

**STUDIES ON ROOT-KNOT AND WILT COMPLEX IN
Coleus forskohlii (Wild.) Briq. CAUSED BY
Meloidogyne incognita (Kofoid and White) Chitwood
AND *Fusarium chlamydosporum* (Frag. and Cif.)
Booth**

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1. INTRODUCTION

India is one of the twelve mega biodiversity centers in the world with a wealth of 15,000 to 20,000 species of medicinal plants. The world of naturals is storming the globe with scientific rationale and trends that are fast emerging to support better health and life through plant and plant products. The demand for the products from these medicinal plants such as phytochemical, steroidal, biologically active compounds, alkaloids *etc.* is increasing in the national and international market.

According to the Hindu Survey of Indian Agriculture report, there was about US \$ 60 billion sales of herbal medicines in the world and projected to increase at an average annual growth rate of 6.4 per cent reaching US \$ trillion by the end of 2050 (Anon., 2007). India being the second largest exporter, next to China account for about 13 per cent of global imports. United States is the principal market for Indian medicinal plants accounting to 50 per cent of exports. The domestic market of medicinal plants or related products is about Rs. 4,000 crores correlated to about quantitative consumption level of 2 lakh tonnes. This, along with an export level of Rs. 1,200 crores makes the commercialization of Indian medicinal plants sector of Rs. 6,200 crores expected to be Rs. 7500 crores by 2010. To meet this demand, medicinal plants are being collected indiscriminately from forests resulting in dwindling supplies and endangering the survival of the species themselves.

Coleus forskohlii (Wild.) Briq. is a plant of Indian origin (Valdes *et al.*, 1987) and belongs to the family Lamiaceae. It is the most important species of genus *Coleus* popularly known as 'Manganiberu' or 'Makhandi beru' in Kannada, Pathachur in Hindi. It is a subtropical and warm temperate crop naturally grown at an altitude of 2500 M elevation. It is cultivated in Arabia, Brazil, Egypt, Ethiopia, India, Pakistan, Sri Lanka, and Tropical East Africa. In India, the crop is cultivated in the parts of Gujarat, Karnataka, Maharashtra, Rajasthan, and Tamil Nadu.

Traditionally, the roots have been used for preparation of condiments and pickling (Anon., 1950) and also for medicinal purposes by the Ayurvedic schools of medicines (Ammon and Muller, 1985). Root juice is given to children suffering from constipation (Singh *et al.*, 1980). Kothas, the native tribes of Trichigadi in Nilgiri, South India consider the decoction of tuberous roots as tonic (Abraham, 1981). Roots are eaten for treating cough in Kumaon Himalayas and one to three tea spoonful of root decoction is recommended for treatment of Asthma in Maharashtra, paste prepared from the roots is mixed with mustard oil and used in the treatment of skin infection and eczema by the natives in the Kumaon Himalayas (DeSouza and Shah, 1988).

Coleus forskohlii, the only known source of forskolin, an important drug used in the treatment of glaucoma, asthma, hypertension, congestive cardiomyopathy and certain types of cancers (Shah *et al.*, 1980). Forskolin content has been found to vary from 0.01 to 0.59 per cent of dry tubers and just 1 g of forskolin cost US \$ 110, reveals economic importance of this crop. It is a diterpenoid (active principle) and has the unique property of activating all hormone-sensitive adenylate cyclase enzymes in biological systems.

In addition, forskolin is reported to have been used in the preparation of medicines preventing grey hair and restoring grey hair to its normal colour. Its foliage is employed in treating intestinal disorders and used as condiment since times immemorial.

The novel feature of forskolin is its unique mechanism of generating cyclic adenosine monophosphate (AMP) in the cells through the direct activation of the catalytic unit of adenylate cyclase enzyme, which made the pharmaceutical industry to recognize the plant as most important (DeSouza and Shah, 1988).

Because of continuous collection of tubers from its wild sources, this plant has been included in the list of endangered species. But, now-a-days people have started cultivating the crop, because of its economical potential (Vishwakarma *et al.*, 1988).

Recently, with increase in the demand of forskolin, its cultivation has picked up in southern states with annual production of about 100 tonnes from 700 ha in India. In Karnataka, it occupies an area of 7 ha with production of 11 tonnes (Anonymous, 2004).

The crop is subjected to attack by many diseases viz., stem blight caused by *Phytophthora nicotianae* var. *nicotianae*, wilt caused by *Sclerotium rolfsii*, leaf spot caused by *Botrydiplodia theobromae*, bacterial root rot caused by *Pseudomonas aeruginosa*, dry root rot caused by *Macrophomina phaseolina*, aerial blight caused by *Rhizoctonia solani*, collar rot complex caused by *Rhizoctonia bataticola*, *Fusarium chlamydosporum*, *Sclerotium rolfsii* and root-knot nematode (*Meloidogyne* species). Among these, root-knot and wilt disease complex is occurring in severe form and cause heavy losses.

Shyla (1998) and Bobby and Bagyaraj (2003) reported that the fungus *Fusarium chlamydosporum* causes root rot of *C. forskolii*. Kamalakannan *et al.* (2003) reported the occurrence of pathogens like *Rhizoctonia solani* and *Macrophomina phaseolina* on root rot of *C. forskolii*. Senthamarai *et al.* (2006b) observed nematode fungal disease complex involving *Meloidogyne incognita* and *Macrophomina phaseolina* on *C. forskolii*.

Although, the disease has assumed economic importance in India and Karnataka, no detailed work has been carried out in root-knot and wilt disease complex of *C. forskolii* caused by *Meloidogyne incognita* and *Fusarium chlamydosporum*.

“Nature does not work with pure cultures, many plant diseases are influenced by associated organisms to a much more profound degree than we have yet realized, not only as to inhibition, but also as to acceleration of the “process” stated Fawcett (1931) almost half a century back. This word stands as true as or truer than ever, even now. In nature we rarely find plants being subjected to the attack of single pathogen. This seems to be more so in case of soil borne pathogens. Obviously these soil borne organisms do influence each other besides, influencing the responses in their common host. The rhizospheres of agricultural crops are exposed to microflora and microfauna, which are intrinsically involved in interactions in a common pathosystem. In the disease complex incidences, the role of nematode in majority of cases has been “primary pathogen”. While other component like fungi and bacteria are considered as “secondary pathogen”. Thus, for the management of disease complex, it is primary to study the nematodes and interaction with fungi.

In view of this, it was felt necessary to study, screening for resistance to root-knot and wilt disease complex and their interaction between *Meloidogyne incognita* and *Fusarium chlamydosporum* and their management in *Coleus forskohlii*.

Keeping in view the above gaps in research, the present investigation on root-knot and wilt disease complex was undertaken with the following objectives.

1. To survey for the incidence of root-knot and wilt disease complex in Northern Karnataka in *Coleus forskohlii*.
2. To study the effect of interaction between *Meloidogyne incognita* and predominant fungi in *Coleus*.
3. To screen different genotypes of *Coleus* for their resistance to *Meloidogyne incognita* and predominant fungi
4. Management of root-knot and wilt disease complex using plant products, bioagents and chemicals.

2. REVIEW OF LITERATURE

Coleus forskohlii is subjected to attack by many soil borne pathogens. Among these, root-knot nematode *Meloidogyne incognita* and *Fusarium chlamydosporum* are believed to be associated with wilt disease complex. Not much work has been done on this disease and the pathogens and literature available on these aspects is meager. However, work on *Meloidogyne incognita*, *Fusarium chlamydosporum* and other soil borne pathogens viz., *Rhizoctonia bataticola* and *Sclerotium rolfsii* on *Coleus* and other crops are reviewed below.

2.1 Survey for the incidence of root-knot and wilt disease complex in Northern Karnataka

Plant parasitic nematodes are found in all agricultural regions of the world and any crop is likely to suffer from these parasites. They may also be additive to other combination of plant parasitic nematodes with fungal pathogens, which is sufficient to induce heavy crop losses (Zacheo Giuseppe, 1993).

2.1.1 Plant parasitic nematodes

Sharma (1988) reported that presence of 39 and 29 species of plant parasitic nematodes associated with pigeonpea and chickpea respectively and also found that presence of nematode enhanced the severity of fungal diseases caused by *Fusarium* and *Rhizoctonia*.

Neeta Sharma (1990) isolated significant number of root-knot nematode larvae along with *Fusarium* from wilted plants of soybean in microplot of Botany Department, Lucknow University.

Krishna Rao and Krishnappa (1996) reported occurrence of *Meloidogyne-Fusarium* wilt disease complex in chickpea in most of the districts of Karnataka except in Mandya and Kolar. The population levels of nematode and fungus ranged from 11.7 to 132.5 juveniles per 250 cc soil and 5 g root and 4.8 to 34.8×10^3 cfu per g soil, respectively. Wilting that resulted from *Meloidogyne-Fusarium* complex was categorized as low to high ranging from 1 - 5 to 11 - 25 per cent.

Patil *et al.* (2003) reported that the prevalence and severity of different diseases and nematode problems in north eastern Karnataka, on medicinal crops such as *Coleus*, Long pepper, Ashwagandha *etc.* revealed the presence of root- knot, damping off, rhizome rot, seed rot, seedling blight and dieback diseases and estimated the loss to the tune of 5-20 per cent.

The survey carried out by Ramaprasad Shresti (2005) to know the incidence of root-knot disease in northern districts of Karnataka revealed the association of root-knot nematode (*Meloidogyne* spp.) with fungi namely *Sclerotium rolfsii*, *Rhizoctonia bataticola*, and *Fusarium chlamydosporum*.

The literature on survey of disease complexes on crop plants involving plant parasitic nematodes and soil borne fungal pathogens under field conditions is very limited (Sharma and McDonald, 1990).

2.1.2 Plant pathogenic fungi

2.1.2.1 *Fusarium chlamydosporum*

Mesterhazy and Vojtovics (1977) reported *Fusarium fusarioides* on maize for the first time from Hungary.

Sherkar and Utikar (1982) noticed leaf spot disease caused by *Fusarium fusarioides* on pomegranate (*Punica granatum*). Bazalar and Delgado (1981) observed that the *Fusarium fusarioides* caused typical wilt symptoms on the terminal leaflets of cotton in Peru.

Fusarium graminearum, *Fusarium moniliforme*, *Fusarium solani*, *Fusarium equiseti*, *Fusarium fusarioides* (*Fusarium chlamydosporum*) and *Fusarium oxysporum* were isolated and identified as the pathogens of maize stalk rot from 10 locations in Northern China (Bai *et al.*, 1988).

Pandey and Singh (1990) conducted a survey for root diseases of chickpea in Allahabad region and revealed the association of *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia bataticola*. The results of the survey also indicated that 83 per cent of villages recorded 26 to 50 per cent wilting. The average intensity of wilt and root-knot nematode was 19 and 49 per cent respectively.

Sharma and McDonald (1990) carried out survey in many countries reported that species of *Meloidogyne*, *Pratylenchus* and *Rotylenchulus* on legumes, species of *Haplolaimus*, *Pratylenchus* and *Xiphinema* on cereals were strongly suspected of increasing the severity of diseases caused by fungal pathogen *Sclerotium rolfsii* on groundnut, *Fusarium udum* on pigeon pea and *Fusarium oxysporum* f. sp. *ciceri* on chickpea caused very important and wide spread disease problems and the presence of *Meloidogyne* spp. aggravated the disease situation.

Aneja *et al.* (1993) conducted a series of surveys throughout Haryana state during 1988-92 to identify naturally occurring fungal pathogens of water hyacinth leaves. It was mainly caused by *Fusarium chlamydosporum*. Oladram and Iwu (1993) reported several fungi associated with fruit rot of tomato including *Fusarium equiseti*, *F. chlamydosporum*, *Alternaria solani*, *Geotrichum candidum*, *Acramonium recifci*, *Aspergillus flavus* and *Aspergillus niger*.

El-Arabi and Abughania (1998) conducted the study on *Fusarium* wilt disease of tomato on some coastal regions of Libya. Several *Fusarium* spp. were isolated from tomato plants and soils and identified including *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium fusarioides* (*Fusarium chlamydosporum*). Shyla (1998) isolated pathogen from diseased roots of *Coleus forskohlii* which consistently yielded *Fusarium chlamydosporum*.

Ina-Mul-Haq *et al.* (1999) reported six fungi associated with root rot of cotton including *Rhizoctonia solani*, *R. bataticola*, *Fusarium chlamydosporum*, *Alternaria alternata*, *Helminthosporium nodulosum* and *Curvularia lunata*.

Association of *Fusarium chlamydosporum*, which was isolated from pustules of groundnut rust caused by *Puccinia arachidis* and was non-pathogenic to groundnut plants (Mathivanan and Murugesan, 2000).

Boby and Bagyaraj (2003) isolated *Fusarium chlamydosporum* from diseased root fragments of *C. forskohlii*. Sachidananda (2005) isolated the pathogens by following standard tissue isolation technique from infected plant parts and identified as *F. chlamydosporum* and *R. bataticola*.

2.1.2.2 *Rhizoctonia bataticola*

The fungus *R. bataticola* is known to infect a wide range of host plants including both monocots and dicots with a high aggressiveness which have made this fungus economically important pathogen. The genus *Rhizoctonia* was erected by de Candolle (1815) for the violet root rot pathogen, *Rhizoctonia erocorum*.

Several species of *Rhizoctonia* have been recorded (Saksena and Vaasteraja, 1961). Among them, *Rhizoctonia bataticola* (Taub), Butler, the important species, which has very wide host range and causes root rot, charcoal rot and ashy blight in most of the cereals, pulses, oilseeds, fibre crops, vegetables, medicinal and aromatic plants, plantation and horticultural crops (Likhite and Kulkarni, 1934; Uppal, 1934; and Young, 1949).

Rhizoctonia is predominantly subterranean in habit living both as a parasite and saprophyte. According to ecological grouping of soil fungi (Garrett, 1956), it is placed under soil inhabitants and characterized as unspecified parasite, generally distributed worldwide.

2.1.2.3 *Sclerotium rolfsii*

Sclerotium rolfsii Sacc. is a well known polyphagous, ubiquitous, omnivorous and most destructive soil borne fungus. This was first reported by Rolfs (1892) as a cause of tomato blight in Florida. Later, Saccardo (1911) named the fungus as *Sclerotium rolfsii*. In India, Shaw and Ajrekar (1915) isolated the fungus from rooted potatoes and identified as *Rhizoctobia destruens*. But, later, studies showed that the fungus involved was *Sclerotium rolfsii* (Ramakrishnan, 1930). Higgins (1927) worked in detail on physiology and parasitism of *Sclerotium rolfsii*.

2.2 Symptomatology

2.2.1 *Fusarium chlamydosporum*

Fungro (1990) reported that, during rainy season a severe outbreak of stem canker was observed in okra (*Arka anamika*) at Wakawali, Maharashtra. Dark brown to black, circular to elongated lesions developed on the stems of young okra seedlings. Many infected seedling showed girdling at the point of infection, which ultimately led to death of seedlings. Under high rainfall and humid conditions, many infected plants showed splitting of the bark exposing inner cortex tissues. The leaf petioles, flower buds, calyx and immature pods were also infected showing small, circular to elongated dark brown lesions. The causal agents was isolated and identified as *Fusarium chlamydosporum* and its pathogenicity was confirmed.

Shyla (1998) described the symptoms on *Coleus* plants caused by *F. chlamydosporum*. In the field, infected plants were characterized by gradual yellowing, marginal necrosis and drying of leaves followed by loss in vigour and premature death. Such plants showed discoloration of roots and complete decaying of tap and lateral root system. The bark of such plants could be easily peeled off. There was extensive sloughing off and shredding of affected bark. Such affected plants were finally killed due to severe root and collar rots. The infected tubers showed rotting and emitted bad odour.

In 1990, an unreported blight occurred on Kangaroo Paw (*Anigozanthos* spp.) plants in southern Japan. Initially the disease developed in roots and the symptoms of wilt and browning progressed upwards. The root growth was suppressed and the internal part of the root was changed to brown. Eventually, leaves and flowering stalks showed a brown to black discolouration exhibiting blight symptoms. Two species of *Fusarium* were obtained from the diseased plant. The same symptoms appeared in plants inoculated artificially with the two species of *Fusarium*. Based on the cultural characteristics and morphology, the two fungi were identified as *F. chlamydosporum* and *F. semitectum* (Satou *et al.*, 2001).

2.2.2 *Rhizoctonia bataticola*

Chi and Childers (1964) observed cellular collapse of host cells after intracellular penetration by the fungus *R. bataticola* accompanied by a browning of the host protoplast.

Amar Singh and Bhowmik (1979) recorded an outbreak of safflower charcoal rot caused by *R. bataticola* at IARI Farm, New Delhi in January 1976. They reported that, the disease appeared mostly in patches resulting in death of about 20-25 per cent of the plants. The disease was characterized by yellowing and drying of leaves. This was followed by wilting and finally the entire plant dried up. The affected plants could easily be pulled out from the soil.

Prashanthi (1994) reported that the safflower plant infected by *R. bataticola* were characterized by gradual yellowing and drying of leaves, followed by loss of vigour and premature death. The bark of such plants could be easily peeled off. There was extensive sloughing off and shredding of affected bark.

Hibiscus schizopetalus plants in Kerala exhibited collar rot disease. The initial symptoms included varying sizes of water-soaked brown spots on the stem and collar regions. These areas were soon covered with cottony white mycelium of the fungus and after

3-4 days, sclerotial bodies appeared. The causal organism was isolated and identified as *R. bataticola* and its pathogenicity was confirmed by Santhakumari *et al.* (2002).

2.2.3 *Sclerotium rolfsii*

Bisht (1982) described the symptoms of *Sclerotium* infection in potato. The mycelium infected at the collar region causing wilt which ultimately lead to drying of plants. White or brown Sclerotia developed at maturity in the root and collar region of the infected plants. Infection spread within few days either by irrigation water or farm implements used for cultural practices, pathogen damaged stem, root or tuber.

Kulkarni *et al.* (1995) reported the damage caused by pathogen in potato, it exhibited stem, root or tuber rot of potato on infected stem it produced dark brown lesions at the collar region causing wilt and ultimately drying of plants. Brownish matured seed like *Sclerotia* developed at later stages on the root and collar region of the infected plants.

Somani and Chauhan (1996) observed a thin white mycelial mat on the tuber surface in fresh harvest and at times thicker mat mustard seed like sclerotia and thin white mycelial mat on almost all the daughter tubers. If the plants wilted after tuber formation, they observed wilting at early stage before tuberization and thus white mycelial mat and sclerotial development was observed.

The infected seedlings become stunted with chlorotic leaves and ultimately withered and died. Sclerotia developed on the surface of soil and infected stems (Baruah *et al.*, 1980). Nyvall (1989) also described the symptoms of wilting of the plants due to infection of stem at base level. Further, soil level near the infected stem overgrowth of white mycelium was observed. The wilted plants remained upright. The infected areas of stem shredded and covered with numerous sclerotial bodies. Stem base was covered with elongated and over crowded brown lesions. These turned to reddish colour. During dry weather brown lenticular lesions occurred on stems just below the soil surface.

2.2.4 *Meloidogyne incognita*

Rajendran and Vadivelu (1991) showed that newly introduced *C. forskohlii* plant with medicinal value exhibited stunted growth and yellow patches and severe galling of roots due to *Meloidogyne incognita*. Tuber formation was found commensurate with the degree of galling and Yield reduction was 65 per cent under field condition. The pathogenic study revealed that the effective damage to the plant was caused by 1000 larva per plant.

Senthamarai *et al.* (2006a) conducted two pot culture experiments to study the pathogenicity of *Meloidogyne incognita* (at 10, 100, 1000, 10000 and 100,000 J₂ per plant containing 5 kg of soil) on *C. forskohlii*. A pot without inoculate was included as the control. The inoculum level of 100 or more J₂ per 5 kg of soil was pathogenic causing significant reduction in shoot length, root length and shoot weight. At the initial levels of 10,000 and 100,000 J₂, the reduction in root weight was 22.22 and 43.05 per cent, respectively over the untreated control.

A significant reduction in tuber yield was observed at and above 10 J₂ per plant with maximum reduction at highest inoculum level of 100,000 J₂ per plant and maximum inoculum level of 100,000 J₂ per plant only 646.7 galls per plant were produced. The galling decreased at highest inoculum levels of 10,000 or 100,000 J₂ per plant. The nematode population in soil increased with increasing inoculum levels upto 1000 J₂ per plant.

2.3 Interaction between nematode and fungus at different inoculum levels

Observations indicated that predisposition of fungal diseases by plant parasitic nematodes requires a minimum level of nematode infestation. Garber *et al.* (1979) observed that low population density of either of the organism fungus 650 propagules per g of soil and 50 larvae per 500 g of soil in combination with high population density (fungus 647×10^3 to 77×10^3 propagules/g of soil) of either could result in marked wilted symptoms in cotton.

Krishna Rao and Krishnappa (1994) observed that inoculation of nematode (*Meloidogyne incognita*) along with fungus (*Fusarium oxysporum* f. sp. *ciceri*) at lower levels resulted in 19.88 per cent wilt incidence when compared to 6.66 per cent with fungus alone. However, maximum wilt incidence (66.70%) was observed when inoculated with 2 juveniles per g of soil and 25 g inoculum per 500 g soil of nematode and fungus respectively. Presence of root-knot nematode along with fungus adversely affected the root-knot disease.

2.3.1 Plant parasitic nematode fungal interactions

In nature plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognized that “nature does not work with pure culture” and that many plant diseases are influenced by associated organisms. Plant parasitic nematodes often play a major role in disease interactions and interactions involving nematodes is important because they contribute substantially to variability in crop growth and resulting heavy crop losses. It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Zacheo Giuseppe, 1993).

2.3.2 Interaction of nematodes with wilt inducing fungi

Atkinson (1892) for the first time reported the presence of *Meloidogyne incognita* along with *Fusarium oxysporum* in the rhizosphere of cotton plants which increased the severity of wilt caused by fungus. Since then nematode fungus complexes have been receiving increasing attention during the recent years and investigations covering this subject have been reviewed by several workers (Sasser, 1989; Khan and Parvatha Reddy, 1993).

Padilla *et al.* (1980) studied the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi* on pea and found that the concomitant inoculation of the nematode and the fungus at planting caused the death of plants after 45 days.

Studies were made by Ramanath and Dwivedi (1981) to determine the role of *Meloidogyne javanica* on the development of wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Plants grown in soil infested with *Fusarium + Meloidogyne* advanced the appearance of wilt by 15 days in chickpea cultivar T-3, than plants grown in soil infested by *Fusarium* alone. More than 56 per cent plants wilted by combined infection of both nematode and fungus and only 32 per cent plants wilted in fungus alone treatments.

Singh *et al.* (1981) observed that simultaneous inoculation of *Meloidogyne incognita* and *Fusarium oxysporum* or inoculation of *Meloidogyne incognita* 10 days prior to fungus drastically reduced plant height and fresh shoot weight with high wilt incidence. While, inoculation of fungus either alone or prior to nematode inoculation resulted in moderate incidence in frenchbean.

Sharma and Cerauskas (1985) observed greater reduction in shoot and root weights in chickpea due to simultaneous inoculation of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *ciceri* than by inoculation of either of them alone.

Goel and Gupta (1986) observed that inoculation of *Meloidogyne javanica* one week prior to *Fusarium oxysporum* inoculation to seedling of chickpea or simultaneous inoculation of both pathogens or inoculation of fungus one week prior to nematode resulted in reduced plant growth when compared to individual inoculations. Maximum and significant reduction of shoot length and fresh root weight were observed when both were inoculated simultaneously as compared to uninoculated checks.

Mani and Sethi (1987) studied the effect of combined inoculation of *Meloidogyne incognita*, *Fusarium oxysporum* and *Fusarium solani* on growth of chickpea cv. JG-62, which was found to be additive in nature. However, when nematode was established one week prior to fungus the resultant effects was more than additive. Occurrence of *Meloidogyne incognita* in combination with both the fungi not only increased the severity of diseases but also shortened the incubations period by 6 – 11 days for disease expression when nematode preceded *Fusarium solani*. The nematode development and multiplication was adversely

affected by *Fusarium solani* irrespective of time of inoculation. *Fusarium oxysporum* did not affect the nematode population significantly.

Marimuthu, (1991) reported that the nematode may predispose the vine to *Phytophthora palmivora*, which is able to infect through wounds caused by *Meloidogyne incognita*

Umamaheshwari (1991) reported that inoculation of nematode prior to fungus reduced the incubation period for the appearance of wilt symptom by a week in a late wilt variety of chickpea K-850. The fungus did not affect the development of nematode in the roots.

Singh *et al.* (1994) observed that inoculation of nematode alone, simultaneously with fungus and 10 days prior to the fungus drastically reduced the plant height and fresh shoot weight in frenchbean and also plants showed maximum wilt. Fungus alone and both the pathogens in different combinations severally decreased the fresh root weight but plants showed moderate wilting and maximum root-knot index was observed when both pathogens were inoculated either in different combinations or alone. Inoculation of both the pathogens simultaneously and nematode inoculation prior to fungal inoculations gave maximum percentage of wilting, while inoculation of fungus alone and fungus inoculation prior to nematode inoculation gave moderate percentage of wilting in frenchbean.

Patel *et al.* (2000) observed interaction of *Fusarium oxysporum* f. sp. *ciceri* with *Meloidogyne incognita* on chickpea cv. Dahood yellow revealed that the organisms either individually or in combinations reduced plant height and fresh root and shoot weights. Significantly, but the reduction was made by *Meloidogyne incognita* as compared to *Fusarium oxysporum* f. sp. *ciceri*. Among combined inoculations, simultaneous inoculation of both pathogens and maximum suppressing effect on growth of chickpea plants as compared to preceding or succeeding inoculation of fungus and nematodes. Root gall index and nematode multiplications on chickpea were maximum when nematodes were inoculated alone but it was reduced in the presence of fungus. The severity of disease increased when root-knot nematode was present with the fungus. Maximum wilting of plant was observed when the fungus and nematode were inoculated simultaneously.

Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* was studied on blackgram cv. T-9. The combined inoculation, plant growth along with rhizobial nodulation was more adversely affected. Simultaneous inoculation of both the pathogens and nematode inoculation prior to fungus synergistically reduced the plant growth and nodulation. It was accompanied by an increase the disease intensity. These effects were less significant when fungus preceded the nematode inoculation, the multiplication of nematodes and galling on roots increased/decreased with pre-inoculation of fungus (Mahapatra and Swain, 2001).

Sethamarai *et al.* (2006b) carried a glasshouse experiment to study the interaction of *Meloidogyne incognita* and *Macrophomina phaseolina* on *Coleus forskohlii*. The nematode multiplication was adversely affected when fungus was inoculated prior to nematode. Simultaneous inoculation of nematode and fungus as well as nematode followed by fungus 15 days later, caused 100 per cent root-knot disease and significant reduction in plant growth compared to the inoculation of fungus alone or fungus inoculation prior to nematode.

Akhtar Haseeb *et al.* (2007) studied individually as well as in combinations of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi* causing significant reduction in all the growth parameters of *Pisum sativum* as compared to uninoculated plants. Simultaneous inoculation of both the pathogens and nematode inoculation prior to fungus significantly reduced the plant growth. The reproduction of nematode and galling on roots decreased with pre-inoculation of fungus, while the infection of fungus increased in the presence of nematode.

2.4 Screening of different genotypes of *Coleus* for their resistant to *Meloidogyne incognita* and predominant fungi (*Fusarium chlamydosporum*)

Plant parasitic nematodes are known to break the disease resistance of crop variety to a given pathogen. Endoparasitic nematodes such as root-knot and cyst nematodes have long been known as primary pathogens due to their ability to predispose plants to infection by secondary pathogens such as several species of *Fusarium*, *Phytophthora* and *Rhizoctonia*. However, a vast majority of nematode infections involved especially root-knot nematode and wilt inducing fungi (Francl and Wheeler, 1993).

Young (1939) indicated that root-knot nematodes greatly decreased the resistance of the tomato cultivars to *Fusarium solani*. Twenty tomato lines and varieties were inoculated with a *Fusarium* + *Meloidogyne* complex. All the varieties and lines tested, susceptible to the complex including those resistant to *Fusarium oxysporum* (Almeida, 1972).

Hyer *et al.* (1979) studied the resistance and control of root-knot nematode – *Fusarium* wilt disease complex in cotton and reported that tolerant variety gave good yield compared to susceptible and resistant varieties.

Thakur *et al.* (1986) reported a change in the wilt incidence in chickpea genotypes ICC 12275 to *Fusarium oxysporum* f. sp. *ciceri* by *Meloidogyne incognita*.

Upadhyay and Dwivedi (1987) also observed loss of wilt resistance in chickpea genotype Avrodhi to *Fusarium oxysporum* f. sp. *ciceri* in the presence of *Meloidogyne javanica*.

Das *et al.* (1988) observed that brinjal cv. Local round was moderately resistant to *Meloidogyne incognita* in pot culture. El-Hawawi *et al.* (1990) tested 48 tomato varieties for their resistance to *Meloidogyne incognita* in pot experiments, of these only Pearson VFN, VFN Bush and VFN 8 proved to possess factors for immunity to *Meloidogyne incognita*.

Umamaheshwari (1991) evaluated 13 wilt resistance genotypes of chickpea for their resistance to *Fusarium oxysporum* in the presence of *M. javanica* and found vascular discolouration in the roots of limited number of genotype ICC-12275, completely wilted due to combined infection of both pathogens. ICC-12429 and Avrodhi were only two genotypes where both fungus and nematode inoculated either alone or together did not effect the plant growth.

Carniero and Moraes (1993) reported that out of eight cultivars of fresh market tomatoes, Mountecarlo, Master 3, Floradade, Tropic Bejya, Caruso, Colorado and Duke for their resistance to root-knot nematodes under greenhouse. Montecarlo and Master 3 showed high resistance to *Meloidogyne javanica* and *Meloidogyne aranaria* by preventing gall formation and reduced egg production by 90 per cent. Further, the cultivar Floradade was moderately resistance by supplying indeterminate levels of nematode reproduction, whereas the cultivars Tropic, Beijyu, Carsuo, Colorado and Duke were susceptible and supported high nematode reproduction.

Soomro *et al.* (1993) observed that five tomato lines viz., 220, 231, 262, 572 and 573 were highly resistant to a mixture of *Meloidogyne incognita* and *M. javanica* in pot experiments conducted in Islamabad, Pakistan, while line 142 and cv. Roma were highly susceptible. However, the same six lines were resistant to Italian populations of both nematode species tested in Naples, Italy.

Krishna Rao (1994) evaluated 13 popular cultivars of chickpea for their reactions to *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* in two soil types viz., red and black and found that Radhey and Avrodhi were least affected by nematode and fungus individually or in combination and highest reduction in plant growth was observed in JG-62 and average percentage wilt incidence with fungus alone was 28.7 per cent while it was 45.1 per cent in the presence of nematode.

Studies were carried out to find the combined effect of root-knot nematode, *Meloidogyne javanica* and wilt pathogen, *Fusarium udum* in ten wilt resistant/tolerant accessions of pigeonpea. Presence of *M. javanica* with *F. udum* increased wilting from 8 to 33 per cent in KPL-44, 15 to 0 per cent in AWR-74/15, 25-50 per cent in ICP 8859 and ICPL 89049 and 15-50 per cent in ICP 12745. Whereas, other five accessions wilting did not increase much in presence of nematodes. The plant weight, fresh shoot and root weight and dry shoot and root weight were significantly lower in combined inoculations of nematode and fungus compared to nematode or fungus alone. The root-knot index varied from 3.00 to 4.50. In these accessions in both treatments having nematode alone and nematode fungus together. The lowest root-knot index was observed in KPL 43 (1.50) and GPS 33 (1.75) (Bansa Singh *et al.*, 2004).

Akhtar Haseeb *et al.* (2005a) evaluated 33 cultivars of chickpea against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* disease complex and they found cultivars like BGM-547, RSJ-823 and RSJ-865 were found resistant against disease complex. The root-knot index and disease index varied from 0.5 to 0.75 and 5.6 to 10.00 per cent, respectively. However, cultivars viz., PUSA-372, CSJ-103, CSJ-353, CSJ-313, CSJ-390 and CSJD-125 were shown moderately resistant against both the pathogens.

Haseeb *et al.* (2006) evaluated 33 cultivars of lentil for resistance, against *Meloidogyne incognita* and *F. oxysporum* f. sp. *lentis* complex. Among 33 cultivars, only six cultivars viz., L-45-97, L-4147, L-4666, HUL-60, LL-887 and PL-01 were found resistant to moderately resistant against disease complex. The root-knot index and disease index varied from 0.5 to 1.50 and 10.0 to 29.70 per cent, respectively.

2.5 Management of root-knot and wilt disease complex by plant products, bioagents and chemicals

The issue facing agriculture, particularly disease control is managing the disease severity below economic threshold following ecologically safe, economically viable and easily operational procedures. The Integrated Disease Management (IDM) strategy is targeted to achieve this objective (Paroda, 2000).

2.5.1 Plant products/botanicals

Among the several methods of managing the plant diseases, soil amendments is one of the effective method Amendments in the form of plant debris, green manures, farmyard manures, compost, oil cakes and fertilizers are known to improve crop productivity by improving nutrient status and soil tilth. Addition of amendments to soils might have increase microbial activities in soil to suppress diseases (Sivaprakasam, 1991).

Sivakumar and Marimuthu (1986) reported that a significant reduction of 44.40 per cent over control in nematode population with neem cake applied at 100 kg per ha.

Groundnut and neem cakes along with carbofuran were used to manage *Meloidogyne incognita* on okras in India. Groundnut cake + carbofuran gave significantly higher okra fruit yield compared to control (Reddy and Khan, 1991).

The efficacy of decomposed products of groundnut, mustard and neem seed cake was investigated. The results indicated that organic amendments showed fluctuations in the mortality of *M. incognita*. Among, organic amendments mustard was the most nematotoxic (Goswami *et al.*, 1993).

Neem cake was more effective in reducing nematode population and improving tomato yield in pot experiments. Lower dose of neem showed a reduction in nematode numbers compared to untreated control (Jain and Gupta, 1997), Vijayalakshmi (2000) reported aqueous extracts of neem seed and neem cake as root dip treatments were effective against *Meloidogyne incognita* infection in tomato.

2.5.2.1 Effects of plant products on nematodes

Amendments of soil with decomposable organic matter is recognized as the most efficient method of changing soil and rhizosphere environment, thereby adversely affecting the life cycle of pathogens and enabling the plant to resist the attack of pathogens through better vigour or altered physiology. It was also reported that chemicals like ammonia (Khan *et al.*, 1974) and fatty acids (Sitaramaiah and Singh, 1978a) liberated during the decomposition of neem cake could be one of the factors involved in nematode control. Apart from this, the neem cake itself contains formaldehyde (0.25%), which is another factor responsible for nematode control. Similarly, in the soil amended with saw dust, phenols are liberated which inhibit the growth and development of root-knot nematode (Sitaramaiah and Singh, 1978b).

2.5.1.2 Effect of plant products on fungal wilt pathogens

Chauhan (1963) reported significant reduction in the incidence of *Fusarium oxysporum* var. *ciceri* in soil amended with cakes of groundnut, sesamum and mustard.

Peanut oil cake suppressed the *Fusarium* population in soil, when amended at more than one per cent, while at lower dosages stimulated the population (Bhatt, 1966).

Singh and Singh (1982) noticed neem cake inhibited radial growth of *F. udum*, when exposed to one or two weeks after amendments.

Dasgupta and Gupta (1989) reported the effect of different soil amendements on wilt of pigeon pea. In an experiment with potted soil inoculated with *Fusarium udum* and amendments of green manure (*Sesbenia aculeolata*), oil cakes (mustard and *Azadirachta indica*) and urea reduced both the pathogen population and the number of wilted plants.

Patil (1993) observed maximum per cent seedling survival of cowpea in neem cake (92.50) followed by safflower oil cake (82.50), groundnut oil cake (82.50) and farm yard manure (71.60) and this was significantly more than the untreated control (63.33).

Devi and Das (1998) reported that neem cake was superior to reducing population of root-knot nematode, gall index and increasing the yield attributing characters of carrot at dosage of 1.0 and 1.5 tonnes per ha followed by saw dust, poultry manure and water hyacinth at higher doses.

Mansoor (2006) reported neem leaf, seed powder, oil cake and two nematicides viz., carbofuran and phorate alone and in combination reduced the root-knot development caused by *Meloidogyne incognita*. Highest reductions in the nematode infections and corresponding improvement in plant growth was noted in pots treated with cake combined with carbofuran.

2.5.2 Biological control

Garret (1956) defined biological control of plant diseases as “any condition under which or practice whereby, survival and activity of a pathogen is reduced through the agency of any other living organisms with the result that there is reduction in the incidence of disease caused by the pathogen”.

2.5.2.1 Effect of antagonists on nematodes

Paecilomyces lilacinus has been reported to be very effective in controlling root-knot nematode, *Meloidogyne* spp. on several crops (Zaki and Maqbool, 1992 and Sosamma and Koshy, 1997).

Hoffman and Sikora (1998) treated seeds of tomato with the rhizobacterium *Pseudomonas* spp. W34 and found that it significantly reduced root galling and seedling growth.

Three applications of *Trichoderma viride* along with oil cake at quarterly intervals was found second best in reducing the nematode population and leaf yield in AAU centre after carbofuran treatment compared to control. However, both the treatments, carbofuran and

three applications of *T. viride* were on par in leaf yield at Jawaharlal Nehru krishi Vishwa Vidyalaya (Anon., 2003).

2.5.2.2 Effect of antagonists on fungal wilt pathogen

Sychev and Shadoshnik (1982) found that under laboratory conditions, *Trichoderma viride* inhibited the growth of *Rhizoctonia solani* and *Fusarium oxysporum* isolated from cucumber. *Trichoderma viride*, *Streptomyces griseus* and *Bacillus subtilis* inhibited the linear growth of *F. solani*, a fababean rot pathogen (Yahia *et al.*, 1985).

Fusarium crown rot of tomatoes was reduced to 80 per cent when *T. harzianum* was applied at 75 days after sowing either as seed coating or as wheat bran peat preparations and there was further increase in yield of tomatoes in *T. harzianum* treated plots over *Fusarium* infected plots (Sivan *et al.*, 1987)

Kaur and Mukhopadhyay (1992) reported that chickpea wilt complex caused by *Fusarium oxysporum* f. sp. *ciceri*, *R. solani*, *S. rolfsii* was effectively controlled by *T. harzianum* alone and in combination with fungicides. Soil applications of *T. harzianum* gave 93.5 to 85.7 per cent disease control in the greenhouse. In field, integrated use of *T. harzianum* with fungicidal seed treatment recorded higher crop yield.

Shamin *et al.* (1998) reported that seed dressing with *Pseudomonas aeruginosa*, *Paecilomyces lilacinus* and *Trichoderma koningii* significantly reduced the infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* on cotton roots in pot experiments. In the field combined use of *P. aeruginosa* strain CMG63 with *T. koningii* produced greater plant height and fresh weight of shoots in the field.

2.5.3 Integrated disease management in nematode-fungal disease complex

A field experiment was conducted to study the effect of organic amendments, nematicides, fungicides and their combination on the crop yield, wilt and root-knot disease of betelvine. The results revealed that maximum yield (13.81 kg = 4270 leaves) was obtained in the bed treated with carbofuran at 1.5 kg a.i. per ha + neem cake urea at 50 + 50 μ per ha + 0.5 per cent Bordeaux mixture. The wilt percentage and root-knot index were minimum in the two treatments viz., small carbofuran and aldicarb at 1.5 kg a.i. per ha both with the combination of neem cake and 0.5 per cent Bordeaux mixture. A significant yield reduction increasing wilt percentage and root-knot index was noticed in untreated control (Anon., 1984).

Carbofuran was effective in reducing the population and development of *Meloidogyne incognita* in tomato and improving plant growth at all concentrations tested. But, soil drench at 50, 100 and 500 ppm gave effective control (Haq and Saxena., 1986). Darekar *et al.* (1990) studied the efficacy of granular nematicides against *Meloidogyne incognita* in tomato nursery. Carbofuran and aldicarb at 0.6 g a.i. per m² had reduced nematode populations to a greater extent with highest yields of 27.40 and 25.67 per cent, respectively over control.

Sivakumar and Vidyasekaran (1990) reported that performance of *Paecilomyces lilacinus* @ 2g/kg, farmyard manure @ 4% (W/W) their combination for control of *Meloidogyne incognita* on *C. forskohlii*. A significant increase was found in all plant growth characters viz., shoot weight, root weight, tuber yield. Minimum egg masses was observed in treatment with FYM + *P. lilacinus*, followed by treatment with FYM alone.

Rakesh Pandey (1995) studied the efficacy of oil cake, pesticides and dry leaf powder on the reproduction potential of *Meloidogyne incognita* and yield of Japanese mint (Hy-77) and reported that application of neem seed cake at 5, 10, 20 g per kg, aldicarb at 0.0020 g a.i. per kg, carbofuran at 0.0015 g a.i. per kg, powdered leaves of *Adhatoda vasica* and *Murraya koenigii* at 50 and 100 g per kg of soil significantly reduced root-knot nematode population in soil and roots. However, maximum reduction in nematode multiplication was found in higher doses of neem cake followed by *A. vasica* leaf powder, aldicarb, neem cake, carbofuran, bavistin and powdered leaves of *M. koenigii*, respectively.

Singh and Vinod Kumar (1995) reported the efficacy of neem cake at two per cent w/w and carbofuran at two kg a.i. per ha in reducing the population of *Meloidogyne incognita*

as well as increasing shoot length, shoot dry weight, root fresh weight, number of leaves of Japanese mint. Neem cake at two per cent reduced number of root galls and population densities of *Meloidogyne incognita*.

De *et al.* (1996) reported coating of chickpea with biocontrol agents viz., *B. subtilis*, *G. virens*, *T. harzianum* and *T. viride* and carboxin (Vitavax) significantly controlled *F. oxysporum* f. sp. *ciceri* wilt by 30 to 45.8 per cent. Integration of biocontrol agents and carboxin significantly increased seed yield by 25.4 and 42.6 per cent as did carboxin treatment alone.

A field study was undertaken to study the possibility of controlling of root rot disease of *C. forskohlii* caused by *Fusarium chlamydosporum* using the biocontrol agents, Inoculation with *Trichoderma viride* + *Glomus mosseae* gave the best result in controlling the disease and resulted in maximum growth, yield and root forskolin concentration of Coleus. The next best treatment was *Pseudomonas fluorescens* + *Trichoderma viride* followed by *Glomus mossae* + *Pseudomonas fluorescens* and *Trichoderma viride* alone. Application of Emisan resulted in better growth than control but it was less when compared to single or dual inoculation of biocontrol agents (Boby and Bagyaraj, 2003).

Chaitali *et al.* (2003) reported that efficacy of groundnut cake and neem cake at 5 per cent w/w and *T. viride* at 2 g mycelial mat of fungus per 500 g of soil for control of disease complex caused by *Rhizoctonia bataticola* and *Meloidogyne incognita* on okra. A significant increase in plant growth parameters viz., shoot length, shoot weight, root weight and considerable reduction in nematode population observed on treatment with neem cake alone followed by *T. viride* alone. However, treatment with groundnut cake alone showed least effective.

Experiments were conducted to find out comparative performance of biocontrol agents and fungicide (carbendazim) in control of grape vine wilt caused by *Fusarium moniliformae*. Under glasshouse conditions, among different biocontrol agents tested in different combinations the treatments with *Pseudomonas fluorescens* + *B. subtilis* and *Pseudomonas fluorescens* + *Trichoderma viride* were found the best. Under field conditions, the treatments with *Pseudomonas fluorescens* + *Trichoderma viride* were found to be the best (Karunakaran *et al.*, 2003).

Senthilkumar and Ramakrishnan (2004) studied the effect of *Pseudomonas fluorescens*, *Trichoderma viride* and carbofuran 3G independently and in combinations for the management of *Meloidogyne incognita* in okra var. Co-3 under glasshouse condition. Independent application of *P. fluorescens* (2.5 kg/ha) and in combination with carbofuran 3G significantly improved plant growth. *P. fluorescens* (1.25 kg/ha) + carbofuran (0.5 a.i. /ha) treated plants recorded highest fruit weight per plant (184.88%).

Akhtar Haseeb *et al.* (2005b) reported the efficacy of bavistin + carbofuran followed by *A. indica* seed powder, *T. harzianum* + *P. fluorescens*, *M. koenigii* leaves, carbofuran, *T. harzianum*, *P. fluorescens*, bavistin, neem leaves against *Meloidogyne incognita* – *Fusarium oxysporum* disease complex on *Vigna mungo*. Highest increase in the fresh and dry weight of root due to severe galling was observed in plants treated with *P. fluorescens* followed by *T. harzianum* and *T. harzianum* + *P. fluorescens*, respectively.

A field experiment was conducted to study the management of collar rot disease of *Coleus forskohlii* using bioagents, organic amendments and chemicals in different combinations. Treatments with *T. viride* + nemato recorded lower per cent wilt incidence (12.76), followed by treatment with *Trichoderma harzianum* alone (18.87) and *Pseudomonas fluorescens* alone (19.98) and lowest population of root-knot nematode recorded in treatment with *Trichoderma viride* + nemato (873.33) followed by treatment with carbofuran alone (1066.67) and nemato alone (1180.00) and lowest number of galls per 5 g root recorded in treatment with *Trichoderma viride* + nemato (10.13) followed by treatment with carbofuran alone (14.93) (Ramprasad Shresthi, 2005).

Experiments were conducted to find out comparative performance of bioagents, botanicals and fungicides in management of root rot of *Coleus forskohlii* (Wild.) Briq. caused by *Fusarium chlamydosporum* and *Rhizoctonia* in pot culture. Among different bioagents,

botanicals and fungicides tested in different combinations, the treatments with parthenium + propiconazole + *T. viride* and parthenium + captan + *T. viride* were found better with least per cent mortality (33.33%). The next best treatment was carbandazim alone, neem + propiconazole + *T. viride*, Pongamia + propiconazole + *T. viride* and neem + captan + *T. viride* recorded 41.66 per cent mortality (Sachidananda, 2005).

Nisha and Sheela (2006) reported that integration of soil solarization in the nursery for 15 days and application of either *P. lilacinus*, *B. mucerans* or neem cake in the main field are the better treatments in improving biometric characters and reducing the nematode population in soil and root on *Coleus* (*Solenostemon rotundifolius*).

Tripati and Singh (2006) reported effect of compatible biocontrol agent along with mustard cake and furadon against *Meloidogyne incognita* in tomato. A significant increase in length and weight of root and shoot and considerable reduction in root galling as well as nematode population in soil and root was observed when plants were treated with *P. lilacinus* and *T. viride* in combination with mustard cake and furadon.

Senthamarai *et al.* (2006c) tested the efficacy of bioagents against *M. incognita* in *Coleus forskohlii* under pot culture experiment. Results of investigation indicated that soil application of *Pseudomonas fluorescens* at the rate of 2.5 kg a.i. per ha showed increased plant growth and root-knot nematode population both in soil and root. Soil application of *T. viride* @ 2.5 kg a.i. /ha recorded increased plant growth and reduced nematode population compared to control followed by *P. fluorescens*.

Sunilkumar *et al.* (2006) reported that *T. harzianum* @ 5 g/kg of soil was highly effective against *M. incognita* when egg masses were inoculated 15 days prior to transplanting and fungus at transplanting time in absence or presence of neem cake.

Paramasivan *et al.* (2007) studied antagonistic effect of fungal and bacterial antagonists against *Macrophomina phaseolina* on *Coleus forskohlii*. Results indicated that *T. viride* @ 2 g/kg of soil was effective in suppression of disease and enhanced the plant stand and tuber yield, followed by *T. harzianum* and *P. fluorescens*.

3. MATERIAL AND METHODS

The materials used and the techniques adopted in accomplishing the objectives of the present investigations are described here under.

The experiments were conducted in the Department of Plant Pathology, Kittur Rani Chennamma, College of Horticulture, Arabhavi, Belgaum district. The place is located in the Northern dry zone of Karnataka state at 16°15' north latitude and 74°45' east longitude.

3.1 Survey for incidence of root-knot and wilt disease complex in Northern Karnataka

Fixed plot survey was undertaken during 2007-08 in the major *Coleus* growing districts of Northern Karnataka. The area of *Coleus* growing cultivation considered for fixed plot survey comprised, Wadagere, Gugi, Mannur, Ratkal, Naron, of Gulbarga districts, Kanamadi, Bableswar, Arakari, Timbagi, Nadagundi, Tangadgi of Bijapur district, Dharwad, Tadakoda and Garag of Dharwad district, Manna E-Khalli, Chitagoppa, Matula, Janwad, Siddeswar, Kamalanagar of Bidar district, Badigwad, Rakshi, Yamakanamardi, Kakmari, Ingali, Ankali, Bommanal, Nayanagar, Inchala, Betageri, Kotabagi, Gurlapur of Belgaum district and Gaddanakeri, Kadapatti, Rabakavi, Savalgi, Yandageri of Bagalkot district. From planting to harvesting stage was taken into consideration for survey.

Samples of soil and roots were collected from infected fields from the rhizosphere of *Coleus* crop to the root depth, in the similar manner totally about 10-15 spots were selected randomly for taking soil and root samples representing the whole field. Later from this, composite samples of 200 g of soil and 5 g of root were formed. Randomly 100 plants were selected in different locations in a field and numbers of plants wilted were counted and the mean wilt incidence was expressed in percentage. Whenever required, the complete wilted plants are also collected. The per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Each sample was filled in polythene bag and tied with a rubber band labeled immediately. Information pertaining to the locality, crop history, *etc.* was also obtained along with the samples. Samples of soil and roots were analyzed on the day of collection or after keeping for a few days under refrigerator condition.

The nematode populations from soil and root samples were estimated. Fungal propagules were isolated from soil samples, while root samples were used for detection of the fungus associated with wilted plants. The wilt symptoms were identified based on the key provided by Maiti and Sen (1979).

Galled root system was scored by using disease rating scale (0 to 5 scale) given by Taylor and Sasser (1978). The severity of root-knot was calculated for each village by using the following formula.

$$\text{Disease Severity} = \frac{\text{Number of infested root samples from a village}}{\text{Total number of root samples collected from a village}} \times 100$$

3.1.1 Estimation of nematode population in soil

Cobb's sieving and decanting technique was followed for which 200 cc of the soil samples was taken in a container and mixed thoroughly with water. Hard particles and

stones, if any, were removed by stirring the suspension and was then passed through a set of sieves of 250, 45 and 37 μm pore size.

The sievates were collected on a tissue paper spread over a coarse mesh, which was then placed in a petridish containing enough water as to keep the tissue paper (placed on coarse mesh) always moist. This assembly was kept still for three days, care was taken to prevent drying of the tissue paper. The nematode suspension collected in the Petri dish was examined by means of research stereobinocular microscope. The different plant parasitic nematodes present in the suspension were identified. Their number present in the suspension were determined by taking the average number of nematodes present in five different one milliliter aliquots of nematode suspension.

3.2 Sterilization of soil, glasswares and earthen pots

Red soil free of lumps and stones was collected from Arabhavi. It was thoroughly mixed with sand 1:1 proportion and autoclaved at 1.05 kg per cm^2 pressure for two hours on two successive days. The sterilized soil was allowed to cool to room temperature and was later used to fill the pots for various experiments.

Glassware were washed cleanly with washing powder and then rinsed with tap water. They were then autoclaved at 1.05 kg per cm^2 for 20 minutes and used for further experiments.

Innerside of the earthen pots was swabbed with one per cent mercuric chloride (HgCl_2) solution.

3.3 Isolation of fungi from plant material

Tissue isolation technique was employed for isolation of *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium chlamydosporum* from plants showing typical symptoms of wilt.

The infected plant parts (from roots and collar region) were washed thoroughly in running tap water repeatedly till they were free of adhering soil. The infected parts were cut into bits of 5 to 6 mm size and surface sterilized in 0.1 per cent mercuric chloride for a minute. This was followed by repeated washing in sterile water. The bits are transferred aseptically on to a sterilized Potato Dextrose Agar (PDA) slants. The slants were incubated at room temperature ($27 \pm 1^\circ\text{C}$) for 72 hours. Pure culture of *Fusarium chlamydosporum* *Rhizoctonia bataticola*, *Sclerotium rolfsii* obtained and maintained on PDA medium. Subsequent sub culturing was done once in 30 days on PDA slants which were preserved in refrigerator at 4°C for further experimental use.

The following medium was used for isolation of fungi from soil samples

Potato Dextrose Agar (PDA)

Potato (peeled and sliced)	200.0 g
Dextrose	20.0 g
Agar	20.0 g
Water	1000 ml

Two hundred grams of peeled, sliced and washed potato was boiled in 500 ml of water. At the same time, agar molten separately in 500 ml of water. The potato decoction was then stained into the molten agar. Dextrose was added to the mixture and volume was made up to one litre and sterilized.

3.4 Isolation of fungi from soil samples

The propagules of fungi were estimated by plating the rhizosphere soil using dilution plate technique. *Fusarium* specific medium was used for plating fungi and *Fusarium* spp. from soil respectively at a dilution of 10^3 . All the Plates were incubated at room temperatures. Counts were made in all the dilution in three replicate plates and fungal colonies developed on the agar plates were counted 5-7 days after incubation and the number of colony forming units (cfu) were calculated and expressed per gram of oven dry soil.

The following medium was used for isolation of fungi from soil samples.

Fusarium Specific Medium (Nash and Snyder, 1962)

Difco peptone	1.50 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.10 g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$)	0.05 g
Streptomycin	500 ppm
Agar-agar	20 g
MBC	300 ppm
Distilled water	1000 ml
pH	6.4

Agar-agar was melted in 500ml distilled water. The other ingredients were dissolved in remaining 500ml of distilled water except streptomycin and PCNB. The two solutions were mixed thoroughly and the volume was made up to one litre. The medium was sterilized at 1.1kg / cm^2 pressure for 15 minutes, Streptomycin was added after autoclaving.

3.4.1 Identification of pathogen

3.4.1.1 *Sclerotium rolfsii*

The fungus was identified based on the morphological and cultural characters described by Domsch *et al.* (1980)

3.4.1.2 *Fusarium Chlamydosporum*.

The morphological, cultural and formation of chlamydospores by *F. chlamydosporum* were the principle characters to identify the pure culture of *F. chlamydosporum*. The character was compared with those described by Booth (1971) and fungus was identified as *F. chlamydosporum*

3.4.1.3 *Rhizoctonia bataticola*

The morphological, cultural and formation of sclerotia were the principle characters to identify the pure cultures of *R. bataticola*. The characters were compared with those described by Ashby (1927) and the fungus was identified as *R. bataticola*.

3.4.2 Multiplication of fungal inoculum

The fungal inoculum was multiplied on sterilized sand maize meal medium in 1000 ml flasks containing 400 g in each flask. A loopfull of fungus from actively growing culture was inoculated into each flask and incubated at room temperature for 15 days. The fungal culture multiplied on sand maize meal medium was used for inoculation to the soil.

3.4.3 Fungus inoculation method

At the time of inoculation the fungus culture maintained on sand maize meal medium was added around the root system by removing a small quantity of soil and replacing it again after inoculation.

3.5 Proving the pathogenicity of *M. incognita* on *Coleus forskohlii* by using different inoculum levels

The experiment was conducted by using cultivar K-8. The cuttings of the above mentioned cultivar were planted in 15 cm diameter pots. Twenty five days after the date of planting (after rooting), suspension containing desirable number of infective juveniles of the nematode were inoculated to the root region. The nematode suspension was determined by taking the average number of juveniles present in five different one milliliter aliquots of the suspension. Uniform quantity of the suspension containing desired number of juveniles was used for inoculation.

The inoculum levels maintained for the pathogenicity test: a check, 10, 100, 1000, 5000, 10,000 infective juveniles, six replications were maintained for each treatment and the pots were placed in glasshouse and suitably randomized (CRD). The temperature during the experimentation was $27 \pm 2^{\circ}\text{C}$. The pots were watered daily and observations were recorded in respect of shoot height, fresh shoot weight, dry shoot weight, dry root weight, tuber yield per plant and number of juveniles (average number present in each of five adequate of suspension and computed for total quality of suspension obtained by processing 200 CC of soil). These were recorded at the time of termination of experiment. Root length, root weight and shoot weight were determined at the end of the experiments after depotting. The root system was gently retrieved after loosening the soil.

Nematode population in the soil was recorded immediately after termination of experiments.

3.6 Collection of cultures of root-knot nematode maintenance and build-up of inoculum

Root-knot infested *Coleus* plants were collected from the experimental plots belonging to Department of Medicinal and Aromatic plants, Kittur Rani Channamma College of Horticulture, Arabhavi. Root portion was carefully removed from the soil and washed gently under running tap water. Egg masses were picked and kept for hatching in a petri dish. After 24-36 hours, juveniles hatched and the same were used to inoculate *Coleus* grown in sterilized soil sand mixture in green house. These plants were served as culture plants. After giving sufficient time to complete 3-4 generations of the nematode, the plants were depotted carefully. The root systems were washed free of soil. The galls containing egg masses were used to get inoculum of the pathogen for further studies.

3.6.1 Identification of prevailing root-knot nematode species

The galled root system from the above culture plants was immersed in a beaker containing boiling 0.1 per cent cotton blue in lactophenol and left over night for clearing (Southey, 1986). The roots infected by root-knot nematode were washed. The females were dissected out from the well developed galls of the roots under the stereo binocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide. The portions of the females were carefully cut with a sharp razor blade and body content were cleaned. The perineal region was trimmed and mounted for observations under immersion objective. At least ten slides were prepared containing the perineal pattern of the nematode. The identification of species was made on the basis of characters of perineal pattern described by Eisenback *et al.* (1981).

3.6.2 Hatching of juveniles and inoculation

The egg masses from stock culture were transferred carefully to a wire gauge sieve containing two layers of facial tissue paper trimmed down to edge of wire gauze and kept in a petri dish holding sufficient water to remain in contact with the bottom of petri dish. After 24 hours, the content of a petridish was emptied into a beaker, diluted to a suitable volume and population counts were made with the help of Fenwicks multi chamber counting slide. Based on the requirement the suspension was diluted with sterile water.

Second stage juveniles were obtained from egg masses collected from heavily infested Coleus plants by incubating large number of egg masses at room temperature in water. After 48 hours of incubation the second stage juveniles in water were collected in a 100 ml beaker and volume of water was made upto 50 ml. The nematode suspension was bubbled with the help of 10 ml pipette and an aliquot of one ml was transferred to counting dish for counting the juveniles under stereobinocular microscope. Five aliquots were examined from each samples and average population was calculated. For inoculation, larval levels were adjusted with water. So as to add equal volume of nematode suspension in each treatment to give desired inoculum level. The required numbers of juveniles in the water were added to potted seedlings to the two cm deep holes made on the rhizosphere. A similar treatment given to the inoculated check plants except that only water was used without nematode.

3.6.3 Estimation of nematode population from root samples

Root samples of known quantity (5 g) were directly observed under stereobinocular microscope for counting adult females of sedentary nematodes and same was processed using blending and Baermann's funnel method for the extraction of active forms of sedentary as well as migratory nematodes. After incubation of 48 hours, the volume of suspension was made to 250 ml out of which 10 ml pipetted out and used for counting nematode. Nematode count from this was finally converted to 5 g root by multiplying with a common factor of 25. To observe presence of second stage larvae and other developing stages. The roots were stained in a boiling solution of 0.05 per cent lactophenol cotton blue for one minute and allowed to cool for few minutes before washing gently under running tap water. Stained roots were then kept in plain lactophenol for few to 48 hours for differentiation and examined under stereobinocular microscope. The stained nematodes were counted and converted to 5 g root. The total nematode counts were expressed per 5 g root. The nematodes were identified based on key provided by Taylor and Sasser (1978).

3.6.4 Counting egg masses

The number of egg masses of root-knot nematode per root system was counted by exposing the infected roots to 0.25 per cent trypan blue for three minutes as per the procedure given by Sharma and Ashok Kumar (1991)

3.7 To study the effect of interaction between *Meloidogyne incognita* and predominant fungus (*Fusarium chlamydosporum*) in Coleus

To study the effect of simultaneous inoculation of *M. incognita* and *F. chlamydosporum* with singly or in sequential on plant growth, host infestation, nematode multiplication and disease development, a pot culture experiment was designed under green house condition by adding 15 g of fungal gaint culture or thousand freshly hatched second stage juveniles of root-knot nematode was applied individually to 25 days old rooted cuttings of Coleus grown in 1:1 sterile sand soil moisture in 45 cm diameter earthen pots. Inoculation of all the said pathogens was applied singly or in combination in sequence as per the following treatments.

1. Inoculation with *M. incognita* alone
2. Inoculation with *F. chlamydosporum* alone

3. Inoculation with *M. incognita* seven days prior to inoculation of *F. chlamydosporum*
4. Inoculation with *F. chlamydosporum* seven days prior to inoculation of *M. incognita*
5. Inoculation with *M. incognita* + *F. chlamydosporum* simultaneously
6. Control

Observations were recorded on plant growth parameters like shoot length, root length, number of branches, fresh shoot weight, dry shoot weight, dry root weight, tuber yield per plant, and number of galls per plant, final nematode population in soil as well as in roots and per cent decrease incidence. Whereas, rating of root-rot index was done following the scale suggested by Chidananda Prabhu (1987) as given below.

0 – Healthy, no symptoms at all

1 – A slight discolouration at the collar regions – roots healthy

2 – Discolouration upto 1-1 ½ inch from ground level, tap roots discoloured, secondary root still healthy

3 – Complete discoloration of tap root 2-2 ½ inch discoloration of stem, roots started getting discoloured.

4 – Majority of secondary roots discoloured, root tips disinfected

5 – All roots rotting – complete discoloration

3.8 To screen different genotypes of *Coleus* for their resistance to *Meloidogyne incognita* and predominant fungi (*Fusarium chlamydosporum*)

Pot culture studies were conducted in the glass house to test the reaction of 11 different genotypes of *Coleus* to root-knot nematode and *F. chlamydosporum*. These genotypes were selected based on their popularity in Northern Karnataka. The cuttings of *Coleus* grown in the 45 cm diameter pot containing 2.5 kg of soil and sand (1:1) proportions and 500 freshly hatched second stage juveniles and 15 g of fungal giant culture were inoculated to each pot 30 days after planting and covered with the soil. The observations were recorded at 160 days after inoculation with respect to shoot length, root length, number of galls/root, nematode population. Root rot index and root-knot index was measured using (1-5 rating scale) (Anon., 1993).

Infection scale: Based on number of galls developed the varieties were rated as follows.

Number of galls per plant Scale (Reaction)

0	1 - Highly resistant
1-10	2 - Resistant
11-30	3 - Moderately resistant
31-100	4 - Susceptible
101 and above	5 - Highly susceptible.

Genotypes used for screening against wilt complex

1. Manna E Kheli	2. Birur local	3. Nimbanur local	4. Gurlapur local
5. K-8	6. Yamakanamardi	7. Rabakavi local	8. IIHR collection
9. Yellow tubers	10. Orange tubers	11. Sunadolli local	

3.9 Management of root-knot and wilt disease complex using plant products, bioagents and chemicals

A pot culture experiment was carried out for the management of root-knot and wilt disease complex of *Coleus* in the greenhouse of KRC, College of Horticulture, and Arabhavi. Terminal *Coleus* cuttings were grown in the earthen pots containing sick soil, the commercially available plant products like Neem seed kernel powder, bioagents like *Trichoderma viride*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and chemicals like carbosulfan and Vitavax Power (Carboxin + Thiram) were used in the experiment.

Sterilized pot mixture was taken in six kg capacity pots and rooted terminal cuttings of *Coleus* were planted separately @ 2 cuttings per pot. After proper establishment of cuttings, commercially available plant products viz., Neem seed kernel powder, bioagents like *Trichoderma viride*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* applied to the soil and chemicals like carbosulfan and Vitavax Power at their respective dosages dipped in the solution at the time of transplanting and drenched individually as well as in combinations into the respective sets of pots. Simultaneously *Meloidogyne incognita* and *Fusarium chlamydosporum* were inoculated @ 1000 J₂ per kg and 15 g of joint culture per kg of soil, respectively around the roots of *Coleus* plants. The treatments were replicated thrice in completely randomized design as follows.

3.9.1 Treatment details

T₁ – Neem seed kernel powder @ 5 g/kg of soil

T₂ – *Paecilomyces lilacinus* @ 10 g/kg of soil

T₃ – *Trichoderma viride* @ 10 g/kg of soil

T₄ – *Pseudomonas fluorescens* @ 10 g/kg of soil

T₅ – Carbosulfan 25 ST @ 3%

T₆ – Neem seed kernel powder @ 5 g/kg of soil + *P. lilacinus* @ 10 g/kg of soil

T₇ – Neem seed kernel powder @ 5 g/kg of soil + *T. viride* @ 10 g/kg of soil

T₈ – Neem seed kernel powder @ 5 g/kg of soil + *P. fluorescens* @ 10 g/kg of soil

T₉ – *Paecilomyces lilacinus* @ 5 g/kg of soil + *T. viride* @ 10 g/kg of soil

T₁₀ – Neem seed kernel powder @ 5 g/kg of soil + *T. viride* @ 10 g/kg of soil + *P. lilacinus* @ 10 g/kg of soil

T₁₁ – Carbosulfan 25 ST @ 3% + Vitavax power @ 0.2%

T₁₂ – Neem seed kernel powder @ 5 g/kg of soil + *T. viride* @ 10 g/kg of soil + *P. lilacinus* @ 10 g/kg of soil + *P. fluorescens* @ 10 g/kg of soil

T₁₃ – Uninoculated control

T₁₄ – Inoculated control

3.9.2 Observations recorded

Observations were recorded on plant growth parameters viz., shoot length (cm), number of leaves, root length (cm), fresh and dry shoot weight (g), dry root weight (g), tuber yield per plant (g), number of galls per plant, number of egg masses per plant, final nematode population, root rot index and root-knot index was measured using following scale (Taylor and Sasser, 1978).

Scale	Number of galls per root
0	No galls per plant root system
1	1-2 galls per plant root system
2	3-10 galls per plant root system
3	11-30 galls per plant root system
4	31-100 galls per plant root system
5	More than 100 galls per plant root system

3.9.3 Statistical analysis of the experimental data

The data obtained in the present investigation regarding parameters such as shoot and root length (cm), fresh and dry shoot weighs (g), dry root weight (g), tuber yield per plant, number of galls per 5g roots, final nematode populations, root-knot index and root- rot index on *Coleus* were subjected to ANOVA for a completely randomized design for *in vitro* studies.

4. EXPERIMENTAL RESULTS

The results on the survey of wilt disease complex of *Coleus*, their pathogenicity, their interaction effects, the reaction of important genotypes of *Coleus* and management of disease complex under glasshouse are presented under the following headings.

4.1 To survey for incidence of root-knot and wilt disease complex in Northern Karnataka

A survey was carried out in Gulbarga, Bijapur, Dharwad, Bidar, Belgaum and Bagalkot districts of Northern Karnataka during 2007-08 to find out the incidence and severity of the wilt disease complex of *Coleus forskohlii*. Five villages in Gulbarga, six villages in Bijapur, three villages in Dharwad, six villages in Bidar, twelve villages in Belgaum and five villages in Bagalkot districts were surveyed as explained in Material and Methods and the data are presented in Table 1 and Plate 1a and 1b.

4.1.1 Root-knot disease

The root-knot disease caused by *Meloidogyne incognita* as noticed in all the districts surveyed with a gall index ranging from 0 to 4. In Belgaum district, maximum per cent disease incidence was observed in Kakmari (68.00%) followed by Yamakanamardi (55.00%) and low per cent disease incidence in Kotabagi (38.00%). In Gulbarga district, maximum per cent disease incidence observed in Mannur (31.00%), followed by Ratkal (25.00%). Whereas, lowest per cent disease incidence was observed in Wadagere (24.00%). In Bijapur district maximum per cent disease incidence observed in Tangadgi (38.00%) followed by Kanamadi (36.00%). Low per cent disease incidence observed in Bableshwar (30.00%). In Dharwad district, maximum per cent disease incidence observed in Garag (32.00%), followed by Dharwad (28.00%). In Bidar district, maximum per cent disease incidence observed in Kamalanagar (36.00%) followed by Janwad (34.00%). In Bagalkot district, the highest (45.00%) and lowest (20.00%) PDI was observed in Kadapatti and Savalagi villages respectively. Root-knot disease incidence was not observed in Rakshi, Ingali, Bommanal and Nayanagar of Belgaum districts, Gugi and Narona of Gulbarga districts, Arakari and Timbagi of Bidar district, Tadakoda of Dharwad district, Matula and Siddeshwar of Bidar district and Gaddanakeri of Bagalkot district (Fig. 1).

4.1.2 Root-knot nematode population

Maximum root-knot nematode population per 200 cc of soil was recorded in Kakmari (526.60) followed by Kamalanagar (266.80) and lowest root-knot nematode population per 200 cc was recorded in Savalagi (46.20) of Bagalkot district and Ratkal (83.50) of Gulbarga district (Plate 2a and 2b).

4.1.3 Wilt inducing fungi

From the survey, it was found that the root rot or wilt caused by *Fusarium chlamydosporum* (Plate 3) was more severe in all the districts surveyed followed by *Sclerotium rolfsii* and *Rhizoctonia bataticola*. Maximum per cent disease incidence of above mentioned diseases was observed in Belgaum district, followed by Bijapur and Gulbarga district.

4.1.4 Population of *F. chlamydosporum* from soil

The soil samples collected from Inchala of Belgaum district recorded a high population of *Fusarium* (55.3×10^3 cfu/g soil) followed by Badigwad (43.2×10^3 cfu/g soil) as against nil population in samples collected from Gugi and Narona of Gulbarga district. Janwad of Bidar district, Rakshi and Nayanagar of Belgaum district and Gaddanakeri, Savalagi and Yandageri of Bagalkot districts. Out of 37 root samples, 28, 24 and 20 yielded *F. chlamydosporum* and *S. rolfsii* and *R. bataticola* respectively. Hence, *F. chlamydosporum* used was identified as predominant fungi in further studies.

Table 1: Incidence of root-knot and wilt disease complex of *Coleus forskohlii* in Northern Karnataka during 2007-08

Place	Per cent disease incidence				Disease grade (0-5) scale	Population of <i>FC</i> from soil (cfu/g)	Nematode population per 200 cc soil <i>FC</i>	Isolation from root		
	<i>FC</i>	<i>SR</i>	<i>RB</i>	<i>MI</i>				<i>SR</i>	<i>RB</i>	<i>MI</i>
Gulbarga districts										
Wadagere	29.00	20.00	12.00	24.00	2.30	14.4	119.60	+	+	+
Gugi	0.00	15.00	0.00	0.00	0.00	0.0	0.00	-	+	-
Mannur	27.00	0.00	18.00	31.00	3.00	22.1	192.60	+	-	+
Ratkal	24.00	0.00	10.00	25.00	1.25	24.2	83.50	+	-	+
Narona	0.00	15.00	0.00	0.00	0.00	0.00	0.00	-	+	-
Mean	16.00	10.00	8.00	16.00	1.31	12.1	79.14			
Bijapur district										
Kanamadi	10.00	0.00	0.00	36.00	2.25	15.5	102.64	+	-	-
Bableshwar	22.00	20.00	20.00	30.00	2.00	22.5	92.40	+	+	+
Arakari	15.00	25.00	0.00	0.00	0.00	12.6	0.00	+	+	-
Timbagi	40.00	20.00	35.00	0.00	0.00	28.8	0.00	+	+	+
Nadagundi	22.00	0.00	20.00	34.00	2.50	22.2	196.50	+	-	+
Tangadagi	14.00	28.00	0.00	38.00	3.00	10.8	216.50	+	+	-
Mean	20.50	15.50	12.50	23.00	1.62	18.7	101.34			
Dharwad district										
Dharwad	27.00	14.00	10.00	28.00	2.50	25.6	146.50	+	+	+
Tadakoda	9.00	16.00	0.00	0.00	0.00	8.6	0.00	+	+	-
Garag	7.00	0.00	8.00	32.00	2.80	10.2	185.60	+	-	+
Mean	14.33	10.00	6.00	20.00	1.76	14.8	110.7			

Contd.....

Place	Per cent disease incidence				Disease grade (0-5) scale	Population of FC from soil (cfu/g)	Nematode population per 200 cc soil	Isolation from root		
	FC	SR	RB	MI				SR	RB	MI
Bidar district										
Manna E Khelli	10.00	25.00	0.00	29.00	2.50	10.8	129.80	-	+	-
Chitaguppa	22.00	8.00	10.00	25.00	1.30	16.2	112.64	+	+	+
Matala	6.00	0.00	0.00	0.00	0.00	12.1	0.00	+	-	-
Janwad	0.00	15.00	4.00	34.00	2.75	0.0	236.50	-	+	+
Siddeshwar	20.00	0.00	0.00	0.00	0.00	16.8	0.00	+	-	-
Kamalanagar	28.00	5.00	4.00	36.00	3.25	23.8	266.80	+	+	+
Mean	12.66	8.83	6.00	20.66	1.63	13.2	124.89			
Belgaum district										
Badigwad	35.00	29.00	0.00	45.00	2.75	43.2	183.60	+	+	-
Rakshi	0.00	36.00	0.00	0.00	0.00	0.0	0.00	-	+	-
Yamakanamar adi	25.00	0.00	0.00	55.00	3.00	25.6	210.70	+	-	-
Kakmari	25.00	32.00	25.00	68.00	3.80	25.6	526.60	+	+	+
Ingali	38.00	0.00	44.00	0.00	0.00	48.7	0.00	+	-	+
Ankali	27.00	0.00	0.00	48.00	2.50	23.6	94.50	+	-	-
Bommanal	30.00	25.00	0.00	0.00	0.00	25.7	0.00	+	+	-
Nayanagar	0.00	29.00	30.00	0.00	0.00	0.00	0.00	+	+	+
Inchala	48.00	0.00	0.00	39.00	2.00	55.3	112.20	-	-	-
Betageri	25.00	0.00	28.00	46.00	2.75	18.6	136.40	+	-	+
Kotabagi	18.00	20.00	0.00	38.00	2.50	12.4	115.60	+	+	-
Gurlapur	35.00	16.00	26.00	46.00	3.25	24.8	221.20	+	+	+
Mean	25.50	15.58	12.75	32.08	1.87	25.2	133.40			

Contd.....

Place	Per cent disease incidence				Disease grade (0-5) scale	Population of <i>FC</i> from soil (cfu/g)	Nematode population per 200 cc soil	Isolation from root		
	<i>FC</i>	<i>SR</i>	<i>RB</i>	<i>MI</i>				<i>SR</i>	<i>RB</i>	<i>MI</i>
Bagalkot district										
Gaddanakeri	0.00	0.00	23.00	0.00	0.0	0.0	0.0	-	-	+
Kadapatti	38.00	16.00	0.00	45.00	2.50	28.2	218.80	+	+	-
Rabakavi	40.00	15.00	8.00	26.00	1.75	34.6	103.75	+	+	+
Savalagi	0.00	16.00	17.00	20.00	1.25	0.0	46.20	-	+	+
Yandageri	0.00	25.00	15.00	28.00	1.30	0.0	98.50	-	+	+
Mean	15.60	14.20	12.60	23.80	1.30	12.5	93.45			

* *FC*- *Fusarium chlamydosporum*, *SR*- *Sclerotium rolfsii*, *RB*- *Rhizoctonia bataticola*, *MI*- *Meloidogyne incognita*

* + = Presence, - = Absence



Plate 1a. Healthy roots of *Coleus forskohlii* (Wild.) Briq.



Plate 1b. Root-knot infected *Coleus forskohlii* (Wild.) Briq.

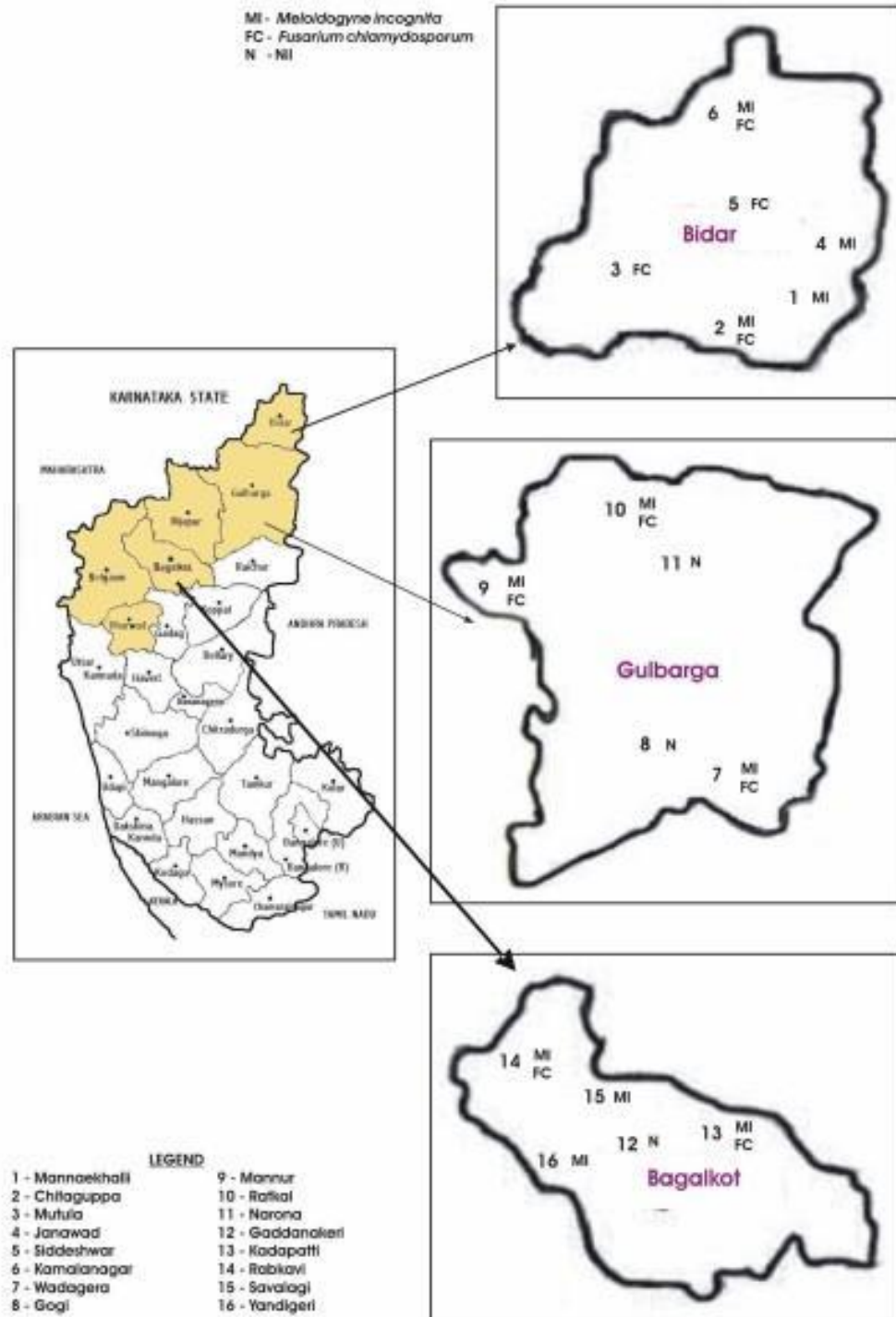


Fig. 1a. Map showing *Meloidogyne incognita* and *Fusarium chlamydosporum* complex in Northern Karnataka

Contd....

MI - *Meloidogyne incognita*
FC - *Fusarium chlamydosporum*
N - NI

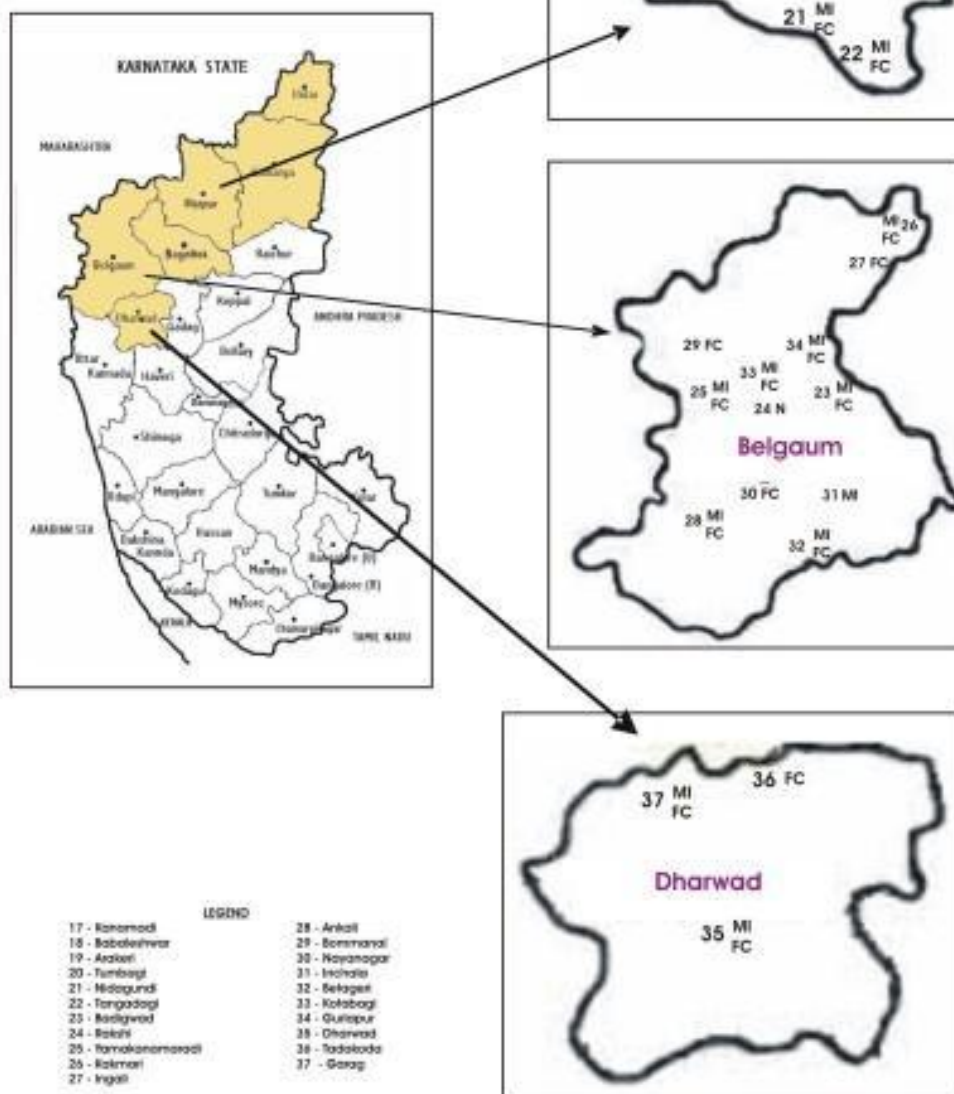


Fig. 1b. Map showing *Meloidogyne incognita* and *Fusarium chlamydosporum* complex in Northern Karnataka



Plate 2a. Coleus plot severely infected with wilt complex at Kakmari village of Belgaum district



Plate 2b. Severe infestation of root-knot nematode at Sunadolli village of Gokak taluk



Dark brown discoloration on stem portion
Due to *Fusarium Chlamydosporum*



Girdling of stem portion



Complete defoliation due to wilt complex

Plate 3a. Symptomatology of *Fusarium chlamydosporum* on *Coleus forskohlii* under field condition

4.2 Isolation of pathogens associated with disease complex in *Coleus forskohlii*

4.2.1 Identification of prevailing root-knot nematode species influencing *Coleus forskohlii* in Northern Karnataka.

For morphological studies, perineal pattern is the most important morphological characters used for reliable species identification. Using the perineal pattern preparations of the root-knot nematode infesting *Coleus* grown in Northern Karnataka. The prevailing *Meloidogyne* species was identified as *M. incognita*. Important diagnostic character of the perineal pattern of this and identified species, *M. incognita* is summarized in Table 2 and Plate 4. The observed characters were computed with the descriptions given by Eisenback *et al.* (1981).

The prevailing root-knot nematode species in Northern Karnataka were characterized by the presence of high, squarish dorsal arch that often continued a distinct whorl in the tail terminal area. The striae were smooth to wavy. Sometimes, zig-zaged and distinct lateral lines.

4.2.2 Isolation of fungi from wilt complex affected samples

In order to determine the fungi associated with disease complex standard tissue isolations were made as described in Material and Methods. *Fusarium chlamydosporum*, *R. bataticola* and *S. rolfsii* were found to be most commonly associated fungi. However, their frequency of occurrence varied with different locations.

4.3 Pathogenicity of *Meloidogyne incognita* on *Coleus forskohlii* and the effect of different inoculum densities on plant growth parameters and nematode multiplication

Pathogenicity of *Meloidogyne incognita* on *Coleus forskohlii* was studied under pot culture using a series of initial inoculum densities of 10, 100, 1000, 5000, 10,000 and a check. Observations on the effect of nematode on growth parameters were recorded at 150 days after inoculation and the data are presented in Table 3 and Fig. 2.

There was significant difference between check and all the nematode inoculum levels with respect to shoot height, fresh shoot and root weight, dry shoot and root weight and tuber yield per plant.

Significant decrease in shoot weight was noticed in plants receiving inoculum levels of 1000, 5000 and 10,000 juveniles compared to uninoculated control. These treatments significantly differed from each other.

With respect to fresh shoot weight, there was a significant differences with respect to plants receiving 10, 100, 1000, 5000 and 10,000 infective juveniles over check and these differed among themselves. Similarly, there was a significant differences with respect to dry shoot weight in case of T₂ (10J₂/plant), T₃ (100 J₂/plant), T₄ (1000 J₂/plant), T₅ (5000 J₂/plant) and T₆ (10000 J₂/plant) over the check and these differed among themselves.

In case of dry root weight, there is significant differences between T₄, T₅ and T₆ over check and these treatments differed among themselves. T₁, T₂ and T₃ were on par to each other.

There were significant differences with respect to tuber yield per plant in case of T₂, T₃, T₄, T₅ and T₆ over the check and these differed among themselves. All the nematode inoculum levels were differed from check.

With respect to nematode populations, there was seven fold (or more) increase in the final juvenile numbers when compared to plant receiving only 10 J₂.

Table 2: Important characters of perineal pattern of prevailing root-knot nematode species in Northern Karnataka

Feature	Characters observed	Original description (Eisenback <i>et al.</i>, 1981)
Dorsal arch	High, squarish	High, squarish
Lateral field	Lateral ridges absent, marked by breaks and forks in striae	Lateral ridges absent, marked by breaks and forks in striae
Striae	Coarse, smooth to wavy	Coarse, smooth to wavy some times zigzag
Tail terminus	Often with distinct whorl	Often with distinct whorl

Table 3: Effect of different inoculum levels of *Meloidogyne incognita* on plant growth parameters and nematode multiplication in *Coleus forskohlii*

Treatments	Shoot height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Dry root weigh (g)	Tuber yield per plant (g)	Nematode population per 200 cc soil
T ₁ – Check	38.60	134.20	40.51	18.95	145.77	0.00
T ₂ – 10 J ₂ / plant	33.10	130.10	39.03	17.04	142.21	618.18
T ₃ – 100 J ₂ / plant	30.92	105.50	31.65	15.71	112.19	10620.00
T ₄ – 1000 J ₂ / plant	24.00	96.00	28.80	15.47	103.15	7946.65
T ₅ – 5000 J ₂ / plant	23.84	90.80	27.24	12.26	94.30	7323.33
T ₆ – 10000 J ₂ / plant	21.63	87.10	26.13	14.67	91.68	6830.26
SEm±	0.80	0.73	0.23	0.74	0.83	13.61
CD @ 1%	2.42	2.19	0.70	2.22	2.57	41.92

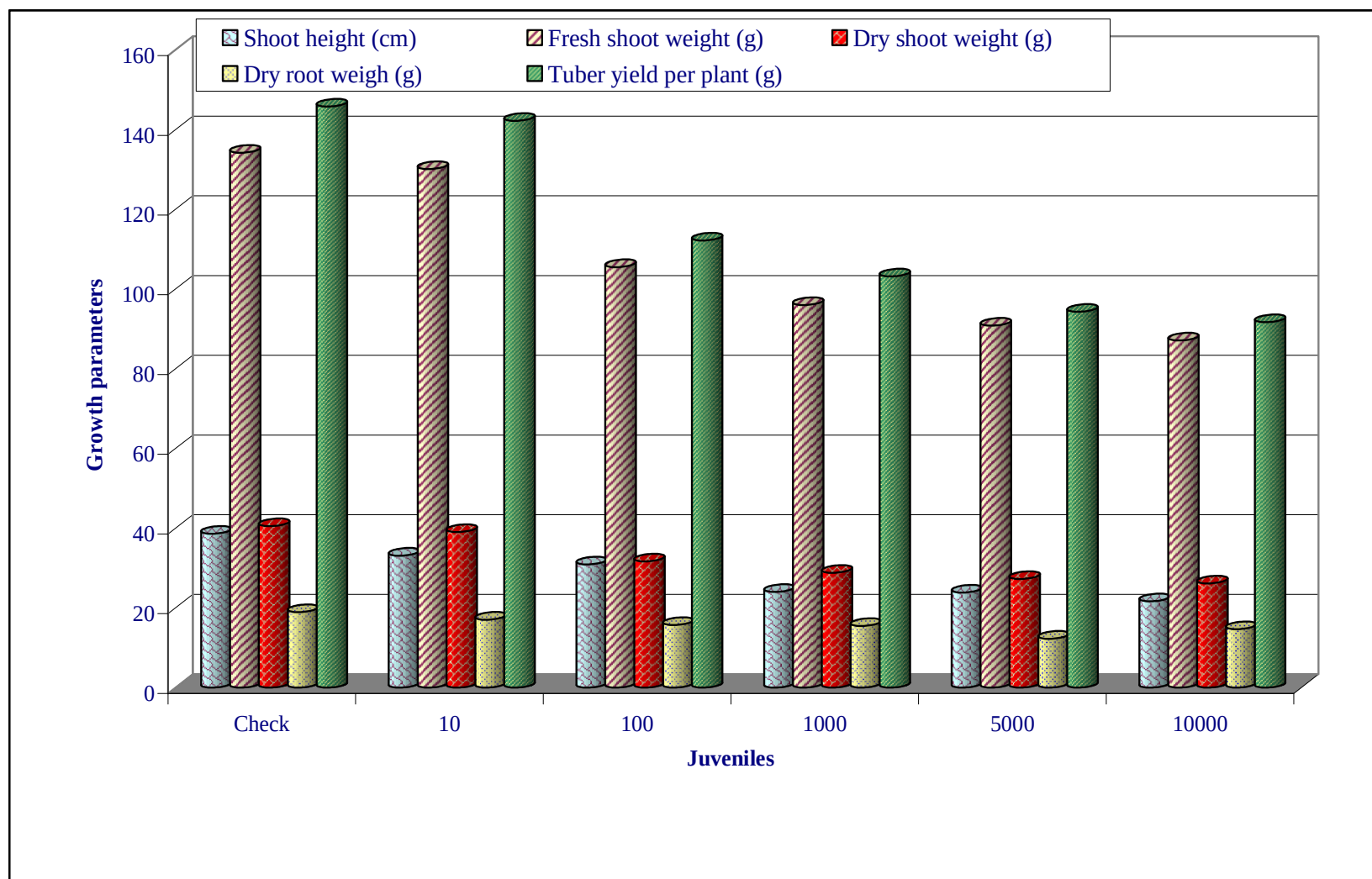


Fig. 2: Effect of different inoculum levels of *Meloidogyne incognita* on plant growth parameters in *Coleus forskohlii*



Perennial pattern of *Meloidogyne incognita*



Culture of *Fusarium chlamydosporum*



Photo micrograph showing 1) microconidia 2) macroconidia of *Fusarium chlamydosporum*

Plate 4. Identification of species of root-knot nematode and fungi

4.4 Interaction between *Meloidogyne incognita* and *Fusarium chlamydosporum* on Coleus

Disease complexes involving nematode and fungi have gained momentum in the recent years leading to considerable yield loss. The data on interaction between *Meloidogyne incognita* and *Fusarium chlamydosporum* are presented in Table 4, Fig. 3 and Plate 5.

Significant reduction in shoot length, root length, number of branches, fresh shoot weight, dry shoot weight, dry root weight and tuber yield per plant was noticed in all the treatments in comparison to uninoculated control.

Data presented in Table 4 revealed that significant reduction in number of branches, shoot length, root length, fresh shoot weight, dry shoot weight, dry root weight and tuber yield per plant observed in simultaneous inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum* followed by *Meloidogyne incognita* seven days prior to inoculation *Fusarium chlamydosporum* over untreated control.

However, of two organisms inoculated individually, *Meloidogyne incognita* cause greater reduction with respect to plant growth parameters over uninoculated control than either by *Fusarium chlamydosporum*. In case of sequential inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum* greatest reduction in plant growth parameters was observed in inoculation of *Meloidogyne incognita* seven days prior to *Fusarium chlamydosporum* over untreated control.

While reduction in plant growth parameters caused by *Meloidogyne incognita* alone, *Meloidogyne incognita* seven days prior to inoculation *Fusarium chlamydosporum* and *Fusarium chlamydosporum* seven days prior to inoculation of *Meloidogyne incognita* were on par to each other.

In these interactions, were inoculations were simultaneous wherein resultant effect on growth parameters was almost equal to sum of individual effects.

Number of galls per plant (830.50), juvenile population in soil (1586.70) and juvenile population in roots (230.00) were maximum in nematode alone treatment. However, lowest number of galls per plant (119.20), Juvenile population soil (210.50) and Juvenile population in root (102.30) was recorded in concomitant inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum*. However in sequential inoculation, highest number of galls per plant (402.50) and juvenile population in soil (1170.20) and roots (193.60) were recorded in inoculation of *Meloidogyne incognita* seven days prior to *Fusarium chlamydosporum* compared to inoculation of *Fusarium chlamydosporum* seven days prior to *Meloidogyne incognita*.

Root rot index (4.70) and per cent disease incidence (85.50%) were maximum in simultaneous inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum* treatments followed by *Meloidogyne incognita* seven days prior to *Fusarium chlamydosporum* treatment (Table 5).

4.5 Screening of popular Coleus genotypes for their reaction to *Meloidogyne incognita* and *Fusarium chlamydosporum*

During the survey, 11 popular Coleus genotypes were collected to test the reaction to *Meloidogyne incognita* and *Fusarium chlamydosporum* wilt complex disease and the data are presented in Table 6, Fig. 4 and Plate 6.

The results indicated that yellow tubers which showed maximum shoot length (42.50 cm) and root length (29.50 cm) followed by Yamakanamaradi local (38.00 cm). Whereas, minimum shoot length (23.60 cm) and root length (18.00 cm) was observed in Sunadolli local genotype. The cultivar K-8 showed maximum number of galls per root (98.00), root-knot index (4.00) and juvenile population (496.10), minimum number of galls, root-knot index (1.00) and juvenile population (90.40) were seen in yellow tuber genotypes.

Table 4: Influence of single or sequential inoculation with *Meloidogyne incognita* and *Fusarium chlamydosporum* on plant growth parameters in *Coleus forskohlii*

Treatments	Shoot length (cm)	Root length (cm)	No. of branches	Fresh shoot weight (g)	Dry shoot weight (g)	Dry root weight (g)	Tuber yield per plant (g)
T ₁ – <i>M. incognita</i> alone	31.00	19.10	15.20	112.37	35.15	28.21	184.29
T ₂ – <i>F. chlamydosporum</i> alone	35.50	21.99	16.00	129.33	39.09	31.80	212.10
T ₃ – Inoculation of <i>M. incognita</i> 7 days prior to inoculation of <i>F. chlamydosporum</i>	29.86	18.28	14.60	107.50	32.18	27.60	176.30
T ₄ – Inoculation of <i>F. chlamydosporum</i> 7 days prior to inoculation of <i>M. incognita</i>	32.60	20.06	17.00	118.01	37.76	28.95	193.54
T ₅ – <i>M. incognita</i> and <i>F. chlamydosporum</i> simultaneously	25.00	15.39	13.00	90.05	28.05	22.20	148.52
T ₆ - Control	37.43	23.04	17.60	135.50	44.00	33.90	226.50
Mean	31.89	19.64	15.56	115.46	36.03	22.78	190.20
SEm±	0.60	0.40	0.23	2.25	0.80	1.17	3.85
CD @ 1%	1.79	1.20	0.69	6.64	2.50	3.48	11.94

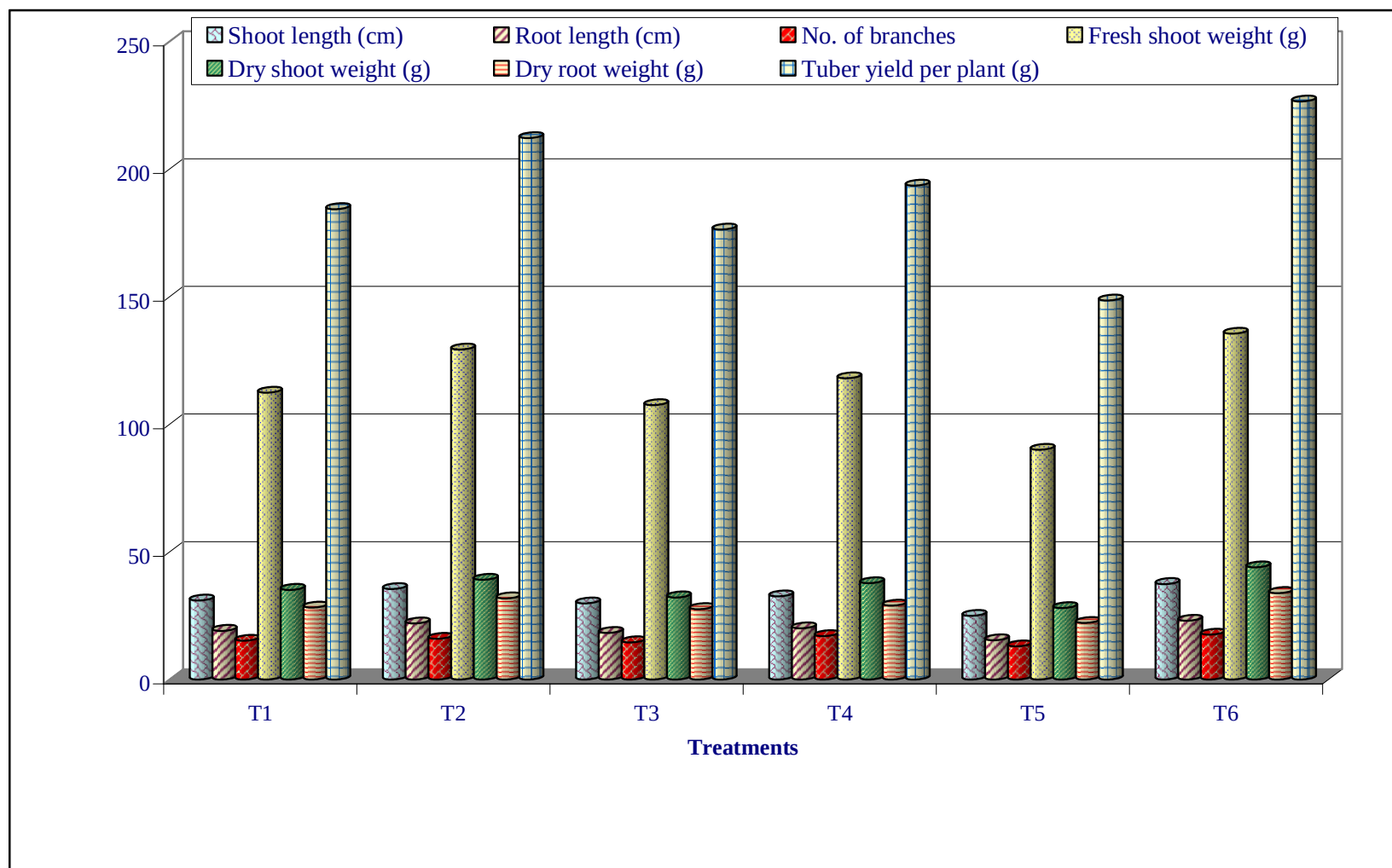


Fig. 3: Influence of single or sequential inoculation with *Meloidogyne incognita* and *Fusarium chlamydosporum* on plant growth parameters in *Coleus forskohlii*



Plate 5. Complete rotting of root system due to concomitant inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum*

Table 5: Influence of single or sequential inoculation with *Meloidogyne incognita* and *F. chlamydosporum* on number of galls, final nematode population, per cent disease incidence and root rot index in *Coleus forskholii*

Treatments	Number of galls per plant	Final nematode population/ 200 cc soil	Nematode population per g of root	Per cent disease incidence	Root rot index
T ₁ – <i>M. incognita</i> alone (2 J2/ g soil)	830.50	1586.70	230.00	1.00	0.00
T ₂ – <i>F. chlamydosporum</i> (15 g/kg soil)	0.00	0.00	0.00	38.40	3.20
T ₃ – Inoculation of <i>M. incognita</i> 7 days prior to inoculation of <i>F. chlamydosporum</i>	402.50	1170.20	193.60	76.70	4.10
T ₄ – Inoculation of <i>F. chlamydosporum</i> 7 days prior to inoculation of <i>F. chlamydosporum</i>	345.00	720.70	151.97	42.00	3.80
T ₅ – <i>M. incognita</i> and <i>F. chlamydosporum</i> simultaneously	119.20	210.50	102.30	85.50	4.70
T ₆ - Control	0.00	0.00	0.00	0.00	0.00
Mean	282.86	614.68	112.97	-	2.63
SEm±	0.87	0.95	0.38	7.59	0.18
CD @ 1%	2.63	2.85	1.14	23.16	0.53

Table 6: Reaction of coleus genotypes against *Meloidogyne incognita* and *Fusarium chlamydosporum*

Genotype	Shoot length (cm)	Root length (cm)	Number of galls per root	Root-knot index (1-5 scale)	Reaction	Nematode population per 200 cc soil	Root rot index
Manna E Khelli	38.63	19.18	68	4.00	S	490.60	5.00
Birur local	33.00	20.03	90	4.00	S	260.00	3.75
Nimbanur	28.00	19.93	25	3.00	MR	299.80	2.50
Gurlapur local	37.60	26.28	48	4.00	S	442.80	5.00
K-8	35.80	23.21	98	4.00	S	496.10	5.00
Yamakanamaradi	38.00	28.20	70	4.00	S	473.60	5.00
Rabakavi local	29.00	28.00	8	2.00	R	130.50	2.50
IIHR collection	36.30	19.00	73	4.00	S	410.20	5.00
Yellow tubers	42.50	29.50	6	1.00	R	90.40	1.25
Orange tubers	33.00	18.60	20	3.00	MR	272.00	4.25
Sunadolli local	23.60	18.00	82	4.00	S	760.00	5.00

R – Resistant MR – Moderately resistant

S - Susceptible

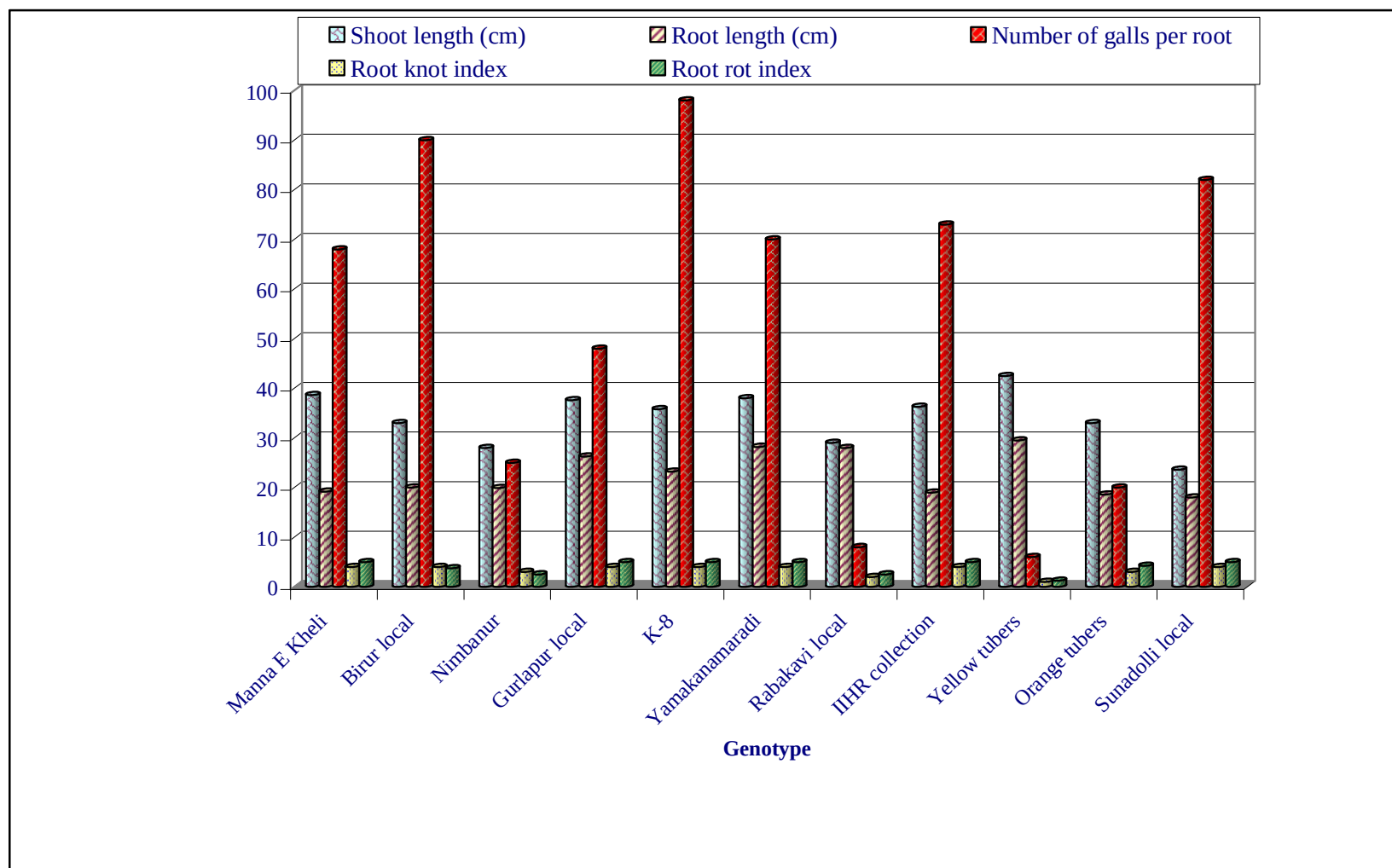


Fig. 4: Reaction of coleus genotypes against *Meloidogyne incognita* and *Fusarium chlamydosporum*



Genotype yellow tubers highly resistant to *Meloidogyne incognita* and *Fusarium chlamydosporum*



Genotype yellow tubers highly susceptible to mealy bug infestation



Genotype K-8 susceptible to *Meloidogyne incognita* and *Fusarium chlamydosporum*



Root system of yellow tubers showing resistant reaction to *M. incognita* and *F. chlamydosporum*

Plate 6. Screening of different genotypes of coleus for their resistance *Meloidogyne incognita* and *Fusarium chlamydosporum*

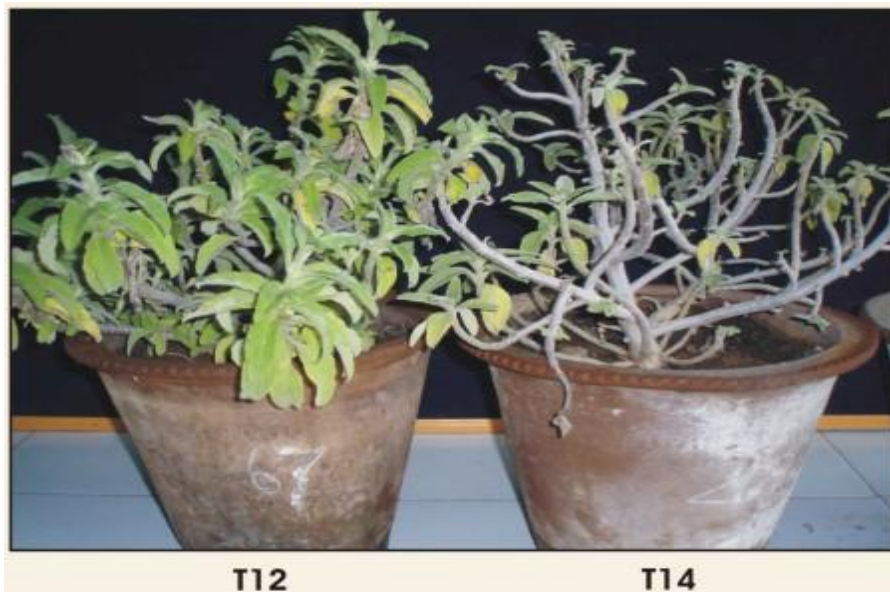


Plate 7. Management of root-knot and wilt complex using plant product, bioagents and chemicals

Based on Root-knot index and Root rot index the genotypes, yellow tubers showed minimum root rot index (1.25) followed by Rabakavi local (2.50), maximum root rot index (5.00) was noticed in MannaEkheili, Gurlapur local, K-8, IIHR collections, Sunadolli local and Yamakanamaradi genotypes.

Based on Root-knot index and root-rot index, genotypes yellow tubers and Rabakavi local showed resistant reaction, orange tubers and Nimbanur genotypes showed moderately resistant reaction and Mannekhalli, Birur local, Gurlapur local, K-8, Yamakanamaradi, IIHR collections and Sunadolli local showed susceptible reaction.

The genotype Yellow tubers showed resistant reaction, but it is highly susceptible to Mealy bug infestation.

4.6 Management of root-knot and wilt disease complex by using plant products, bioagents and chemicals

The data on the effect of various treatments on growth parameters of Coleus are presented in Table 7, Fig. 5 and Plate 7.

All the treatments significantly increased with respect to number of leaves, shoot and root length, fresh shoot weight and dry shoot weight, dry root weight and tuber yield per plant over inoculated control.

4.6.1 Number of leaves

Among individual treatments, the plants treated with carbosulfan (T₅) showed maximum number of leaves (142.30) followed by *Pseudomonas fluorescens* (T₄) (132.60) and they were on par to each other. Whereas, minimum number of leaves were recorded in *Paecilomyces lilacinus* (T₂) (95.50), followed by *Trichoderma viride* (T₃) (103.000) and neem seed kernel powder (T₁) (108.50) and they were on par with each other.

Among combination treatments, neem seed kernel powder + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) which showed maximum number of leaves (212.00) followed by Carbosulfan + Vitavax Power (T₁₁) (192.60) and they differed significantly over inoculated control. The minimum number of leaves were recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (102.10) followed by neem seed kernel powder + *Paecilomyces lilacinus* (T₆) (128.00) and neem seed kernel powder + *Pseudomonas fluorescens* (T₈) (129.00) and they were on par to each other.

4.6.2 Shoot length (cm)

Among individual treatments, Carbosulfan (T₅) showed maximum shoot length (35.04 cm) followed by *Pseudomonas fluorescens* (T₄) (28.91 cm) and neem seed kernel powder (T₁) (28.00 cm) and they differed significantly over inoculated control.

Minimum shoot length recorded in *Paecilomyces lilacinus* (T₂) (27.15 cm) followed by *Trichoderma viride* (T₃) (27.89 cm). Neem seed kernel powder (T₁) (28.00 cm) and *Pseudomonas fluorescens* (T₄) (28.91 cm) were on par to each other.

Among combination treatments, neem seed kernel powder + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) showed maximum shoot length (40.33 cm) followed by Carbosulfan + Vitavax Power (T₁₁) (37.40 cm) and they were on par to each other.

Minimum shoot length recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (30.00 cm) followed by neem seed kernel powder + *Paecilomyces lilacinus* (T₆) (32.00 cm), neem seed kernel powder + *Pseudomonas fluorescens* (T₈) (32.67 cm) and neem seed kernel powder + *Trichoderma viride* (T₇) (34.00 cm) which were on par to each other.

Table 7: Effect of different plant products, bioagents and chemicals on growth parameters of *Coleus forskohlii* infected by *M. incognita* and *F. chlamydosporum*

Treatment	Number of leaves	Length (cm)		Shoot weight (g)		Root weight (g)	Tuber yield per plant (g)
		Shoot	Root	Fresh	Dry	Dry	
T ₁ – Neem seed kernel powder @ 5 g/kg soil	108.50	28.00	16.29	98.63	30.82	30.60	156.10
T ₂ – <i>Pacilomyces lilacinus</i> @ 10 g/kg of soil	95.50	27.15	14.95	98.22	30.45	26.83	137.11
T ₃ – <i>Trichoderma viride</i> @ 10 g/kg of soil	103.00	27.89	16.00	98.52	30.51	32.54	157.15
T ₄ – <i>Pseudomonas fluorescens</i> @ 10 g/kg of soil	132.60	28.91	17.45	104.81	32.79	33.99	171.14
T ₅ – Carbosulfan 25 ST @ 3%	142.30	35.04	20.06	125.42	38.10	34.55	187.39
T ₆ – Neem seed kernel powder @ 5 g/kg + <i>P. lilacinus</i> @ 10 g/kg of soil	128.00	32.00	18.60	112.54	34.98	37.46	191.05
T ₇ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg of soil	136.50	34.00	19.55	118.63	34.50	36.84	175.00
T ₈ – NSKP @ 5 g/kg + <i>P. fluorescens</i> @ 10 g/kg of soil	129.00	32.67	19.18	120.80	34.83	32.88	178.73
T ₉ – <i>P. lilacinus</i> @ 10 g/kg + <i>T. viride</i> @ 10 g/kg of soil	102.10	30.00	17.00	104.00	32.50	32.36	165.04
T ₁₀ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg + <i>P. lilacinus</i> @ 10 g/kg of soil	145.20	34.20	19.93	113.38	37.45	39.06	195.30
T ₁₁ – Carbosulfan 25 ST @ 3% + Vitavax Power @ 0.2%	192.60	37.40	23.21	136.80	40.46	40.44	218.39
T ₁₂ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg + <i>P. lilacinus</i> @ 10 g/kg + <i>P. fluorescens</i>	212.00	40.33	33.80	146.25	48.34	46.42	241.31
T ₁₃ – Uninoculated Control	216.50	41.55	34.10	152.69	47.33	51.63	250.00
T ₁₄ – Inoculated control	76.20	25.63	12.64	90.50	28.06	10.13	51.66
Mean	137.14	32.48	20.19	115.79	35.79	34.69	176.81
SEM±	6.18	0.97	3.11	0.96	2.51	0.65	7.68
CD @ 1%	19.04	2.95	9.31	2.99	7.53	2.08	22.90

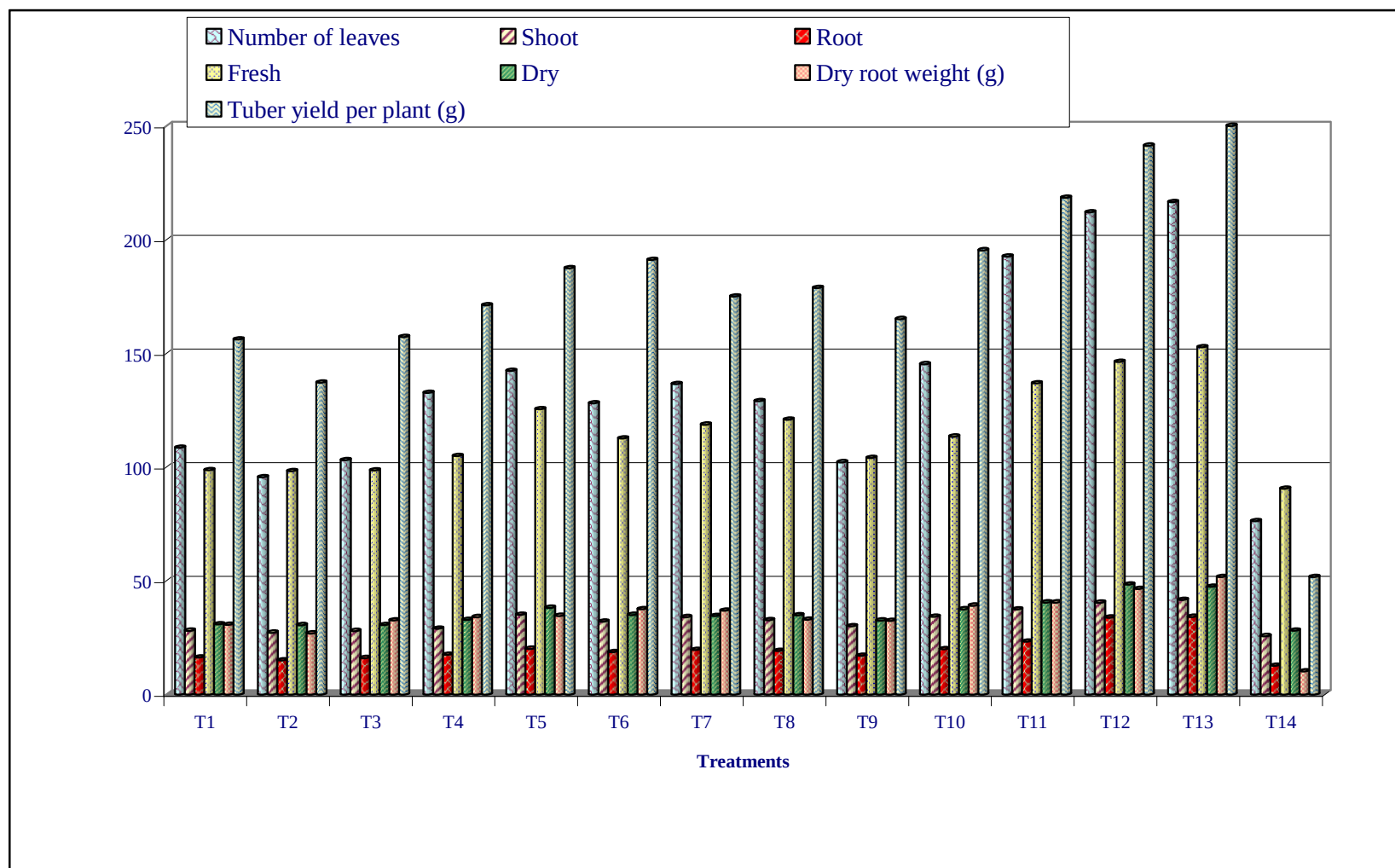


Fig. 5: Effect of different plant products, bioagents and chemicals on growth parameters of *Coleus forskohlii* infected by *M. incognita* and *F. chlamydosporum*

4.6.3 Root length (cm)

Among individual treatments, Carbosulfan (T₁₀), which showed maximum root length (20.06 cm) followed by *Pseudomonas fluorescens* (T₄) (17.45 cm) and they were on par with each other.

Minimum root length was recorded in *Trichoderma viride* (T₃) (14.95 cm) followed *Paecilomyces lilacinus* (T₂) (16.00 cm) and neem seed kernel powder (T₁) (16.29 cm) which were on par to each other.

In case of combination treatments NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂), which showed maximum root length of (24.20 cm) followed by Carbosulfan + Vitavax Power (T₁₁) (23.21 cm) and NSKP + *Trichoderma viride* (T₇) (19.93 cm) and they were on par to other.

Minimum root length was noticed in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (17.00 cm) followed by NSKP + *Paecilomyces lilacinus* (T₆) (18.60 cm), NSKP + *Pseudomonas fluorescens* (T₈) (19.18 cm) and NSKP + *Trichoderma viride* (T₇) (19.55 cm) and they were statistically on par to each other.

4.6.4 Fresh shoot weight (g)

Among individual treatments Carbosulfan (T₅) recorded maximum fresh shoot weight of 125.42 followed by *Pseudomonas fluorescens* (T₄) (104.81 g) and they were significantly differed over inoculated control.

Minimum shoot weight was recorded in *Paecilomyces lilacinus* (T₂) (98.22 g), followed by *Trichoderma viride* (T₃) (98.52 g) and neem seed kernel powder (T₁) (98.63 g) and these treatments were on par to each other.

Among combination treatments, NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) recorded maximum shoot weight (146.25 g) followed by Carbosulfan + Vitavax Power (T₁₁) (136.80 g) and they differed significantly over inoculated control.

Minimum shoot weight recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (104.00) followed by NSKP + *Paecilomyces lilacinus* (T₆) (112.54 g) and NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* (T₁₀) (113.38 g) and these treatments were on par to each other.

4.6.5 Dry shoot weight (g)

Among individual treatments Carbosulfan (T₅) showed maximum shoot weight (38.10 g) followed by *Pseudomonas fluorescens* (T₄) (32.79 g) and these treatments were on par each other.

Minimum shoot weight was recorded in *Paecilomyces lilacinus* (T₂) (30.45 g) followed by *Trichoderma viride* (T₃) (30.51 g) and NSKP (T₁) (30.82 g) and they were on par with each other.

With respect to combination treatments, NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) recorded maximum shoot weight (45.34 g) followed by Carbosulfan + Vitavax Power (T₁₁) (42.46 g) and NSKP + *Paecilomyces lilacinus* + *Trichoderma viride* (T₁₀) (37.45 g) and they were on par with each other.

Minimum dry shoot weight was recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (32.50 g) followed by NSKP + *Trichoderma viride* (T₇) (34.50 g), NSKP + *Pseudomonas fluorescens* (T₈) (34.83 g) and NSKP + *Paecilomyces lilacinus* (T₆) (34.98 g) and these treatments were on par with each other.

4.6.6 Dry root weight

With respect to individual treatments, Carbosulfan (T₅) showed highest dry root weight of 34.55, followed by *Pseudomonas fluorescens* (T₄) (33.99 g) and *Trichoderma viride* (T₃) (32.54 g) and these treatments were on par with each other.

Minimum dry root weight was noticed in *Paecilomyces lilacinus* (T₂) (26.83 g), followed by NSKP (T₁) (30.60 g) and these treatments significantly differed over inoculated control.

With respect to combination treatments, NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) showed maximum dry shoot weight of 46.42 cm followed by Carbosulfan + Vitavax Power (T₁₁) (40.44 g), NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* (T₁₀) (39.06 g) and NSKP + *Paecilomyces lilacinus* (T₆) (37.46 g) and these were on par with each other. Minimum dry root weight was recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (32.36 g) followed by NSKP + *Pseudomonas fluorescens* (T₈) (32.88 g) and these treatments were on par with each other.

4.6.7 Tuber yield per plant

Among the individual treatments, Carbosulfan (T₅) showed significantly maximum tuber yield per plant (187.39 g) followed by *Pseudomonas fluorescens* (T₄) (171.14 g) and *Trichoderma viride* (T₃) (157.15 g) and these were on par with each other.

Minimum tuber yield per plant recorded in *Paecilomyces lilacinus* (T₂) (137.11 g) followed by NSKP (T₁) (156.10 g) and these were on par with each other.

With respect to combination treatments, NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* + *Pseudomonas fluorescens* (T₁₂) recorded significantly highest tuber yield per plant (241.31 g) followed by carbosulfan + Vitavax Power (T₁₁) (218.39 g) and NSKP + *Trichoderma viride* + *Paecilomyces lilacinus* (T₁₀) (195.30 g) and these treatments were on par with each other.

Minimum tuber yield per plant was recorded in *Paecilomyces lilacinus* + *Trichoderma viride* (T₉) (165.04 g), followed by NSKP + *Trichoderma viride* (T₇) (175.00 g) and NSKP + *Pseudomonas fluorescens* (178.73 g) and these treatments were statistically on par with each other.

4.7 Effect of plant products, bioagents and chemicals on number of galls, egg masses, root-knot index, root rot index and nematode population

4.7.1 Number of galls per 5 g of root

Among individual treatments, Carbosulfan T₅ (10.68) recorded lower number of galls followed by *Pseudomonas fluorescens* T₄ (18.13) and neem seed Kernal powder T₁ (19.68). These treatments differed significantly over inoculate control. However, Neem seed kernel powder T₁ (19.68), *Trichoderma viride* T₃ (20.13) and *Pseudomonas fluorescens* T₄ (18.13) which were on par with each other. With respect to combination treatments, NSKP+ *T. viride* + *P. lilacinus* + *P. fluorescens* T₁₂ (7.38) recorded lowest number of galls followed by Carbosulfan + Vitavax Power T₁₁ (9.68) and NSKP + *T. viride* + *P. lilacinus* T₁₀ (12.13) these treatments differ among themselves. Treatments NSKP + *P. lilacinus* T₆ (24.68), NSKP + *P. florascens* T₈ (23.80), *P. lilacinus* + *T. viride* T₉ (26.00) were on par with each other. However, significantly highest number of galls per 5 g of root recorded in linoculated Control T₁₄ (105.13) (Table 8 and Fig. 6).

4.7.2 Root-knot index

With respect to individual treatments Carbosulfan T₅ (2.86) recorded lowest root-knot index followed by *Trichoderma viride* T₃ (3.00) and *Pseudomonas fluorescens* T₄ (3.13) and

Table 8: Effect of different plant products, bioagents and chemicals on number of galls, number of egg masses, root-knot index, nematode population and root rot index

Treatments	Number of galls per root	Number of egg masses per root	Root-knot index	Nematode population per 200 cc soil	Per cent reduction over control	Root rot index
T ₁ – Neem seed kernel powder @ 5 g/kg soil	19.68	46.84	3.70	2510.40	64.18	4.23
T ₂ – <i>Pacilomyces lilacinus</i> @ 10 g/kg of soil	32.38	45.68	4.00	1986.20	71.66	4.50
T ₃ – <i>Trichoderma viride</i> @ 10 g/kg of soil	20.13	60.00	3.00	2775.60	60.40	2.84
T ₄ – <i>Pseudomonas fluorescens</i> @ 10 g/kg of soil	18.13	49.03	3.13	2168.00	69.07	2.30
T ₅ – Carbosulfan 25 ST @ 3%	10.68	43.16	2.86	1736.30	75.23	4.69
T ₆ – Neem seed kernel powder @ 5 g/kg + <i>P. lilacinus</i> @ 10 g/kg of soil	24.68	50.22	3.74	2010.50	71.31	4.10
T ₇ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg of soil	10.13	65.42	2.83	2022.00	71.15	2.42
T ₈ – NSKP @ 5 g/kg + <i>P. fluorescens</i> @ 10 g/kg of soil	23.80	80.61	3.00	2130.00	69.61	2.13
T ₉ – <i>P. lilacinus</i> @ 10 g/kg + <i>T. viride</i> @ 10 g/kg of soil	26.00	51.13	3.13	2079.70	70.33	3.90
T ₁₀ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg + <i>P. lilacinus</i> @ 10 g/kg of soil	12.13	43.60	3.70	1940.30	72.32	2.36
T ₁₁ – Carbosulfan 25 ST @ 3% + Vitavax Power @ 0.2%	9.68	58.96	2.76	2066.80	70.51	1.33
T ₁₂ – NSKP @ 5 g/kg + <i>T. viride</i> @ 10 g/kg + <i>P. lilacinus</i> @ 10 g/kg + <i>P. fluorescens</i>	7.38	39.30	2.70	1650.00	76.46	1.80
T ₁₃ – Uninoculated Control	0.00	0.00	0.00	0.00	0.00	0.00
T ₁₄ – Inoculated control	105.13	88.00	5.00	7010.00	-	5.00
Mean	22.85	51.56	3.11	2291.84	-	2.97
SEM+	0.99	0.83	0.16	31.60	-	0.10
CD @ 1%	2.98	2.53	0.49	94.48	-	0.30

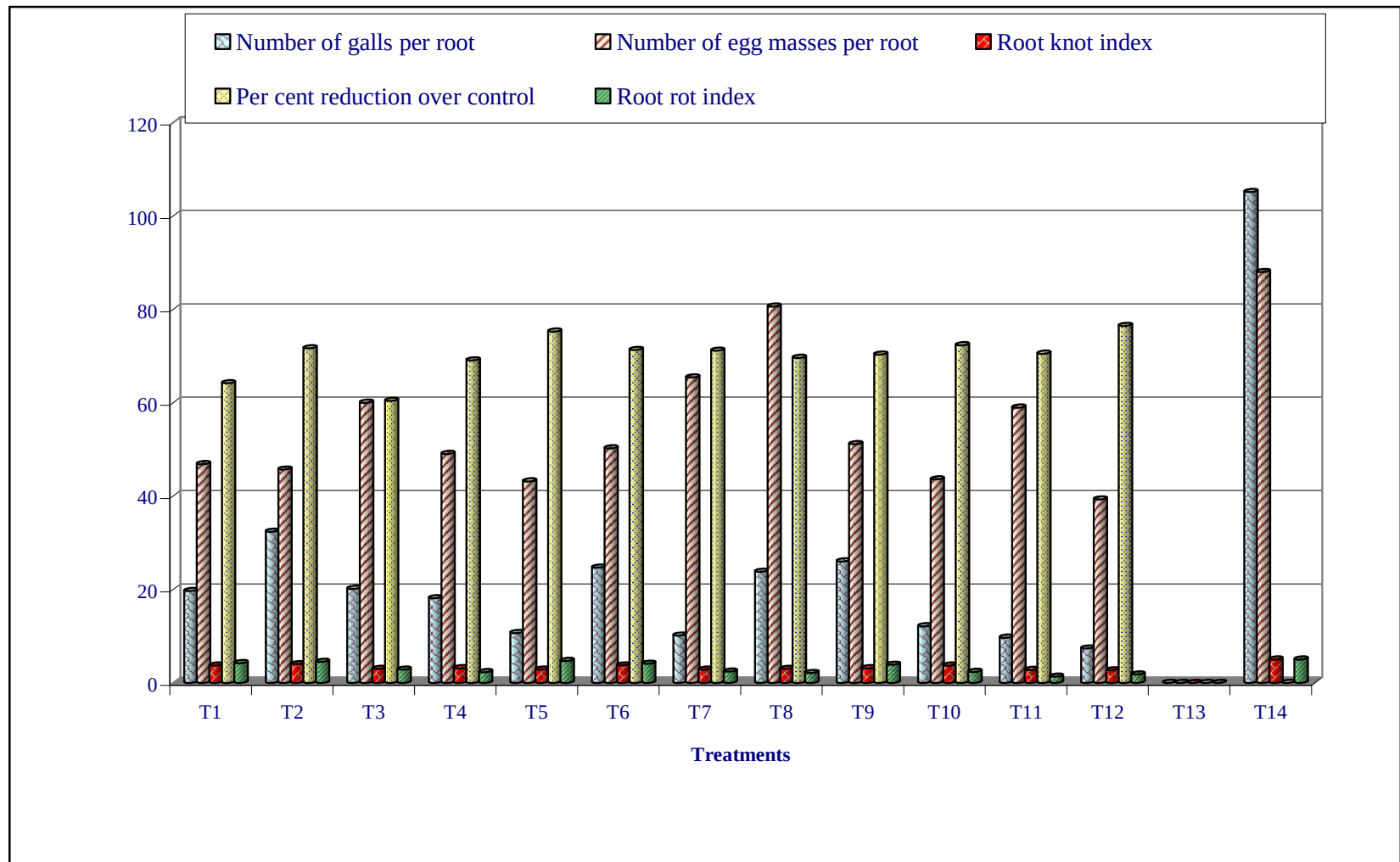


Fig. 6: Effect of different plant products, bioagents and chemicals on number of galls, number of egg masses, root knot index and root rot index

these treatments differ significantly over inoculated control. However, treatments, *Trichoderma viride* T₃ (3.00) and *Pseudomonas fluorescens* T₄ (3.13) which were on par with each other.

Among combination treatments, lowest root-knot index was recorded in NSKP + *T. viride* + *P. lilacinus* + *P. fluorescens* T₁₂ (2.70) followed by Carbosulfan + Vitavax Power T₁₁ (2.76). However NSKP + *P. fluorescens* T₈ (3.00) and *P. lilacinus* + *T. Viride* T₉ (3.13) which were on par with each other.

However significantly highest root-knot index recorded in Inoculated Control T₁₄ (5.00) treatment.

4.7.3 Number of egg masses per root

Among individual treatments, number of egg masses/root significantly lowest in treatment Carbosulfan T₅ (43.16), followed by *Paecilomyces lilacinus* T₂ (45.68) and Neem seed kernel powder T₁ (46.84). These treatments differed significantly over inoculated control. However, Neem Seed kernel powder T₁ (46.84), *Paecilomyces lilacinus* T₂ (45.68) and *Pseudomonas fluorescens* T₄ (49.03) were on par with each other. Significantly highest number of egg masses per root (88.00) was recorded in T₁₄ (control) as compared to other treatments.

With respect to combination treatments, number of egg masses per root was significantly highest in control T₁₄ (88.00), whereas significantly lowest in NSKP + *T. viride* + *P. lilacinus* + *P. fluorescens* T₁₂ (39.30), followed by NSKP + *T. viride* + *P. lilacinus* T₁₀ (43.60) these treatments differed significantly over inoculated control. Whereas, treatments NSKP + *P. lilacinus* T₆ (50.22), *P. lilacinus* + *T. viride* T₉ (51.13) were on par with each other.

4.7.4 Population of root-knot juveniles per 200 cc of soil

With respect to individual treatments, significantly lowest number of juveniles were recorded in treatment Carbosulfan T₅ (1736.30), followed by *Paecilomyces lilacinus* T₂ (1986.20), *Pseudomonas fluorescens* T₄ (2168.00) and these treatments differed among themselves.

Among combination treatments, the number of juveniles per 200 cc of soil was significantly lowest in NSKP + *T. viride* + *P. lilacinus* + *P. fluorescens* T₁₂ (1650.00), followed by NSKP + *T. viride* + *P. lilacinus* T₁₀ (1940.30), NSKP + *P. lilacinus* T₆ (2110.50). These treatments differed significantly over inoculated Control.

However treatments NSKP + *P. lilacinus* T₆ (2010.50), NSKP + *T. viride* T₇ (2022.00) and *P. lilacinus* + *T. viride* T₉ (2079.70) on par with each other. Significantly higher number of juveniles per 200cc of soil is recorded in T₁₄ Control (7010.00).

4.7.5 Root rot index

Among individual treatments, *Pseudomonas fluorescens* T₄ (2.30) recorded lowest root rot index followed by *Trichoderma viride* T₃ (2.84) and these treatments differed significantly over Inoculated control. Where as, treatments Neem seed kernel powder T₁ (4.23), *Paecilomyces lilacinus* T₂ (4.50) and Carbosulfan T₅ (4.69) were on par with each other.

With respect to combination treatments, treatments Carbosulfan + Vitavax Power T₁₁ (1.33) recorded lowest root rot index, followed by NSKP + *T. viride* + *P. lilacinus* + *P. fluorescens* T₁₂ (1.80) and NSKP + *P. fluorescens* T₈ (2.13) these treatments differ significantly over Inoculated control. However, treatments NSKP + *T. viride* T₇ (2.42), NSKP + *P. fluorescens* T₈ (2.13), NSKP + *T. viride* + *P. lilacinus* T₁₀ (2.36) were on par with each other. However, significantly highest root rot index (5.0) was recorded in T₁₄ (control) as compared to other treatments.

5. DISCUSSION

In the soil ecosystem, plants are constantly exposed to multiple variety of organisms, many of which are common components of the soil biosphere. As they occupy the same environmental niche, such organisms besides influencing the plants are likely to influence each other as well. According to Fawcett (1931), nature does not work with pure cultures and several plant diseases are affected by associated organisms which results in disease syndromes of complex nature. Many plant parasitic nematodes involve themselves in diseases caused by fungi, bacteria and viruses and indirectly contribute to the losses attributed to the better known pathogen with nematodes, none are more damaging to the crops worldwide than that caused by the combined effects of wilt inducing fungi and plant parasitic nematodes. The combinations of nematodes and fungus often results in synergistic interaction wherein, the crop loss is greater than expected from either of the pathogens alone or an additive effect of the two together (Franci and Wheeler, 1993).

Out of several nematodes of economic importance, root-knot nematodes are the most widely studied and are commonly found involved in synergistic interactions with wilt inducing fungi. In *Coleus forskohlii* association of *Fusarium chlamydosporum*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* with *Meloidogyne incognita* was noticed. Association of a single fungus with *Meloidogyne incognita* or combination of two or more fungi with *Meloidogyne incognita* was also observed.

A study was, therefore, carried out with the objectives viz., 1) To survey the incidence of root-knot and wilt disease complex in Northern Karnataka 2) To study the effect of interaction between *Meloidogyne incognita* and *Fusarium chlamydosporum* in *Coleus* 3) To screen different genotypes of *Coleus* for their resistance to *Meloidogyne incognita* and predominant fungi *Fusarium chlamydosporum* and 4) Management of root-knot and wilt disease complex using plant products, bioagents and chemicals.

5.1 Survey for incidence of root-knot and wilt disease complex in Northern Karnataka

In general, root-knot disease caused by *Meloidogyne* spp. is one of the major constraints in the productivity of several crops. Very little information was available with regard to the incidence and severity of the disease on *Coleus* in Northern Karnataka. Hence, a survey was undertaken to assess the root-knot and wilt disease complex on *Coleus* crop in Bagalkot, Belgaum, Bidar, Bijapur, Dharwad, Gulbarga districts.

The findings of the present study revealed that root-knot caused by *Meloidogyne incognita* was observed in all the districts surveyed. But, overall incidence was more in Belgaum district (32.08%), followed by Bagalkot (23.80%) and Bijapur districts (23.00%).

However, earlier surveys in Northern Karnataka revealed that presence of *Meloidogyne* spp. with an average disease incidence of 34.50 per cent in some areas (Ramprasad Shresthi, 2005).

The findings of the present study revealed that high root-knot nematode population was recorded in Kakamari (Belgaum district) followed by Bidar and Bijapur districts.

The present survey also indicated that associations of *Meloidogyne incognita* with fungi namely *Fusarium chlamydosporum*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* in most of locations surveyed with a high frequency of occurrence of both the pathogens (*Meloidogyne incognita* and *Fusarium chlamydosporum*) from soil and root samples collected from Belgaum district. Similar observations were also made in survey conducted by Krishna Rao and Krishnappa (1994) on chickpea in Karnataka. Pandey and Singh (1990) from Allahabad on chick pea. Sharma and McDonald (1990) from parts of the world also reported the associations of nematode fungi in legumes and cereals, respectively.

5.1.1 Isolation of pathogens associated with disease complex in *Coleus*

The most characteristics feature having taxonomic importance in case of females of *Meloidogyne* species is the presence of pereneal pattern in the posterior body region. This area comprised the tail terminus, plasmids, lateral lines, anus and vulva surrounded by cuticular folds or striae. The pereneal patterns of individuals and populations within species slightly vary, but basic species characteristics do not change significantly over an extended period of culturing and in no case, do pattern characteristics change from one species to those of another.

The perennial pattern of the prevailing root-knot nematode species from parts of Northern Karnataka characteristically showed high squarish dorsal arch, absence of lateral ridges in lateral field marked by breaks and forks in striae. Striae were coarse, smooth to wavy and the tail terminus contained distinct whorl (Table 2). These findings are in confirmation with those mentioned by Eisenback *et al.* (1981) for the root-knot nematode *Meloidogyne incognita*. By studying pereneal patterns of these populations which showed high squarish dorsal arch, zig zag striae around the vulva. It was seen that these characters were similar to those of *Meloidogyne incognita*. Thus, prevailing pathogens was identified as *Meloidogyne incognita*.

The causal organism *Fusarium chlamydosporum* was isolated from diseased plants of *Coleus* by following standard tissue isolation method. The pure culture obtained was sub-cultured on petri plates and slants containing *Fusarium* specific media and stored in refrigerator for further studies. Sachidananda (2005), Ramaprasad Shresti (2005), Bobby and Bagyaraj (2003) and Shyla (1998) were also isolated *Fusarium chlamydosporum* associated with wilt of *Coleus*.

5.1.2 Effect of different inoculum levels of the root-knot nematode on *Coleus forskohlii*

Effect of different inoculum levels of *Meloidogyne incognita* on shoot height, shoot and root weight and tuber yield revealed that, there was positive relationship between the initial inoculum levels of *Meloidogyne incognita* and reduction in shoot height, shoot and root weight and tuber yield. Maximum reduction in corresponding parameters was recorded at the highest inoculum levels (10,000 J₂) as compared to uninoculated control. These results are in conformation with Patnaik and Das (1986). Similarly, highest nematode population was increased up to 100J₂ per plant. While it decreased at higher levels of 5000 or 10000 J₂ per plant. This might be attributed to the competition among the nematode juveniles. These results are in conformity with Raut and Sethi (1980) in soybean infested with *Meloidogyne incognita*.

5.2 Interaction between *Meloidogyne incognita* and *Fusarium chlamydosporum*

Nematode and fungi cause severe damage to *Coleus*. However, the effect on plant growth and wilt incidence increased when both pathogens were present together, Increased incidence in the presence of nematode on *Coleus* was also reported by Senthamarai *et al.* (2006b), Marimuthu (1991) on betel vine, Krishna Rao and Krishnappa (1994) on chickpea.

Plants receiving simultaneous inoculations of *M. incognita* and *Fusarium chlamydosporum* showed the greatest reductions in plant growth parameters as compared to other treatments. These findings are in conformity with those recorded by Bhabesh Bhagawati *et al.* (2007) who suggested that the greater damage in simultaneous inoculation of fungus and nematode might be attributed to the prior invasion of nematodes into the roots, thereby making the host more favourable for fungal infection by offering a metabolically rich substrate and/or nematode might also modify the rhizosphere, thus favouring the fungal growth.

Similar results were also obtained by Patel *et al.* (2000) on Chick pea, Mahapatra and Swain (2001) on Black gram and Akhtar Haseeb *et al.* (2007) on Pea. Senthamari *et al.*

(2006b) also recorded similar reduction in growth parameters of Coleus where as, *M. Incognita* and *M. phaseolina* were present together.

Number of galls per plant, juvenile population in soil and root were maximum in nematode alone treatment, but were significantly less in other treatments receiving fungi. However, the treatment receiving concomitant inoculation of *M. incognita* and *F.chlamydosporum* recorded significantly lowest number of galls and nematode population over other treatments.

The reduction in galling and nematode population could be possibly attributed to deleterious effects of metabolites of *Fusarium chlamydosporum* on the juveniles of root-knot nematode. This is further supported by greater reductions when fungi and nematodes were inoculated simultaneously. These results are in conformity with the results recorded by Akhtar Haseeb *et al.* (2007), who reported that reduction of nematode population build up, number of galls might be due to reduced root system. Thus, the nematode faced competition for food. In addition to fungal disruption of nematode feeding sites, plants affected by disease complex may be more prone to early senescence and death which in turn might prevent nematodes from completing life cycles leading to reduced reproduction. Similar results were reported by Mani and Sethi (1987), Patil *et al.* (2000) and Senthamarai *et al.* (2006b).

5.3 Screening of popular Coleus genotypes for their reaction to *Meloidogyne incognita* and *Fusarium chlamydosporum*

In view of the long range programmes in management of complex diseases based on the use of resistant cultivars, present study helps in identifying promising cultivars having some degree of resistance/ tolerance to the nematode and/or fungus disease complex.

Eleven genotypes were tested for their reaction to *M. incognita* and *F. chlamydosporum* together under glasshouse condition. Among 11 genotypes, yellow tubers showed maximum shoot and root length. Whereas, minimum shoot and root length was noticed in Sunadolli local.

Yellow tubers which showed least number of galls, root-knot index, nematode population, as compared to other genotypes. With respect to root rot index minimum root rot index observed in genotype like Rabakavi local and yellow tubers.

Among 11 genotypes, yellow tuber and Rabakavi local genotypes showed resistant reaction, Orange tuber and Nimbanur local genotypes showed moderately resistant reaction. Remaining seven genotypes showed susceptible reactions. But the Yellow tuber is highly susceptible to Mealy bug infestation.

Caperton *et al.* (1986) found that interaction between *Fusarium chlamydosporum* and *Meloidogyne incognita* on summer squash cultivar is specific where root-knot nematode induced the susceptibility to *Fusarium* wilt only in limited number of cultivars. The present study indicated that the ability of nematode to induce wilt susceptibility was not uniformly consistent. This is the first report on screening of popular genotypes of Coleus to *M. incognita* and *F. chlamydosporum* in glasshouse conditions.

5.4 Management of root-knot and wilt disease complex by using plant products, bioagents and chemicals

Protection of crop plants from disease causing agents has been the focal point of the scientists in dealing with plant pathogens. In depth analysis and realization about the starting features of microbial ecology have impelled workers to replace the term 'control' with 'management'. No single method of management has given a lasting solution. Prohibitive costs of chemicals and their adverse ecological impacts of major compulsion invariably ask for diversions of research priorities from chemical method to other alternatives. The successive shifting of priorities from chemical to cultural and currently to integrated strategies demonstrates the elasticity of the scientific ideas (Nagarajan, 1990; Khan and Parvatha Reddy, 1993).

Integrated management systems constitutes best management strategies and tactics for the disease complex at hand (Brodie, 1970 and Khan and Parvatha Reddy, 1993). There is hardly any comprehensive effort of highlight the significance of multi-pathogenic scenario vis-a-vis their integrated management.

The results of the present study clearly indicated that with respect to individual treatments, Carbosulfan 25 ST at 3 per cent as a soil application found to increase the plant growth parameters like number of leaves, shoot and root length. Fresh and dry shoot weight, fresh and dry root weight, tuber yield per plant. It was followed by *Pseudomonas fluorescens* (10 g/kg) as soil treatment.

Significant and improved growth parameters may be attributed to the increased activity of phytohormones. Similar increase in growth with Carbosulfan (25 ST) at 3 per cent and neem seed kernel powder at 10 per cent in gladiolus was observed by Ravishankar and Singh (2005) and Ali (1994) reported that Carbosulfan 4 per cent as seed treatment gave maximum shoot length, shoot weight, root weight and root length in chickpea against *Meloidogyne incognita*.

In the present study, carbosulfan 25 ST at 3 per cent as soil treatment recorded minimum number of galls, root-knot index, and nematode population, number of egg masses. These findings are in conformity with the findings of Narayana and Prasad (1999) and Hong and Sethi (1988) who also reported carbosulfan to be most effective chemical in reducing the root-knot galls and juvenile population of *Meloidogyne incognita* on sunflower and French bean, respectively.

The reduction in the number of juvenile population may be due to reduction in root invasion and development. This may be due to systemic action of chemicals in the plant altering the juvenile behaviour in terms of orientation towards roots and root penetration. Senthamarai *et al.* (2006c) also reported *Pseudomonas fluorescens* is best soil application followed by *Trichoderma viride*.

With respect to combination treatments, neem seed kernel powder 5% (w/w) + *Paecilomyces lilacinus* 10 g per kg + *Trichoderma viride* 10 g per kg + *Pseudomonas fluorescens* 10 g per kg significantly improved the plant growth parameters, followed by Carbosulfan 25 ST @ 3 per cent + Vitavax Power @ 0.2 per cent as drenching.

Similar increase in growth parameters was observed in combination of *Paecilomyces lilacinus*, *T. harzianum* and neem cake in pointed gourd (Verma *et al.*, 2005). The reduction in growth parameters due to antinematode effect of neem cake might be attributed to the phenol compounds released during its degradation and the fungus. *P. lilacinus* has been reported to produce peptidic antibiotic viz., lilacin, paecilotoxin and *T. harzianum* is potential biocontrol agent which poses growth hormone and all these combinations resulted in improved growth parameters. Pant and Pandey (2002) also reported better growth of chickpea amended with neem cake + *T. harzianum*.

In the present study, combination of NSKE 5 g per kg + *Paecilomyces lilacinus* 10 g per kg + *Trichoderma viride* 10 g per kg + *Pseudomonas fluorescens* 10 g per kg as soil application recorded lowest number of galls, root-knot index, root rot index, number of egg masses and nematode population compared to other treatments. These findings are conformity with those Tripathi and Singh (2006), who also reported combination of fungal biocontrol agents along with oil cakes is an ideal for controlling nematode population and increasing plant growth. Ramaprasad Shresti (2005) also reported application of *Trichoderma viride* + nemato for controlling wilt incidence, nematode population of root-knot nematode and number of galls on *Coleus forskohlii* under field conditions. Similar results were obtained by Spiegel and Chet (1998). They reported suppression of root-knot nematode with application of *Trichoderma* species. Krishna Rao (1994) also reported the efficacy of integration of physical, chemical and biological methods on nematode fungal complex of chickpea.

Field evaluation of fungicides by several workers have shown that carbendazim or propiconazole as soil drench was effective against *Fusarium* spp. on many crops (Mishra and Ghosh, 1978; Tarabeih and Attarackehi, 1979 and Pal and Chowdhary, 1983).

However, treatment receiving combination of NSKP, *Paecilomyces lilacinus*, *Trichoderma viridae* and *Pseudomonas fluorescens* significantly managed wilt disease complex compared to either individual or combination of plant products, bioagents and chemicals.

5.5 Future line of work

During the present investigation certain thrust areas have been identified which could be taken up for future investigation.

1. To conduct systematic and comprehensive surveys of all the Coleus growing areas of Karnataka for wilt disease complex and estimation of their economic importance and involvement of bacterial pathogen.
2. Detailed studies on the influence of various agroecological factors on the distribution and incidence of disease complex.
3. To understand the genetic basis of mechanism of interaction, physiological and histological changes in the disease complex.
4. Characterization of translocatable metabolites in nematode-fungal interaction and determination of their biochemical nature and mode of action and specificity.
5. Further studies on interaction of several other methods of management and exploring the indigenous biocontrol agents.
6. Detailed studies with respect to population variation of both nematode and fungus in relation to different agro-climatic conditions.

6. SUMMARY AND CONCLUSIONS

Coleus forskohlii is an important medicinal plant, Forskolin content of dry tubers varies from 0.01 to 0.59 per cent. Among the different diseases affecting coleus, root-knot and wilt disease complex caused by *Meloidogyne incognita* and *Fusarium chlamydosporum* observed in severe form. The information available on this disease as well as pathogens involved are very meager. In India, research on this disease and pathogen is virtually a non-starter. Hence, it demanded an early investigation into the various aspects of the pathogen and also the disease.

The present investigation included different studies viz., survey for the disease incidence in Northern Karnataka, isolation, proving the pathogenicity of nematode on coleus, interaction studies of the pathogens involved. Screening of popular genotypes of coleus against *Meloidogyne incognita* and *Fusarium chlamydosporum* complex, management of the disease.

Survey on the occurrence of root-knot and wilt disease complex in Gulbarga, Bijapur, Dharwad, Bidar, Belgaum and Bagalkot districts revealed 0.0 to 68.00 per cent root-knot nematode incidence. High density of root-knot nematode was noticed in Belgaum and Bagalkot districts.

The present survey also indicated the high density of *Fusarium* propagules from soil in Inchala and Badigwad (Belgaum dist.). The association of *Meloidogyne* with fungi namely *Fusarium chlamydosporum*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* in most of the locations surveyed with a high frequency of occurrence of *Meloidogyne incognita* and *Fusarium chlamydosporum* from soil and root samples collected from Belgaum district.

The prevailing *Meloidogyne* species infecting Coleus in Northern Karnataka was identified as *Meloidogyne incognita* based on perineal pattern studies. An initial inoculum level of 100 juveniles of *Meloidogyne incognita* per plant was found to be pathogenic to coleus and were responsible for the reduction in growth parameters of same and increase in nematode multiplication.

In the interaction studies, *Meloidogyne incognita* was the most aggressive pathogen compared to *Fusarium chlamydosporum*. However simultaneous inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum* caused greater reduction in plant growth as well as nematode multiplication. However, with respect to individual pathogen, *Meloidogyne incognita* caused greater reduction in plant growth compared to *Fusarium chlamydosporum*. In case of sequential inoculation of *Meloidogyne incognita* 7 days prior to *Fusarium chlamydosporum* caused reduction in plant growth parameters.

The effect of simultaneous inoculation of *Meloidogyne incognita* with *Fusarium chlamydosporum* of Coleus was additive in nature. However, when *Meloidogyne incognita* was inoculated with *Fusarium chlamydosporum* the resultant effect was almost equal to sum of individual effect.

These results suggest that the nematode can predispose the Coleus to infection by fungus namely *Fusarium chlamydosporum* and can aggravate the disease. Reduction in number of galls per plant and final nematode population was observed in simultaneous inoculation of nematode and fungi.

Studies on screening of different genotypes of Coleus against *Meloidogyne incognita* and *Fusarium chlamydosporum* disease complex showed that the genotypes viz., yellow tubers and Rabakavi local showed resistant reaction. Orange tubers and Nimbanur local genotype showed moderately resistant reaction and Manna E Kheli, Birur local, Gurlapur local, K-8, Yamakanamaradi, IIHR collection and Sunadolli local, showed susceptible reaction.

The genotype Yellow tubers was resistant to *M. incognita* and *F. chlamydosporum*, but highly susceptible for Mealy bug infestation.

In disease management study conducted in pot culture under glasshouse condition, it was found that a combined application of plant products (Neem seed kernel powder) with biocontrol agents (*Paecilomyces lilacinus*, *Trichoderma viride*, *Pseudomonas fluorescens*) was found effective in reducing the number of galls, nematode population, number of egg masses, root-knot index, root rot index and improving the plant growth parameters as compared to inoculated control.

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**STUDIES ON ROOT-KNOT AND WILT COMPLEX IN
Coleus forskohlii (Wild.) Briq. CAUSED BY
Meloidogyne incognita (Kofoid and White) Chitwood
AND *Fusarium chlamydosporum* (Frag. and Cif.)
Booth**

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2008

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ABSTRACT

Coleus forskohlii is subjected to be attacked by several diseases, among them Root-knot and wilt complex caused by *Meloidogyne incognita* and *Fusarium chlamydosporum* is the most important.

A survey on the occurrence of root-knot disease in northern district of Karnataka revealed zero to 68.00 percent root-knot incidence conducted during 2007-08. Survey also indicated the association of *Meloidogyne* spp. with fungi namely *Fusarium chlamydosporum*, *Sclerotium rolfsii* and *Rhizoctonia bataticola* in most of locations surveyed with high frequency of occurrence of *Meloidogyne incognita* and *Fusarium chlamydosporum* from soil and root samples collected from Belgaum district.

In interaction studies, simultaneous inoculation of *Meloidogyne incognita* and *Fusarium chlamydosporum* cause a greater reduction in plant growth parameters as well as nematode multiplication. However, individually, *Meloidogyne incognita* which caused greater reduction in plant growth parameters as well as nematode multiplication compared to *Fusarium chlamydosporum*. In case of sequential inoculation of *Meloidogyne incognita* seven days prior to *Fusarium chlamydosporum* caused reduction in plant growth parameters.

Genotypes viz., yellow tubers and Rabakavi local showed resistant reaction. Orange tubers and Nimbanur local genotype showed moderately resistant reaction and MannaEKhalli, Birur local, Gurlapur local, K-8, Yamakanamaradi, Local IIHR collection and Sunadolli local showed susceptible reaction against *Meloidogyne incognita* and *Fusarium chlamydosporum*.

In the management study in pot culture, combined application of plant product (Neem seed kernel powder @ 5 g/kg of soil) + biocontrol agents (*Paecilomyces lilacinus*, *Trichoderma viride* + *Pseudomonas fluorescens* @ 10 g/kg of soil respectively) was found effective in reducing the number of galls, nematode population, number of egg masses, root-knot index, root-rot index and improving the plant growth parameters when compared to inoculated control.