, 1971). Nash and Snyder (1962) devised excellent selective media for the direct isolation of *Fusarium* spp. from freshly collected plant tissue and soils. Ellanska
(1969) studied the effect of 26 carbon sources on growth and sporulation of *F. gibbosum*, *F. lateritium*, *F. sambucium* and *F. semitectum*. Maltose and inulin were best for growth and sporulation of all the species. Osman *et al.* (1992) investigated various culture conditions of *Fusarium* spp. and stated that the best culture media were Czapek's Dox Agar and Potato Dextrose Agar. The *Fusarium* belongs to the class Deuteromycetes, sub class: Hypomycetes, order Moniliales, family Tuberculariaceae and Division Mycota. Members of the genus typically produced three types of conidia that are termed as macroconidia, microconidia and chlamydospores. The macroconidia are long, multiseptate, terminal, spindle to sickle shaped, born on nonphilides inside the sporodochium. The microconidia are small, usually one celled, spindle to kidney shaped and born on long phialides. The chlamydospores are formed from the hypha, often from the cells of the macroconidium in old cultures. These spores are thick walled, oval to spherical in shape, yellowish to golden in colour and intercalary occur singly or in chains of two (Butler, 1981; Nelson *et al.*, 1983; Burgess *et al.*, 1994; Singh, 1998).

Perusal of literature reveals that the pathogenicity of root knot nematode, *Meloidogyne* spp. was conducted by various workers through artificial inoculation of second stage juvenile (*J_2*) larvae in soil and proved the pathogenicity on various crops by judging the reduction of plant growth parameters and formation of galls/knots on root system (Bhagawati and Phukan, 1991; Kalita and Phukan, 1993; Eapen, 1994; Rao and Krishanappa, 1995 a). Similarly, various workers have tested the pathogenicity of species of *Botryodiplodia* and *Fusarium* by artificial inoculation in the soil and found that the method was effective to induce the disease in certain crops including mulberry (Sivamani and Gnanamanickam, 1988; Philip *et al.*, 1995; Sharma *et al.*, 1996; Gupta *et al.*, 1997; Shukla *et al.*, 2001). Prasad *et al.* (1999) adopted the hydroponic method by keeping the seedlings in spore suspension of fungal isolates for rapid induction of the fusarial wilt in groundnut caused by *F. oxysporum*. Similarly, Latin and Snell (1986) observed that dipping of seedlings in spore suspension was found to be effective to induce the *Fusarium* wilt in muskmelon. Lima and Aguillar (1978) also proved the pathogenicity of *F. solani* (groundnut wilt) by dipping the seeds and seedlings in a spore suspension.
Several reports are available with regard to the reduction of the plant growth in various crops with increasing population density of nematode and soilborne pathogens. Measurable level of damage occurs only when the population density of the nematodes exceeds certain limits (Seinhorst, 1965). The magnitude of crop losses depends upon various factors such as the population density of the nematodes present in soil, susceptibility of the crop and environmental conditions (Hanounik et al., 1975). Mahapatra et al. (1999) conducted the pathogenicity of *M. incognita* and *F. oxysporum* on black gram by artificial inoculation of nematode and fungus. The results revealed that significant reduction of growth parameters was recorded in black gram (cv. T-9) at an initial inoculum level of 1000 second stage juvenile (J2) of *M. incognita* per kg of soil. Similarly, the pathogenic effect of *F. oxysporum* was recorded at the initial inoculum level of 4 g mycelial mat per/kg soil. Pathogenic effect of root knot nematode (*M. incognita*) on banana, *Musa* spp. was studied in greenhouse conditions. Results revealed that significant reduction occurred in plant growth parameters viz., plant height, pseudo stem girth, number of leaves, leaf area, root length and weight in plants inoculated with 1000 and 10000 second stage juvenile (J2)/kg soil. The highest gall indices of 4.6 and 5.0 were recorded at inoculum levels of 1000 and 10000, respectively (Jonathan and Rajendran, 2000).

It has been reported that the root knot nematode, *M. incognita* is pathogenic to more than 2000 host plants belonging to crops like agricultural, horticultural, vegetable, plantation, ornamental, cash, etc. (Sasser, 1980; Sasser and Carter, 1985; Swarup and Dasgupta, 1986; Rangaswami and Mahadevan, 1999). Several workers have reported that *M. incognita* causing root knot disease is highly pathogenic to mulberry (Saha et al., 1983; Govindaiah et al., 1991; Sharma et al., 2003; Naik et al., 2005). Similarly, the fungus, *B. theobromae* causes several diseases such as stem canker, die-back, death of stump, root rot, etc. and poses a serious threat to various crops including mulberry during cultivation (Luke and Paul, 1982; Radhakrishnan et al., 1995; Gupta et al., 1997; Sukumar et al., 2000; Channamma 2001; Chowdary et al., 2003). Further, species of *Fusarium* are widespread in nature, common in soil, aerial and subterranean plant parts. They cause many diseases such as necrotic leaf spots, blights, wilts and root rots in certain crops including mulberry (Gangwar and Thangavelu, 1991; Philip et al., 1995; 1997; Sridhar,
Apart from mineral nutrition, the depletion of starch reserves caused by overbearing or over pruning or in tea, by over plucking has been observed to predispose the plantation crops to damage from certain weak parasites such as the root and stem infecting fungus *B. theobromae* (Butler and Jones, 1961).

### 2.3 Economic Threshold Level (ETL) of nematode and virulent fungi

The term "threshold" indicates the point at which some effect begins to loss of crops (Ferris, 1987). Bruehl (1987) defined that plants tolerate certain population of pathogen with no detectable economic loss. At some threshold density, the losses begin and they are proportional to increased number of propagules. At some point, the host no longer suffers in proportion to the increase in number of pathogens and it is essential to know the threshold level of inoculum as a measure of biological stress imposed by pathogen. Generally, Integrated Disease Management (IDM) should be adopted when the infestation has crossed the Economic Threshold Level of pathogens. Various workers have determined the ETL of nematodes and soilborne fungal pathogens for adopting the IDM strategies against soilborne diseases in various crops.

Gupta *et al.* (1995) studied the effect of initial inoculum levels of *Meloidogyne* spp. on some cucurbitaceous crops. It was observed that 100 larvae of *M. javanica* per pot, as initial inoculum in bitter gourd (*Momordica charantia*) and 1000 larvae in smooth gourd (*Luffa cylindrica*), ridge gourd (*Luffa acutangula*) and squash melon (*Benincasa hispida*), significantly reduced the growth parameters. Rao and Krishnappa (1995 a) reported that initial inoculum of 1000 larvae of *M. incognita* also significantly reduced the growth parameters. Galling and nematode reproduction was directly related to the initial inoculum level. An inoculum level of 2 larvae per gram soil was found as optimum damaging threshold level in chickpea (cultivar Annegiri-1) under green house conditions. Increase in the level of larval inoculum resulted in proportional decrease in plant growth and an increase in root knot disease in chickpea.

Determination of threshold levels of *M. incognita* and *Rotylenchulus reniformis* on black gram revealed that *M. incognita* was more devastating, as
it caused 47.87 % reduction in dry shoot weight in comparison to 41.31 %
reduction caused by *R. reniformis* at the inoculum level of 4000 larvae/plant.
However, significant reduction in growth parameters over control were found
when 1000 or more infective stages of either *M. incognita* or *R. reniformis* per
plant per kg soil were inoculated (Fazal, *et al*., 1996). Rao and Krishnappa
(1997) observed that the percentage of wilted plants due to *F. oxysporum* f.
sp. *ciceri* varied in proportion to the quantum of inoculum added to the soil.
Five per cent inoculum (w/w) containing 15 x 10^2 CFU/g soil was found as
optimum threshold level resulting in wilt incidence to the extent of 53 % in
chickpea (cv. Annegiri-1).

An attempt was made by Reddy *et al.* (2001) to know the minimum
inoculum levels at which *M. incognita* and *P. aphanidermatum* pathogens affect
seedling emergence and damping off in tomato. Results revealed that the
presence of nematode significantly increased pre-emergence damping off,
incidence increased from 37.6 to 43.8 % with increased inoculum levels of
nematode (@ 1000 J2/500 g soil) and fungus (@ 1000 oospores/g soil). The
presence of nematode along with fungus increased pre-emergence damping off,
from 42.0 - 50.0 % at the same inoculum levels of both.

In mulberry, the effect of inoculum levels of second stage juveniles (J2)
of *M. incognita* is significant in reducing the plant growth, leaf yield and
moisture content. The shoot and root weight showed a progressive decrease
with increasing inoculum levels. It is proved that the population density of
10000 J2/cutting is pathogenic to mulberry and affects the plant growth
reported reduction in growth of seedling and grafting of mulberry when
inoculated with more than 2500 second stage juveniles of *M. incognita*. A year
after inoculation, 30 - 60 % of those trees infected by nematode were dead.
Thangavelu *et al.* (1989) recorded the symptoms produced in *Morus alba* L.
due to infection by the dagger nematode *X. basiri*. The infested trees exhibited
varying degrees of chlorosis followed by dropping of leaves. The affected trees
wilting and dried in the case of severe infection. An inoculum density of 1000 J2
of *M. incognita*/plant caused significant reduction in plant growth and leaf yield
(52.58 %). In the case of *X. basiri*, the nematode inoculum density 10000
nematodes was recorded as pathogenic level which affects the plant growth
severely (Vadivelu, 1996). Govindaiah *et al.* (1991) and Sharma (1999) have
reported that the Economic Threshold Level of *M. incognita* on mulberry was 150 larvae/250 cc soil (1000 larvae/plant). Naik *et al.* (2004) reported that the inoculum load of *F. solani* $1.8 \times 10^5$ spores/g soil and 165 *M. incognita*/250 cc soil was considered as the damaging threshold level in mulberry against root knot disease complex.

Much attention has not been paid on ETL of soilborne pathogens (Naik, 2003). Rao and Rao (1963; 1966) showed that the tempo of disease development on a susceptible variety of cotton against *F. oxysporum* f. sp. *vasinfectum* increased with a rise in the inoculum level from 0.5 to 10%. Netzer (1976) estimated 200-800 propagules of *F. oxysporum* f. sp. *niveum* per gram of soil in a field cropped with watermelon and he also noticed that saturation point for infection reached with a population of 300 propagules/g in the infested soil. The ETL is an important epidemic tool particularly for soilborne pathogens and is helpful for evaluation of several management strategies such as cropping pattern, biocontrol, agronomic manipulation, soil solarization, etc. (Naik *et al.*, 1996). Thus, it can be used as a decision-making tool for management of soilborne pathogens. The ETL has an underlying economic basis and is a function of relationship between cost and stress reduction (Naik, 2003).

In mulberry, the inoculum density of 6.25% is considered as the damaging threshold level for *B. theobromae* having a spore load of $2.6 \times 10^6$/g soil against root rot disease (Naik *et al.*, 2005).

### 2.4.1 Study the interaction effects of nematode and virulent fungi on disease severity

Fawcett (1931) recognized that “nature does not work with pure cultures” and many plant diseases are influenced by an array of associated organisms. Plant parasitic nematodes have often played a major role in disease interactions. Interactions of nematodes with fungi, bacteria and viruses are well established (Powell, 1971; Khan, 1993 a; Siddiqui *et al.*, 2005). Powell (1979) and Wallace (1983) reported that all plant parasitic nematodes caused wound in roots while feeding and these wounds are sites through which other microbes can enter. Certain nematodes predispose the plants susceptible to other soilborne pathogens and such phenomenon are considered as synergistic interactions, which results in more extensive damage to crop than the sum of
the effects, when both are acting independently. Many attempts have been made to review the work done on nematode interactions with other microbes (Gupta, 1987; Chand, 1987; Bajaj, 1987; Khan, 1993; Khan and Khan, 1995; Siddiqui et al., 2005).

Synergistic interactions between nematodes and fungi have been recognized as early as 1892, when Atkinson reported that infection of cotton by the root knot nematode increased the severity of *Fusarium* wilt (Atkinson, 1892). Later it was reported that various other fungi viz., *F. oxysporum*, *F. solani*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Pythium aphanidermatum*, *Phytophthora infestans*, *Verticillium albo-atrum*, etc. have also been associated with root knot nematode causing the disease complex in several agricultural and horticultural crops, which resulted in increasing the disease severity and causing considerable damage (Khan, 1993). Of several nematodes, root knot especially the species of *Meloidogyne* are thoroughly studied and commonly found to involve in synergistic interactions with wilt or root rot causing fungi (Evans and Haydock, 1993; Francl and Wheeler, 1993; Siddiqui et al., 2005).

In chickpea (*Cicer arietinum* L.), Mani and Sethi (1987) studied the role of root knot nematode (*M. incognita*) in combination with *F. oxysporum* f. sp. *ciceri* and *F. solani* on wilt disease complex and observed that the combined inoculum of nematode alongwith both the fungal pathogens increased the severity of disease rapidly. They also observed that root rot index was very high when the nematode preceded to *F. solani*. The nematode development and multiplication was adversely affected by the fungal pathogens. Similar observations were also made by Rao and Krishnappa (1995 b) and Patel et al. (2000) on interaction between *M. incognita* and wilt inducing fungus *F. oxysporum* f. sp. *ciceri* on chickpea. An attempt was made by Rao and Krishnappa (1996 b) to study the interaction of *F. oxysporum* f. sp. *ciceri* with *M. incognita* on chickpea cultivar Annegiri in two-soil types viz., alfisol and vertisol. They reported that inoculation of fungus alongwith nematode, for seven days before or after, resulted in significant reduction of fresh and dry weight of shoot and root as well as increase in the wilt incidence compared to plant inoculated with fungus alone. Nematode multiplication was high in alfisol soil while fungal infection in the vertisol soil resulting in high root knot in the former and more wilt incidence in the latter. Rahaman et al. (2000) studied the
effect of root knot (*M. javanica*), black root rot (*F. solani*), dry root rot (*R. bataticola*) and wilt (*F. oxysporum* f. sp. *ciceri*) pathogens alone and in different combinations, on four chickpea genotypes (C - 104, BG - 212, JG - 62 and WR - 315) under green house conditions. The interaction between *F. oxysporum* f. sp. *ciceri* and *M. javanica* was synergistic on wilt susceptible genotypes (C-104 and JG -62). The reaction of wilt resistant genotypes (BG - 212 and WR - 315) was not altered by the presence of any of the three pathogens. Presence of *F. solani* had a mild but significant suppressive effect on nematode Induced root galling in genotype JG - 62. The *F. oxysporum* f. sp. *ciceri* increased the dry and black root rots on JG - 62.

In banana (*Musa paradisiaca* L.), Pathak et al. (1999) studied the interaction among the *M. incognita*, *F. oxysporum* f. sp. *cubense* and *Pseudomonas solanacearum* on plant growth parameters and disease severity. Results revealed that maximum banana wilt was increased when nematode was inoculated 10 days prior to fungus and bacterium. The presence of all the three pathogens together had a more deleterious effect on the banana plants, than when one or two pathogens were inoculated, indicating a positive interaction between all the three pathogens.

In tomato (*Lycopersicon esculentum* Mill.), Chahal and Chabra (1984) made an attempt to study the interaction of *M. incognita* and *Rhizoctonia solani* on disease severity. Results indicated that *M. incognita* and *R. solani* alone and in combination significantly reduced the shoot length, shoot weight and root weight as compared to uninoculated control. The synergistic effect of simultaneous inoculation was apparent from the significant reduction of shoot weight in comparison to either of the pathogens alone. Bhagawati and Goswami (2000) studied the interaction between *M. incognita* (N) and *F. oxysporum* f. sp. *lycopersici* (F) and observed that though both the pathogens alone significantly reduced the plant vigour compared to uninoculated check, synergistic effect was recorded in the treatments with either simultaneous inoculation of the pathogens (N+F) or where nematode inoculation preceded 10 days to fungi (N< F10). The possible explanation for comparatively greater damage in these two treatments as compared to F < N10, may be attributed to the prior invasion of nematode into the roots thereby making the host more favourable for fungal infection by offering metabolically rich substrate. Interaction effects of *M. incognita* and *Phytophthora aphanidermatum* on root knot
The disease complex of tomato and damping off incidence in greenhouse was made by Reddy and Rao (2003). Results revealed that per cent reduction in shoot length, dry weight of shoot and root was maximum in nematode inoculation, one week prior to fungus and was minimum in the fungus treatment by itself. The root knot index (5.0) was maximum in nematode alone and minimum (3.3) in fungus inoculation, one week prior to nematode. The pre-emergence and total damping off was maximum with nematode inoculation, one week prior to fungus as against minimum when fungus was inoculated, one week prior to the nematode. Nematode and fungus independently caused damage to tomato. However, the effect on growth and damping off incidence aggravated when both the pathogens were present together. Saini et al. (2005 a) studied the interaction between F. oxysporum f. sp. lycopersici (Fol) and M. incognita (Mi) in wilt resistant and susceptible tomato genotypes. Results revealed that there was a significant reduction in the plant growth when Fol and Mi were inoculated simultaneously. However, the effect was more pronounced when Mi was inoculated 7 days prior to Fol inoculation. All test genotypes exhibited increased wilt symptoms (yellowing, wilting and vascular browning) in simultaneous and prior inoculation of Mi than that of Fol alone. The wilt symptoms were more pronounced in susceptible cultivar (Hisar Arun) than in resistant genotype (NF - 31). The interaction between Fol and Mi had a synergistic effect leading to reduced plant growth and enhanced wilt incidence in tomato genotypes.

In cowpea (Vigna sinensis Endl.), Singh and Goswami (2001) studied interactive effects between M. incognita and F. oxysporum in cultivars like Pusa Komal (susceptible to both nematode and fungus) and C - 152 (resistant to nematode and susceptible to fungus). They reported that M. incognita enhanced wilting in cultivar Pusa Komal when inoculated in combination with F. oxysporum (simultaneously), while nematode inoculation preceded by fungal inoculation showed maximum enhancement of the wilt incidence (synergistic). Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression. Both, the pathogens interacting simultaneously and nematode infecting prior to fungus, affected nodulation.

In black gram (Vigna mungo L. Hepper), Mahapatra and Swain (1999) assessed the combined effect of M. incognita and F. oxysporum on disease severity in naturally infested fields for two consecutive years. Results revealed that the total plant stand at the time of harvest, irrespective of the years of
study, decreased significantly (16.4 %) in untreated, over treated ones. On the other hand, both root knot and root rot indices significantly enhanced in untreated plots, with a corresponding increase of 89.9 and 120.4 over the treated plots. The grain yield was significantly less in both the years under untreated conditions.

In green gram (*Vigna radiata* L.), Sharma *et al.* (2005) studied the effect of *M. incognita* (2000 J2/kg of soil) and *F. oxysporum* (2 ×10<sup>8</sup> spores/kg of soil) alone and in combination on the multiplication of the nematode, wilt disease development and plant growth parameters. Results indicated that highest reduction in all growth and yield parameters was observed in plants inoculated with nematode and fungus simultaneously, followed by nematode 7 days prior + fungus post, fungus 7 days prior + nematode post and nematode and fungus alone, respectively. Root colonization by the fungus was significantly increased in the presence of nematode, whereas the reproduction factor of the nematode was found significantly decreased in the presence of fungus.

Zaidi and Tiyagi (1989) studied the interaction between root knot nematode, *M. incognita* and fungus, *F. solani* on chilli (*Capsicum annuum* L.). The results revealed that growth of the plants reduced significantly when inoculated with both the pathogens after sequential and simultaneous inoculations. The reduction was maximum in simultaneous inoculation. The rate of multiplication of *M. incognita* was adversely affected when fungus was inoculated earlier than nematode.

In mulberry, it has been observed that root knot nematodes predispose the plants, making them vulnerable to various other soilborne pathogens and viruses causing the disease complex. In Thailand, Somkuan *et al.* (1989) and Toida and Keereewan (1991) reported that nematodes like *Ecphyadophora quadralata* and *Hoplolaimus seinhorsti* are found to be associated with root rot pathogens. In Japan, Yagita and Kamura (1992) reported that mulberry ring spot virus (MRV) is transmitted through the nematode (*Longidorus martini*), and observed that nematode acted as a vector to spread the viral disease. In India, the interaction between *M. incognita* with *F. solani/Rhizoctonia bataticola* cause disease complex in mulberry and *M. incognita* provides a good site for easy entry of developing hyphae of these pathogens into the roots. It was also found that simultaneous inoculation of *M. incognita* and soilborne fungal
pathogens reduced the plant growth parameters and increased the disease severity rapidly (Bhagyarathy et al., 2000; Sukumar et al., 2000; Naik et al., 2004).

2.5 Management of nematode-fungal disease complex

Based on changing scenarios and archaic scientific knowledge on one hand and the economic needs, ecological repercussions and technical compulsions on the other hand management of nematode-fungal disease have had a gradual shift from chemical to non-chemical methods. Other than this, the prohibitive costs of chemicals and their adverse ecological impacts are major compulsions for diversion of research priorities from chemical to ecofriendly (biological or cultural) integrated management. This aspect involves the various methods adopted to deal with management of different plant pathogens involved in complex diseases.

2.5.1 Efficacy of botanicals, oil cakes, bioagents and chemicals

Efficacy of botanicals and oil cakes:

Some of the wild vegetations are known for the active principle of having medicinal properties (Bambode and Shukla, 1973). Certain plants containing products such as alkaloids, tannins, quinines, coumarins, phenolic compounds, phytoalexins, ipomeamarone in the extracts and exudates, are known for antifungal activities (Fawcett and Spencer, 1966). The active toxic compounds like diallyl disulphide, diallyl trisulphide and allicin have been reported in garlic (De Groot, 1972; Arya et al., 1995; Gautam and Chauhan, 2003; Gupta and Shirkot, 2004) and lawsone in mehendi (Sakarkar et al., 2004). Hedge and Babu (2004) reported that niger oil cake produces compounds like oleic acid, linoleic acid, ferric oxide, calcium oxide, etc. while neem oil cake produced compounds like nimbidin, thionemone and phenolics (Khan et al., 1974; Alam, 1993).

Gupta and Sharma (1991) reported that garlic bulb extracts exhibited strong ovicidal and larvicidal action against M. incognita as compared to extracts of garlic leaves. Further, they also stated that dry powder of garlic bulbs has also showed nematicidal activity like bulb extracts. Arya et al. (1995) reported that allicin a major constituent of garlic containing sulphur, has strong toxic properties against various soilborne pathogenic fungi. Gupta and Shirkot
(2004) studied the nematicidal activity of allicin, an active principle of garlic and its effect on other soil micro-organisms in tomato. A juvenile mortality of 87 - 100 per cent in allicin at 2.5 - 5.0 ppm was recorded within 72 hrs under in vitro conditions. In the soil, there was complete mortality as low as 10 ppm concentration. Besides, the allicin also resulted in drastic reduction in harmful fungal and bacterial populations in soil. Various workers have tested the efficacy of garlic and mehendi (L. inermis) against nematode and various soilborne fungal pathogens and were found to be effective (Arya et al., 1995; Niza, 1995; Chandrasekaran et al., 1998; Sindhan et al., 1999; Datar, 1999; Naik et al., 2004; 2005; Iyer et al., 2004; Mohanty and Sahoo, 2005).

Dried powder of some botanicals was studied by Pandey (2002) and the results showed that the application of dried plant materials of specific plants is successful in reducing the nematode population and improving the plant growth characters. The extent of nematode reduction depends on qualitative and quantitative application of specific plant materials. Use of natural materials like dried parts of specific plants is more beneficial than the use of chemicals. The natural materials are generally non-toxic to plants and animals as compared to chemicals.

Marigold is a popular ornamental plant grown all over the world including India. The antagonistic effect of this plant was first realized by Tyler (1938) against root knot nematode. Steiner (1941) also marked the same effect of Tagetes spp. to Meloidogyne. Zechmeister and Sease (1947) first reported the active nematicidal principle involved in the root exudates of Tagetes (L-terthienyl derivative). Based on nematicidal property of Tagetes, subsequently this crop was included in the cropping system for effective management of phytonematodes, either as a sole crop preceding cucumber (Siyanand et al., 1995); brinjal (Ray et al., 2000) or as an inter-crop with brinjal (Jain et al., 1990; Dhangar et al., 1995) and mulberry (Govindaiah et al., 1991 a). Saini et al. (2005) reported that neem leaf powder (as root coating) provides less expensive, environmentally safe and easy to adopt method for transplanted crops like tomato for the management of Fusarium wilt in the presence of M. incognita.

Mehta, et al. (1995) studied the different oil cakes of groundnut, neem, mustard, cotton and oat meal on reduction of disease complex caused by R. bataticola and root knot nematode (M. javanica) in tomato. Mustard oil cake
amendment resulted in maximum plant weight compared to the rest of the oil cakes. The number of galls was least in mustard oil cake amendment soil. *R. bataticola* disease was decreased in all the amended soils except in simultaneous inoculation of nematode and fungus in amended soil of neem and mustard cakes; whereas *R. solani* was decreased only in cotton seed cake and oat meal oil cake amended soils. Studies conducted at Aligarh and Patnagar demonstrated that root knot can be successfully controlled by the application of oil cakes. Tiyagi et al. (2002) studied the biodegradable effect of oilseed cakes of neem, castor, groundnut and mustard against plant parasitic nematodes and soil inhabiting fungi infesting fenugreek and mungbean, in field condition. The resultant biodegradation effect of oilseed cakes effectively reduced the population of plant parasitic nematodes such as *M. incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, *Helicotylenchus indicus*, etc. and the frequency of parasitic-fungi *M. phaseolina*, *F. oxysporum*, *R. solani*, *Phyllosticta phaseolina*, *S. rolfsii*, etc. A pot experiment was conducted to investigate the effect of mustard oil cake and *Glomus etunicatum* (VAM) in different combinations of *M. incognita* and *F. oxysporum* f. sp. lycopersici infecting tomato cultivar Pusa Ruby. Both mustard oil cake and *G. etunicatum* were equally effective in reducing the damage caused by the nematode and/ or the fungus. When both the management components were applied together on the infected area, the combined efficacy was much more than the single application (Bhagawati et al., 2000 a).

Jain et al. (1990) reported that garlic intercropped with brinjal had shown a significantly lower number of galls and reduced *M. javanica* population in brinjal, which was due to the nematicidal property of garlic.

In mulberry, neem/pongamia oil cake @ 2 MT/ha/year in four equal split doses at an intervals of 3 months around the basin of the plants during intercultural operations/fertilizer application reduces 65 - 70 % root galls and egg mass of nematode, resulting in an increase of 16 - 18 % in leaf yield (Govindaiah et al., 1989; 1997; Sharma et al., 1998). The aqueous extract of leaves and stems of Mexican marigold (*Tagetes minuta*) was effective to kill the plant parasitic nematodes associated with mulberry within 48 hours (Toida, 1972). Leaf extracts of *Carica papaya* (papaya), *Euphorbia synadenium* (Kalli) and *Calotropis gigantean* (Madar) completely suppressed the egg hatching of root knot nematode. Papaya and kalli extracts resulted in 100 and 96 %
mortality of *M. incognita* juveniles, respectively when exposed for 48 hours (Philip *et al.*, 1993). Philip and Sharma (1997) tested the extracts of dry leaves and oil cakes of neem and pongamia against root rot pathogens of mulberry like *F. solani* and *F. oxysporum* under *in vitro* conditions. The maximum inhibition of radial mycelial growth of both the pathogens (77 - 86 %) was observed in neem leaf and its oil cake extracts. Naik and Sharma (2004) screened 6 different plant extracts and 5 oil cakes against pathogens, causing root knot disease complex in mulberry (*M. incognita* and *F. solani*) under *in vitro* conditions. Amongst all, the extract of *Allium sativum* and pongamia oil cake showed strong fungi/nematicidal effect against the above pathogens.

**Efficacy of bio-agents:**

Soluble root exudates released in the rhizosphere, support the growth of fungi, bacteria and actinomycetes. There are many genera of fungi and bacteria that have the potential to control plant parasitic nematode and pathogenic fungi. In nature, microbial interaction involve competition; hyper parasitism or antibiosis and these phenomena play an important role in striking ecological balance and keeping several plant pathogens in check. In recent times, biological control of plant pathogenic fungi has received considerable attention as it has several advantages such as possibility of multiple pathogen suppression, low cost and promotion of soil fertility. There are a number of antagonists, pathogens and parasites that attack the nematode in the rhizosphere (Kerry and Jaffee, 1997).

Isolate of *T. harzianum* can affect *Meloidogyne* spp. by various modes viz., direct parasitism on second stage juveniles & eggs, and metabolites produced by fungus affect second stage juveniles and egg hatching. Culture filtrate paralyzed and killed second stage juveniles (Sharon *et al.*, 2001). *T. harzianum* has been reported to induce systemic resistance to plants (Harman, 2000; Yediadia *et al.*, 2000). Chahal and Chahal (1994) reviewed the potential of *Trichoderma* as a biocontrol agent against soilborne pathogens. Further, they emphasized that *T. harzianum* and *T. viride* have been reported to parasitize eggs of nematodes. Bourne *et al.* (1996) reported that *V. chlamydosporium* is mainly confined to the rhizosphere and is more abundant on nematode infected roots. The extent of colonization depends on the strain of the fungus and plant species.
For the biological control of root knot nematode disease, the hyperparasites/egg parasites, *P. lilacinus*, *V. chlamydosporium* and *Pasteuria penetrans* (syn. *Bacillus penetrans*) have been paid much attention by various workers because of their potential hyperparasitic activities against the nematode, *M. incognita*. *P. lilacinus* and *V. chlamydosporium* are the facultative parasites which parasitize the nematode eggs and cysts and kill them. *P. penetrans* is an obligate parasite of the second stage juveniles of nematode which prevents the nematode from reproduction and reduce the infectivity of juveniles (Jatala, 1986; Davies et al., 1991; Stirling, 1991). Chahal and Chahal (1998) reported that beneficial effects of *T. harzianum* in reducing the number of galls caused by root knot nematode and disease complexes formed by *M. incognita* with soilborne fungal pathogens. Khan et al. (1997) observed the beneficial effects of *P. lilacinus* and *T. harzianum* when applied against *M. incognita* and *F. solani* on potted papaya in steam sterilized soil. The combined effect of both the biocontrol agents was more effective than individual biocontrol agents in reducing nematodes and decreasing the incidence of root rot.

Various workers have reported that species of *Trichoderma* are able to reduce the growth of pathogenic species of wilt causing *Fusarium* and root rot causing *Botryodiplodia* to a maximum extent under *in vitro* conditions (Katragadda and Murugesan, 1996; Chattopadhyay and Sastry, 1997; Karunanithi and Usman, 1999; Sonawane and Pawar, 2001; Jayalaksmi et al., 2003; Prameela et al., 2005).

In mulberry, *P. lilacinus* is reported to infest eggs of *M. incognita* in the soil in Brazil (Silva et al., 1992). Sharma (1998 a; 1999 a) evaluated eight bioagents on hatching of *M. incognita* eggs and larval mortality under *in vitro* conditions and *T. harzianum* (Th-2), *V. chlamydosporium* and *P. lilacinus* were found to be effective in suppression of egg hatching and increasing the larval mortality upto 70.0 - 80.0 %. On evaluation of various antagonistic microbes under *in vitro* and *in vivo* conditions, an isolate of *T. harzianum* isolated from rhizosphere of mulberry was found have the most potential against root rot pathogens like *F. solani* and *F. oxysporum* (Philip et al., 1996; Philip et al., 2001). Naik and Sharma (2004) screened five bioagents against pathogens causing root knot disease complex (*M. incognita* and *F. solani*) under *in vitro* conditions. Among them, *T. harzianum* (Th-o) showed strong fungi/nematicidal
effect against nematode and fungal pathogens. Besides, Vesicular Arbuscular Mycorrhizal (VAM) fungi, *Acaulospora laevis* and *Glomus fasciculatum* are also reported to reduce the disease severity of root knot and nematode population in mulberry (Chandrashekar *et al.*, 1997).

**Efficacy of chemicals:**

Use of chemical nematicides was effective in management of nematode problems but their hazardous effect on environment, ground water contamination and ill effect on human health creates a necessity to search cheaper as well as ecofriendly methods of nematode control. Hague and Gowen (1987) stated that the chemical control of nematodes, though very effective, has proved to be expensive for developing countries. In developed countries, nematicides and other chemical pesticides, have been under pressure, time and again due to associated problems of residual toxicity, environmental pollution and public health hazards (Thomason, 1987). Application of certain nematicides/ insecticides like Carbofuran, Phenomiphos, Aldicarb, Ethophos, Phorate, Sebofos, Benfurocarb, etc. have been tested against root knot nematode in various crops and have successfully reduced the number of galls, egg masses and nematode population in soil besides improving the plant growth characters (Meher, 1990; Patel *et al.*, 1992; Verma, 1993; Poornima and Vadivelu, 1993).

Sirohi and Pankaj (2005) studied the induction of systemic resistance by Salicylic acid (SA) and Giberrellic acid against root knot nematode (*M. incognita*) under glass house conditions in tomato. The results of SA spray @ 100 µg/ml decreased galling in roots of nematode infested tomato significantly, when compared to inoculated control. Pankaj *et al.* (2005) had screened eleven promising genotypes of chickpea against root knot (*M. incognita*) nematode. Out of 11 genotypes, all were moderately resistant except Pusa - 362, which was susceptible. Moreover, the SA content in roots (24.00 - 18.14 ppm/g sample) was more as compared, in the shoots, which ranged between 16.44 - 14.75 ppm. Therefore, the SA has a positive role in the resistant mechanism in chickpea.

Non-volatile nematicides, Carbofuran and Phorate each at 4, 6 and 8 kg a.i./ha dosages were tested as pre and post inoculation treatments for the
control of *Phytophthora - Meloidogyne* disease complex in tobacco. Carbofuran was found to be the most effective treatment followed by Phorate as pre-inoculation treatment and decreased root knot severity and the black shank disease incidence at higher dosages (@ 6 and 8 kg a.i./ha) of application than in the post inoculation treatment compared to the untreated control (Sitaramaiah and Kumari, 1997).

In mulberry, application of Organophosphates and Carbamates nematicides like Furadan 3G (Carbofuran) @ 40 kg/ha/year or Rugby 10G (Sebufos) @ 30 kg/ha/year or Aldicarb (Temik 10 G) @ 30 kg/ha/year in four equal split doses at intervals of 3 months can effectively reduce 70 - 75 % root galls and egg masses of the nematode, resulting in an improvement in leaf yield to an extent of 12 - 16 %. After 40 - 45 days of nematicide application, the leaves can be used for silkworm rearing (Sikdar et al., 1986; Govindaiah et al., 1993; 1997). Philip et al. (1995) and Sharma (1999 b) suggested the chemical method for the control of dry root rot, which involves the root dipping of saplings in 0.1 % Bavistin (Carbendazim 50 % WP) solution for half an hour and planting in pits dusted with 10 g of Dithane M-45 (Mancozeb 75 % WP). Black root rot (*B. theobromae*) can be controlled through spray of 0.2% Bavistin or Captan 50 % WP on the stumps immediately after pruning, followed by soil application at the rate of 8 kg/acre. The soil application was given after 10 days of aerial spray (Sukumar et al., 2000).

### 2.5.2 Preparation of formulations, standardization of application procedure, dose and compatibility test

Successful introduction of biocontrol agents in IDM package is dependent on a number of factors such as compatibility, mass production, formulation, field efficacy, toxicity evaluation, quality assurance, etc. Being living organisms, biocontrol agents may have limited shelf life because of their sensitivity to changes in environment. Besides, it must also be remembered that biocontrol agents themselves are not exempt from the restrictions that are imposed on chemical pesticides. Therefore, while developing a bioformulation for field application and commercialization, it must be taken care that the bioformulation should be cheap and easy to handle and use, as well as safe for the environment, plants, animals and human. Further, it is most important to
provide a food-based substrate to promote the growth of biocontrol agent (Gupta, 2005 a).

Mass production of bioagent is a dynamic process. Therefore, it is most important to provide a food-based substrate that not only can promote the growth of bioagent but also allow its continuous production of anti-microbial compounds during its growth. Utility of sorghum or husk based medium for the mass multiplication of various species of \textit{Trichoderma} to get the maximum growth and propagules in preparation of bioformulation have been proved earlier by various workers (Upadhyay and Mukhopadhyay, 1986; Padmanabhan and Alexander, 1987; Lewis \textit{et al.}, 1991; Sawant and Sawant, 1996; Mathivanan \textit{et al.}, 1998; Prasad and Rangeshwaran, 1999; Saju \textit{et al.}, 2002; Gaur \textit{et al.}, 2005). Some of them were in the opinion that the use of solid inert materials to be the best preferred approach in preparations of bioformulation to get the maximum CFU of antagonistic microbes in bioformulation.

Suitable carrier helps to maintain the viability of bioagent colonies in bioformulation for longer duration without loosing its vitality. The carriers, which have been used successfully for the production of bioformulation by \textit{Trichoderma} spp., are talc, lignite, peat, kaolin, gypsum, etc. (Howell, 1982; Jones \textit{et al.}, 1984; Sivan \textit{et al.}, 1984; Papavizas, 1985; Gupta, 2005 a). Talc has been used as a carrier in various \textit{Trichoderma} species by many workers for production of bioformulation for the control of certain soilborne diseases including root rot of mulberry (Jeyarajan and Ramakrishnan, 1991; Jeyarajan \textit{et al.}, 1991; Ramakrishnan \textit{et al.}, 1994; Philip \textit{et al.}, 2000; Gaur \textit{et al.}, 2005). Many workers have reported that using talc as a carrier for \textit{T. harzianum} provides to maintain the maximum number of viable colonies in the soil for a longer duration (Philip \textit{et al.}, 2000; Gaur \textit{et al.}, 2005).

Establishment of a bioagent in the soil and its spore load/g soil play a commendable role in controlling the plant diseases. De Leij \textit{et al.} (1992) reported that more than 5000 \textit{chlamydospores} of \textit{V. chlamydosporium/g soil} are required for the control of \textit{M. incognita} in tomato plants. Kerry and De Leij (1992) and De Leij \textit{et al.} (1993) also stated that about $10^7$ CFU of \textit{V. chlamydosporium/g soil} have established the fungus well in the soil and proved to be effective for the control of root knot nematode in tomato.
Gupta and Sharma (2003) evaluated six effective bioagents (from in vitro study) for standardization of their dosage and application method by applying in the form of bioformulations in different dosages (high @ 20 kg/ha in two splits and lower @ 10 kg/ha in two splits) in furrows and individual plant, for the control of leaf spot and leaf rust diseases, through inducing systemic resistance in perennial crop (mulberry). Results revealed that the higher dosage applied for application of plant growth promoting rhizobacteria viz., Azotobacter chroococcum (Azc-3) and Pseudomonas fluorescens (Psf-4) by making the furrows near the plant root was found to be effective for reducing the disease severity in mulberry considerably.

It is better to know the compatibility of a potent/ideal bioagent before integration with other components for its inhibitory effects so that it can be used in IDM package safely. Various workers have determined the compatibility of bioagent with certain pesticides, botanicals and other bioagents. Sharma et al. (1999; 2003) and Gupta et al. (1999) reported that T. harzianum and T. pseudokoningii were found to be compatible with neem oil cake, phytohormones, Furadan (Carbofuran) and Dithane M-45 (Mancozeb) and can be used for formulating the IDM strategies alongwith oil cake or sub-lethal dose of agrochemicals for the management of root rot and nursery (stem canker and cutting rot) diseases of mulberry. Similarly, several workers have also reported the compatibility of many isolates of Trichoderma spp. with Carbofuran and various fungicides for safe use in IDM package for the control of many plant diseases (Chandra et al., 1981; Muthamilan and Jeyarajan, 1996; Goudar et al., 1999; Sushir and Pandey, 2001; Sharma et al., 2001 a; Gupta, 2004; Vijayaragahavan and Abraham, 2004; Pandey et al., 2006).

Host resistant:

One of the most effective and safe ways of controlling the plant parasitic nematodes is to evolve the resistant cultivars. Although it is very laborious initially and time consuming, it is most useful as a non-recurring cost. In the roots of resistant plants, nematode larvae may either fail to enter, or may enter in reduced numbers (Christie, 1949).

An effort was made to test the relative resistance/susceptibility of 13 popular chickpea cultivars against disease complex (wilt and root knot disease
complex) and the results revealed that all the tested cultivars were found susceptible to root knot disease caused by *M. incognita* and the reaction was altered even in the presence of the fungus. However two cultivars viz., BDN-9-3 and Avrodhi lost their resistance in the presence of nematode. Further, two cultivars namely L - 550 and ICCY - 2 were found moderately susceptible to fungus, but became susceptible in the presence of nematode while Radhey, ICOG - 4, Jyothi, ICCC - 37, which showed tolerant reaction to the fungus, were found to be susceptible or moderately so, in the presence of nematode (Rao and Krishnappa, 1995 b). Out of 62 cultivars of black gram screened against mixed inoculation of *M. incognita* and *F. oxysporum*, none of them were recorded resistant. Four varieties viz., DPU-88-2, WBU-104, DPU-88-5 and NDU-88-9 exhibited moderate resistance (Mahapatra et al., 1999). Rahaman et al. (2000) studied the effect of root knot (*M. javanica*), black root rot (*F. solani*), dry root rot (*R. bataticola*) and wilt (*F. oxysporum f. sp. ciceri*) pathogens alone and in different combinations on four chickpea genotypes (C - 104, BG - 212, JG - 62 and WR - 315) in the green house. The reaction of wilt resistant genotypes (BG - 212 and WR-315) was not altered by the presence of any of the three pathogens. Presence of *F. solani* had a mild but significant suppressive effect on nematode induced root galling on JG - 62. Presence of the nematode did not enhance the severity of dry and black root rots.

In mulberry, the reports on screening the varieties for their resistant/susceptible reaction against disease complex (*M. incognita* with soilborne pathogens) are negligible. However, Campos et al. (1974) have screened several varieties against nematode (*Meloidogyne* spp.) and the variety Calabresa was found to be fairly resistant. Govindaiah et al. (1996) and Sharma et al. (2001 a) screened the various varieties for resistance against root knot (*M. incognita*) and none of them were found to be immune or resistant to the disease. However, varieties like S - 13, S - 30, S - 1096, RFS - 135 and Assambola were found to be moderately resistant to the disease. Vadivelu (1996) screened the mulberry varieties against *X. basiri* and found Kanva - 2, MR - 2, S - 13 and S - 36 to be resistant. Mohana (2003) reported that varieties namely, Kanva - 2, V - 1, DD and S - 54 were susceptible to *M. incognita* among 10 varieties screened for their reaction to root knot nematode.
2.5.3 Integrated Disease Management for Disease complex

Although there are several successful examples of biological control of diseases of many crops, it will perhaps be better for the greater success of biocontrol when it is coupled with other management practices. Several antagonistic microbes have shown their compatibility with various chemical pesticides and other agrochemicals. Therefore, for enhancing the level of disease control, bioformulations may be tried in combination with chemical and also with other methods of disease control. For the development of an effective integrated system for disease control, all components of the system (cultural, mechanical, chemical, etc.) must be considered as independent units, and emphasis must be given to integrate them without causing complexity in their application procedure and with minimum cost of application (Gupta, 2005 a). As mulberry is a perennial crop, it is difficult to control the disease by a single method. Therefore, it is required to use a variety of techniques involving cultural, chemical and biological methods to keep the population of nematode and other soilborne pathogens below Economic Threshold Level.

Studies on the IDM of disease complex caused by root knot nematode, *M. incognita* and wilt fungus, *F. oxysporum* on cowpea cv. Pusa Komal (PK) were conducted by the application of neem oil cake and Carbofuran. The combination of neem oil cake and Carbofuran (sub lethal dose) revealed the best response in reducing the nematode multiplication with an increase in plant vigour (Singh and Goswami, 2001 a).

Rao et al. (2003) studied the management of nematode induced wilt disease complex in tuberose (*Polianthus tuberosa*) cultivar Prajwal by application of neem based formulation, *Pochonia chlamydosporia* (40 g @ 10^6 CFU/g) and *Trichoderma harzianum* (40 g @ 10^6 CFU/g) per 2 x 2 m sized plots before planting and repeated subsequently once in 4 months, decreased the incidence of root knot nematode (*M. incognita*) and wilt disease complex significantly. These treatments also significantly increased number of florets/spike and spikes/plot. These findings form the basis for sustainable management of nematode induced wilt disease complex in tuberose under open field conditions. Haseeb et al. (2005) studied the comparative efficacy of Carbofuran at 1 mg a.i./kg soil, Bavistin at 1 mg a.i/kg soil, neem seed powder at 50 mg/kg soil, green mould (*T. harzianum*) at 50 ml/kg soil, rhizobacteria...
(P. fluorescens) at 50 ml/kg soil against root knot nematode, M. incognita-wilt fungus and F. oxysporum disease complex of green gram cultivar ML-1108 which revealed that all the treatments significantly improved the growth of the plants as compared to untreated inoculated plants. Analysis of data showed that Carbofuran and A. indica seed powder increased the plant growth and yields significantly more in comparison to Bavistin and P. fluorescens. Carbofuran was highly effective against nematode, Bavistin against fungus, A. indica seed powder against both the pathogens and both the bioagents were moderately effective against both the pathogens. Shreenivasa et al. (2005) studied the integrated management of nematode complex (Rodopholus similis, Helicotylenchus multicinctus and M. incognita) on banana by physical, chemical and cultural methods in the farmers' field. Integration of paring and hot water treatment of suckers along with application of Carbofuran and neem oil cake at the time of planting, reduced the soil and root population of nematode besides increasing growth, development and yield of banana.

Rao and Krishnappa (1995 c) made an attempt under field conditions to formulate an effective and economic IDM against wilt disease complex (M. incognita and F. oxysporum) in chickpea and observed that integration of soil solarization (for 6 weeks), Glomus fasciculatum inoculation (12 g/hill) and seed treatment with Carbosulfan (3 % w/w) was highly effective in reducing population levels of both the pathogens. Begum and Kumar (2005) reported that soil application of Carbofuran @ 3 kg a.i/ha and neem oil cake and P. fluorescens as seed treatment were effective in the management of the disease complex involving Heterodera cajani and M. phaseolina. Combined treatments of P. fluorescens as seed treatment and neem oil cake as soil application and T. viride as seed treatment have resulted in lowest root rot incidence of 11.1 and 22.2 % respectively, with significant increase in plant growth and pod yield.

In mulberry, the IDM strategy against disease complex was not formulated. However, IDM was developed against root knot and root rot diseases separately. An ecofriendly IDM strategy has been developed involving application of Bionema produced by V. chlamydosporium along with neem oil cake for effective management of the root knot disease. The Bionema is applied after mixing with neem oil cake and FYM (1:24:200) @ 200 g/plant around the roots (3 times/year at intervals of 4 months) during cultural operations/fertilizer application followed by irrigation. The technology reduces the disease
severity by 85 - 90 % and increases the leaf yield by 23 % (Sharma, 1999).

Similarly, the IDM package was developed by combined application of Dithane M-45 and talc-based biofungicide Raksha (*T. harzianum*) for the control of root rot disease and the technology controlled the disease upto 80 % (Philip et al., 1996; Sharma, 1999 b).

2.5.4 Bio-assay study on silkworm

Since silkworms are highly sensitive to any toxic substance, the safe period of treated leaves must be maintained, before using the mulberry leaves. In case, the safe period is not followed properly and the treated leaves are fed directly to the silkworm, the silkworm larvae may have toxic symptoms like vomiting of the digestive juice, swinging of the anterior half of the body, shrinkage/shortening of the body due to loss of body fluid, muscle contraction and paralysis followed by death of the silkworm larvae resulting in loss of silkworm crop considerably (Watanabe, 1978; Sengupta et al., 1990; Sharma, 2005 a). Several workers have tested the residual toxicity of various nematicides and pesticides in different crops viz., betelvine and grapes and found no residual effect after 40 - 45 days of application (Naganathan, 1988; Acharya, 1989).

Yokoyama (1962) tested the residual toxicity of Bordeaux mixture in mulberry and its effect on silkworm mortality. Considerable time gap between spraying and feeding of leaves was advocated, since Bordeaux mixture was lethal to silkworms even 15 days after treatment on the leaves. Siddaramaiah et al. (1978) advocated Dino-cap at 0.1% for the control of powdery mildew disease with 10 days of safe period. Later, it was reported that Dithane M-45 was toxic at 2000 ppm upto 10 days after spraying. Similarly, Bavistin has no toxic effect on silkworm after 3 days of spraying (Sikdar et al., 1979). Govindaiah et al. (1994 a) studied the effect of some systemic and non-systemic fungicides for the control of powdery mildew and their residual toxicity. They found that feeding silkworms with leaves sprayed with fungicides, did not affect silkworm growth and development even if given immediately after 3 days of spray. Govindaiah et al. (1997) have conducted a bioassay study by feeding the treated mulberry leaves with chemical (Rugby 10G), oil cakes of neem and pongamia, organic manures and various botanicals to silkworm larvae. Results revealed that Rugby 10 G did not show any residual
effect on silkworm after its application of 40 - 45 days, while application of oil cakes, botanicals and organic manures did not have any toxic effect on silkworm after 2 days of their application. Sharma et al., (2004) reported that the technology involving using of Bionema (V. chlamydosporium) alongwith neem oil cake for the control of root knot nematode in mulberry applied at any time of the crop period does not show any residual toxic effects in mulberry or in silkworms.

Thus, the literature cited in this chapter indicates that though the disease complex has been reported on mulberry on the association of M. incognita with R. bataticola and M. incognita and F. solani, not much work has been carried out on management of the disease complex. Whereas, in other crops, a lot of work has been done on the management of disease complex. Therefore, the present study undertaken may help to formulate an effective IDM strategy against disease complex in mulberry.