STUDIES ON COLLAR ROT COMPLEX OF Coleus forskohlii (Wild.) Briq.

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I. INTRODUCTION

India is one of the twelve mega biodiversity centers in the world with a wealth of 8000 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 obtained from these plants such as phytochemical, steroidal, biologically active compounds, alkaloids, etc. is increasing in the national and international market.

According to a report there was about US $ 62 billion sales of herbal medicines in the world and it is expected to increase upto US $ 3 trillion by 2020. Exports from India have increased from Rs. 460 crores in 1995 to 1200 crores in 2000. (Ghosh, 2000). 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 [Syn. *C. barbatus* (Andr.) Benth.] is a plant of Indian origin (Valdes *et al*. 1987) and belongs to the family Lamiaceae (previously Labiatae). It is the most important species of genus *Coleus* popularly known as *Mainamool* or *Manganiberu* or *Makandi beru* in Karnataka and *garmar* in Maharashtra. It is distributed in sub-tropical Himalayas from Gharwal to Nepal up to an altitude of 2500 m above mean sea level including Pakistan and Sri Lanka. Traditionally, the roots have been used for preparation as condiments in pickles and preparation of pickles (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 curing cough in Kumaon Himalayas and one to three teaspoonful 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 by the natives of Kumaon Himalayas.

In India the crop is cultivated in the parts of Gujarat, Maharashtra, Rajasthan, Karnataka and Tamil Nadu and is being grown in an area of more than 2500 hectares for its tuberous roots.

Extensive investigations of the pharmacological effect of *C. forskohlii* led to the isolation of a diterpene active principle called forskolin (Bhat *et al.,* 1977). The therapeutic properties of forskolin are utilized in treating cardiac insufficiency, hypertension, glaucoma, thrombosis, asthma and metastatic conditions (Seamon, 1984). Pharmacological and biochemical investigations established that forskolin possesses multifaceted biological activities such as positive inotropic, antihypertensive, branchospasmolytic, anti-thrombatic, platelet aggregation inhibiting, antiglaucoma, adenylate cyclase stimulation (Rupp *et al.,* 1986). 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 medicinally and economically important.

It is said that the Indian herb *C. forskohlii* is the only known source of forskolin (De souza and Shah, 1988). Though almost all the plant parts are found to have traces of forskolin, the roots are the main source possessing 0.1 to 0.5 per cent and preferred for its extraction (Valdes *et al.,* 1987).

The pharmaceutical industries are mainly dependent upon the wild population of the plant for the supply of tuberous roots for forskolin extraction. The large scale and indiscriminate collection of the wild material from the forests and inadequate attempts either to allow its replenishment or its cultivation has led to *C. forskohlii* being listed as endangered species (Gupta, 1988, Vishwakarma *et al.,* 1988).

The crop is subjected to attack by many diseases *viz.,* leaf spot caused by *Botryodiplodia theobromae,* stem blight caused *Phytophthora nicotianae* var. *nicotianae,* bacterial root rot caused by *Pseudomonas aeruginosa,* collar rot complex by root knot nematode i.e. *Meloidogyne incognita, Sclerotium rolfsii, Fusarium chlamydosporum* and

Rhizoctonia bataticola. Among these collar rot complex is occurring in severe form and causes heavy losses.

Several pathogens have been reported from different places causing root rot of *C. forskohlii.*

Shyla (1998) and Boby and Bagyaraj (2003) reported that, the fungus *Fusarium chlamydosporum* causes root rot of *C. forskohlii.* Kamalakannan (2003) reported the occurrence of pathogens like *Rhizoctonia solani* and *Macrophomina phaseolina* on root rot of *Coleus forskohlii.*

Though the disease has assumed importance in India and Karnataka, no detailed work has been carried out on collar rot complex of *C. forskohlii.*

In view of this it was felt necessary to study the cultural, nutritional and physiological aspects of pathogens.

Biological control is a potential non-chemical means for plant disease management by reducing the harmful effects of a parasite or pathogen through the use of other living entities. Since the disease is caused by soil borne fungus, soil antagonists may play a significant role in reducing the inoculum level in the soil. Hence, it was found necessary to test the antagonistic microflora from the amended soil against *F. chlamydosporum, R. bataticola* and *S. rolfsii.* The easiest and quickest way to keep pace with the pathogen and to manage the disease, such a study was thus inevitable.

Keeping in view the above gaps in research, the present investigations on collar rot complex was undertaken with the following objectives:

1. Survey for incidence of collar rot complex disease in northern districts of Karnataka.
2. Isolation, identification and proving pathogenicity of pathogens.
3. Cultural, nutritional and physiological studies of (predominant) fungal pathogens.
4. Interaction studies involving these pathogens.
5. Management of collar rot complex using bioagents, amendments and chemicals.

II. REVIEW OF LITERATURE

The crop *Coleus forskohlii* is subjected to attack by many soil borne pathogens and nematodes. Out of them, root knot nematode and pathogens *viz. Fusarium chlamydosporum* [*Fusarium fusarioides* (Frag. and Cif.) (Booth)], *Rhizoctonia bataticola* and *Sclerotium rolfsii* are suspected to be involved in causing collar rot complex.

However, much work has not been done on this disease and the pathogens. Hence, literature available on this aspect is scanty. Therefore, work on *Meloidogyne incognita, Fusarium chlamydosporum, Rhizoctonia bataticola* and *Sclerotium rolfsii* on coleus and other crops are reviewed below.

SURVEY

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 stress factors can induce predisposition of their hosts to attack by other combination of plant parasitic nematodes with fungal pathogens which is sufficient to induce heavy crop losses (Zacheo, 1993).

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

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).

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) reported that the *Fusarium fusarioides* caused typical wilt symptoms on the terminal leaflets of cotton in Peru.

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

Aneja *et al.* (1993) conducted a series of surveys throughout Haryana during 1988-92 to identify naturally occurring fungal pathogens of water hyacinth leaves. It was mainly caused by *Fusarium chlamydosporum.*

Shyla (1998) isolated pathogen from diseased roots of *C. forskohlii* which consistently yielded *Fusarium chlamydosporum*. El-Arabi and Abughania (1998) conducted the study on *Fusarium* wilt disease of tomato in 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*).

Boby and Bagyaraj (2003) isolated *Fusarium chlamydosporum* from diseased root fragments of *C. forskohlii.*

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

Rhizoctonia bataticola

The fungus *Rhizoctonia bataticola* is known to infect a wide range of host plants such as both monocots and dicots with a high aggressiveness and these attributes have made this fungus economically important pathogen. The genus *Rhizoctonia* was erected by de Candole (1815) for the violet root rot pathogen, *Rhizoctonia erocorum* D.C. ex. Fr.

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.

Several species of *Rhizoctonia* have been recorded (Saksena and Vaasteraja, 1961). Among them, *Rhizoctonia bataticola* (Taub.) Butler. is 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, aromatic and medicinal plants, plantation and horticultural crops (Uppal, 1934, Likhite and Kulkarni, 1934 and Young, 1949).

Sclerotium rolfsii

Sclerotium rolfsii Sacc. is a well known polyphagus 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 rotted potatoes and identified as *Rhizoctonia 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.*

SYMPTOMATOLOGY

Fusarium chlamydosporum

Shyla (1998) described the symptoms on coleus plants caused by *Fusarium chlamydosporum.* In the field, the infected plants were characterized by gradual yellowing marginal necrosis and withering 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 was 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.

Rhizoctonia bataticola

Prashanthi (1994) reported that, 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, India 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 a fungus and after 3-4 days, a white mass of fungal sclerotia appeared on the infected portions. The causal organism was isolated and identified as *R. bataticola* (*Macrophomina phaseolina* and its pathogenicity was confirmed by Santa Kumari *et al.* (2002).

Sclerotium rolfsii

Bisht (1982) described the symptoms of sclerotium infection in potato. The mycelium infected at the collar region of potato plants causing wilt which ultimately leads 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 irrigated water or farm implements used for cultural practices. Pathogen damaged stem, root or tuber.

The infected seedlings became stunted with chlorotic leaves and ultimately withered and died. Sclerotia developed on the surface of soil and infected stem (Baruah *et al.,* 1980). Nyvall (1989) also described the symptoms as wilting of the plants due to infection of stem at base level. Further, soil level near the infected stem was over grown with white mycelium. 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.

Meloidogyne incognita

Rajendran and Vadivelu (1991) showed that the 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 which was carried out in controlled condition revealed that the effective damage to the plant was caused by 1000 larvae/plant.

GROWTH ON SOLID MEDIA

Fusarium chlamydosporum

Sherkar and Utikar (1982) reported that, Richard's agar and potato dextrose agar followed good growth and sporulation of *Fusarium fusarioides* (*Fusarium chlamydosporum*).

Cultural studies of *Fusarium oxysporum* f. sp. *sesame* the causal agent of wilt of sesame were carried out on six different media *viz.,* Asthana and Hawker's medium, Czapek's Dox agar medium, Potato dextrose agar, Richard's medium and Wakman's medium. The fungus showed luxuriant growth and maximum sporulation on potato dextrose agar (Raghuwanshi, 1995).

In vitro studies were carried out to find out the effect of different media on growth and sporulation of *Fusarium chlamydosporum* causing stem canker of okra. It was observed that Richard's (Synthetic) and potato dextrose agar (non-synthetic) media were found most suitable for growth and sporulation of the test fungus (Jadhav *et al.,* 2000).

Rhizoctonia bataticola

Vasudeva (1937) reported that Richard's agar supported best growth and sclerotial formation of *R. bataticola* as compared to potato dextrose agar, Czapek's Dox and Sabouraud's agar. On the contrary, Rose bengal agar was found to be the best medium for isolation of *Macrophomina phaseolina* propagules from infected soil (Shekar, 1983). Similarly Ramamurthy (1982) recorded the maximum radial growth of *R. bataticola* on Richard's agar, Sabouraud's agar and potato dextrose agar while the least radial growth was recorded on oatmeal agar whereas, Waseer *et al.* (1990) isolated *Macrophomina phaseolina* from soybean and reported that, the pathogen grew best on potato dextrose agar and potato dextrose juice.

Kulkarni *et al.* (1992) reported that, *R. bataticola* grew best on cotton root extract agar, potato dextrose agar, Richard's agar and Sabouraud's agar. Sclerotial production was excellent on the dextrose agar, Richard's agar and Sabouraud's agar and on Czapek's agar. Sahi *et al.* (1992) observed that, potato dextrose agar supported the maximum colony diameter (9 cm) of *Macrophomina phaseolina* whereas Richard's agar supported maximum sclerotial production by the pathogen.

Four isolates of *Macrophomina phaseolina* on pigeon pea were compared for cultural variability. Potato dextrose agar and Czapek's agar were found best for the growth of all the isolates (Lokesha, 2002).

Sclerotium rolfsii

Sulladmath *et al.* (1977) reported that out of the six media used potato dextrose agar and malt extract agar supported very good growth, while the growth was least in Czapek's agar. Isolates from wheat and tobacco made slow growth on oat meal agar as compared to others. Sunflower isolate grew faster than the other isolates in most of the media used.

Lingaraju (1977) reported that the isolate from the sunflower showed maximum growth in potato dextrose broth on tenth day.

Sulladmath *et al.* (1977) reported that the time taken to reach the maximum growth varied with the isolate. Pigeon pea and tobacco isolates reached maximum growth on the seventh day. Sunflower and groundnut on the ninth day while wheat and potato isolates continued to grow upto eleventh day.

GROWTH PHASE OF THE PATHOGEN

Lilly and Barnett (1951) discussed the growth pattern of fungi and outlined the following growth phases a) stationary phase, b) phase of accelerated growth, c) maximum stationary phase and d) phase of decline or autolysis. They attributed these phases of fungus to the environmental and nutritional conditions in which it grows.

Fusarium chlamydosporum

Shyla (1998) used potato dextrose broth to study the growth phase of *Fusarium chlamydosporum* and observed maximum growth at either 18th or 20th day after inoculation. The autolysis stage of the fungus was recorded after 20 days of inoculation.

Rhizoctonia bataticola

Rhizoctonia follows the curve in a liquid medium having an initial period of accelerating growth followed by a phase of very rapid growth and then decreased in weight due to autolysis (Israel and Ali, 1964). Satishchandra (1977) reported that the fungus *Macrophomina phaseolina* attained maximum growth after 11 days of inoculation in Richard's liquid medium.

Ramamurthy (1982) reported that, *R. bataticola* reached maximum growth after 12 days of incubation on potato dextrose broth.

GROWTH ON LIQUID MEDIA

Fusarium chlamydosporum

Among the media tested for growth and sporulation of *Fusarium chlamydosporum,* maximum growth was obtained on potato dextrose agar and rose bengal agar while the least growth was observed on host extract agar (Shyla, 1998).

Desai (1982) reported that, *Fusarium moniliforme* was able to grow very well in Czapek's-Dox broth while, the least growth was recorded in Elliott's broth.

Richard's medium was found to be best suited medium for the growth of *Fusarium udum* the causal agent of *Fusarium* wilt on pigeon pea (Sataraddi *et al.,* 2003).

Rhizoctonia bataticola

Shanmugam and Govindswamy (1973) and Desai (1982) reported that Richard's medium gave the maximum growth of groundnut isolate of *Macrophomina phaseolina*. Among the liquid media tried, Richard's broth and potato dextrose broth gave the maximum growth and sclerotial production (Ramamurthy, 1982).

Byadgi and Hegde (1985) observed variation in dry mycelial weight of six isolates of *R. bataticola* to be significant. Bean isolates produced maximum growth with mean mycelial weight of 190 mg followed by Bengal gram, cowpea, sorghum, soybean and glyricidia isolates at 12 days after incubation.

NUTRITIONAL STUDIES

EFFECT OF CARBON SOURCES

Fusarium chlamydosporum

Brannon (1923) observed that, glucose and fructose were utilized equally by *Fusarium* spp. for tissue formation, when grown on Czapek's modified solution. Moore (1924) reported that, the weight of mycelium varied with the source of sugars. Moore and Chupp (1952) working with *Fusarium oxysporum* f. sp. *lycopersici, Fusarium conglutinans* and *Fusarium oxysporum* f. sp. *niveum* reported that, all the three were able to utilize a number of carbon sources and hydrolyzed starch.

Sowmya (1993) studied the effect of carbon sources on growth of *Fusarium oxysporum* f. sp. *cubense.* Maximum growth was noticed in medium with glucose as carbon sources while, Sataraddi *et al.* (2003) reported that the maximum growth of *Fusarium udum* was obtained on media with mannitol followed by fructose as carbon sources.

Rhizoctonia bataticola

Luthra and Vasudeva (1938) reported that, all the carbohydrates such as maltose, glucose, sucrose, lactose, galactose, dextrin and soluble starch supported fairly abundant growth of *R. bataticola.* Similarly, Moniz and Bhide (1963) working with the Gujarat strain of *Macrophomina phaseolina* observed that, dextrose, sucrose, raffinose, levulose and maltose supported good growth and sclerotial formation of the fungus. Patil and Kulkarni (1965) reported that, arabinose, dextrin, glucose, lactose and sucrose supported good growth of *Macrophomina phaseolina* isolated from cotton, sesame, groundnut and castor.

Singh *et al.* (1974) noticed that abundant growth and Sclerotial production in media with carbon sources as sucrose, glucose and fructose while, galactose, sorbose, lactose and mannitol permitted poor growth of *R. bataticola.* Similarly, among the carbon sources, sucrose was utilized most efficiently followed by maltose. The fungus *R. bataticola* did not utilize organic acids such as citric acid and mallic acid (Ramamurthy, 1982).

EFFECT OF NITROGEN SOURCES

Fusarium chlamydosporum

Subramanian and Srinivasa Pai (1953) reported good growth of *Fusarium vasinfectum* on potassium nitrate, while ammonium sulphate was found to be poor source of nitrogen. Of the seven nitrogen sources tested, maximum growth of *Fusarium oxysporum* f. sp. *niveum* was obtained on potassium nitrate, ammonium nitrate, ammonium oxalate, ammonium sulphate and ammonium phosphate (Jhamaria, 1972).

Arshad *et al.* (1990) reported the effect of different nitrogen sources, among them galactose and potassium nitrate were found to be the best sources for *Fusarium.* Among the different nitrogen sources tested, asparagine was better utilized by *Fusarium oxysporum* f. sp. *cubense* (Sowmya, 1993).

Rhizoctonia bataticola

Shanmugam and Govindswamy (1973) obtained significantly higher growth of *Macrophomina phaseolina* in asparagine followed by glutamine, peptone and potassium nitrate. *R. bataticola* metabolized a number of nitrogen compounds for the growth and that the amount of growth varied with the type of nitrogen source. The best growth of the fungus occurred with glutamine, glutamic acid and alanine (Singh *et al.* 1974). Similarly, among the nitrogen sources, glutamic acid supported the maximum growth and sclerotial production of the fungus *R. bataticola* while least growth was supported by ammonium chloride (Ramamurthy, 1982).

PHYSIOLOGICAL STUDIES

EFFECT OF TEMPERATURE

Fusarium chlamydosporum

Clayton (1923) tested the behaviour of *Fusarium lycopersici* at different temperature ranges. He found that, the minimum temperature required for growth was 9-10^{0}C, the optimum was 28^{0}C and maximum was 37^{0}C.

Bai *et al.* (1988) isolated and identified six *Fusarium* spp. from maize stalk rot in China including *Fusarium fusarioides* (*Fusarium chlamydosporum*). The optimum temperature required for growth was found to be 25^{0}C with a range of 8-30^{0}C.

Sowmya (1993) reported that the pathogen *Fusarium oxysporum* f. sp. *cubense* causing panama disease of banana produced maximum growth at 35^{0}C. But, at 40^{0}C growth was drastically reduced. Profuse growth and sporulation of *Fusarium oxysporum* f. sp. *sesami* was recorded at 27^{0}C.

Rhizoctonia bataticola

Singh and Mehrotra (1980) observed that the incubation temperature of 35^{0}C stimulated the mycelial growth of *R. bataticola.*

Sahi *et al.* (1992) reported that 30^{0}C was ideal for the growth of *M. phaseolina* causing dry root rot of mungbean. They also noticed maximum colony diameter (9 cm) and sclerotial formation at this temperature. (Sandhu *et al.,* 1999) reported that 30^{0}C favored maximum disease development. Kulkarni (2001) noticed variation in growth of *Macrophomina phaseolina* due to temperature. Significant growth of all the isolates was observed at 35^{0}C and 40^{0}C indicating their preferential range to be between 35 and 40^{0}C.

Sclerotium rolfsii

Sulladmath *et al.* (1977) studied variation in requirement of temperature by different isolates and found that all isolates grew well between 23 and 25^{0}C. The optimum temperature for groundnut isolate was 25^{0}C and 30^{0}C for tobacco and potato but 35^{0}C for rest of the isolates. Hari *et al.* (1988) reported that, 26^{0}C was optimum temperature for growth of *Sclerotium rolfsii.* Further, they observed maximum growth of *Sclerotium rolfsii* at 30^{0}C. Maximum number of sclerotial bodies production was observed at 25^{0}C.

Dalvi and Raut (1986) found that the optimum temperature and relative humidity for the *Sclerotium rolfsii* of groundnut, in culture were 28±1^{0}C and 77 per cent respectively.

EFFECT OF HYDROGEN-ION CONCENTRATION

Fusarium chlamydosporum

Bai *et al.* (1988) isolated and identified six *Fusarium* spp. from maize stalk rot in China, including *Fusarium chlamydosporum.* The optimum pH range required for growth was found to be 6.5 – 7.5 however, the fungi grew on medium with a pH range of 4.3 to 9.8.

Fusarium oxysporum f. sp. *niveum* could grow well on wide range of pH varying from 3.2 to 8.3 with the optimum lying between pH 5.0 and 6.5. As the pH increased or decreased from the optimum, the rate and amount of growth as well as the extent of sporulation gradually decreased (Jhamaria, 1972). Marras *et al.* (1981) reported that the optimum pH for *Fusarium roseum* var. *avenaceum* was 7.0.

Jadhav *et al.* (2000) reported that, the fungus *Fusarium chlamydosporum* recorded maximum growth at pH 6.5 which was followed by pH 6.0 and 5.5. Pokhar Rawal *et al.* (2003) noticed that, the pH 6.5 favoured maximum growth and sporulation of *Fusarium* spp.

Rhizoctonia bataticola

Shanmugam and Govindswamy (1973) studied the physiological aspects of *Macrophomina phaseolina* causing groundnut root rot and found that, *Macrophomina phaseolina* grew best on Richard's medium at optimum pH of 5.0.

Kulkarni (2001) reported that variation due to change in pH level was evident in *Macrophomina phaseolina* isolates. Highest growth was observed at pH 7.0 closely followed by pH 6.5 indicating preferential range to be between pH 6.5 and 7.0.

Lokesha (2002) reported that, four isolates of *Macrophomina phaseolina* on pigeon pea were isolated and compared for physiological variability on potato dextrose agar at different hydrogen ion concentrations, where in the neutral pH (7.0) favoured good growth of fungus.

Sclerotium rolfsii

Lingaraju (1977) found that optimum pH for growth of *Sclerotium rolfsii* ranged from 2.8 to 5.8 and from 5.8 onwards the growth decreased suddenly and at pH 7.6 a little rise in growth was seen.

Hari *et al.* (1988) observed maximum growth of *Sclerotium rolfsii* at pH 6.0 when supplied with starch and dextrin as carbon sources and peptone and potassium nitrate as nitrogen sources.

Prasad *et al.* (1986) found a maximum mycelial growth at pH 7.0. Dey *et al.* (1992) recorded mycelial growth and sclerotial formation at pH 4.0 – 7.0.

MANAGEMENT STUDIES

IN VITRO EVALUATION OF BIOAGENTS

Biological control of soil borne diseases is a popular and challenging goal and has been a focus for research since many years. Biological control is attractive in an environmental and economic sense because it offers safe and cost effective alternative to fungicides.

Fusarium chlamydosporum

Fungi in the genus *Trichoderma* are the most promising biocontrol agents of plant pathogenic fungi. Specific strains have the ability to control a range of pathogens, under a variety of environmental conditions (Chet, 1987).

Beltaief (1995) studied the biological control of *Fusarium oxysporum* f. sp. *niveum* by biological soil additives such as *Trichoderma harzianum, Trichoderma koningii* and *Streptomyces griseoviridis. Trichoderma* spp. recorded decreased disease incidence by 75 per cent on aerial parts of plants and by 77 per cent on the vascular browning and tyloses in the host Xylem vessels.

Ram *et al.* (1997) reported that, biocontrol agents, *Trichoderma harzianum* and *Pseudomonas fluorescens* when introduced to soil for control of rhizome rot of ginger caused by *Fusarium solani* and *Phytophthora myriotylum,* inhibited the growth of pathogen.

The fungal antagonist *Trichoderma harzianum* inhibited the growth of *Fusarium moniliforme* significantly higher than *Trichoderma viride.* Similarly, the bacterial antagonistic *B. subtilis* showed maximum inhibition compared to *Pseudomonas fluorescens* in controlling *Fusarium moniliforme* (Karunakaran *et al.,* 2003).

Rhizoctonia bataticola

Sanford (1926) was the first to realize that, soil microorganisms themselves exert a natural biological control on root diseases. Then on wards there has been a dramatic increase in research efforts and several books (Baker and Cook, 1974; Burges, 1981 and Cook and Baker, 1983) and review articles (Naik and Sen, 1985 and Jayashree *et al.,* 2000) have come up covering the use of specific microbes, their mechanisms and commercial evaluation.

The fungus antagonists like *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma longibrachiarum* and *Gliocladium virens* were evaluated against black gram root rot fungus *Macrophomina phaseolina. Trichoderma harzianum* and *Trichoderma longibrachiarum* were on par in controlling *Macrophomina phaseolina* (Indra *et al.,* 2003).

Kamalakannan *et al.* (2003) studied the effects of volatile and diffusible compounds of two *Trichoderma* spp. (*Trichoderma harzianum* and *Trichoderma viride*), two *Pseudomonas fluorescens* (Pf C6 and Pf1) isolates and two *B. subtilis* (BSC 7 and BSC 8) isolates against coleus root rot pathogens like *R. solani* and *Macrophomina phaseolina.* Volatile compounds from all the biocontrol agents were less effective in inhibiting the mycelial growth of the pathogens except volatile compounds of *Trichoderma harzianum* and Pf 1 which effectively reduced the sclerotial production of *R. solani.* Similarly, volatile compound of Pf 1 and *Trichoderma harzianum* effectively inhibited the mycelial growth and sclerotial production of *Macrophomina phaseolina.* The diffusible compound from all the biocontrol agents effectively inhibited the mycelial growth and sclerotial production of *R. solani* and *Macrophomina phaseolina.* Diffusible compound of Pf C 6 recorded maximum inhibitory effect on mycelial and sclerotial production of test fungi. The other biocontrol agents were less effective.

Sclerotium rolfsii

Singh and Singh (1994) reported that, all the isolates of *Trichoderma harzianum, Trichoderma viride* and *Gliocladium virens* were found to be effective against *Sclerotium rolfsii* in dual culture technique and culture filtrate methods. Inhibitory activity of autoclaved culture filtrate was less effective compared to filter sterilized culture filtrates.

Bagwat (1997) tested the antagonistic organisms against *Sclerotium rolfsii.* Among them, *Trichoderma harzianum* was found to be superior and it produced the inhibition zone of 3.27 mm. Maximum reduction of sclerotial bodies was observed in *Trichoderma harzianum* followed by *Trichoderma viride.*

IN VITRO EVALUATION OF BOTANICALS

Fusarium chlamydosporum

Effectiveness of leaf extracts of *Pongamia pinnata, Calotropis giganta* L., *Azadirachta indica* L. against *Fusarium pallidoroseum* and *Fusarium oxysporum* was reported by Gupta *et*

al. (1996). The inhibitory effects of essential oils extracts from 10 Indian plants were evaluated against *Fusarium chlamydosporum.* The plants used for extraction of essential oils were *Eucalyptus* spp. *Ocimum basilicum, Prosopis cineraria Derris indica* and *Pongamia pinnata.* The paper disc method and the serial dilution techniques were followed to test the susceptibility of the *Fusarium* species and the results were compared with miconazole. The essential oils extracted from eucalyptus markedly inhibited fungal growth (Rai *et al.,* 1999).

Farrukh Aqil (2003) obtained essential oils of pepper mint (*Mentha* sp.), clove (*Syzygium aromaticum*) and eucalyptus (*Eucalyptus globulus*) and evaluated for their antifungal activity against soil borne fungi, including *Aspergillus niger, Alternaria alternata* and *Fusarium chlamydosporum* by agar well diffusion method. Maximum antifungal activity was detected in essential oil of clove oil followed by those of peppermint and eucalyptus. *Fusarium chlamydosporum* was found to be most susceptible to essential oils in liquid as well as solid media.

Rhizoctonia bataticola

Sindhan *et al.*(1999) reported the efficacy of leaf extracts of neem (*Azadirachta indica*), mint (*Mentha arvensis* L.), Eucalyptus (*Eucalyptus globulus* L.), tulsi (*Ocimum sanctum* L.), datura (*Datura alba* L.), bougainvillea (*Bougainvillae spectabilis* L.), rhizome extract of ginger (*Zingiber officinalis*), bulb extract of garlic (*Allium sativum*) and onion (*Allium cepa*) against the mycelial growth of *R. solani* and *R. bataticola* (*Macrophomina phaseolina*) *in vitro* at 5, 10 and 20 per cent concentrations. The results showed that all the plant extracts were inhibitory to *R. solani* and *R. bataticola* even at 5 per cent concentration. In general all the extracts were more inhibitory to *R. solani* and *R. bataticola* at all the concentrations.

Ahamad *et al.* (2000) evaluated the activity of various plant products (0.1% Palmarosa oil, 0.3% neem oil, 10% *Ocimum tenuiflorum* leaf extract) along with various antagonistic microorganisms (*Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride, Aspergillus flavus*) against dry root rot of chickpea caused by *R. bataticola* in pot culture experiment. Palmarosa oil at 0.1 per cent concentration gave the best control (22-32 per cent disease incidence compared with >66 per cent in control), which was followed by soil inoculation plus seed treatment method.

Sclerotium rolfsii

Annapurna *et al.* (1983) noticed that leaf extract of *Polyalthia longiflora* was having broad spectrum antimicrobial activity. Hyphal dry weight and sclerotial production of *Sclerotium rolfsii* were significantly reduced by bark extracts of *Rauvolfia serpentana, Datura stramonium, Eucalyptus globulus* and *Azadirachta indica* and rhizome extracts of *Cucumis domestica* and *Zingiber officinale* (Singh and Dwivedi, 1987).

Mishra and Tewari (1990) reported antifungal activity of leaf extracts of *Azadirachta indica* and *Datura stromanium* against *Rhizoctonia solani*, *Aspergillus niger* and *Sclerotium rolfsii.* Mycelial growth and sclerotial production were completely inhibited by different concentrations of *Ageratum conyzoides, Eupatorium cannabinum* and *Crotalaria medicaginea* (Kumar and Tripathi, 1991). Dayaram and Tewari (1994) observed that soil application of green leaves *Azadirachta indica, Anisomeles ovata* and *Adathoda vasaka* were very effective in controlling of collar rot of chickpea caused by *Sclerotium rolfsii.*

IN VITRO EVALUATION OF FUNGICIDES

Fusarium chlamydosporum

Of nine fungicides and three nitrogenous compounds tested *in vitro,* the fungicides bavistin (carbendazim) and difolatan (Captafol) effectively checked the growth of *Fusarium oxysporum* f. sp. *lycopersici.* Whereas PCNB (*Quintozene*) stimulated the growth (Dwivedi and Pathak, 1981).

Eleven fungicides were assayed *in vitro* and in the field against *Piper beetle* decline due to *Fusarium solani.* Bavistin (Carbendazim) was found to be the most effective chemical under prevailing soil conditions followed by captan and blitox (Copper oxy chloride) Hiremath *et al.,* 1987).

Dilip (1989) reported that, *Fusarium oxysporum.* f. sp. *nicotianae* causing wilt of tobacco, completely inhibited the growth by carbendazim at 1000 ppm concentration which was followed by ziram and captafol substantially.

The laboratory assay of fungicides against *Fusarium oxysporum* f. sp. *cubense* showed cent per cent inhibition with bavistin, dithane M-45, and emisan at all concentrations tested (Sowmya, 1993). Among the six concentrations Carbendazim, captan, copper oxy chloride at 1000 ppm gave total inhibition of the fungal growth.

Pokhar Rawal *et al.* (2003) reported that systemic fungicides *viz.,* (at 10, 25, 50 and 75 ai/ha) Carbendazim, tridemifon and kitazin were found effective in inhibiting the growth of *Fusarium solani* which caused *Fusarium* rot of sponge gourd fruits.

Rhizoctonia bataticola

Chattannavar *et al.* (1988) reported the percentage inhibition of growth of *R. bataticola* (*Macrophomina phaseolina*) (isolated from roots of *Casurina equisetifolia*) in pure culture on potato dextrose agar, by 11 fungicides tested at four concentrations (100, 250, 500 and 1000 ppm). Rovrol (Iprodione) inhibited growth by 80-90 per cent at all concentrations tested. Similarly, bayleton (Triademiphon), penoctine and penotil at 500-1000 ppm and vitavax (carboxin) at 1000 ppm were also effective. Alliette (fosetyl-Al) was least effective. The other fungicides tested were baytan (triademinol), brassicol (Quintozene), captan and dithane M-45.

Bavistin as seed treatment at three g/kg seeds and soil drench at 0.2 per cent effectively controlled the root rot of cotton caused by *R. bataticola* (Kulkarni *et al.,* 1992). Peshney *et al.* (1992) reported that growth and sclerotial germination of *R. bataticola* were effectively controlled by thiram (0.2%), captan (0.2%), mancozeb (0.2%), Iprodione (0.2%), carbendazim (0.2%) and tridemorph (0.7%). Six fungicides as soil drenching treatment were tested against *R. bataticola* causing root rot of safflower. Among them, carbendazim (0.01%), propiconazole (0.05%), captan (0.2%) and chlorothalonil (0.2%) were found to be effective (Prashanthi, 1994).

Four systemic and three non-systemic fungicides were evaluated against the *Macrophomina phaseolina in vitro.* Benomyl significantly inhibited the growth of the fungus at 250 ppm followed by carbendazim among systemic fungicides. Among non-systemic fungicides, mancozeb and thiram at 500 ppm inhibited significantly the fungal growth followed by captan (Lokesha, 2003).

Sclerotium rolfsii

Propiconazole was found to be highly effective in inhibiting the growth of *Sclerotium rolfsii* (Waterfield and Sisler, 1985 and Hagan *et al.,* 1992). Hagan *et al.* (1992) also reported the efficacy of tebuconazole, diniconazole and flutonil against *Sclerotium rolfsii,* a causal agent of southern stem rot peanut.

Virupaksha Prabhu (1994) tested both systemic and non-systemic fungicides against *Sclerotium rolfsii*. Among systemic fungicides Hexaconazole, Propiconazole and Tridemorph were found highly effective with per cent inhibition of 98.89, 96.35 and 86.31 respectively and among non-systemic thiram proved to be effective against *Sclerotium rolfsii* in *in vitro* studies.

INTERACTION BETWEEN NEMATODE AND FUNGUS AT DIFFERENT INOCULUM LEVELS

Observations indicate that plant predisposition to 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 organisms fungus 650 propagules/g of soil and 50 larvae / 500 g of soil in combination with high population density (Fungus 647 x 10^3 to 77 x 10^3 propagules / g of soil) of either could result in marked wilted symptoms in cotton.

In nature plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognised that “nature does not work with pure cultures” and that many plant diseases are influenced by associated organisms. Plant parasite 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, 1993).

Of all the interactions of pathogens with nematodes none are more damaging to crops world wide as the combined effects of wilt producing fungi and plant parasitic nematodes. The combination of nematode and fungus often results in a synergistic interaction wherein the crop loss is greater than expected from either pathogens alone or an additive effect of the two together. The result for a cultivar sensitive to the interaction can be total failure (Francl and Wheeler, 1993).

Atkinson (1892) for the first time reported that presence of *Meloidogyne incognita* along with *Fusarium oxysporum* in the rhizosphere of cotton plants 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; Gopal Swarup, 1990; Khan and Parvathareddy, 1993 and Francl and Wheeler, 1993).

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 french bean.

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 javanica, Fusarium oxysporum* and *Fusarium solani* on growth of chickpea CU-JG-62, which was found to be additive in nature. However when nematode was established one week prior to fungus the resultant effect was more than additive. Occurrence of *Meloidogyne incognita* in combination with both the fungi not only increased the severity of disease but also shortened the incubation 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.

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

MANAGEMENT OF NEMATODE FUNGAL DISEASE COMPLEX

The literature available on management of disease complex of coleus involving plant parasitic nematodes and soil borne fungal pathogens under field conditions is very limited. So, the efforts have been made to review the work done on closely related aspects.

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 strategy is targeted to achieve this objective (Paroda, 2000).

Effect of antagonists on fungal wilt pathogens

Weindling (1932) was first to show the antagonistic effects of a soil fungus to *Sclerotium rolfsii.* He observed that, the hyphae of *Trichoderma* sp. secreted some substances which was lethal to *Sclerotium rolfsii.* Later it was identified as gliotoxin. Many workers reported that *Trichoderma viride* was found to be antagonistic to *Sclerotium rolfsii* (Hino and Endo, 1940, Henis *et al.,* 1983, Deb, 1993).

Singh and Vinod Kumar (1995) studied the efficacy of neem cake and carbofuran (2 kg ai/ha) alone and in combination at varying dosages against root-knot nematode *Meloidogyne incognita* infecting Japanese mint (*Menthus arvensis* cv. Shivalik). Results of the investigations indicated that neem cake at two per cent w/w and carbofuran @ two kg ai/ha were very effective 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.*

Carbofuran was effective in reducing the population and development of *Meloidogyne incognita* on tomato and improving plant growth at all concentrations tested. But soil drench at 50, 100 and 500 ppm gave effective control (Haq *et al.* 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./m^2 had reduced nematode population to a greater extent with highest yields of 27.40 and 25.68 per cent respectively over control.

A field study was undertaken to study the possibility of controlling the root rot disease of *C. forskohlii* caused by *Fusarium chlamydosporum* using the biocontrol agents *viz. Glomus mosseae, Pseudomonas fluorescens, Trichoderma viride,* singly and in combination. Planting of coleus cutting was done in wilt sick soil. Inoculation with *Trichoderma viride* + *Glomus mosseae* gave the best result in controlling the disease. The same treatment also resulted in maximum growth, yield and root forskolin concentration of coleus. The next best treatment was *Pseudomonas fluorescens* + *Trichoderma viride* followed by *Glomus mosseae* + *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 inoculations of biocontrol agents (Boby and Bagyaraj, 2003).

Experiments were conducted to find out comparative performance of biocontrol agents and chemicals (Carbendazim) in control of grapevine wilt caused by *Fusarium moniliforme.* Under glasshouse conditions among different biocontrol agents tested in different combinations the treatment with *Pseudomonas fluorescens* + *B. subtilis* and *Pseudomonas fluorescens* + *Trichoderma viride* were found the best. Under field conditions the treatment with *Pseudomonas fluorescens* + *Trichoderma viride* was found to be the best (Karunakaran *et al*., 2003).

Application of *Trichoderma harzianum* to the root zone of tomato controlled *Sclerotium rolfsii* infection in naturally infested soils and further on transplanting the treated plant showed reduced disease incidence upto 93.00 per cent (Elad *et al.,* 1980). Later in 1981 they observed that an isolate of *Trichoderma harzianum* was capable of lysing mycelium of *Sclerotium rolfsii* and *R. solani.*

III. MATERIAL AND METHODS

The materials used and the techniques adopted during the course of investigations are described in this chapter.

The field experiments were conducted in the field of Department of Spices and Plantations Crops, Kittur Rani Channamma, College of Horticulture, Arabhavi and also in the glasshouse and laboratory of the Department of Plant Pathology, College of Agriculture, Dharwad.

SURVEY FOR COLLAR ROT COMPLEX OF *Coleus forskohlii*

An intensive survey was conducted during 2004-05, on the incidence of collar rot complex in *Coleus forskohlii* growing districts of northern Karnataka such as Bellary, Raichur, Dharwad, Belgaum, Bagalkot and Bidar. In Bellary district survey was conducted in the following places *viz.,* Benchikottala, D-Hirehaal, Kappagal and Hitnal. In Raichur district survey was conducted in Gorkal, Toranadinni, Koodlur, Bellatagi and Maski region. In Dharwad district survey was conducted in two places *viz.,* Dharwad and Alnavar. In Belgaum survey was conducted in places *viz.,* Hattargi, Yamakanmaradi, Mamadapur, Arabhavi and Sadalga. In Bagalkot district survey was conducted in places like Katarki, Chikkalagundi, Chikkur, Mantur and Muthur. In Bidar district survey was conducted in places *viz.,* Mannalli, Anadoor, Bawgi and Bidar. Samples of soils 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, a composite samples of 200g of soil and 5 g of root were formed. Randomly 100 plants were selected in different locations in a field and number of plants wilted were counted and the mean wilt incidence was expressed in percentage. Wherever required, the complete wilted plants, were also collected for isolation and other studies. 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 and labelled immediately. Information pertaining to the locality, crop history, etc. was also obtained along with the samples. Samples and roots were analysed on the day of collection or after keeping for a few days under refrigerated conditions. The nematode populations from soil and root samples were estimated. Root samples were used for detection of the fungi associated with collar rotted plants. The wilt symptoms were identified based on the key provided by Maiti and Sen (1979).

Estimation of nematode population in soil

Cobb's sieving and decanting technique was followed, for which 200 cc of the soil sample 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 mesh size.

The sievates were collected on a tissue paper spread over a coarse mesh, which was then placed in a petridish containing enough water and tissue wet paper as to keep the tissue paper just wet and nematode can move from soil samples to water in Petriplate.

The nematode suspension collected in the Petridish was examined by means of research stereobinocular microscope. The root knot nematode and other 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.

Sterilization of soil

Black soil free of lumps and stones, was collected from the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India. It was thoroughly mixed with river bed sand in 1:1 proportion, autoclaved at 1.05 kg/cm^2 pressure for two hours on two successive days. The sterilized soil was allowed to cool to room temperature and was spread on floor for three days, to facilitate the escape of poisonous gases.

Inner side of the earthen pots were swabbed with 0.1 per cent mercury chloride solution before filling up of sterilized soil mixture.

Glassware and cleaning

Borosil and Corning glassware were used for all the experimental studies, wherever required and were kept in the cleaning solution containing 60 g of potassium dichromate ($K_2Cr_20_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in one litre of water for a day. Then they were cleaned by washing with detergent powder followed by rinsing several times in tap water and finally in distilled water.

Sterilization

All the glasswares were sterilized in an autoclave at 1.1 kg per sq cm pressure for 20 minutes. All the media were sterilized for 15 minutes at 1.1 kg per sq cm pressure, except those containing sugars and nitrogen sources which were sterilized at 0.7 kg per sq. cm pressure for 10 minutes and soil used for experiment was sterilized at 1.33 kg/sq cm pressure for two hours.

ISOLATION OF FUNGAL PATHOGENS FROM PLANT MATERIAL

Standard tissue isolation technique was employed for isolation of *Sclerotium rolfsii, Rhizoctonia bataticola* and *Fusarium chlamydosporum* from plants showing typical collar rot.

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 five to six mm size and surface sterilized in 0.1 per cent mercuric chloride for a minute. This was followed by repeated washings in sterile water. The bits were transferred aseptically on to sterilized Potato Dextrose Agar (PDA) slants. The slants were incubated at room temperature (27 ± 1^0C) for 72 hours.

Purification of cultures

Ten ml of clear filtered water agar of strength two per cent was poured into sterile Petriplate and allowed to solidify. Dilute suspensions of spores / sclerotial bodies was prepared in sterile distilled water of which two ml was spread uniformly on water agar plates and the excess suspension was drained off aseptically from the plates. Such plates were incubated at 27 ± 1^0C for few hours. They were examined frequently under microscope, well isolated germinating spores / sclerotial bodies were marked with ink on the glass surface of the plate. These marked agar areas were cut and transferred aseptically to potato dextrose agar slants and incubated at 27 ± 1^0C. Ten such single spore / sclerotial body cultures were made and compared for their growth characters. The isolates were found to be identical. Each culture was multiplied and used for further studies.

Maintenance of culture

The fungus was sub-cultured on potato dextrose agar slants and allowed to grow at 27 ± 1^0C for one week. Such slants were preserved in a refrigerator at 5^0C and renewed once in two months.

IDENTIFICATION OF THE PATHOGEN

Fusarium chlamydosporum

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

Rhizoctonia bataticola

The morphological, cultural and formation of sclerotia were the principal 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.*

Sclerotium rolfsii

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

Mass multiplication of isolated fungi

Sand-corn meal medium was prepared in the proportion of 95:5 in order to get maximum inoculum of the fungus. About 400 g of sand-maize meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.33 kg pressure/sq cm for one hour. The pure cultures of *S. rolfsii, R. bataticola* and *F. chlamydosporum* were inoculated separately to different flasks under aseptic condition and incubated at 27 ± 1^{0}C for 20 days. The flasks were shaken on alternate days to get uniform growth. The giant cultures so obtained were used for further studies.

PROVING THE PATHOGENICITY OF COLLAR ROT PATHOGENS

The coleus cuttings were raised in earthen pots containing sterilized soil to which different inocula (*S. rolfsii, R. bataticola* and *F. chlamydosporum*) maintained on sand-corn meal medium, were added @ 4 per cent in different pots. A control treatment was maintained in which no inoculum was added.

1. *Sclerotium rolfsii* alone.
2. *R. bataticola* alone.
3. *F. chlamydosporum* alone.
4. Control (No. inoculum was added).

The pots were maintained at 25 per cent moisture holding capacity and the moisture loss was maintained by adding water on weight basis. Observations were made every alternate day regarding development of wilt symptoms. After the plants showed wilt symptoms such plants were carefully uprooted and the fungi were reisolated by standard tissue isolation method. The fungi reisolated were compared with original culture.

Collection of cultures of root knot nematodes, maintenance and buildup of inoculum

Root knot infected coleus plants were collected from the orchards of KRC College of Horticulture, Arabhavi and University of Agricultural Sciences, Dharwad. Root portion was carefully removed from the soil and washed gently under running tap water. Egg masses were picked and kept for hatching in water in a petridish. After 24-36 hours, juveniles hatched and the same were used to inoculate tomato plants grown in sterilized soil-sand mixture in greenhouse. These plants served as culture plants. After giving sufficient time so as 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 throughout.

Identification of prevailing root knot nematode species

The galled root system from the above culture plant was immersed in a beaker containing boiling 0.1 per cent cotton blue in lactophenol and left overnight 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 stereobinocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide. The posterior portions of the females were carefully cut with a sharp razor blade and body contents were cleaned. The perineal region was trimmed and mounted for observation under immersion objective. At least ten slides were prepared containing the perineal pattern of the nematode. The identification of the species was made on the basis of characters of perineal pattern described by Eisenback *et al.* (1981).

Hatching of juveniles, inoculation and proving the pathogenicity

The egg masses from stock culture were transferred carefully to a wire gauze sieve containing two layers of facial tissue paper trimmed down to edge of a 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 contents of a petri dish were emptied into a beaker, diluted to a suitable volume and population counts were made with the help of Fenwick's multi chamber counting slide. Based on the requirement, the suspension was diluted with sterile water.

Thirty days after planting coleus cuttings in test pots, the suspension containing pre-determined number of juveniles was pipetted and spread uniformly over the surface of roots which were carefully exposed earlier. Then the roots were covered with soil. The plants were lightly watered to keep the soil moist. A similar treatment was given to the uninoculated check plants except that only water was used instead of the nematode suspension. After giving sufficient time (60 to 100 days) to complete 2 to 3 generations of the nematode, the root systems of the depotted plants were washed free of soil and were examined for presence of galls. For further confirmation, staining of root material was done with acid fuchsin (Mc Beth *et al.,* 1941).

CULTURAL STUDIES

Growth characters on solid media

The growth characters of the fungi were studied on 14 solid media as mentioned below:

1. Czapek's Dox agar
2. Elliot's agar
3. Rhichard's agar
4. Sabouraud's agar
5. Tochinai's agar
6. Brown's agar
7. Asthana and Hawker's agar
8. Potato dextrose agar
9. Carrot agar
10. Malt extract agar
11. V8 juice agar
12. Cornmeal agar

13. Host leaf extract agar

14. Host root extract agar

Composition and preparation of different media

1. Czapek's Dox agar

Sodium nitrate ($NaNO_3$)		3.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.5 g	
Magnesium sulphate ($MgSO_4$, $7H_2O$)	0.5 g	
Potassium chloride (Kcl)	0.5 g	
Ferrous sulphate ($FeSO_4$, $7H_2O$)	0.01 g	
Sucrose ($C_{12}H_{22}O_{11}$)	20.0 g	
Agar agar		20.0 g
Distilled water (to make up)	1000 ml	

2. Elliot's agar

Potassium dihydrogen phosphate (KH_2PO_4)	1.36 g	
Sodium carbonate (Na_2CO_3)	1.0 g	
Magnesium sulphate ($MgSO_4$, $7H_2O$)	0.5 g	
Dextrose ($C_6H_{12}O_6$)	1.0 g	
Asparagine ($NH_2CO\text{-}CH_2NH_2\text{-}COOH$)	1.0 g	
Agar agar		15 g
Distilled water (to make up)	1000 ml	

Agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter by adding distilled water.

3. Richard's agar

Potassium dihydrogen phosphate (KH_2PO_4)	5 g	
Potassium nitrate (KNO_3)	10 g	
Magnesium sulphate ($MgSO_4$ $7H_2O$)	2.5 g	
Ferric chloride ($Fecl_3$ $6H_2O$)	0.02g	
Sucrose ($C_{12}H_{22}O_{11}$)	50 g	
Agar agar		15 g
Distilled water (to make up)	1000 ml	

All the above ingredients except potassium dihydrogen phosphate and agar were dissolved in 450 ml of distilled water. Agar-agar was dissolved in 450 ml of distilled water.

Agar-agar was melted separately in 400 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml by adding distilled water. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were autoclaved and subsequently mixed together.

4. Sabouraud's agar

Peptone	10 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar	20 g
Distilled water (to make up)	1000 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter by adding distilled water.

5. Tochinai's agar

Potassium dihydrogen phosphate (KH_2PO_4)	0.5 g
Potassium nitrate (KNO_3)	2.0 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	1.0 g
Ferric chloride ($Fecl_3$ $6H_2O$)	Trace
Sucrose ($C_{12}H_{22}O_{11}$)	30 g
Agar agar	15 g
Distilled water (to make up)	1000 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one litre by adding distilled water.

6. Ashtana and Hawker's agar

Glucose ($C_6H_{12}O_6$)	5.0 g
Potassium nitrate (KNO_3)	3.50 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.75 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.75 g
Agar agar	15.0 g
Distilled water (to make up)	1000 ml

7. Brown's medium

Glucose ($C_6H_{12}O_6$)	2.00 g
Asparagine	2.00 g
Dipotassium hydrogen phosphate (K_2HPO_4)	1.25 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.75 g
Trace element mixture	1 ml
Agar agar	20 g
Distilled water (to make up)	1000 ml

Trace element mixture was prepared by dissolving 0.1 g of ferrous sulphate ($FeSO_4$ $7H_2O$) 0.1 g of zinc sulphate ($ZnSO_4$ $7H_2O$) 0.1 g of manganese sulphate ($MnSO_4$ $4H_2O$) and 0.01 g of copper sulphate ($CuSO_4$ $5H_2O$) in 100 ml of water.

1. Potato dextrose agar

Potato	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar agar	20 g
Distilled water (to make up)	1000 ml

Potatoes were peeled off and cut into small pieces then 200 g of peeled off cut pieces of potato were boiled in 800 ml of water and extract was filtered through muslin cloth. The dextrose was dissolved in about 1000 ml of distilled water and agar was added and boiled until it was properly dissolved. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water.

2. Carrot agar

Carrot	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar agar	20 g
Distilled water (to make up)	1000 ml

Carrots were peeled off and cut into small pieces then 200 g of peeled pieces were boiled in 800 ml of water and extract was filtered through muslin cloth. The dextrose was dissolved in the filtrate and agar agar was added and boiled until it properly dissolved. Further, volume was made to 1000 ml by adding distilled water.

3. Malt extract agar

Malt extract	20 g
Agar agar	20 g
Distilled water (to make up)	1000 ml

All the ingredients were mixed thoroughly in 400 ml of water except agar agar. Agar agar was melted in 400 ml of water. Both the solutions were mixed thoroughly and the column was made upto 1000 ml by adding distilled water.

4. Corn meal agar

Corn meal	200 g
Dextrose (C6H12O6)	20 g
Agar agar	20 g
Distilled water (to make up)	1000 ml

Corn meal was boiled in 400 ml water and later dextrose was added and mixed thoroughly agar agar was melted in 400 ml water. Both the solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water.

5. V-8 juice agar

V-8 juice	8.30g
L-asparagine	10g
Yeast extract	2g
Calcium carbonate	2g
Glucose	2g
Agar agar	20g
Distilled water (to make up)	1000ml

V-8 juice agar of 44.3 g obtained from High media was suspended in 1000ml distilled water and sterilized at 1.1kg/cm^2 pressure for 15 minutes.

6. Host leaf extract agar

Coleus forskohlii leaves	200 g
Sucrose	20 g
Agar agar	20 g
Distilled water (to make up)	1000 ml

Coleus leaves were boiled in 400 ml of water for one hour at 100^0C then extract was filtered through muslin cloth and mixed with the sucrose (20 g). Agar agar was melted in 400 ml of water. Both the solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water.

7. Host root extract agar

Coleus forskohlii roots	200 g
Sucrose ($C_{12}H_{22}O_{11}$)	20 g

Agar agar	20 g
Distilled water (to make up)	1000 ml

Coleus tubers were boiled in 400 ml of water for one hour at 100^0C and then the extract was filtered through muslin cloth and mixed with the sucrose. Agar agar was melted in 400 ml of water. Both the solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water.

Twenty ml of each of the above medium was poured aseptically into Petriplates. Each treatment was replicated thrice. Five mm discs of eight day-old culture were used for inoculation. Such plates were incubated at 27 ± 1^0C for seven days.

The colony diameter was recorded by taking mean of the radial growth of the colony in two directions for each plate of the fungus. The various cultural characters *viz.,* rate of growth, type of margin, colour and sporulation / sclerotial production on different media were also recorded. The data on radial growth were analysed statistically.

GROWTH PHASE IN LIQUID MEDIA

The growth phase study was conducted on potato dextrose broth. Thirty ml of broth was added to 100 ml flasks and sterilized at 1.1 kg per sq cm pressure for 15 minutes. These flasks were allowed to cool. Separate sets of flasks were maintained for each pathogen. Five mm discs of *F. chlamydosporum, Rhizoctonia bataticola* and *Sclerotium rolfsii* of seven day culture of the individual isolate was inoculated and incubated at room temperature (27 ± 1^0C). Each treatment was replicated three times. Culture was filtered through Whatman number 42 filter paper disc of 12.5 cm diameter, which was dried at a constant temperature of 60^0C in an electrical oven prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with it. One set of flasks was harvested on second day. Subsequent harvesting was done at an interval of two days upto 24^{th} day. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60^0C, cooled in a desiccator and weighed immediately on an analytical electric balance. The results were analysed statistically.

GROWTH STUDIES IN DIFFERENT LIQUID MEDIA

The liquid media used were same as that of solid media except that agar agar was not added to the liquid media.

All the liquid media were sterilized at 1.1 kg per sq cm for 15 minutes. Cultures of *F. chlamydosporum, R. bataticola* and *S. rolfsii* were inoculated separately and incubated at 27 ± 1^0C for 12, 16 and 10 days respectively. Each treatment was replicated thrice. The mycelial mat was filtered through Whatman number 42 filter paper discs of 12.5 cm diameter, dried to a constant weight at 60^0C prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with the mycelium and dried to a constant weight in an electrical oven at 60^0C, cooled in a desiccator and weighed immediately on an analytical electric balance. The weight of dry mycelium was recorded and the data were statistically analysed. The best synthetic medium was found out and used for further studies as basal medium.

NUTRITIONAL STUDIES

Carbon utilization

The carbon requirement of the fungi i.e. *F. chlamydosporum, R. bataticola* and *S. rolfsii* was studied by replacing sucrose with different carbon compounds in Richard's solution. The carbon compounds used in present study were lactose, citric acid, starch, galactose, maltose, fructose, glucose, dextrose and sucrose. The quantity of each carbon compound to be added was determined on the basis of their molecular weight so as to provide equivalent amount of carbon as that of sucrose present in basal medium. Each treatment was replicated thrice. All the sugars were dissolved properly. They were sterilized at 1.1 kg per sq cm for 15 minutes. Separate sets of flasks were maintained for each pathogen. Cultures of *F. chlamydosporum, R. bataticola* and *S. rolfsii* were inoculated separately to the respective sets of flasks and incubated at 27 ± 1^0C for 12, 16 and 10 days respectively. Dry mycelial weight of the fungus was recorded and results were analysed statistically.

Nitrogen utilization

The nitrogen requirement of the fungi was studied by replacing potassium nitrate of Richard's medium for *F. chlamydosporum, R. bataticola* and *S. rolfsii* with different nitrogen sources. The nitrogen sources used in the present study were *viz.,* ammonium chloride, ammonium sulphate, sodium nitrate, peptone, glycine, potassium nitrate, asparagine, methionine, calcium nitrate, ammonium nitrate.

The quantity of the nitrogen sources was determined on the basis of molecular weight so as to provide equivalent amount of nitrogen as that in the basal medium.

All the nitrogen sources were dissolved properly and each source was replicated thrice. Further they were sterilized at 1.1 kg per sq cm for 15 minutes. The flasks were inoculated and incubated as described earlier. The dry mycelial weight of the fungus was recorded and data were analysed statistically.

PHYSIOLOGICAL STUDIES

Temperature requirement

For *F. chlamydosporum, R. bataticola* and *S. rolfsii* Richard's medium was used in this experiment. The different temperatures tried for the growth of the fungi were 10, 15, 20, 25, 30, 35 and 40^0C. For each treatment level, three replications were maintained. The flasks were inoculated and incubated as described earlier. The dry mycelial weight was recorded and results were analysed statistically.

Hydrogen ion concentration

The liquid medium used in this experiment was Richard's broth / solution for *F. chlamydosporum, R. bataticola* and *S. rolfsii.* Adjustment of pH was done by adding 0.1 N alkali (NaoH) or acid (HCL). Reaction of the medium was adjusted to the desired pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Twenty ml of the medium was pipetted out into each 100 ml conical flask. Each treatment was replicated thrice. The flasks were sterilized at 1.1 kg per sq cm for 15 minutes. They were inoculated and incubated as described earlier. The dry mycelial weight was recorded and the data were analysed statistically. pH of the culture filtrate was determined by using precision pH meter.

MANAGEMENT STUDIES

In vitro evaluation of fungicides

Five non-systemic and five systemic fungicides were tested *in vitro.* The systemic fungicides were tested at 500 and 1000 ppm and non-systemic fungicides at 1000 and 2000 ppm concentration.

Poison food technique (Sharvelle, 1961) was followed to test the efficacy of the above mentioned fungicides. Fungal cultures of *F. chlamydosporum, R. bataticola* and *S. rolfsii* were grown separately on potato dextrose agar medium in Petriplates for 7, 5 and 5 days respectively, prior to setting the experiment. Required quantity of respective fungicides were added separately to sterilized potato dextrose agar cooled at 45^0C to obtain the desired concentration on the basis of active ingredient present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized Petriplates. Suitable checks were also maintained without addition of any fungicides. Mycelial disc of 5 mm was taken from the periphery and placed in the center and incubated at 27 ± 1^0C till growth of the fungus touched the periphery in control plate. Four replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I : Per cent inhibition

C : Radial growth in control

T : Radial growth in treatment

The data were analysed statistically.

Sl. No.	Common name	Chemical name	Trade name
	Non-systemic fungicides		
1	Captan	N (Thichloromythyl thio) cyclohelix 4-ere –1, 2 dicarboximide	Captaf 50% WP
2	Copper oxy chloride	Copper oxy chloride	Blitox 50% WP
3	Mancozeb	Manganese + zinc ethylene bis dithiocarbamate	Indofil 75% WP M-45
4	Chlorothalonil	Tetrachloro isopthalonitrile	Kavach 75% WP
5	Thiram	Tetramethyl thiuram disulphide (TMTD)	Thiram 75%WP
	Systemic fungicides		
1	Carbendazim	2 – (Methoxy – Carboxyl) – benzimidazole	Bavistin 50% WP
2	Hexaconazole	(RS)-2-(2-4-dichlorophenyl) –1-(14-1, 2, 4-triazole – 1-yl) hexane –2-ol)	Contaf 5% EC
3	Propiconazole	1-(2 (2, 4 dichlorophenyl) – 4-propyl –1, 3-dioxolanyl methyl)-1H –1-4 triazole	Tilt 25% EC
4	Benomyl	Methyl (butyl carbomoyl) benzimidazole carbamate	Benlate
5	Tridimifan	1-(4-Chlorophenoxy)-3, 3-dimethyl-1-1H-(1, 2, 4-triazole-1-41)-2-butanone	Bayletan 25% WP

In vitro evaluation of bioagents

Five antagonists such as *T. viride, T. harzianum, T. koningii, T. virens* and *P. fluorescens* were used to study their interaction with *R. bataticola, F. chlamydosporum* and *S. rolfsii* separately.

Twenty ml of potato dextrose agar was poured into 9 cm diameter Petriplates and allowed to solidify. Five mm disc of fungal cultures were taken and placed at one end of Petriplates and antagonistic organisms were inoculated at the opposite side. A control was maintained. Each treatment was replicated four times and incubated till the culture reached periphery of Petriplate in control at 27 ± 1^{0}C. The interactions were recorded by measuring inhibition zone between fungi and antagonistic fungi or bacterium and compared with control. Data were analysed statistically.

In vitro evaluation of botanicals

In the present study the plant leaf extracts of the following plants were selected for testing their efficacy against various pathogens causing collar rot complex in *Coleus.*

Sl. No.	Botanical name	Common name	Family
1	*Azadirachta indica* A. Juss	Neem	Meliaceae
2	*Duranta repens* L.	Duranta	Verbenaceae
3	*Eupatorium oduratum* L.	Communist weed	Asteraceae
4	*Parthenium hysterophorus* L.	Congress grass	Asteraceae
5	*Ocimum sanctum* L.	Tulsi / Holybasil	Labiateae
6	*Eucalyptus globulus* Labill	Eucalyptus	Myrtaceae
7	*Tridax procumbens* L.	Tridax / Deer foot	Compositae
8	*Clerodendron inerme* Gaertn	Kashmir bouquet	Verbenaceae
9	*Cassia occidentalis* L.	Negro coffee	Caesalpiniaceae
10	*Prosopis juliflora* L.	Bellary jali	Mimosaceae
11	*Pongamia pinnata* L.	Honge	Simarubaceae
12	*Glyricidia maculata* L.	Glyricidia	Leguminaceae

Preparation of cold aqueous extract

Fresh leaves of each test plant were collected and washed first in tap water and then in distilled water. Then 100 g of fresh sample was crushed in a surface sterilized pestle and mortar by adding 100 ml sterile distilled water (1:1 w/v). The extract was used as stock solution.

To study the anti-fungal activity of plant extract the poisoned food technique was followed.

Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten potato dextrose agar media respectively so as to get 5 and 10 per cent concentrations. The medium was thoroughly shaken for uniform mixing of the extract.

Twenty ml of medium was poured into each of the 90 mm sterilized Petriplates. Each plate was seeded with 5 mm mycelial discs taken from the periphery of fungal culture and incubated at 27 ± 1^{0}C till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the centre of the Petriplate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration.

Three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs were inoculated into the centre of potato dextrose agar plates without plant extracts. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1927).

The data were analysed statistically.

Interaction studies

To study the effect of sequential inoculation of *M. incognita* followed by fungal pathogen (*F. chlamydosporum, R. incognita* and *S. rolfsii*) singly or in various combinations and vice versa on plant growth, host infestation, nematode multiplication and disease development, a pot culture experiment was designed under greenhouse conditions.

Four per cent of each fungal giant culture or one thousand freshly hatched second stage juveniles of root knot nematode was applied individually to 30 days old cutting of coleus grown in 1:1 sterile sand soil mixture in 45 cm diameter earthen pots. Incoulum of all the said pathogens was applied singly or in combinations in sequence as per the following treatments.

1. Control
2. *Meloidogyne incognita* alone.
3. *Fusarium chlamydosporum* alone.
4. Inoculation of *M. incognita* seven days prior to inoculation of *F. chlamydosporum.*
5. Inoculation of *F. chlamydosporum* seven days prior to inoculation of *M. incognita.*
6. *Rhizoctonia bataticola* alone.
7. Inoculation of *M. incognita* seven days prior to inoculation of *R. bataticola.*
8. Inoculation of *R. bataticola* seven days prior to inoculation of *M. incognita.*
9. *Sclerotium rolfsii* alone.
10. Inoculation of *M. incognita* seven days prior to inoculation of *S. rolfsii.*
11. Inoculation of *S. rolfsii* seven days prior to inoculation of *M. incognita.*
12. Inoculation of *M. incognita* seven days prior to inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii.*
13. Inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii* seven days prior to inoculation of *M. incognita.*

Observation on wilting and other symptoms were recorded till 150 days after inoculation with the said organisms.

Data on shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, per cent galled area, final population of nematodes in soil and per cent wilt incidence were recorded, whereas, rating for gall index was done following the scale used by Sivakumar and Vidyasekaran (1990).

Per cent galled area	**Scale (based on percentage of root system galled**
0	1 (No galling)
1-25	2 (Light galling)
25-50	3 (Moderate galling)
51-75	4 (Heavy galling)
76-100	5 (Severe galling)

MANAGEMENT OF COLLAR ROT COMPLEX DISEASE OF *Coleus forskohlii* USING DIFFERENT BIOCONTROL AGENTS, ORGANIC AMENDMENTS AND CHEMICALS

A field trial was carried out (during *Kharif,* 2004) in natural wilt disease complex plot of Department of Spices and Plantation Crops, Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka. In the experimental plot initial population of root knot nematode and colony forming units of fungal pathogens were recorded. Eleven treatments were laid out with three replications, each plot of size 3.0 m x 1.6 m (gross size) and net size of each treatment was 1.8 m x 1.2 m. In each treatment planting of coleus cuttings was done at a spacing of 60 cm x 20 cm. Cuttings of coleus were planted in the experimental plot and treatment imposed 30 days after planting. The following treatments were imposed in randomized block design.

Treatment details

T_1 – *Trichoderma viride* @ 10 ml / plant ($8x10^3$ cfu/ml).
T_2 – *Trichoderma harzianum* @ 10 ml/plant ($8x10^3$ cfu/ml).
T_3 – *Pseudomonas fluorescens* @ 10 ml/plant ($24x10^5$ cfu/ml).
T_4 – Pronto @ 5% soil drench.
T_5 – Neemto @ 500 g/5 m^2.
T_6 – Carbofuran 3G @ 15 g a.i./5 m^2.
T_7 – Farm yard manure @ 5 kg/5 m^2.
T_8 – *Trichoderma viride* @ 10 ml/plant ($8x10^3$ cfu/ml) + Neemto @ 500 g/5 m^2.
T_9 – Carbendazim @ 0.1% soil drench.
T_{10} – Propiconazole @ 0.1% soil drench.
T_{11} – Control.

Observations recorded

Observations on per cent wilt incidence, nematode population, number of galls/5g of root and colony forming units of *F. chlamydosporum* and *Rhizoctonia bataticola* were recorded at harvest (150 days after imposing the treatments).

Statistical analysis of the experimental data

The data obtained in the present investigations for various parameters such as shoot and root lengths, fresh and dry shoot weights, fresh and dry root weights, root gall index and final nematode population were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized block design for *in vivo* studies.

IV. EXPERIMENTAL RESULTS

The results of the survey on collar rot complex disease of coleus, isolation of participating pathogens, their pathogenicity, cultural, nutritional and physiological characters of the pathogens, their interaction effects and the management of disease complex in naturally sick plot are presented under the following headings.

SURVEY OF COLLAR ROT COMPLEX DISEASE OF COLEUS

A survey was carried out in Raichur, Bellary, Dharwad, Belgaum, Bagalkot and Bidar districts of northern Karnataka during 2004-05 to find out the incidence and severity of the collar rot complex disease of *Coleus forskohlii.* Five villages in Raichur, four villages in Bellary, two villages in Dharwad, five villages in Belgaum, five villages in Bagalkot and four villages in Bidar districts were surveyed as explained in 'Material and Methods' and the data are presented in Table 1. General view of the crop and healthy and diseased plant are shown in Plates 1a and 1b.

Table 1. Incidence of collar rot complex disease of *Coleus forskohlii* in northern Karnataka during 2004-05.

Plate 1a. General view of the crop (*Coleus forskohlii* (Wild.) Briq.

Plate 1b. Healthy and diseased plant of *Coleus*

Root knot disease

The root knot disease caused by *Meloidogyne incognita* was noticed in all the districts surveyed. In Bellary district, maximum per cent disease incidence was observed in Hitnal (72.00%) followed by Benchikottala (45.00%). A low per cent disease incidence was observed in Koodlur (4.00%). In Raichur district maximum per cent disease incidence was observed in D-Hirehaal (21.00%). In Dharwad district two villages were surveyed. Maximum per cent disease incidence was observed in Dharwad (42.00%) and no nematode disease incidence was observed in Alnavar. In Belgaum district, maximum per cent disease incidence was recorded in Yamakanamaradi (21.00%) followed by Arabhavi (19.00%). A low per cent disease incidence was observed in soundaga (11.00%). In Bagalkot district, maximum per cent disease was recorded in Katarki (17.00%) and a low per cent disease incidence observed in Chikkalgundi (08.00%). In Bidar district, maximum per cent incidence was recorded in Bidar (45.00%) and a low per cent disease incidence in Mannalli (10.00%). There was no root-knot nematode disease incidence in Toranadinni, Kappagal, Alnavar, Hattargi, Kallolli and Muthur.

Root knot nematode and other plant parasitic nematode population

Maximum root-knot nematode population per 200cc of soil was recorded in Hitnal (512.80) followed by Dharwad (375.60). A low root knot nematode population per 200cc was recorded in Chikkalgundi (40.00) of Bagalkot district and Mannalli (40.00) of Bidar district. Whereas other plant parasitic nematodes per 200 cc of soil was maximum in Dharwad (715.80) followed by Hitnal (591.20). Next high other plant parasitic nematode population per 200 cc of soil was recorded Bidar (475.60) and Benchikottala (375.40) of Bellary district.

Wilt inducing fungi

From the survey, it was found that the root rot or wilt caused by *Fusarium chlamydosporum* was more severe in all the districts surveyed followed by *Rhizoctonia bataticola* and *Sclerotium rolfsii.* A maximum per cent disease incidence of above mentioned pathogens was observed in Bellary district, followed by Bidar and Raichur districts.

During the course of survey, collection and analysis of the infected root samples showed that fungi namely, *F. chlamydosporum, R. bataticola* and *S. rolfsii* were found to be associated with root-knot nematode.

IDENTIFICATION OF PREVAILING ROOT-KNOT NEMATODE SPECIES INFECTING *Coleus forskohlii* IN NORTHERN KARNATAKA

The morphology of perineal pattern is the most important morphological character used for reliable species identification. Important diagnostic characters of perineal patterns of

Table 1. Incidence of collar rot complex disease of *Coleus forskohlii* in northern Karnataka during 2004-05.

Place	Per cent disease incidence				Nematode population / 200cc of soil	
	Fusarium chlamydosporum	***Rhizoctonia bataticola***	***Sclerotium rolfsii***	***Meloidogyne incognita***	***M. incognita***	**Other plant parasitic nematodes***
RAICHUR DISTRICT						
Bellatagi	19.00	6.00	24.00	32.00	92.60	79.50
Gorkal	35.00	12.00	3.00	14.00	105.50	91.50
Koodlur	24.00	19.00	4.00	4.00	60.40	133.30
Maski	12.00	18.00	8.00	40.00	214.70	119.70
Toranadinni	15.00	7.00	0.00	0.00	0.00	40.20
Mean	21.00	12.40	7.80	18.00	94.64	92.84
BELLARY DISTRICT						
Benchi kottala	46.00	29.00	0.00	45.00	127.20	376.40
D. Hirehaal	18.00	31.00	8.00	21.00	74.40	112.80
Hitnal	22.00	40.00	0.00	72.00	512.80	591.20
Kappagal	0.00	0.00	48.00	0.00	0.00	29.20
Mean	21.50	25.00	14.00	34.50	178.60	277.40
DHARWAD DISTRICT						
Alnavar	18.00	19.00	0.00	0.00	0.00	0.00
Dharwad	21.00	15.00	0.00	42.00	375.60	715.80
Mean	19.50	17.00	0.00	21.00	187.80	357.90

BELGAUM DISTRICT						
Arabhavi	26.00	19.00	0.00	19.00	108.30	267.50
Hattaragi	19.00	25.00	7.00	0.00	0.00	0.00
Mamadapur	12.00	0.00	0.00	14.00	68.00	20.00
Sadalga	24.00	8.00	0.00	11.00	50.00	124.20
Yamakanamaradi	31.00	14.00	0.00	21.00	75.00	241.7
Mean	22.40	13.20	1.40	13.00	60.26	130.68
BAGALKOT DISTRICT						
Chikkalgundi	17.00	10.00	24.00	8.00	40.00	157.50
Kallolli	27.00	21.00	29.00	0.00	0.00	75.40
Katarki	8.00	24.00	0.00	17.00	78.60	90.60
Mantur	12.00	9.00	5.00	12.00	60.40	116.70
Muthur	19.00	17.00	0.00	0.00	0.00	0.00
Mean	16.60	16.20	11.67	7.4	35.8	88.04
BIDAR DISTRICT						
Anadoor	14.00	23.00	0.00	15.00	92.00	208.30
Bawgi	21.00	16.00	11.00	23.00	67.60	175.00
Bidar	34.00	27.00	19.00	45.00	216.00	475.60
Mannalli	18.00	12.00	0.00	10.00	40.00	191.70
Mean	21.75	19.50	7.50	23.25	103.90	262.65

*Other plant parasitic nematodes includes *Aphelenchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Longidorus* spp., *Pratylenchus* spp., *Roty lenchulus reniformis, Tylenchus* like plant parasitic nematodes, *Xiphinema* spp. and other *Dorylaimid* plant parasitic nematodes

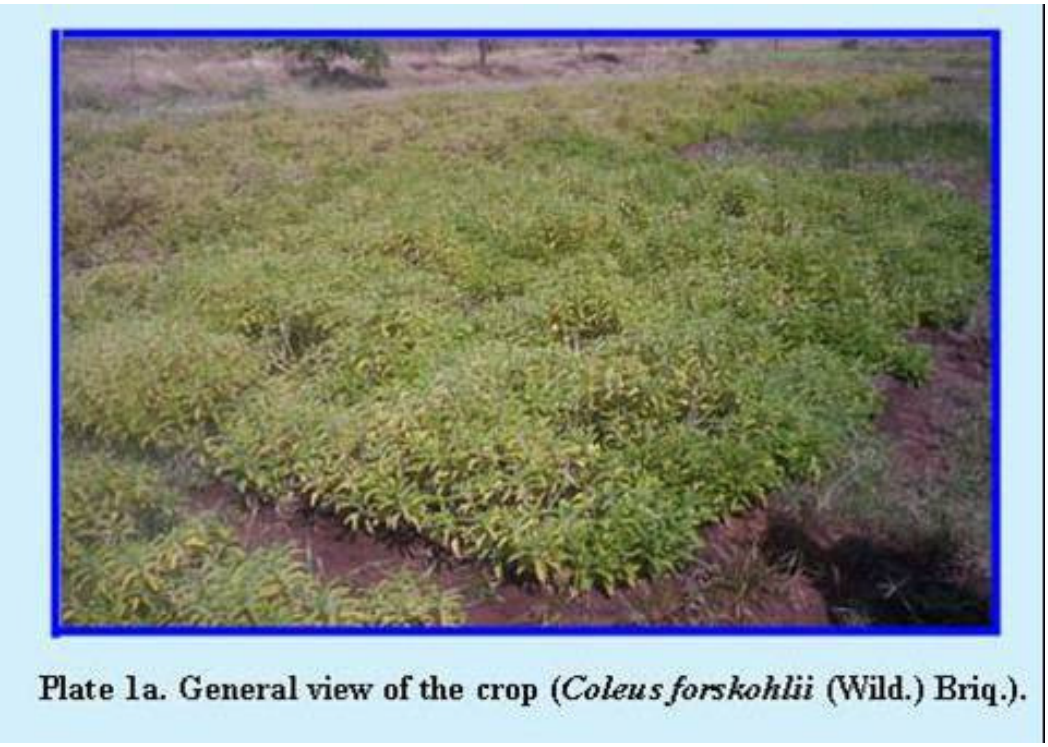


Plate 1a. General view of the crop (*Coleus forskohlii* (Wild.) Briq.).

Plate 1a. General view of the crop (Coleus forskohlii (Wild.) Briq.)

Collar rot complex diseased plant

Healthy plant

Plate 1b. Healthy and diseased plant of Coleus.

the identified species, *M. incognita* are summarized in Table 2 and Plate 2. The observed characters were compared with the descriptions given by the Eisenback *et al.,* (1981).

Table 2. Important characters of perineal pattern of prevailing root – knot nematode species in northern Karnataka.

Plate 2. Identification of species of root knot nematode and fungi.

The prevailing root-knot nematode species in northern Karnataka were characterised by the presence of high, squarish dorsal arch that often contained a distinct whorl in the tail terminal area. The straiae were smooth to wavy, some times zig-zagged and distinct lateral lines were absent.

ISOLATION OF FUNGI FROM COLLAR ROT COMPLEX AFFECTED SAMPLES

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

Identification of pathogenic fungi

The fungi, *Fusairum chlamydosporum*, *R. bataticola* and *S. rolfsii* were identified based on morphological characters mentioned below and in Plate 2.

Fusarium chlamydosporum

The fungus isolated had microconidia, which were fusiform to clavate with, rounded apex, usually with single septa. Macroconidia were curved, 3 to 5 septate. Chlamydospores were usually rough or smooth walled (intercalary) and abundant, fungus produced whitish to pink colonies, wooly and abundant aerial mycelium and on potato dextrose agar growth rate was rapid.

Rhizoctonia bataticola

The growth of the fungal mycelium on PDA was fast. Hyphae were branched at right angle and septate, fluffy and brown to black initially. As the culture grew old, it turned black completely. Abundant production of sclerotia was observed. The sclerotia were spherical, ellipsoidal and dark brown, measuring 70 µm in diameter and was connected to mycelium. The germination of sclerotia and right angled branching of mycelium was noticed.

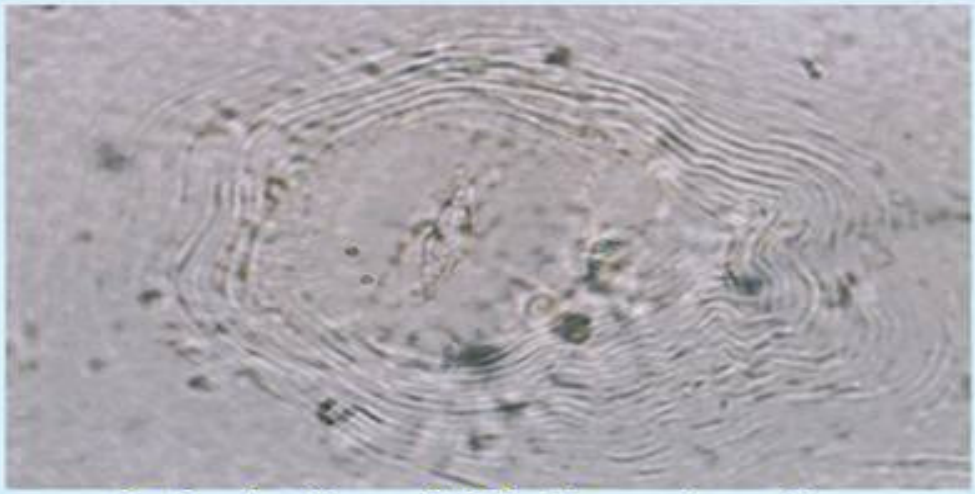
Sclerotium rolfsii

The fungus produced white, dense, radiating mycelial growth on potato dextrose agar medium. In the early stages, the fungus produced silky white mycelium and gradually lost its luster and became dull in appearance. Aerial hyphae was not uniformly distributed. Initiation of sclerotial bodies were obtained from fifth day after inoculation. In the beginning, the sclerotial bodies were white but gradually turned to buff brown colour and then to chocolate brown, at maturity. The fully matured sclerotial bodies were spherical to ellipsoidal and measured 1.5 mm in diameter. The fungus obtained from the affected plant tissue was compared with type species originally described by Domsch *et al.* (1980) and was found to resemble *S. rolfsii* in all morphological characters.

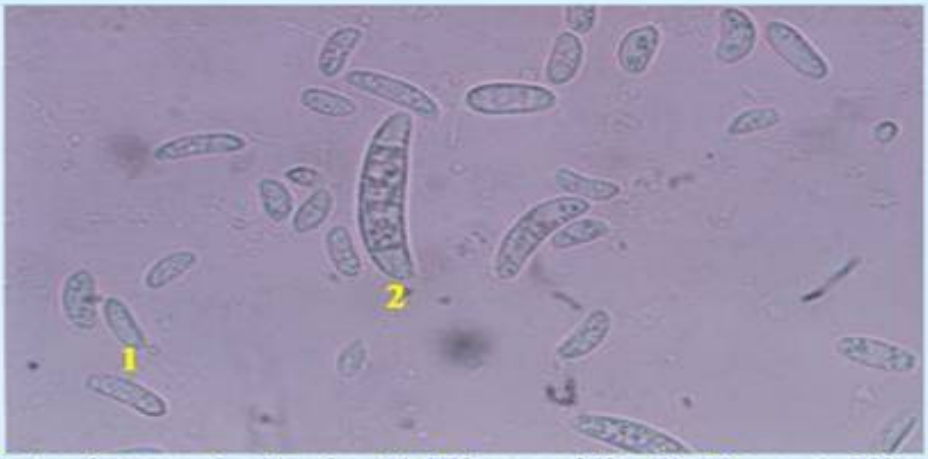
On the basis of these characters the pathogens were identified as *Fusarium chlamydosporum* (Frag and Cif). Booth, *Rhizoctonia bataticola* (Taub.). Butler and *Sclerotium rolfsii* Sacc. and the nematode identified as *Meloidogyne incognita* (Kofoid and White) Chitwood.

Table 2. Important characters of perineal pattern of prevailing root – knot nematode species in northern Karnataka.

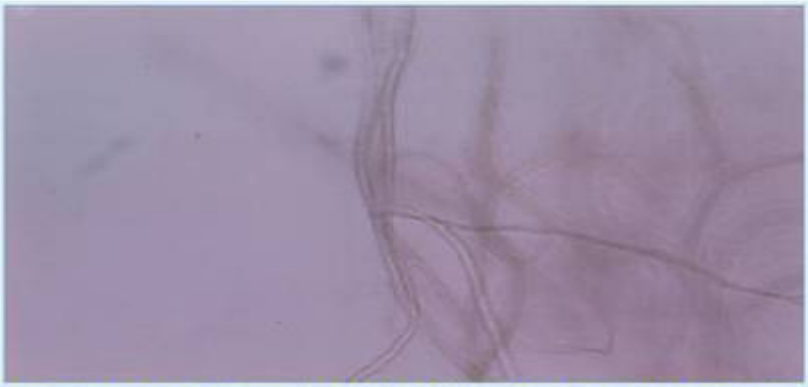
Feature	*Characters observed*	**Original description (Eisenback *et al.,* 1981)**
Dorsal arch	High, squarish	High, squarish
Lateral field	Lateral ridges absent, marked by breaks and forks in striae	Lateral ridge absent, marked by breaks and forks in striae
Striae	Coarse, smooth to wavy	Coarse, smooth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



Perineal pattern of *Meloidogyne incognita*



Photomicrograph showing 1) Microconidia, 2) Macroconidia of *Fusarium chlamydosporum*



Photomicrograph showing characteristic right angled branching with constriction at the point of union of *Rhizoctonia bataticola*.

Plate 2. Identification of species of root knot nematode and fungi.

Plate 2. Identification of species of root knot nematode and fungi.

PATHOGENICITY

Root knot nematode

Pathogenicity of root knot nematode *Meloidogyne incognita* in coleus was studied under glasshouse conditions as per the procedure described in 'Material and Methods' and shown in Plate 3.

Plate 3. Proving pathogenicity of different organisms in *Coleus forskohlii.*

In general, *M. incognita* infected coleus plants were stunted in their growth, showed yellow coloured leaves. When infected plants were uprooted, deformed roots with prominent galls of varying size were noticed. Microscopic examination of the galls revealed the presence of eggs, juveniles and females of nematodes in the vascular bundles of roots. On an average, 3-4 adult females were present along with immature stages in a single gall. The female was pyriform in shape while the males were filiform and in general outline, differed from the juveniles. The presence of egg masses outside the gall was a common phenomenon noticed in coleus roots. The egg mass matrix was observed to be whitish, glistening and round which were exposed on the roots.

Collar rot inducing fungi

Photograph showing the proving pathogenicity of fungal pathogens shown in Plate 3.

Fusarium chlamydosporum

Pathogenicity test for *F. chlamydosporum* was carried out as described in 'Material and Methods' by inoculating with spore suspension and homozenised mycelial bits of *F. chlamydosporum* on 30 days old coleus plants. The infected plants were characterized by gradual yellowing and drying of leaves followed by loss of vigour and premature death. The infected plants pulled off from soil showed discolouraiton of roots and complete destruction of tap and lateral root system. The bark of such plants was easily peeled off. The affected plant were killed finally due to severe rot. The infected tubers showed rotting and emitted bad odour.

Rhizoctonia bataticola

The disease caused by *R. bataticola* was initially expressed as water soaked areas and the affected tissues soon turned into a soft, black, watery mass at the collar region of the plant. The infection was also found on roots and caused decay, which ultimately resulted in collapse of the plant. The infected plant roots showed discolouration followed by rotting of root hairs. Extensive sloughing and pulling off affected bark was also observed. Under conditions of high humidity the disease was found to spread rapidly.

Sclerotium rolfsii

The earliest symptom of the disease was darkening of the stem at collar region of the plant. The leaves became flaccid and dropped off. White, fan shaped mycelial strands crept over the stem portion, developing small light to dark brown sclerotia on the infected portion. The sclerotial initials were white at first, later turned brown with age. Finally the plant wilted and dried.

CULTURAL STUDIES

Growth on solid media

Cultural characters were studied in 14 different solid media. The radial growth of the fungi was measured when the maximum growth was measured when the maximum growth was attained in any of the media tested. Observations on various culture characters were recorded as described in Material and Methods. The results are presented in Table 3, 4, 5 and 6 and Plate 4.

Table 3. Growth of *Fusarium chlamydosporum, Rhizoctonia bataticola* and *Sclerotium rolfsii* on different solid media.

Table 4. Cultural characters of *F. chlamydosporum* on different solid media after seven days of incubation.

Plate 3. Proving pathogenicity of different organisms in Coleus forskohlii

Table 3. Growth of *Fusarium chlamydosporum, Rhizoctonia bataticola* and *Sclerotium rolfsii* on different solid media.

Sl. No.	Medium	Mean colony diameter (mm)		
		Fusarium chlamydosporum	***Rhizoctonia bataticola***	***Sclerotium rolfsii***
1	Asthana and Hawker's agar	66.33	70.00	76.33
2	Brown's agar	71.67	71.00	77.67
3	Carrot agar	81.67	78.33	87.00
4	Corn meal agar	78.00	65.33	71.67
5	Czapeks Dox agar	73.67	74.00	85.00
6	Elliot's agar	77.67	70.00	63.33
7	Host leaf extract agar	27.67	39.33	32.67
8	Host root extract agar	69.00	45.33	35.33
9	Malt extract agar	75.33	81.67	85.33
10	Potato dextrose agar	86.00	90.00	90.00
11	Richard's agar	84.33	89.33	88.67
12	Sabouraud's agar	40.33	41.67	67.67
13	Tochinai's agar	64.33	74.33	84.67
14	V8 juice agar	75.33	73.33	62.33
	Mean	63.38	68.83	71.98
	SEm±	1.13	1.18	0.88
	CD at 1%	4.39	4.61	3.43

Table 4. Cultural characters of *F. chlamydosporum* on different solid media after seven days of incubation**.**

Sl. No.	*Media*	**Growth characters#**	**Sporulation***
1	Asthana & Hawker's agar	Moderate growth, spare mycelium, smooth margin and light white colour	+
2	Brown's agar	Good growth, uniformly dense mycelium, smooth margin, white colour mycelium.	++
3	Carrot agar	Good growth uniformly dense mycelium, smooth margin, light pinkish white coloured mycelium.	++
4	Corn meal agar	Good growth, uniform dense mycelium with distinct concentric rings smooth margin, light pinkish white coloured mycelium.	++
5	Czapeck's Dox agar	Good growth, smooth margin and mycelium white colour.	++
6	Elliot's agar	Good growth, uniform mycelium, smooth margin and light white colour.	++
7	Host leaf extract agar	Slow growth, sparse growth of mycelium, indistinct margin and light white coloured mycelium.	-
8	Host root extract agar	Moderate growth mycelium sparse, irregular margin, hyaline edge and white colour.	+
9	Malt extract agar	Good growth uniformly dense mycelium smooth margin, white coloured mycelium.	+++
10	Potato dextrose agar	Good growth uniformly dense mycelium, smooth margin distinct concentric rings and Pinkish white coloured mycelium.	+++
11	Richard's agar	Good growth, uniformly dense mycelium, smooth margin and mycelium white colour	+++
12	Sabouraud's agar	Slow growth, indistinct margin fluffy dense mycelium mycelium milky white colour.	+++
13	Tochinai's agar	Moderate growth mycelium sparse, smooth margin, mycelium with white colour spores	++
14	V8 juice agar	Good growth, smooth margin, uniform mycelium mycelium white colour.	++

+++: Good sporulation (more than twenty spores per microscopic field)
++: Moderate sporulation (more than five spores per microscopic field)
+: Scanty sporulation (less than five spores per microscopic field)
-: No sporulation
Good growth: Colony diameter more than 70 mm diameter at the end of 7 days.
Moderate growth: Colony diameter between 50 and 70 mm diameter at the end of 7 days.
Slow growth: Colony diameter less than 50 mm attained at the end of 7 days.

Table 5. Cultural characters of *R. bataticola* on different solid media after five days of incubation.

Sl. No.	*Media*	Growth characters#	Sclerotial production*
1	Asthana & Hawker's agar	Good growth irregular margin, sparse mycelium, slightly raised colony, sclerotial the third day.	++
2	Brown's agar	Good growth, irregular margin, raised colony, sclerotial initiation on the third day.	++
3	Carrot agar	Good growth, smooth margin, uniformly dense mycelium initiation on the third day.	++
4	Corn meal agar	Moderate growth, flat colony, sclerotial initiation on the fourth day.	+
5	Czapeck's Dox agar	Good growth, smooth margin, colony raised, sclerotial initiation on the third day and mycelium black colour.	++
6	Elliot's agar	Good growth, irregular margin, colony raised, sclerotial initiation on the fourth day and mycelium black colour.	++
7	Host leaf extract agar	Slow growth, mycelium sporse, colony flat and sclerotia not produced.	-
8	Host root extract agar	Slow growth, sparse mycelium, flat colony and sclerotia not produced.	-
9	Malt extract agar	Good growth, smooth margin, colony raised, sclerotial initiation on the third day.	+++
10	Potato dextrose agar	Good growth, dense mycelium, smooth margin, mycelium black colour and sclerotial initiation on the third day.	+++
11	Richard's agar	Good growth uniformly dense mycelium, colony raised, sclerotial initiation on the third day and mycelium balck coloured.	+++
12	Sabouraud's agar	Slow growth, sparse growth, colony slightly raised, sclerotial initiation on the third day.	+
13	Tochinai's agar	Good growth, irregular margin, colony raised, sclerotial initiation on the third day.	++
14	V8 juice agar	Good growth, margin smooth, colony flat, sclerotial initiation on fourth day.	++

Based on visual observation
+++: Excellent sclerotial production
++: Good sclerotial production
+: Poor sclerotial production
-: No sclerotial production
Good growth: Colony diameter more than 70 mm attained at the end of 5 days.
Moderate growth: Colony diameter between 50 and 70 mm attained at the end of 5 days.
Slow growth: Colony diameter less than 50 mm attained at the end of 5 days

Table 6. Cultural characters of *S. rolfsii* on different solid media after four days of incubation.

Sl. No.	*Media*	Growth characters#
1	Asthana & Hawker's agar	Good growth, margin smooth, flat colony, sclerotial initiation on the 8th day, sclerotia spherical and light brown.
2	Brown's agar	Good growth margin smooth, raised colony, sclerotial initiation on 15th day, sclerotia sub-spherical and dark brown.
3	Carrot agar	Good growth, smooth margin, flat colony, sclerotial initiaition on the 8th day sclerotia spherical and light brown.
4	Corn meal agar	Good growth, fluffy mycelium, raised colony, sclerotial initiation on the 9th day, sclerotia spherical and dark brown.
5	Czapeck's Dox agar	Good growth, margin smooth, flat colony, sclerotial initiation on the 9th day, sclerotia spherical and dark brown.
6	Elliot's agar	Moderate growth spherical and dark brown, slightly irregular margin, raised colony, sclerotial initation on the 10th day, sclerotia spherical and light brown.
7	Host leaf extract agar	Slow growth, irregular margin, flat colony, sclerotial initiation on the 15th day, sclerotial ellipsoidal and light brown.
8	Host root extract agar	Slow growth, irregular margin, glat colony, sclerotial initiaition on the 14th day sclerotia ellipsoidal and light brown.
9	Malt extract agar	Good growth, margin smooth, cottony mycelium, flatcolony, sclerotial initiation on the 8th day, sclerotia spherical and dark brown.
10	Potato dextrose agar	Good growth, smooth margin, raised colony, sclerotial initiation on the 7th day, sclerotia spherical and dark brown.
11	Richard's agar	Good growth, smooth margin, raised colony, sclerotial initiation on the 7th day, sclerotial spherical and dark brown.
12	Sabouraud's agar	Moderate growth, smooth margin, fluffy cotton mycelium, raised colony, sclerotial initiation on the 10th day sclerotia spherical and light brown.
13	Tochinai's agar	Good growth, spare thread like mycelium, flat colony, sclerotial initiation on the 11th day, sclerotia spherical and light brown.
14	V8 juice agar	Moderate growth, irregular margin, sparse mycelium, sclerotial initiation on the 15th day, sclerotia sub-spherical and dark brown.

#Good growth: Mean colony diameter more than 70 mm attained on the fourth day
Moderate growth: Mean colony diameter between 50 and 70 mm attained on the fourth day
Slow growth: Mean colony diameter less than 50 mm attained on the fourth day

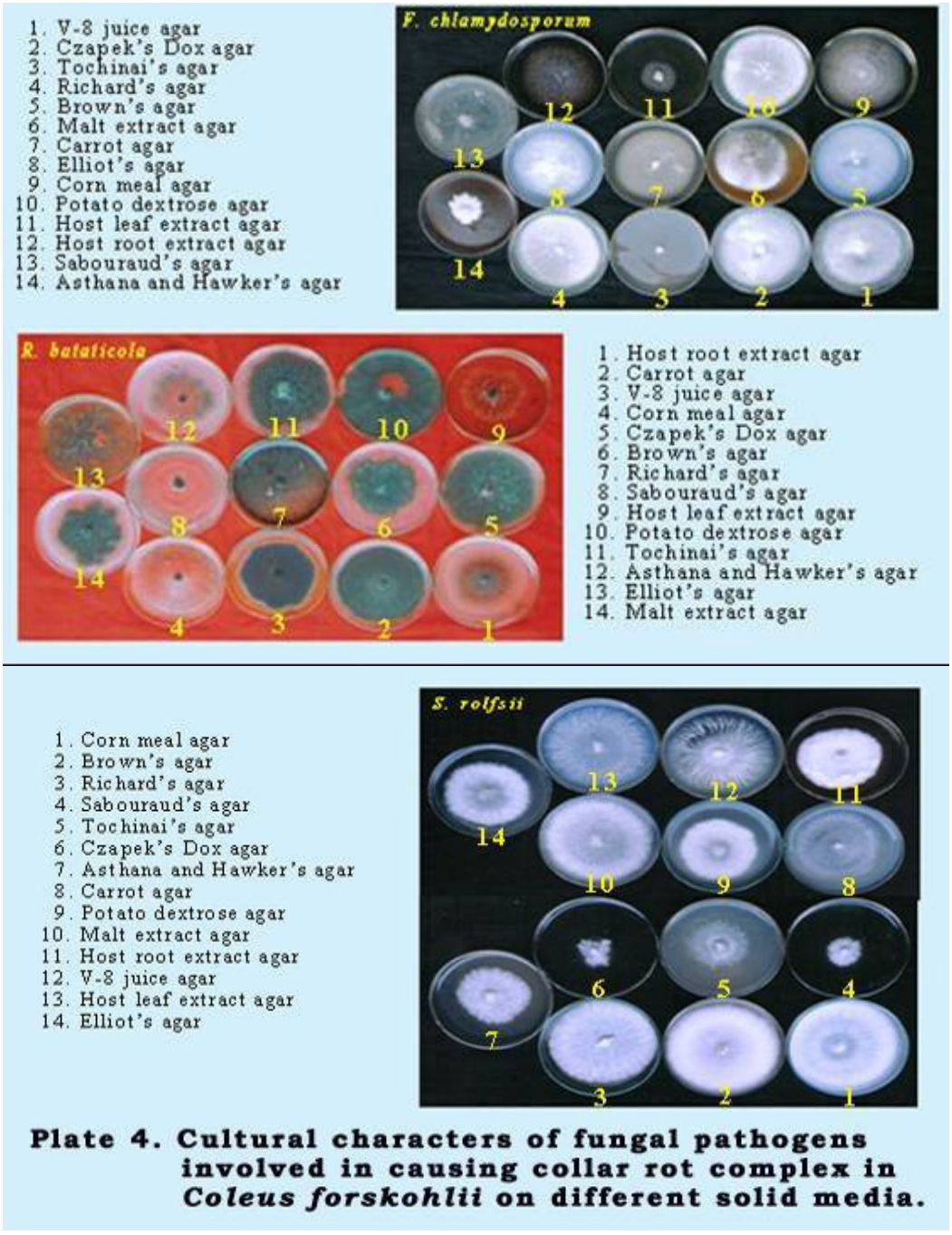


Plate 4. Cultural characters of fungal pathogens involved in causing collar rot complex in Coleus forskohlii on different soild media

Table 5. Cultural characters of *R. bataticola* on different solid media after five days of incubation.
Table 6. Cultural characters of *S. rolfsii* on different solid media after four days of incubation.
Plate 4. Cultural characters of fungal pathogens involved in causing collar rot complex in *Coleus forskohlii* on different solid media.

Fusarium chlamydosporum

The effect of 14 different media on the growth of the fungus was significant. The maximum radial growth was observed in potato dextrose agar (86.00 mm) after seven days of incubation at 27±1^{0}C. This was followed by Richards agar (84.33 mm) and carrot agar (81.67 mm). These were on par with each other and significantly superior over the rest of the media tested. Minimum radial growth was observed in Host leaf extract agar (27.67 mm).

Sporulation was abundant in Richard's agar, sabourauds agar, malt extract agar and potato dextrose agar media. But, there was good sporulaiton in Czapeck's Dox agar, Elliot's agar, Tochinais agar, Brown's agar, carrot agar, V8 juice agar and corn meal agar. sparse sporulation was observed in Asthana and Hawker's agar and Host root extract agar. Host leaf extract agar did not support for sporulation.

Rhizoctonia bataticola

Among fourteen media tested, potato dextrose agar supported maximum radial growth (90.00 mm). However, it was on par with Richard's agar (89.33 mm), followed by Malt extract agar (81.67 mm) and carrot agar (78.33) which were on par with each other. Minimum radial growth was observed in host leaf extract agar (39.33 mm).

Abundant sclerotial production was observed in Richard's agar, Malt extract agar and potato dextrose agar. Good sclerotial production was observed in Czapeck's Dox agar, Elliot's agar, Tochinai's agar, Asthana & Hawker's agar, Brown's agar, Carrot agar, V8 juice agar and sparse sclerotial production in sabouraud's agar and corn meal agar. Host leaf extract agar and host root extract agar did not support sclerotial production of the fungus.

Sclerotium rolfsii

The effect of 14 different media on the growth of the fungus was significant. The maximum radial growth was observed in potato dextrose broth (90.00 mm). This was followed by Richard's agar (88.67 mm) and carrot agar (87.00 mm). These were found to be statistically on par with each other and significantly superior over the rest of the media tested. Minimum radial growth was supported by host leaf extract agar (32.67 mm).

Sclerotial initiation was observed on seventh day in potato dextrose agar and Richard's agar. Whereas in Asthana and Hawker's agar, carrot agar and Malt extract agar it was observed on eighth day of incubation. However, in Brown's agar and Host leaf extract agar sclerotial initiation was on fifteenth day of incubation.

GROWTH PHASE

The experiment was conducted as detailed in Material and Methods to ascertain the period when the maximum growth of the pathogen could occur.

The data is presented in Table 7 and Fig. 1.

Table 7. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in potato dextrose broth.

Fig 1. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in potato dextrose broth.

Fusarium chlamydosporum

Maximum growth of the fungus (205.66 mg) was observed on 16th day of incubation and later the growth decreased significantly. As the maximum growth was observed on 16th day after inoculation, this period was used as a maximum growth period for further studies.

Rhizoctonia bataticola

Maximum growth of the fungus (234.00 mg) was observed on 12th day of incubation. As the maximum growth was observed on 12th day after inoculation, this period was used as maximum growth period for further studies.

Table 7. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in potato dextrose broth.

Sl. No.	Days after incubation	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	2	26.84	32.37	54.33
2	4	40.58	58.76	82.76
3	6	66.45	94.55	123.33
4	8	112.78	141.09	163.66
5	10	127.46	189.66	201.00
6	12	152.49	234.00	198.33
7	14	174.11	228.60	162.33
8	16	205.66	206.70	151.00
9	18	201.46	197.13	136.33
10	20	195.14	191.22	123.00
11	22	195.64	186.09	120.66
12	24	191.85	177.42	116.66
	Mean	140.87	161.47	136.11
	SEm±	2.68	2.80	1.26
	CD at 1%	10.59	11.06	4.98

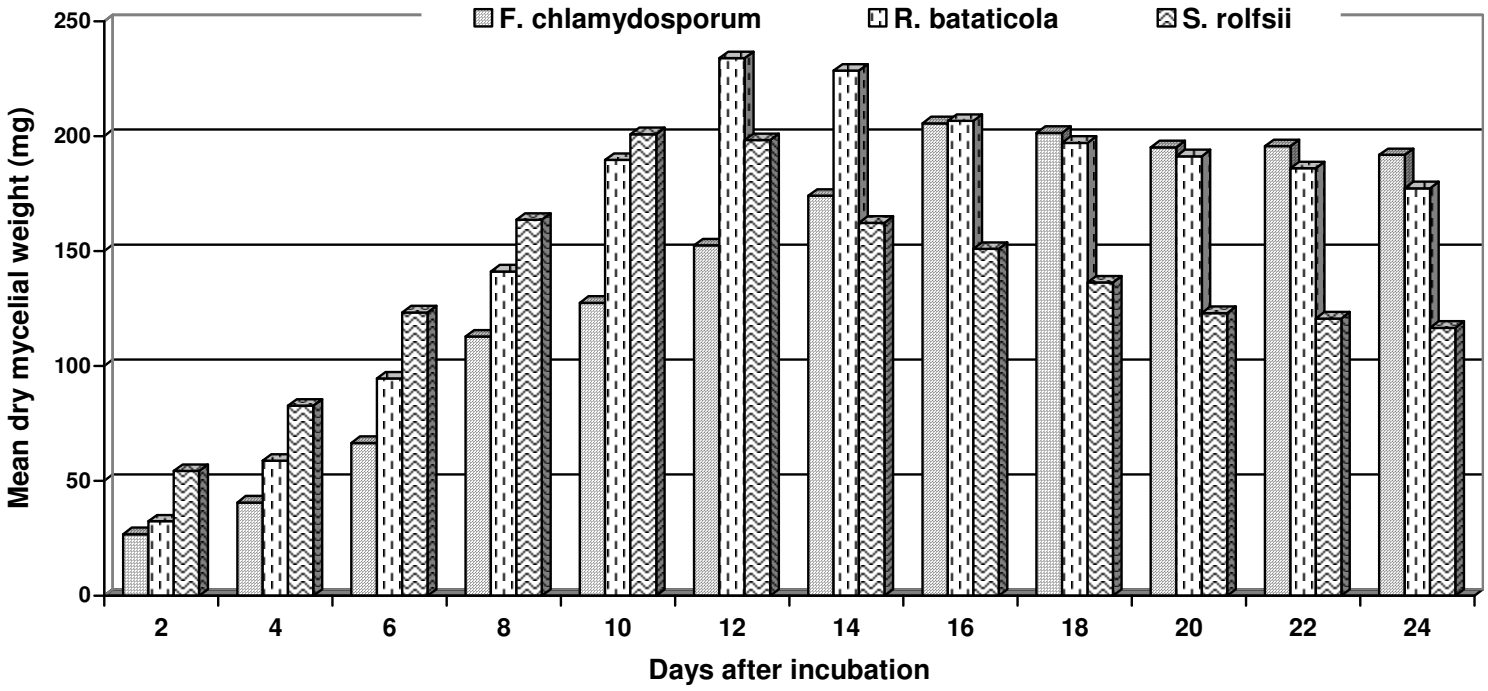


Fig. 1. Growth of F. chlamydosporum, R. botaticola and S. rolfsii in potato dextrose broth

Sclerotium rolfsii

Maximum growth of the fungus (201.00 mg) was observed on 10th day of incubation. As the maximum growth was observed on 10th day of inoculation this period was used as maximum growth period for further studies.

Growth studies on different liquid media

Growth of the fungus was studied in the liquid media to select a medium that would support maximum growth as explained in 'Material and Methods'. The results are presented in Table 8 and Fig. 2.

Table 8. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in different liquid media at 16, 12 and 10 days of incubation respectively.

Fig 2. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in different liquid media (broths) at 16, 12 and 10 days of incubation respectively.

Fusarium chlamydosporum

Among the liquid media tested, maximum growth of the fungus was observed in Richard's broth (193.66 mg) followed by potato dextrose broth (190.33 mg). The least growth of the fungus was observed in host leaf extract (71.00 mg) followed by host root extract (90.66 mg), Asthana & Hawker's medium (92.66 mg) and Brown's medium (92.33 mg).

Rhizoctonia bataticola

Among the liquid media tested, fungus growth was maximum in Richard's broth (209.33 mg) which was statistically significant and was followed by potato dextrose broth (192.33 mg). Growth supported by sabouraud's broth (167.67 mg) and Carrot broth (163.33 mg) were on par with each other. Least growth was observed in Brown's medium (46.67 mg) followed by Czapek's Dox broth (50.33 mg) and Elliot's broth (58.67 mg).

Sclerotium rolfsii

Among 14 different liquid media tested maximum growth of fungus was observed in Richard's broth (433.00 mg) followed by potato dextrose broth (427.66 mg) which were statistically on par. V8 juice broth (306.33 mg) supported next best growth, whereas least growth of fungus was observed in host root extract (73.33 mg) followed by host leaf extract (81.66 mg).

NUTRITIONAL STUDIES

Carbon utilization

This experiment was carried out to study the utilization of various carbon sources by the fungi. Ten carbon sources were used in the study as given in 'Material and Methods' and the results are presented in Table 9 and Fig. 3.

Table 9. Effect of carbon sources on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

Fig 3. Effect of carbon sources on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

Fusarium chlamydosporum

The effect of different carbon sources on the mycelial growth was significant. Maximum dry mycelial weight of 185.26 mg was obtained when sucrose was used as a carbon source. It was found significantly superior to the rest of the sources tested. This was followed by fructose (174.07 mg), dextrose (169.30 mg) and lactose (166.30 mg) which were on par with each other. The least dry mycelial weight (65.27 mg) was observed incase of citric acid.

Rhizoctonia bataticola

Effect of different carbon sources on the growth of the fungus was significant. Maximum mean mycelial dry weight of the fungus (205.67 mg) was obtained when sucrose was used as a carbon source. It was followed by fructose (198.93 mg). Least growth of the

Table 8. Growth of *F. chlamydosporum, R. botaticola* and *S. rolfsii* in different liquid media at 16, 12 and 10 days of incubation respectively.

Sl. No.	Medium	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	Asthana and Hawker's medium	92.66	121.66	196.33
2	Brown's medium	92.33	46.66	97.66
3	Carrot medium	111.33	163.63	133.66
4	Corn meal broth	104.33	128.33	245.00
5	Czapek's Dox medium	130.66	50.33	295.33
6	Elliot's medium	162.33	58.66	97.00
7	Host leaf extract broth	71.00	67.33	81.66
8	Host root extract broth	90.66	88.33	73.33
9	Malt extract broth	123.66	154.00	244.00
10	Potato dextrose broth	190.33	192.33	427.66
11	Richard's medium	193.66	209.33	433.00
12	Sabouraud's medium	147.66	167.66	90.33
13	Tochinai's medium	141.66	71.33	61.33
14	V8 juice broth	132.33	140.00	396.33
	Mean	127.48	118.52	205.19
	SEm±	0.84	1.43	1.63
	CD at 1%	3.25	5.57	6.34

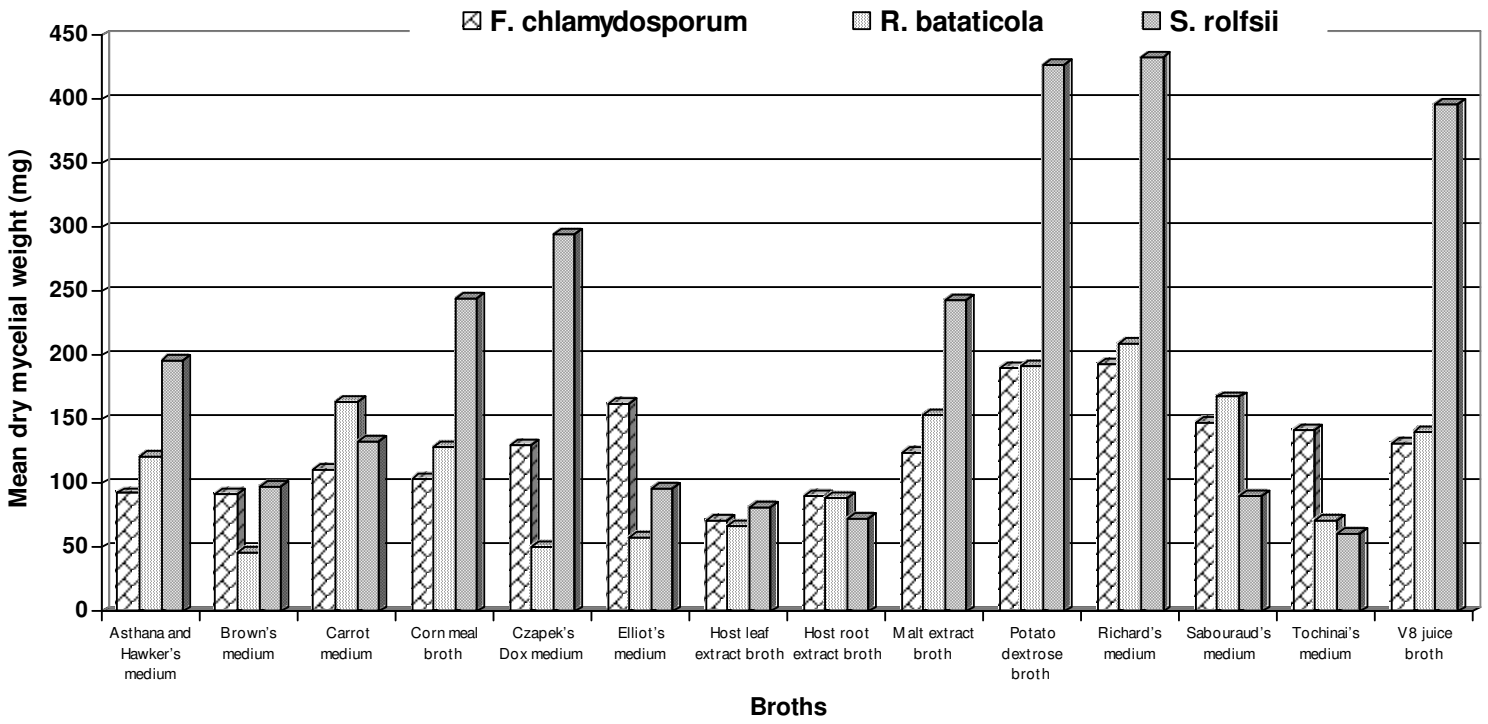


Fig. 2. Growth of F. chlamydosporum, R. botaticola and S. rolfsii in different liquid media (broths) at 16, 12 and 10 days of incubation respectively

Table 9. Effect of carbon sources on the growth of *F. chlamydosporum*, *R. bataticola* and *S. rolfsii*.

Sl. No.	Carbon sources	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	Citric acid	65.26	39.36	62.33
2	Dextrose	169.30	150.30	134.66
3	Fructose	174.06	198.93	86.66
4	Galactose	119.36	121.06	71.33
5	Glucose	136.13	187.43	153.33
6	Lactose	166.26	113.93	75.66
7	Maltose	159.73	193.63	89.66
8	Mannitol	146.50	80.33	91.33
9	Starch	111.26	128.00	115.66
10	Sucrose	185.26	205.66	183.66
	Mean	143.30	141.87	106.43
	SEm±	0.82	1.26	1.38
	CD at 1%	3.30	5.06	5.54

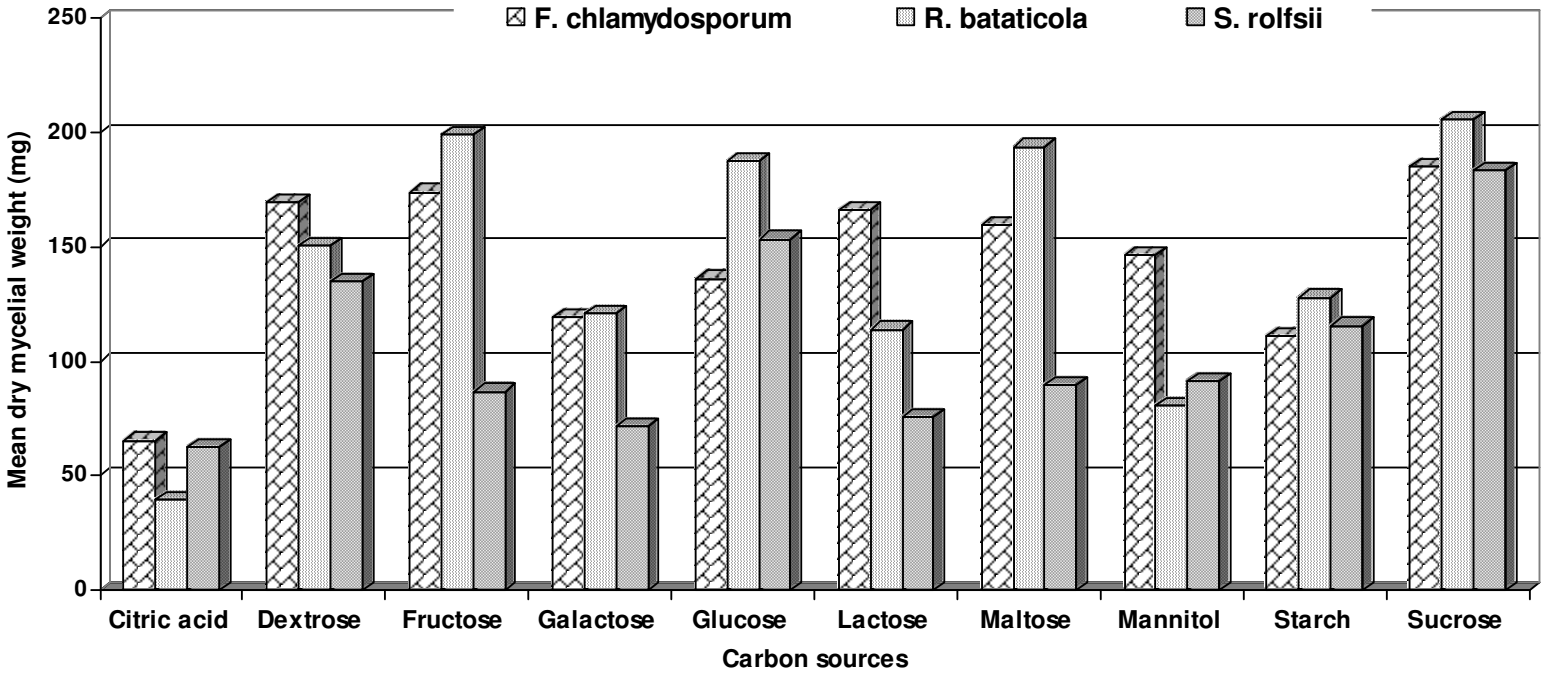


Fig. 3. Effect of carbon sources on the growth of F. chlamydosporum, R. bataticola and S. rolfsii

fungus was in citric acid (39.37 mg) followed by mannitol (80.33 mg) and lactose (113.93 mg).

Sclerotium rolfsii

Among ten different carbon sources used, maximum mean dry mycelial weight of the fungus (183.67 mg) was obtained when sucrose used as a carbon source, which was significantly superior over all other carbon sources. The least mean dry mycelial weight of the fungus was obtained incase of citric acid (62.33 mg).

Nitrogen utilization

The utilization of ten different nitrogen sources by the fungi was tested as described in Material and Methods. The results of the experiments are presented in Table 10 and Fig. 4.

Table 10. Effect of Nitrogen sources on the growth of *F. chlamydosporum*, *R. bataticola* and *S. rolfsii.*

Fig 4. Effect of Nitrogen sources on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

Fusarium chlamydosporum

The effect of different nitrogen compounds on the growth of the fungus was significant. Maximum growth of 190.53 mg was recorded when potassium nitrate was used as a source of nitrogen. Growth supported by Glycine (185.37 mg), L-Asparagine (179.00 mg), calcium nitrate (169.96 mg) and sodium nitrate (162.83 mg) were significantly different amongst each other. Least mean dry mycelial weight (105.40 mg) was observed in case of ammonium chloride.

Rhizoctonia bataticola

The effect of different nitrogen compounds on the growth of the fungus was significant. Potassium nitrate supported the maximum growth of 211.5 mg of the fungus. This was followed by glycine 208.40 mg and L-asparagine (206.60 mg), but there was no significant difference between these treatments. Least dry mycelial weight (150.33 mg) was observed incase of ammonium chloride.

Sclerotium rolfsii

The effect of different nitrogen compounds on the growth of the fungus was significant. Maximum growth of 323.67 mg was recorded when potassium nitrate was used as a source of nitrogen followed by ammonium nitrate (219.33 mg). Least dry mycelial weight (44.33 mg) was observed incase of ammonium chloride.

PHYSIOLOGICAL STUDIES

Effect of temperature

The effect of temperature on the growth of the fungi was studied as explained in 'Material and Methods and the results are presented in Table 11 and Fig. 5.

Table 11. Effect of temperature on the growth of *F. chlamydosporum*, *R. bataticola* and *S. rolfsii.*

Fig 5. Effect of temperature on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

Fusarium chlamydosporum

The effect of different temperature levels on the growth of the fungus was significant. The maximum growth of the fungus (191.30 mg) was observed at a temperature of 30^0C which was significant and superior to the rest of the temperature levels tested. Least temperature which supported the growth was 10^0C (62.10 mg).

Rhizoctonia bataticola

The effect of different temperature levels on the growth of the fungus was significant. Significantly highest growth of the fungus (209.40 mg) was observed at temperature of 30^0C. The next best temperature levels were 35^0C (192.93 mg), 25^0C (190.80 mg) and 40^0C (188.10 mg). The least growth was recorded at 10^0C (48.50 mg).

Sclerotium rolfsii

The effect of different temperatures on the growth of the fungus was significant. The maximum growth of the fungus (278.33 mg) was observed at temperature of 30^0C, which was on par with that of at 35^0C (251.33 mg), but significantly superior over the rest of the temperature levels tested. The least growth was recorded at 10^0C (51.33 mg).

Table 10. Effect of Nitrogen sources on the growth of *F. chlamydosporum*, *R. bataticola* and *S. rolfsii.*

Sl. No.	Nitrogen sources	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	Ammonium chloride	105.46	172.00	44.33
2	Ammonium nitrate	111.80	201.70	219.33
3	Ammonium sulphate	145.63	176.03	83.33
4	Calcium nitrate	169.96	208.46	120.66
5	Glycine	185.36	187.53	140.33
6	L-Asparagine	179.50	206.60	127.00
7	Methionine	126.90	161.66	148.00
8	Peptone	137.66	150.33	203.00
9	Potassium nitrate	190.53	211.53	323.66
10	Sodium nitrate	162.83	189.13	137.33
	Mean	151.57	186.50	154.70
	SEm±	0.96	0.95	1.23
	CD at 1%	3.86	3.80	4.93

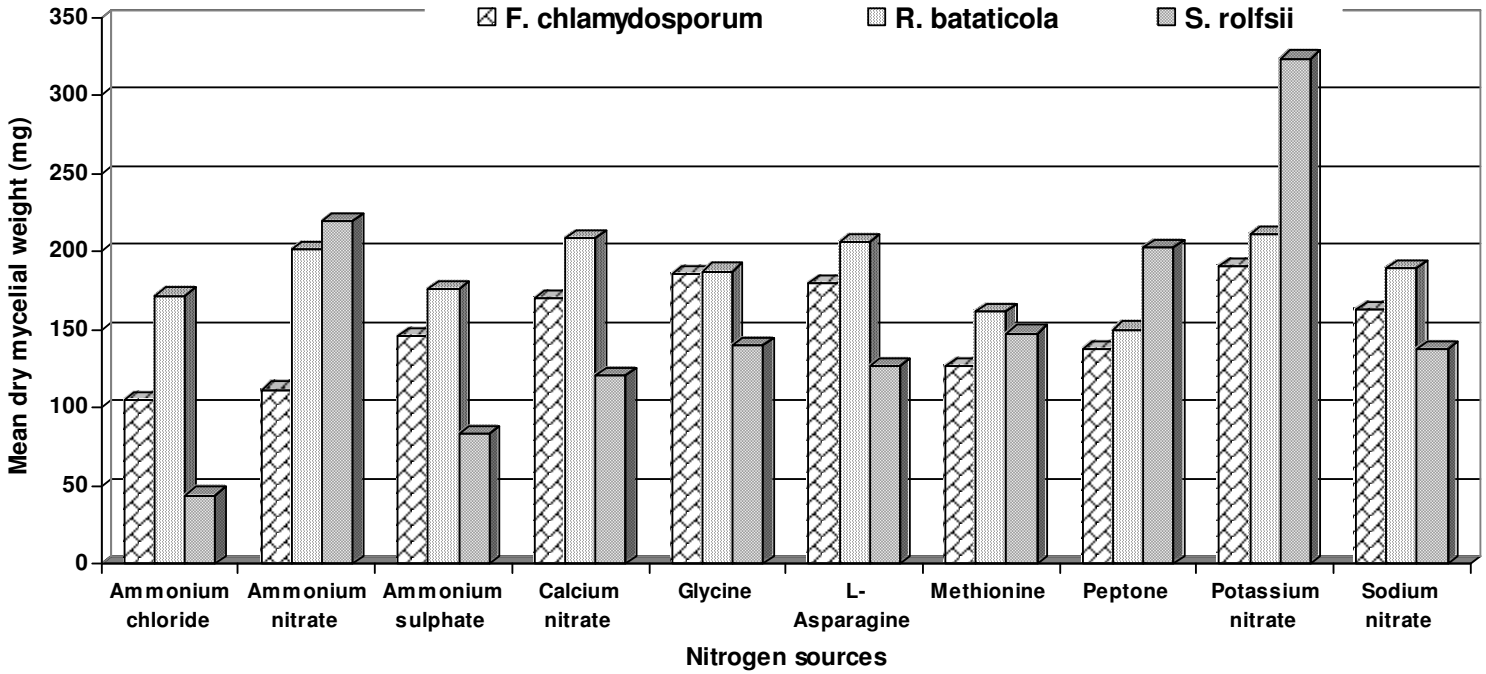


Fig. 4. Effect of Nitrogen source on the growth of F. chlamydosporum, R. batatticola and S. rolfsii

Table 11. Effect of temperature on the growth of *F. chlamydosporum*, *R. bataticola* and *S. rolfsii*.

Sl. No.	Temperature	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	10^0C	62.10	48.50	51.33
2	15^0C	106.53	80.56	71.00
3	20^0C	165.90	139.20	109.00
4	25^0C	187.60	190.80	197.66
5	30^0C	191.30	209.40	278.33
6	35^0C	180.40	192.93	251.33
7	40^0C	149.20	188.10	183.00
	Mean	149.06	149.93	163.10
	SEm±	0.84	0.59	4.05
	CD at 1%	3.49	2.45	16.84

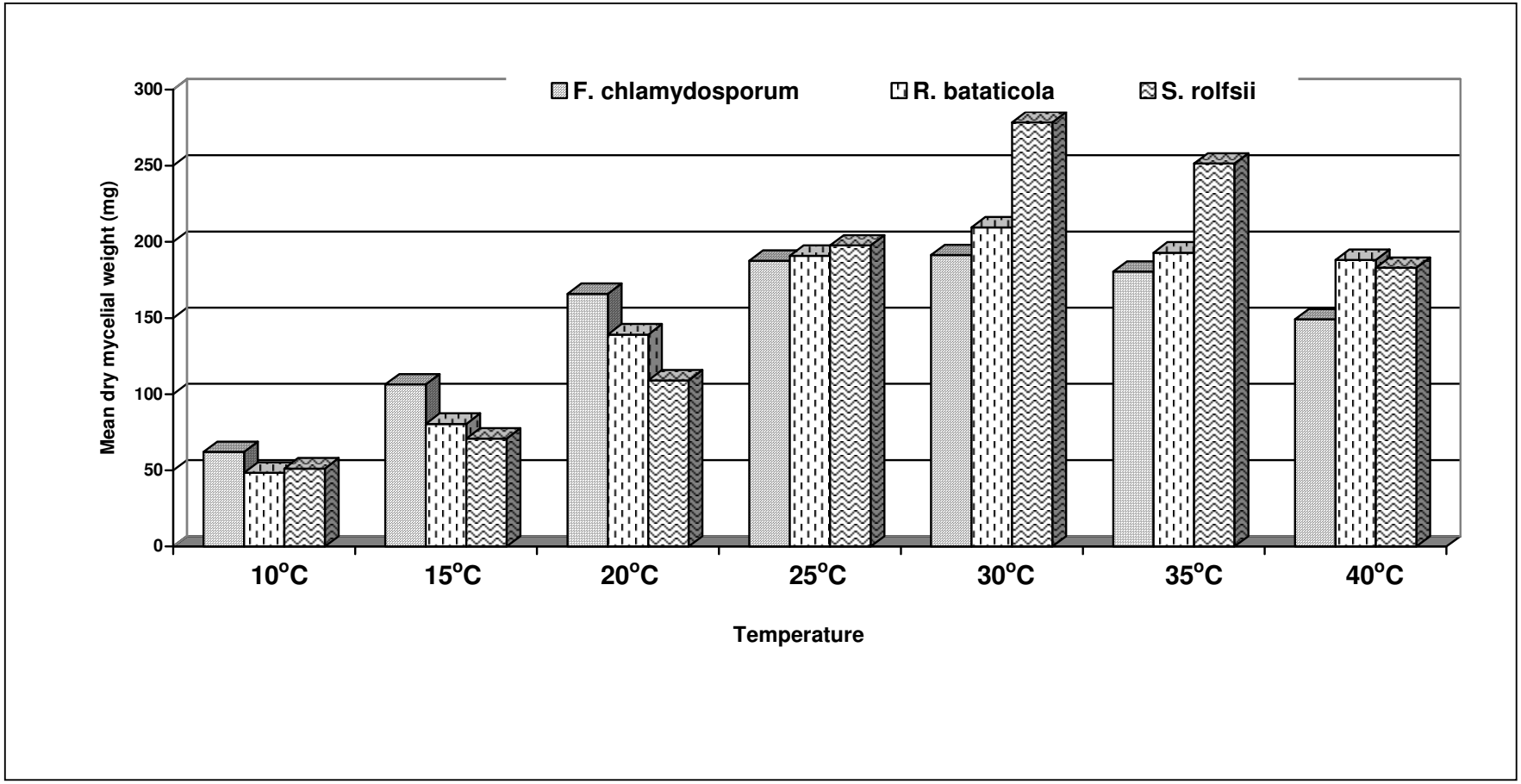


Fig. 5. Effect of temperature on the growth of F. growth of F. chlamydosporum, R. bataticola and S. rolfsii.

Table 12. Effect of pH on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii*.

Sl. No.	pH levels	Mean dry mycelial weight (mg)		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	3.5	68.73	87.93	496.33
2	4.0	117.80	108.03	505.00
3	4.5	140.67	131.57	466.67
4	5.0	159.67	170.03	488.00
5	5.5	169.97	188.13	470.67
6	6.0	177.00	200.67	426.67
7	6.5	182.53	218.13	210.33
8	7.0	188.47	209.27	263.33
9	7.5	179.70	196.83	279.33
10	8.0	168.07	181.33	208.67
	Mean	155.26	169.19	381.50
	SEm±	0.89	0.65	7.59
	CD at 1%	3.58	2.62	30.45

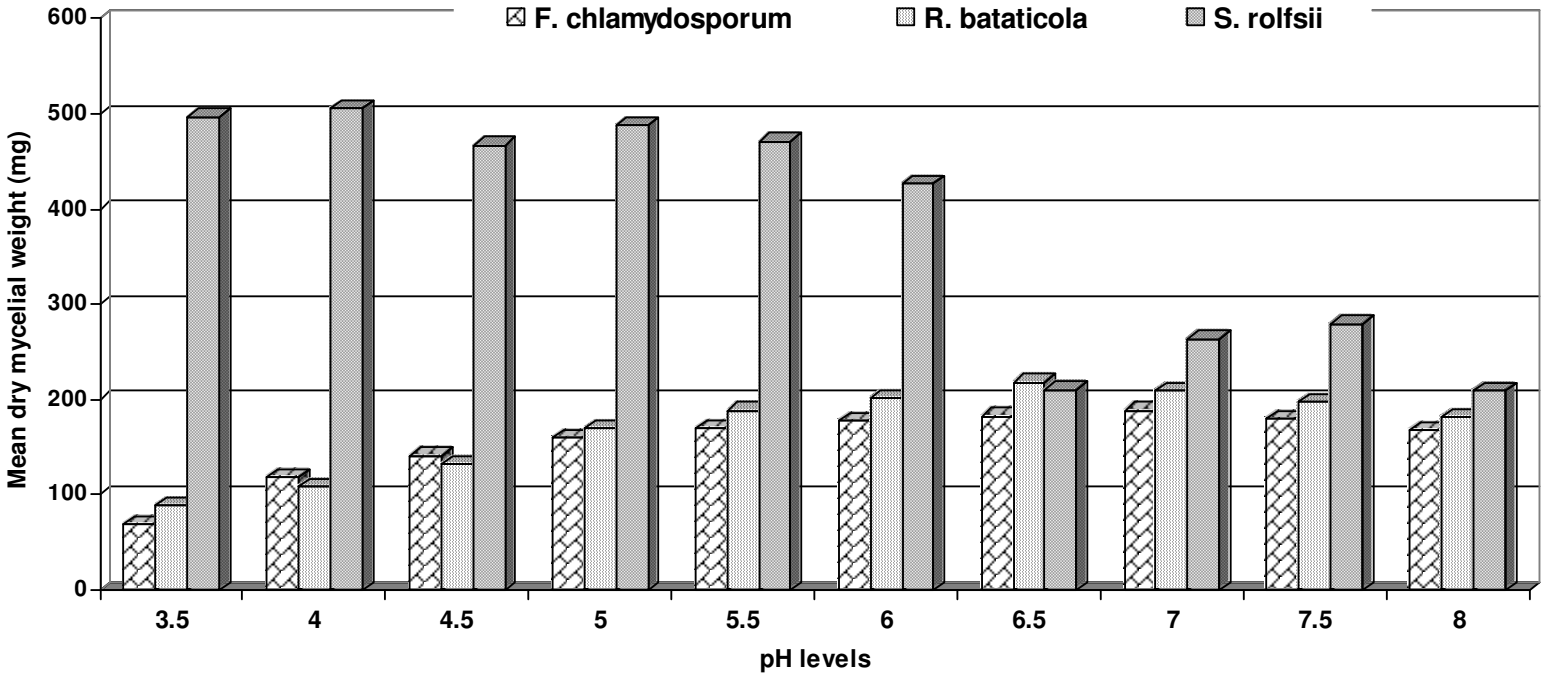


Fig. 6. Effect of pH levels on the growth of F. chlamydosporum, R. bataticola and S. rolfsii

Effect of hydrogen ion concentration

The effects of pH on the growth of the fungi were studied as described in 'Material and Methods. The results obtained are presented in Table 12 and Fig. 6.

Table 12. Effect of pH on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii*.

Fig 6. Effect of pH levels on the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii*.

Fusarium chlamydosporum

The effect of different pH levels on the growth of the fungus was significant. The maximum growth of the fungus was noticed at a pH level of 7.0 (188.47 mg) which was significant over other pH levels. The next best pH level was at 6.5 (182.53 mg). This was followed by pH levels of 7.5 (179.70 mg) and 6.0 (177.00 mg) which were on par with each other. The growth decreased as the pH moved away from 7.0 on either side. Also growth of the fungus at pH 5.5 (169.97 mg) and pH 8.0 (168.07 mg) were on par with each other. Least growth of the fungus was observed at pH 3.5 (68.73 mg).

Rhizoctonia bataticola

The effect of different pH levels on the growth of the fungus was significant. The maximum growth of the fungus was noticed at a pH level of 6.5 (218.13 mg). It was found significantly superior over rest of the pH levels tested. This was followed by pH 7.0 (209.27 mg), 6.0 (200.67 mg) and 7.5 (196.83 mg). The least mean dry mycelial weight of the fungus was observed at pH level 3.5 (87.93 mg).

Sclerotium rolfsii

The effect of different pH levels on the growth of the fungus was significant. The maximum growth of the fungus was noticed at a pH level of 4.0 (505.00 mg) followed by at pH levels 3.5 (496.33 mg), 5.0 (488.00 mg), which were on par with each other and significant over rest of the pH levels tested. The least mycelial growth of the fungus was at pH level of 8.00 (208.67 mg) which was on par with pH level 6.5 (210.33 mg).

MANAGEMENT STUDIES

In vitro evaluation of bioagents

The competitive ability of antagonists against *F. chlamydosporum, R. bataticola* and *S. rolfsii* were studied by dual culture method as decribed in 'Material and Methods'. The results obtained are presented in Table 13 and Plate 5.

Table 13. Inhibition of mycelial growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii* by different biocontrol agents.

Plate 5. Antagonistic effect of bioagents against *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

Fusarium chlamydosporum

There is a significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *F. chlamydosporum. T. harzianum* showed the maximum inhibition of the test fungus (56.17%) and it was found significantly superior over rest of the bioagents tested. This was followed by *T. viride* (52.20%), *T. virens* (48.90%) and *T. koningii* (43.33%). However, they significantly differed with one another. The least antagonistic effect was observed incase of *P. fluorescens* (17.67%).

Rhizoctonia bataticola

There is a significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *R. bataticola.* Maximum per cent inhibition of mycelial growth was obtained when *T. viride* (78.80%) was used as bioagent. It was found to be significantly superior to the rest of the bioagents tested. This was followed by *T.*

Table 13. Inhibition of mycelial growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii* by different biocontrol agents.

Sl. No.	Bioagents	Per cent inhibition of mycelial growth		
		F. chlamydosporum	***R. bataticola***	***S. rolfsii***
1	*Trichoderma viride*	52.20 (46.26)*	78.80 (62.47)	50.60 (45.35)
2	*Trichoderma harzianum*	56.17 (48.55)	64.80 (53.63)	52.90 (46.42)
3	*Trichoderma virens*	48.90 (44.35)	51.03 (45.59)	48.80 (43.86)
4	*Trichoderma koningii*	43.33 (41.17)	56.23 (48.47)	41.67 (40.20)
5	*Pseudomonas fluorescens*	17.67 (24.86)	18.77 (25.65)	19.87 (26.47)
	Mean	43.65 (41.04)	53.93 (47.16)	47.77 (40.46)
	SEm±	0.11	0.16	0.24
	CD at 1%	0.45	0.67	0.99

*Figures in parentheses are arc sin angular transformed values

a. Antagonistic effect of bioagents against *F. chlamydosporum*.
1. *Trichoderma harzianum*
2. *Trichoderma viride*
3. *Trichoderma koningii*
4. *Trichoderma virens*
5. *Pseudomonas fluorescens*
6. Control

b. Antagonistic effect of bioagents against *R. bataticola*.
1. *Trichoderma harzianum*
2. *Trichoderma viride*
3. *Trichoderma koningii*
4. *Trichoderma virens*
5. *Pseudomonas fluorescens*

c. Antagonistic effect of bioagents against *S. rolfsii*.
1. *Trichoderma harzianum*
2. *Trichoderma viride*
3. *Trichoderma koningii*
4. *Trichoderma virens*
5. *Pseudomonas fluorescens*
6. Control

Plate 5. Antagonistic effect of bioagents against *F. chlamydosporum*, *R. bataticola* and *S. rolfsii*.

Plate 5. Antagonistic effect of bioagents against F. chlamydosporum, R. bataticola and S. rolfsii

harzianum (64.80%) and *T. koningii* (56.23%). The least antagonistic effect was observed incase of *P. fluorescens* (18.77%).

Sclerotium rolfsii

There was a significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *S. rolfsii.* Maximum per cent inhibition of mycelial growth was obtained when *T. harzianum* (52.90%) was used as bioagent. It was found to be significantly superior to the rest of the bioagents tested. This was followed by *T. viride* (50.60%) *T. virens* (48.80%). The least antagonistic effect was observed incase of *P. fluorescens* (19.87%).

In vitro evaluation of botanicals

As plant extracts are cost effective means of management, an effort was made to know the efficacy of different plant extracts against *F. chlamydosporum, R. bataticola* and *S. rolfsii.* This was carried out by adopting the poison food technique as described in 'Material and Methods'.

Results relating to the effects of plant extracts on per cent inhibition of *F. chlamydosporum, R. bataticola* and *S. rolfsii* are presented in Table 14, 15 and 16 and Plate 6.

Table 14. Effect of botanicals on growth of *F. chlamydosporum*.

Table 15. Effect of botanicals on growth of *R. bataticola*.

Table 16. Effect of botanicals on growth of *S. rolfsii*.

Plate 6. *In vitro* evaluation of botanicals against the fungal pathogens involved in causing collar rot complex of *Coleus.*

Fusarium chlamydoporum

Twelve botanicals were evaluated against *F. chlamydosporum.* The results revealed that effect of botanicals on the fungal growth was significant. *Parthenium hysterophorus* (34.97%) was found effective in inhibiting mycelial growth which was significantly superior to all other plant extracts evaluated. *Azadirachta indica* (31.81%) and *Eucalytus globulus* (30.87%) were next best which were on par with each other. However, *Ocimum sanctum* (29.78%) and *Eupotorium oduratum* (28.77%) were on par with each other. Least inhibition of mycelial growth of *F. chlamydosporum* was obtained in case of *Tridax procumbens* (21.02%) and *Cassia occidentalis* (19.52) leaf extracts.

The leaf extracts at 10 per cent were significantly superior to five per cent. *P. hysterophorus* (37.91%) at 10 per cent was the best and significantly superior over all other plant extracts. Next best in inhibiting the growth of mycelia was *E. globulus* (34.13%) followed by *A. indica* (33.23%) and *P. hysterophorus* (32.03%) at five per cent. *T. procumbens* at five per cent (19.70%) and *C. occidentalis* at five per cent (18.53%) were least effective in inhibiting the mycelial growth of *F. chlamydosporum.*

Rhizoctonia bataticola

The antifungal activity of twelve leaf extracts were assayed, at two concentrations against *R. bataticola.* The results revealed that, effect of plant extracts on the fungal growth was significant.

P. hysterophorus (49.29%) was most effective in inhibiting the mycelial growth which was significantly superior over all other treatments. This was followed by *A. indica* (47.61%). *Clerodendron inerme* (39.28%) and *O. sanctum* (28.21%) were next best followed by *Pongamia pinnata* (36.56%) and Eucalyptus (36.10%).

The leaf extracts at 10 per cent were significantly superior over five per cent. *P. hysterophorus* (56.06%) at 10 per cent and *A. indica* at 10 per cent (54.66%) were the best and significantly superior over all other plant extracts. Next best was *C. inerme* (42.28%) and *O. sanctum* (40.87%) which were on par with each other followed by *E. globulus* (38.95%) and *P. pinnata* (38.82%) which were on par with each other. *Prosopis juliflora* (17.95%) and *E. oduratum* (16.69%) at five per cent were least effective in inhibiting the mycelial growth of *R. bataticola.*

Table 14. Effect of botanicals on growth of *F. chlamydosporum*.

Sl. No.	Leaf extracts	Per cent inhibition of mycelial growth		Mean
		Concentration		
		5%	10%	
1	*Azadirachta indica* A. Juss	30.38 (33.46)*	33.23 (35.22)	31.81 (34.34)
2	*Cassia occidentalis* L.	18.53 (25.51)	20.50 (26.93)	19.52 (26.22)
3	*Clerodendron inerme* Gaertn	20.57 (26.98)	25.73 (30.50)	23.15 (28.74)
4	*Duranta repens* L.	21.53 (27.66)	23.87 (29.26)	22.70 (28.46)
5	*Eucalyptus globulus* Labill	27.60 (31.71)	34.13 (35.77)	30.87 (33.74)
6	*Eupatorium oduratum* L.	28.00 (31.96)	29.53 (32.94)	28.77 (32.45)
7	*Glyricidia maculata* L.	21.30 (27.50)	25.60 (30.41)	23.45 (28.96)
8	*Ocimum sanctum* L.	28.80 (32.47)	30.77 (33.71)	29.78 (33.09)
9	*Parthenium hysterophorus* L.	32.03 (34.49)	37.91 (38.02)	34.97 (36.25)
10	*Pongamia pinnata* L.	20.40 (26.86)	24.33 (29.57)	22.37 (28.22)
11	*Prosopis juliflora* L.	26.40 (30.93)	28.62 (32.36)	27.51 (31.64)
12	*Tridax procumbens* L.	19.70 (26.36)	22.33 (28.21)	21.02 (27.29)
	Mean	24.60 (29.66)	28.05 (31.91)	26.33 (30.78)
		Botanicals (B)	**Concentration (C)**	**B x C**
	SEm±	0.19	0.08	0.27
	CD at 1%	0.72	0.29	1.02

*Figures in parentheses indicate angular transformed values

Table 15. Effect of botanicals on growth of *R. bataticola*.

Sl. No.	Leaf extracts	Per cent inhibition of mycelial growth		Mean
		Concentration		
		5%	10%	
1	*Azadirachta indica* A. Juss	40.56 (39.58)*	54.66 (47.70)	47.61 (43.64)
2	*Cassia occidentalis* L.	20.28 (26.77)	25.84 (30.57)	23.06 (28.67)
3	*Clerodendron inerme* Gaertn	36.29 (37.06)	42.28 (40.58)	39.28 (38.82)
4	*Duranta repens* L.	20.33 (26.80)	27.94 (31.93)	24.14 (29.36)
5	*Eucalyptus globulus* Labill	33.26 (35.24)	38.95 (38.63)	36.10 (36.94)
6	*Eupatorium oduratum* L.	16.69 (24.13)	25.99 (30.66)	21.34 (27.39)
7	*Glyricidia maculata* L.	21.80 (27.85)	31.29 (34.03)	26.55 (30.94)
8	*Ocimum sanctum* L.	35.54 (36.61)	40.87 (39.76)	38.21 (38.19)
9	*Parthenium hysterophorus* L.	42.52 (40.72)	56.06 (48.50)	49.29 (44.61)
10	*Pongamia pinnata* L.	34.30 (35.84)	38.82 (38.56)	36.56 (37.21)
11	*Prosopis juliflora* L.	17.95 (25.08)	28.11 (32.03)	23.03 (28.59)
12	*Tridax procumbens* L.	25.55 (30.38)	27.15 (31.42)	26.35 (30.90)
	Mean	28.76 (32.17)	36.50 (37.03)	32.63 (34.60)
		Botanicals (B)	**Concentration (C)**	**B x C**
	SEm±	0.24	0.10	0.34
	CD at 1%	0.90	0.37	1.28

*Figures in parentheses indicate angular transformed values

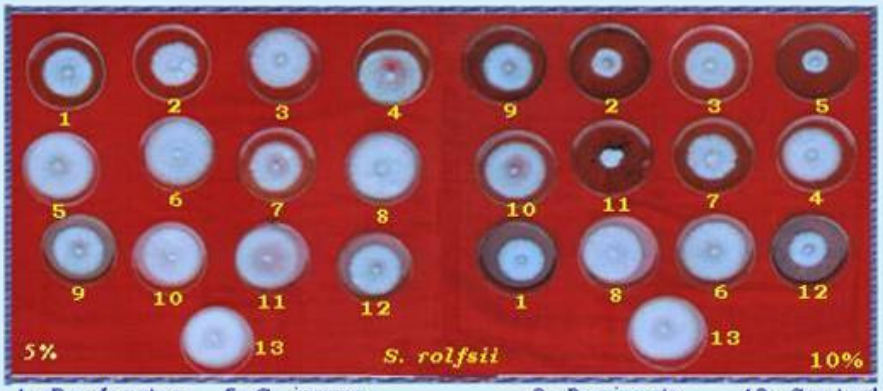
Table 16. Effect of botanicals on growth of *S. rolfsii*.

Sl. No.	Leaf extracts	Per cent inhibition of mycelial growth		Mean
		Concentration		
		5%	10%	
1	*Azadirachta indica* A. Juss	25.62 (30.42)*	51.40 (45.82)	38.51 (38.12)
2	*Cassia occidentalis* L.	36.33 (37.09)	51.50 (45.88)	43.91 (41.48)
3	*Clerodendron inerme* Gaertn	16.32 (23.82)	70.49 (57.13)	43.41 (40.48)
4	*Duranta repens* L.	12.82 (20.97)	21.31 (27.50)	17.07 (29.24)
5	*Eucalyptus globulus* Labill	21.50 (27.63)	79.95 (63.43)	50.72 (45.53)
6	*Eupatorium oduratum* L.	37.57 (37.81)	51.74 (46.02)	44.65 (41.92)
7	*Glyricidia maculata* L.	22.86 (28.57)	25.46 (30.32)	24.16 (29.45)
8	*Ocimum sanctum* L.	56.74 (48.90)	66.55 (54.69)	61.65 (51.80)
9	*Parthenium hysterophorus* L.	28.70 (32.41)	59.37 (50.43)	44.03 (41.42)
10	*Pongamia pinnata* L.	28.39 (32.21)	45.41 (42.39)	36.90 (37.30)
11	*Prosopis juliflora* L.	8.08 (16.51)	30.66 (33.64)	19.37 (25.07)
12	*Tridax procumbens* L.	21.77 (27.83)	25.26 (30.19)	23.52 (29.01)
	Mean	26.39 (30.35)	48.26 (43.95)	37.33 (37.15)
		Botanicals (B)	**Concentration (C)**	**B x C**
	SEm±	0.30	0.12	0.42
	CD at 1%	1.12	0.46	1.58

*Figures in parentheses indicate angular transformed values

1. *B. oduratum*
2. *O. sanctum*
3. *A. indica*
4. *E. globulus*
5. *T. procumbens*
6. *C. inerme*
7. *C. occidentalis*
8. *P. juliflora*
9. *P. pinnata*
10. *G. maculata*
11. *D. repens*
12. *P. hysterophorus*
13. Control

1. *A. indica*
2. *E. globulus*
3. *O. sanctum*
4. *T. procumbens*
5. *G. maculata*
6. *P. pinnata*
7. *P. hysterophorus*
8. *C. inerme*
9. *C. occidentalis*
10. *E. oduratum*
11. *P. juliflora*
12. *D. repens*
13. Control



1. *E. oduratum*
2. *O. sanctum*
3. *A. indica*
4. *G. maculata*
5. *C. inerme*
6. *D. repens P. pinnata*
7. *C. occidentalis*
8. *P. juliflora*
9. *P. pinnata*
10. *T. procumbens*
11. *E. globulus*
12. *P. hysterophorus*
13. Control

Plate 6. *In vitro* evaluation of botanicals against the fungal pathogens involved in causing collar rot complex of *Coleus*.

Plate 6. In vitro evaluation of botanicals against the fungal pathogens involved in causing collar rot complex of Coleus.

Sclerotium rolfsii

Twelve plant leaf extracts were assayed, at two concentrations against *S. rolfsii.* The results revealed that, effect of plant extracts on the fungal growth was significant. *O. sanctum* (61.65) was found effective in inhibiting mycelial growth which was significantly superior over all other plant extracts evaluated. *E. globulus* (50.72%) was next best followed by *E. odurotum* (44.65%) and *P. hysterophorus* (44.03). *C. occidentalis* (43.91%) and *C. inerme* (43.41%) were on par with each other and were next in order. Least growth inhibition of *S. rolfsii* was obtained from *Prosopis juliflora* (19.37%) and *Duranta repens* (17.07%).

The leaf extracts at 10 per cent were significantly superior over five per cent. *E. globulus* (79.95%) at 10 per cent was the best and significantly superior over all other plant extracts. Next best in inhibiting the growth of mycelia were *C. inerme* (70.49%), *O. sanctum* (66.55%) and *P. hysterophorus* (59.37%). However, least growth inhibition was recorded in *Prosopis juliflora* (8.08%) at five per cent.

In vitro evaluation of fungicides

Efficacy of five systemic and five non-systemic fungicides were tested at different concentraiton by poisoned food technique as explained in 'Material and Methods'. The per cent inhibition over control was worked out based on the test fungal growth in control plate. The results thus obtained are presented in Table 17a, 17b, 18a, 18b, 19a and 19b and Plate 7.

Table 17a. Inhibition of mycelial growth of *F. chlamydosporum* by systemic fungicides.

Table 17b. Inhibition of mycelial growth of *F. chlamydosporum* by non-systemic fungicides.

Table 18a. Inhibition of mycelial growth of *r. Bataticola* by different systemic fungicides.

Table 18b. Inhibition of mycelial growth of *r. Bataticola* by different non-systemic fungicides.

Table 19a. Inhibition of mycelial growth of *S rolfsii* by systemic fungicides.

Table 19b. Inhibition of mycelial growth of *S rolfsii* by non-systemic fungicides.

Plate 7. Photograph showing inhibition of mycelial growth of pathogens by different fungicides.

Fusarium chlamydosporum

The data presented in the table revealed that the effect of different fungicides on growth of *F. chlamydosporum,* was significant. Among systemic fungicides. Carbendazim and propiconazole were effective in completely (100%) inhibiting the growth of fungus at both the concentrations (0.05% and 0.10%). Among non-systemic fungicides tested, chlorothalonil (94.15%) at 0.2 per cent found to be significant followed by mancozeb (92.83%) at 0.2 per cent. Least inhibition of fungus growth was recorded in copper oxy chloride (33.72%) at 0.1 per cent. Interaction between fungicides and concentration was found to be significant.

Rhizoctonia bataticola

The per cent inhibition of mycelial growth of *R. bataticola* in different fungicides were found significant. Systemic fungicides, like carbendazim and propiconazole at both the concentrations were found to be most effective and significantly superior which inhibited cent per cent growth of the fungus. Least mycelial growth inhibition was recorded in bayletan (57.08%) at 0.05 per cent. Non-systemic fungicides, like captan at 0.1 per cent inhibited the mycelial growth by cent per cent and found to be significant over others. Least inhibition of mycelial growth was observed in thiram (38.32%) at 0.1 per cent.

The systemic fungicides at 0.1% and non-systemic fungicides at 0.2 per cent were significantly superior over systemic at 0.05 per cent and non-systemic at 0.1 per cent.

Sclerotium rolfsii

Table 19a and 19b revealed that there was significant difference among different chemicals evaluated.

The systemic fungicides at 0.1 per cent and non-systemic fungicides at 0.2% were found significantly superior over systemic fungicides at 0.05 per cent and non-systemic

Table 17a. Inhibition of mycelial growth of *F. chlamydosporum* by systemic fungicides.

Sl. No.	Systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.05%	0.1%	
1	Carbendazim	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)
2	Hexaconazole	65.64 (54.14)	88.22 (69.97)	76.93 (62.06)
3	Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Benomyl	39.82 (39.15)	52.33 (46.36)	46.08 (42.75)
5	Bayleton	47.97 (43.86)	51.51 (45.89)	49.74 (44.87)
	Mean	70.69 (63.45)	78.28 (68.34)	74.48 (65.89)
		Fungicides (F)	**Concentration (C)**	**F x C**
	SEm±	0.18	0.11	0.25
	CD at 1%	0.69	0.44	0.98

*Figures in parentheses indicate angular transformed values

Table 17b. Inhibition of mycelial growth of *F. chlamydosporum* by non-systemic fungicides.

Sl. No.	Non-systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.1%	0.2%	
1	Captan	76.72 (61.19)*	78.49 (62.41)	77.61 (61.79)
2	Thiram	28.39 (32.21)	32.89 (35.01)	30.64 (33.61)
3	Chlorothalonil	67.89 (55.51)	94.15 (76.08)	81.02 (65.79)
4	Mancozeb	91.46 (73.05)	92.83 (74.53)	92.15 (73.79)
5	Copper oxy chloride	33.72 (35.51)	39.06 (38.70)	36.39 (37.11)
	Mean	59.64 (51.49)	67.48 (57.35)	63.56 (54.42)
		Fungicides (F)	**Concentration (C)**	**F x C**
	SEm±	0.25	0.16	0.36
	CD at 1%	0.98	0.62	1.39

*Figures in parentheses indicate angular transformed values

Table 18a. Inhibition of mycelial growth of *R. bataticola* by different systemic fungicides.

Sl. No.	Systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.05%	0.1%	
1	Carbendazim	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)
2	Hexaconazole	91.47 (73.06)	93.33 (75.10)	92.40 (74.08)
3	Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Benomyl	74.43 (59.66)	82.21 (65.10)	78.32 (62.38)
5	Bayleton	57.08 (49.10)	73.06 (58.77)	65.07 (53.94)
	Mean	84.59 (72.38)	89.72 (75.18)	87.16 (74.09)
		Fungicides (F)	**Concentration (C)**	**F x C**
	SEm±	0.30	0.19	0.42
	CD at 1%	1.16	0.73	1.64

*Figures in parentheses indicate angular transformed values

Table 18b. Inhibition of mycelial growth of R. bataticola by different non-systemic fungicides.

Sl. No.	Non-systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.1%	0.2%	
1	Captan	88.14 (69.91)*	100.00 (90.00)	94.07 (79.98)
2	Thiram	38.82 (38.27)	48.45 (44.13)	43.39 (41.20)
3	Chlorothalonil	76.87 (61.29)	85.02 (67.29)	80.94 (64.29)
4	Mancozeb	84.34 (66.76)	84.29 (66.69)	84.31 (66.73)
5	Copper oxy chloride	72.15 (58.19)	75.82 (60.58)	73.99 (59.38)
	Mean	71.96 (58.88)	78.72 (65.75)	75.34 (63.32)
		Fungicides (F)	**Concentration (C)**	**F x C**
	SEm±	0.37	0.23	0.52
	CD at 1%	1.43	0.90	2.02

*Figures in parentheses indicate angular transformed values

Table 19a. Inhibition of mycelial growth of *S rolfsii* by systemic fungicides.

Sl. No.	Systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.05%	0.1%	
1	Carbendazim	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)
2	Hexaconazole	69.34 (56.41)	83.36 (65.99)	76.35 (61.19)
3	Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Benomyl	32.35 (34.68)	37.29 (37.66)	34.82 (36.17)
5	Bayleton	23.01 (28.67)	25.01 (30.02)	24.01 (29.34)
	Mean	64.94 (59.97)	69.13 (62.75)	67.04 (61.36)
		Fungicides (F)	Concentration (C)	F x C
	SEm±	0.27	0.17	0.38
	CD at 1%	1.04	0.66	1.47

*Figures in parentheses indicate angular transformed values

Table 19b. Inhibition of mycelial growth of *S rolfsii* by non-systemic fungicides.

Sl. No.	Non-systemic fungicides	Per cent inhibition of mycelial growth		Mean
		Concentration		
		0.1%	0.2%	
1	Captan	72.25 (58.25)*	100.00 (90.00)	86.13 (74.15)
2	Thiram	73.93 (59.33)	94.56 (76.64)	84.24 (67.77)
3	Chlorothalonil	32.97 (35.06)	37.45 (37.75)	35.21 (36.40)
4	Mancozeb	96.27 (78.97)	100.00 (90.00)	98.14 (84.51)
5	Copper oxy chloride	26.49 (30.99)	67.09 (54.42)	46.29 (42.70)
	Mean	60.38 (52.52)	79.62 (69.78)	70.00 (61.15)
		Fungicides (F)	Concentration (C)	F x C
	SEm±	0.34	0.22	0.48
	CD at 1%	1.33	0.84	1.88

*Figures in parentheses indicate angular transformed values

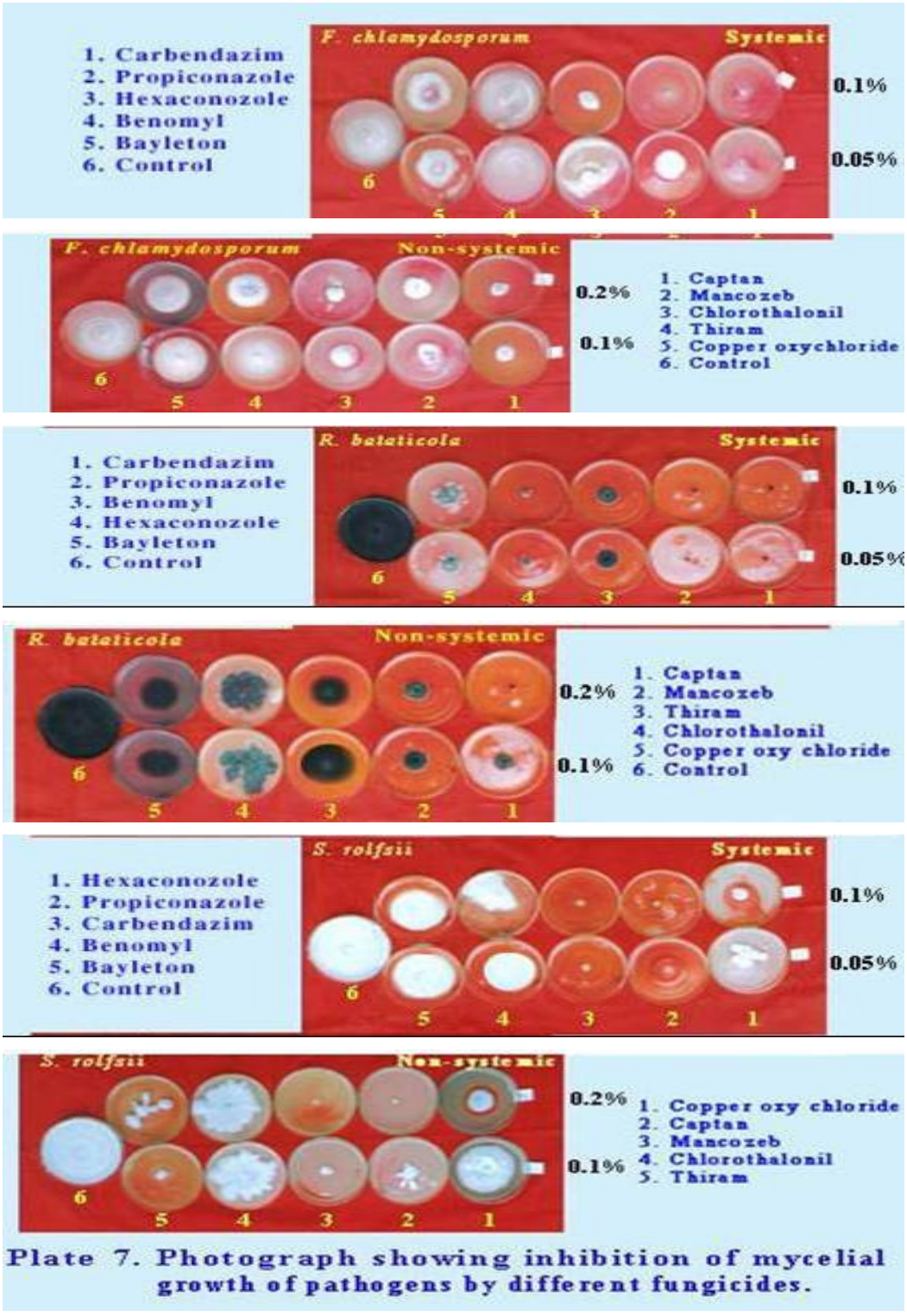


Plate 7. Photograph showing inhibition on of mycelial growth of pathogens by different fungicides

fungicides at 0.1 per cent. Systemic fungicides, carbendazim and propiconazole at both concentrations (0.05% and 0.1%), captan and mancozeb at 0.2 per cent were found to be most effective and significantly superior which inhibited cent per cent growth of the fungus. However, least mycelial growth inhibition was recorded in bayleton (23.01%) at 0.1 per cent among systemic fungicides and among non-systemic fungicides least mycelial growth was recorded in copper oxychloride (26.49%) at 0.1 per cent.

INTERACTIONS BETWEEN ROOT-KNOT NEMATODE AND WILT INDUCING FUNGI

Disease complex involving nematode and fungi have gained momentum in the recent years leading to considerable yield loss. The data on interaction between *M. incognita* and wilt inducing fungi are presented in Table 20 and 21 and Plate 8.

Table 20. Influence of single or sequential inoculations with *m. Incognita, f. Chlamydosporum, r. Bataticola* and *s. Rolfsii* on per cent wilt incidence in *coleus forskohlii*.

Table 21. Influence of single or sequential inoculation with *m. Incognita, f. Chlamydosporum, r. Bataticola* and *s. Rolfsii* on plant growth parameters, root knot index and nematode population in *coleus forskohlii*.

Plate 8. Interaction studies involving *M. incognita* and various fungal pathogens.

Wilt symptoms were first recorded at 45 days after inoculation in treatment inoculated with *M. incognita* seven days prior to inoculation of all the fungal pathogens simultaneously (*F. chlamydosporum* + *R. bataticola* + *S. rolfsii*). Whereas incase of inoculation of root knot nematode followed by individual fungal pathogen wilt symptoms was first recorded in treatment inoculation with root knot nematode seven days prior to inoculation of *S. rolfsii* (75 days after inoculation), followed by treatment inoculated with root knot nematode seven days prior to inoculation of *R. bataticola* (100 days after inoculation) and inoculated with root knot nematode seven days prior to inoculation of *F. chlamydosporum* (100 days after inoculation). Incase of treatment inoculated with individual fungal pathogen seven days prior to inoculation of root knot nematode wilt symptoms were first recorded in treatment inoculated with *S. rolfsii* seven days prior to inoculation of root knot nematode (75 days after inoculation) followed by treatment inoculated with *R. bataticola* seven days prior to inoculation of root knot nematode (100 days after inoculation) and treatment inoculated with *F. chlamydosporum* seven days prior to inoculation of root knot nematode (115 days after inoculation). However, in individual inoculations of fungi, the wilt symptoms were first recorded in *S. rolfsii* (90 days after inoculation) followed by *R. bataticola* (115 days after inoculation) and *F. chlamydosporum* (130 days after inoculation).

Significant reduction in shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was noticed in all the treatments in comparison to uninoculated control. Data presented in Table 21 revealed that the four organisms (*M. incognita, F. chlamydosporum, R. bataticola* and *S. rolfsii*) affected plant growth parameters like shoot and root length, fresh and dry weight of shoot, fresh and dry weight of root per plant. Of the four organisms inoculated individually, *S. rolfsii* caused greater reduction in plant growth parameters over control than caused either by *R. bataticola* or *F. chlamydosporum* or *M. incognita.* While reduction in plant growth parameters caused by *F. chlamydosporum, R. bataticola* were on par with each other. Incase of sequential inoculation of *M. incognita* seven days prior to inoculation of individual fungal pathogen. Greatest reduction in growth parameters was caused by the treatment with *M. incognita* seven days prior to inoculation of *S. rolfsii* followed by the treatment inoculated with *M. incognita* seven days prior to inoculation of *R. bataticola* and the treatment inoculated with *M. incognita* seven days prior to inoculation of *F. chlamydosporum.* However, the treatment inoculated with *M. incognita* seven days prior to inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii,* showed a highest reduction in plant growth parameters like shoot length, fresh and dry weight of shoot, whereas in treatment inoculated with *F. chlamydosporum* + *R. bataticola* + *S. rolfsii* seven days prior to inoculation of *M. incognita,* showed highest reduction in plant growth parameters like root length, fresh and dry weight of roots when compared to other treatments.

In these, interactions, it was observed that the effect of sequential inoculation of the combined inocula (*F. chlamydosporum* + *R. bataticola* + *S. rolfsii*) followed by *M. incognita* or vice versa, on plant growth parameters was additive in nature, where inoculations were simultaneous wherein the resultant effect on growth parameters was almost equal to sum of

Table 20. Influence of single or sequential inoculations with *M. incognita, F. chlamydosporum, R. bataticola* and *S. rolfsii* on per cent wilt incidence in *Coleus forskohlii*.

Treatment	Per cent wilt of plants								
	Days after inoculation								
	30	**45**	**60**	**75**	**90**	**100**	**115**	**130**	**150**
C	-	-	-	-	-	-	-	-	-
N	-	-	-	-	-	-	-	-	-*
F	-	-	-	-	-	-	-	25	50
N→F	-	-	-	-	-	25	50	75	75
F→N	-	-	-	-	-	-	25	50	50
R	-	-	-	-	-	-	25	50	100
N→R	-	-	-	-	-	50	75	100	-
R→N	-	-	-	-	-	25	50	75	100
S	-	-	-	-	25	50	75	100	-
N→S	-	-	-	25	50	100	-	-	-
S→N	-	-	-	25	50	75	100	-	-
N→F+R+S	-	25	75	100	-	-	-	-	-
F+R+S→N	-	-	25	50	100	-	-	-	-

C : Uninoculated control

N : *Meloidogyne incognita* alone.

F : *Fusarium chlamydosporum* alone.

N→F : Inoculation of *M. incognita* seven days prior to inoculation of *F. chlamydosporum.*

F→N : Inoculation of *F. chlamydosporum* seven days prior to inoculation of *M. incognita.*

R : *Rhizoctonia bataticola* alone.

N→R : Inoculation of *M. incognita* seven days prior to inoculation of *R. bataticola.*

R→N : Inoculation of *R. bataticola* seven days prior to inoculation of *M. incognita.*

S : *Sclerotium rolfsii* alone.

N→S : Inoculation of *M. incognita* seven days prior to inoculation of *S. rolfsii.*

S→N : Inoculation of *S. rolfsii* seven days prior to inoculation of *M. incognita.*

N→F+R+S: Inoculation of *M. incognita* seven days prior to inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii.*

F+R+S→N: Inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii* seven days prior to inoculation of *M. incognita.*

*Nematode alone inoculated plants exhibited stunted growth and marginal yellowing of leaves

Table 21. Influence of single or sequential inoculation with *M. incognita, F. chlamydosporum, R. bataticola* and *S. rolfsii* on plant growth parameters, root knot index and nematode population in *Coleus forskohlii*.

Treatment	Length (cm)		Shoot weight (g)		Root weight (g)		Root knot index	Final nematode population /200 cc of soil
	SHOOT	Root	Fresh	Dry	Fresh	Dry		
C	45.75	20.20	40.75	11.35	12.72	3.67	-	-
N	36.12	18.32	26.75	7.35	10.02	2.47	5.0	1625
F	30.37	17.55	25.50	6.17	10.22	2.57	-	-
N→F	22.60	16.02	12.25	4.15	9.42	2.22	3.50	1165
F→N	26.25	13.02	17.50	4.90	10.32	2.70	3.25	610
R	32.30	19.07	25.62	5.95	10.75	2.95	-	-
N→R	23.47	14.87	15.12	4.22	10.05	2.47	3.75	1355
R→N	27.70	12.37	19.00	5.12	8.62	2.05	3.25	735
S	28.42	16.75	20.50	5.65	9.60	2.40	-	-
N→S	21.02	13.87	12.12	3.82	10.32	2.55	3.00	845
S→N	25.65	11.25	16.37	4.60	7.55	1.77	3.00	505
N→F+R+S	19.95	10.07	9.62	3.02	4.45	0.80	2.75	465
F+R+S→N	20.45	9.95	10.25	3.12	4.37	0.67	2.00	415
Mean	27.69	14.87	19.34	5.34	9.112	2.26	2.27	593.85
SEm±	0.64	0.25	0.46	0.13	0.14	0.05	0.19	9.47
CD at 1%	2.45	0.97	1.74	0.50	0.52	0.21	0.75	36.19

a. Inoculation of individual pathogens

1. Uninoculated control, 2. *Fusarium chlamydosporum* alone
3. *Rhizoctonia bataticola* alone 4. *Sclerotium rolfsii* alone
5. *Meloidogyne incognita* alone

b. Sequential inoculation of fungal pathogens seven days prior to inoculation of *M. incognita*.

1. C 2. F → N 3. R → N 4. S → N 5. F+R+S → N

c. Sequential inoculation of *M. incognita* seven days prior to inoculation of fungal pathogens

1. C 2. N → F 3. N → R 4. N → S 5. N → F+R+S

Plate 8. Interaction studies involving *M. incognita* and various fungal pathogens.

Plate 8. Interaction studies involving M. incognita and various fungal pathogens.

individual effects. However, in treatment inoculated with *M. incognita* seven days prior to inoculation of all three fungal pathogens or vice versa, the resultant effect was more than simple additive effect.

Root knot index (5.00) as well as juvenile population in soil (1625.00) were maximum in the treatment where only nematode were inoculated. Whereas, incase of sequential inoculations involving nematode and fungus, the lowest root knot index and nematode population were recorded in the treatment inoculated with *S. rolfsii* seven days prior to inoculation of *M. incognita,* followed by inoculation of *F. chlamydosporum* seven days prior to inoculation of *M. incognita,* inoculation of *R. bataticola* seven days prior to inoculation of *M. incognita.* However, incase of sequential inoculations involving nematode and all the fungi, the lowest root knot index and nematode population were recorded in the treatment inoculated with *F. chlamydosporum* + *R. bataticola* + *S. rolfsii* seven days prior to inoculation of *M. incognita* followed by the treatment of inoculation of *M. incognita* followed by the treatment inoculated with *M. incognita* seven days prior to inoculation of *F. chlamydosporum* + *R. bataticola* + *S. rolfsii.*

MANAGEMENT OF COLLAR ROT COMPLEX OF *Coleus forskohlii* USING DIFFERENT BIOCONTROL AGENTS, ORGANIC AMENDMENTS AND CHEMICALS

The results of the field experiment conducted in a natural sick plot of Department of Spices and Plantation Crops, Kittur Rani Channamma College of Horticulture, Arabhavi (Karnataka) are presented in Table 22.

Table 22. Management of collar rot complex of *coleus forskohlii* using different biocontrol agents, organic amendments and chemicals.

Per cent wilt incidence

The treatment T_8 recorded significantly lowest per cent wilt incidence (12.76) over control (35.52) followed by T_2 (18.87) and T_3 (19.98). Treatments T_1, T_2, T_3, T_4, T_5, T_9 and T_{10} were statistically on par. The highest wilt per cent was recorded in control.

Population of root knot juveniles/200 cc of soil

The number of juveniles per 200 cc of soil was significantly highest in control (2173.33) and significantly lowest number of juveniles was recorded in treatment T_8 (873.33), followed by T_6 (1066.67) and T_5 (1180.00). Treatments T_1 (1640.00), T_2 (1633.33) and T_3 (1533.33) were on par with each other.

Number of galls/5 g of root

The number of galls/5 g of root was significantly lowest in treatment T_8 (10.13), followed by T_6 (14.93) which was on par with each other. However, treatments T_5 (16.07), T_4 (17.33), T_3 (18.27), T_2 (19.53) and T_1 (21.13) were on par with each other. Significantly highest number of galls (5 g) of root was recorded in T_{11} (Control), as compared to other treatments.

Colony forming units of *Fusarium chlamydosporum*

Colony forming units of *Fusarium chlamydosporum* was significantly highest in the control (19.60) whereas significantly lowest in T_9 (3.60) followed by T_{10} (3.80) and T_8 (6.20) were on par with each other.

Colony forming units of *Rhizoctonia bataticola*

Colony forming units of *Rhizoctonia bataticola* was found to be significantly lowest in the treatment T_9 (6.80 x 10^{-3}/g of soil) followed by T_{10} (7.40 x 10^{-3}/g of soil) were on par with each other. Whereas, treatments T_1 (12.20 x 10^{-3}/g of soil), T_2 (12.60 x 10^{-3}/g of soil) and T_3 (14.20 x 10^{-3}/g of soil) were on par with each other. Colony forming units of *Rhizoctonia bataticola* was found to be significantly highest in control (21.60 x 10^{-3}/g of soil).

Table 22. Management of collar rot complex of *Coleus forskohlii* using different biocontrol agents, organic amendments and chemicals.

Treatments	Per cent wilt incidence*	Population of root knot juveniles/200 cc of soil	No. of galls/ 5 g of root	*Cfu***	
				Fusarium chlamydosporum	***Rhizoctonia. Bataticola***
T1	21.09 (27.33)**	1640.00	21.13	7.60	12.20
T2	18.87 (25.74)	1633.33	19.53	8.00	12.60
T3	19.98 (26.51)	1533.33	18.27	8.00	14.20
T4	23.31 (28.84)	1366.67	17.33	10.60	15.60
T5	21.09 (27.24)	1180.00	16.07	12.60	16.40
T6	24.42 (29.57)	1066.67	14.93	16.20	17.60
T7	25.53 (30.38)	1960.00	25.67	15.20	18.80
T8	12.76 (20.93)	873.33	10.13	6.20	9.60
T9	21.09 (27.33)	1933.33	23.33	3.60	6.80
T10	23.31 (28.84)	1906.67	23.00	3.80	7.40
T11	35.52 (36.59)	2173.33	28.40	19.60	21.60
Mean	22.45 (28.12)	1569.69	19.82	10.13	13.89
SEm±	1.18	49.05	1.83	0.87	0.95
CD at 5%	3.48	144.68	5.38	2.49	2.72

*Observations recorded at harvest (150 days after planting).
**Figures in parentheses are arc sin angular transformed values.
***Cfu – Colony forming units $X10^{-3}$/g of soil (Average of 5 replications

V. DISCUSSION

In the soil ecosystem, plants are constantly exposed to multiplicity 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 result 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 pathogens. Of all the interactions of pathogens with nematodes, none are more damaging to the crops worldwide than those caused by the combined effects of wilt inducing fungi and plant parasitic nematodes. The combination of nematode 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 (Francl and Wheeler, 1993).

Of several nematodes of economic importance, root knot nematodes are most widely studied and are commonly found involved in synergistic interactions with wilt inducing fungi. In *Coleus forskholili,* association *F. chlamydosporum, R. bataticola* and *S. rolfsii* with *M. incognita* was noticed. Association of a single fungus with *M. incognita* or combination of two or more fungi with *M. incognita* was also noticed. Hence, studies of pathogenicity and management aspects were carried out.

In general, root knot disease caused by *Meloidogyne* spp. is one of the major constraints in the productivity of several crops. No information is available with regard to the incidence and severity of the disease in coleus in northern Karnataka. Hence, a survey was undertaken to assess the collar rot complex disease incidence on coleus crop in Raichur, Bellary, Bagalkot, Belgaum, Dharwad and Bidar districts.

The findings of the present study revealed that the root knot disease caused by *M. incognita* was observed in all the districts surveyed. But overall disease incidence was more in Bellary district (34.50%) followed by Bidar (23.15%) and Raichur district (18.00%).

In the present survey, high root knot-nematode population was recorded in Hitnal (Ballary dist.) followed by Dharwad and other plant parasitic nematode population was high in Bidar followed by Benchikottala (Bellary dist.).

The present survey also indicated the association of *Meloidogyne* spp. with fungi namely, *F. chlamydosporum, R. bataticola* and *S. rolfsii* in most of the locations surveyed, with a high frequency of both the pathogens (*M. incognita* with either of the three fungi) from soil and root samples collected from Bellary district.

The most characteristic feature having taxonomic importance incase of females of *Meloidogyne* species is the presence of perineal pattern in the posterior body region. This area comprises the tail terminus, phasmids, lateral lines, anus and vulva surrounded by cuticular folds or striae. The perineal 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 perineal 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. Straie 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 *M. incognita.* By studying perineal patterns of these populations which showed high squarish dorsal arch, zigzag straie around the vulva, it was seen that these characters were very similar to those of *M. incognita.* Thus, the prevailing pathogen was identified as *Meloidogyne incognita.*

The causal organisms *F. chlamydosporum, R. bataticola* and *S. rolfsii* were isolated from diseased plants of coleus by following standard tissue isolation method. The pure culture obtained was sub-cultured on Petriplate and slants containing potato dextrose agar and stored in refrigerator for use in further studies. Sachidananda (2005), Shyla (1998) and Boby and Bagyaraj (2003) isolated *Fusarium.* Sacchidananda (2005) and

Kamalakannan *et al.* (2003) isolated *Rhizoctonia* from infected roots of coleus, also isolated *Sclerotium* from collar region of coleus following standard tissue isolation technique.

The fungus produced micro conidia which were fusiform to clavate with rounded apex, usually with single septum. Macro conidia were curved three to five septate. Chlamydospores were usually rough or smooth walled. Fungus produced whitish to pink colonies with wooly and abundant mycelium on potato dextrose agar. Growth rate was rapid. The description of the fungus agreed with the description given for *F. chlamydosporum* by Commonwealth Mycological Institute, Kew, Surrey, England (Booth, 1971), Sachidananda (2005), Shyla (1998) and Boby and Bagyaraj (2003) in coleus.

The fungus grew rapidly and profusely on potato dextrose agar covering the entire Petriplate within five days of inoculation. Hyphae were hyaline at first and gradually turned grey to brownish black. As the culture grew old, abundant tiny black sclerotia were produced and the culture turned completely black. Mycelium of the fungus was septate and was branched at right angles having a septum at the point of origin. The sclerotia were spherical to oblong in shape and were of dark brown to black coloured. The description of the fungus agreed with the description given for *R. bataticola* (Taub.) Butler (Pycnidial stage *Macrophomina phaseolina*) by Ashby (1927). Dingra and Sinclair (1977), Prashanthi (1994) and Sachidananda (2005).

The identification of *S. rolfsii* was made based on the morphological characters described by Domsch *et al.,* (1980). The pathogen produced a white dense cotton like radiating mycelium on PDA medium. Initiation of sclerotial bodies was noticed seventh day after inoculation. In the initial stage, the sclerotial bodies produced were white, but later turned gradually to brown at maturity.

Pathogenicity test of *M. incognita* carriedout under pot culture conditions, showed stunted plants bearing yellowish coloured leaves. When the affected plants were uprooted, deformed roots with prominent galls and swellings were observed. Symptoms expressed in the present study is in agreement with the descriptions of Dhande and Sulaiman (1961).

The first symptom appeared 25 days after planting. The infected plants were well characterized by gradual yellowing and drying of leaves, followed by loss of vigour and pre-mature death. Such plants showed discolouration of roots and complete decaying of tap and lateral root system. The bark of such plants was easily peeled off. Such affected plants were killed finally due to severe rot. The infected tubers showed rotting and emitted bad odour. The symptoms produced by the pathogen were found to be in agreement with Shyla (1998); and Sachidananda (2005).

The symptoms produced by *R. bataticola* were similar to the reports of earlier worker (Sachidananda, 2005). The infection started at the collar region of the plants as water soaked areas and the affected tissues soon turned into a soft, black, watery mass. The infection was found to spread to the roots of the plants and caused decay which ultimately toppled and collapsed the infected plants. These infected plants were easily pulled off from the soil and showed brown discolouration of roots followed by rotting of root hairs. Similar symptoms were observed by Prashanthi (1994); Santha Kumari *et al.* (2002) and Sachidananda (2005) in case of *R. bataticola* in safflower coral hibiscus and *Coleus* respectively.

The symptoms caused by *S. rolfsii* are in agreement with the description given by Chowdhury (1945); Maiti (1977); and Palakshappa (1986). The pathogen reisolated was identified as *S. rolfsii* as it resembled *S. rolfsii* described by Domsch *et al.* (1980) on the basis of morphological characters.

These pathogens were reisolated from infected roots and the identity of the causal organisms were confirmed by comparing with the original cultures.

Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from the substrate upon which they grow. In order to culture the fungi artificially it is necessary to supplement in the medium, those essential nutrients needed for their growth, development and other metabolic processes. To find out the best sources of nutrients for the fungal growth, different synthetic and non-synthetic growth media were tested. The radial growth of the fungus was used to determine growth on solid media. While, dry mycelial weight was used for liquid media.

The fungus was grown on 14 different solid media. The results indicated that the best mycelial growth was made on potato dextrose agar (86.00 mm) followed by Richard's agar (84.33 mm) and carrot agar (81.67 mm). Least growth was recorded in host leaf extract agar (27.67 mm). The results are in confirmation with those of Shyla (1998) and Sachidananda (2005) incase of *F. chlamydosporum.* Abundant sporulation was observed incase of

Richard's, sabouraud's, malt extract and potato dextrose agar media. Similar observations were made by Jhamaria (1972), Sherkar and Utikar (1982), Shyla (1998) and Sachidananda (2005).

The fungus was grown on 14 different solid media. The results indicated that the best mycelial growth was made on potato dextrose agar (90.00 mm) followed by Richard's agar (89.33 mm) and malt extract agar (81.67 mm). Least growth was recorded in host leaf extract agar (39.33 mm). Similar observations were made by Kulkarni *et al.* (1992); Sahi *et al.* (1992); Karunanithi *et al.* (2000). Lokesha (2002) and Sacchidananda (2005) in *R. bataticola.*

Abundant sclerotial production was observed in Richard's agar, carrot agar and potato dextrose agar. Similar observations were made by Vasudeva (1937); Ghosh and Sen (1973); Kulkarni *et al.* (1992), Lokesha (2002) and Sachidananda (2005).

The fungus was grown on 14 different solid media. The results indicated that the best mycelial growth was made on potato dextrose agar (90.00 mm) followed by Richard's agar (88.67) and carrot agar (84.67 mm). Least growth was recorded in host leaf extract agar (32.67 mm). Similar observations were made by Sulladmath (1977); Manjappa (1979); Hari *et al.* (1988) Takahashi (1927) and Weber (1931).

The fungus also differed with regard to time taken for sclerotial initiation on solid media, Sulladmath *et al.* (1977) and Manjappa (1979) have also observed similar type of variation for sclerotial initiation. The earliest sclerotial initiation was observed on seventh day on potato dextrose agar and Richard's agar.

Every living organism has a definite growth pattern, in which it attains a maximum growth and declines thereafter.

In the present study, the fungus *F. chlamydosporum* attained maximum growth after 16 days of incubation in potato dextrose broth and there after a decline in dry mycelial weight was observed. The results of the study indicated that the autolysis stage of the fungus starts from 18^{th} day after inoculation. Similar results were obtained by Shyla (1998) and Sachidananda (2005).

The fungus *R. bataticola* attained maximum growth, after 12 days of incubation in potato dextrose broth and thereafter a decline in dry mycelial weight was observed. The results of the study indicated that the autolysis stage of fungus starts from 14^{th} day after inoculation. Ramamurthy (1982) and Sachidananda (2005) made similar observations.

The fungus *S. rolfsii* attained maximum growth, after ten 10 days of incubation in potato dextrose broth and thereafter a decline in dry mycelial weight was observed. The results of the study indicated that the sclerotial initiation started from 12^{th} day after inoculation. Similar observations were made by Lingaraju (1977).

The fungus *F. chlamydosporum* was grown on 14 different liquid media. The results indicated that the best dry mycelial weight was made on Richard's broth (193.67 mg) followed by potato dextrose broth (190.33 mg) and Elliots broth (162.33 mg). Least mycelial growth of the fungus was observed in Host leaf extract broth (71.00 mg).

In *R. bataticola* the fungus was grown on 14 different liquid media. The results indicated that the best dry mycelial weight was observed on Richard's broth (209.33 mg) followed by potato dextrose broth (192.33 mg). Least growth was recorded in host leaf extract broth (67.33 mg).

The fungus *S. rolfsii* was grown on 14 different liquid media. The results indicated that the best dry mycelial weight was observed in Richard's broth (433.00 mg) followed by potato dextrose broth (427.66 mg). Least mycelial growth was recorded in host root extract broth (73.33 mg).

In the radial measurement, it was not possible to consider the amount of submerged mycelium. Hence, the determination of dry weight was thought to be the best method for precise work. Here, the good growth of fungi i.e. *Fusarium, Rhizoctonia* and *Sclerotium* was noticed in Richard's medium. The ability of these fungi to grow more in Richard's medium indicated the requirement of certain nutrients and vitamins, which might be presented in the medium. This is in conformity with the findings of Sataraddi *et al.* (2003) incase of *F. udum,* Sachidananda (2005) incase of *F. chlamydosporum,* Shanmugam and Govindswamy (1973), Kulkarni (2001) incase of *R. bataticola* and Lingaraju (1977) incase of *S. rolfsii*.

Carbon is the most essential element required by fungi since it comprises of about 50 per cent of total mycelial weight as a component of both structural and functional constituents. The utilization of various carbon compounds may depend on the activity of the fungus to utilize certain simpler norms.

In the present study, sucrose supported the maximum mean dry mycelial weight (185.27 mg) of the fungus followed by fructose (174.07 mg) and dextrose (169.30 mg). Least growth (65.27 mg) was observed incase of citric acid.

Incase of *R. bataticola,* sucrose supported the maximum mean dry mycelial weight (205.67 mg) followed by fructose (198.93 mg). Least growth (39.37 mg) was recorded in citric acid.

Among different carbon sources, sucrose supported the maximum mean dry mycelial weight (183.67 mg) followed by Glucose (153.33 mg). Least mycelial growth was recorded in citric acid (62.33 mg).

The utilization of various carbon compounds, may depend either on the activity of the fungus to utilize certain simpler forms or on its power to convert the complex carbon compounds into simpler forms, which may be easily utilized.

Sucrose was found to be the better carbon source than any other carbon sources tested for *Fusarium, Rizoctonia* and *Sclerotium.* Sucrose being the major component of photosynthesis in plants is generally utilized as a good carbon source by most of the plant pathogenic fungi. Similar observations were made by Luthra and Vasudeva (1938); Singh (1967), Ramamurthy (1982) and Sachidananda (2005).

Nitrogen is very important element for protein synthesis and like carbon, it is used by fungi for functional as well as structural purpose. But all the sources of nitrogen are not equally good for the growth of fungi.

The fungus made the best growth when potassium nitrate (190.53 mg). The next best nitrogen sources were Glycine (185.37 mg) and L-asparagine (179.00 mg). Least mean dry mycelial weight of the fungus was observed in ammonium chloride (105.40 mg).

The fungus made the best growth in potassium nitrate (211.50 mg dry mycelial weight) followed by Glycine (208.40 mg) and L-asparagine (206.60 mg). Least mean dry mycelial weight of the fungus was observed in ammonium chloride (150.33 mg).

The fungus grew well utilizing potassium nitrate (323.67 mg dry mycelial weight) followed by ammonium nitrate (219.33 mg dry mycelial weight). Least mean dry mycelial weight was recorded in ammonium chloride (44.33 mg).

The fungi *F. chlamydosporum, R. bataticola* and *S. rolfsii* showed variation in their ability to utilize different nitrogen sources. They utilized potassium nitrate more efficiently and it was a better nitrogen source than any other nitrogen sources tested. The nitrate compounds are excellent nitrogen sources for imperfect fungi and also ascomycetes. Similar findings were made by Moore (1924); Subramanian and Srinivasa (1953); and Sachidananda (2005) in case of *Fusarium* spp. Moniz and Bhide (1963) and Tandon (1967) made similar observations in case of *R. bataticola.*

The next best nitrogen sources were L-asparagine and glycine. The fact that good growth in these amino acids indicates the direct utilization of these compounds in protein synthesis. Sowmya (1993); Sataraddi *et al.* (2003) and Sachidananda (2005) made similar observations incase of *Fusarium* spp. Paharia and Sahai (1968); Shanmugam and Govindswamy (1973) and Sachidananda (2005) obtained the similar findings in case of *R. bataticola.*

Temperature plays an important role, among the external factors which influence the growth and reproduction of fungi. Each fungus has its own temperature range. In the present study, maximum growth of *F. chlamydosporum* (191.30 mg), *R. bataticola* (209.40 mg) and *S. rolfsii* (278.33 mg) was obtained at 30^0C, whereas optimum range was 25^0C to 30^0C, 25^0C to 35^0C and 30^0C to 35^0C respectively.

Togashi (1949) reported that a number of plant pathogenic fungi have optimum temperature range of 20^0C to 30^0C and about half of these have their optimum temperature between 25^0C and 30^0C. The present findings are in confirmation of with the results of Neal (1927); Chaung and Su (1988); Raghuwanshi (1995); Pokhar Rawal *et al.* (2003), in case of species of *Fusarium,* Uppal (1936); Waseer *et al.* (1990); Rodrigues *et al.* (1997) and Kulkarni (2001) in case of *R. bataticola* and Gondo (1964); Manjappa (1979); Sulladmath *et al.* (1977) and Palaiah (2002) in case of *S. rolfsii.*

The fungi generally utilize substrate in the form of solution only if the reaction of the solution is conducive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for the better fungal growth. In general, the pathogen showed the preference for pH levels towards acidic side to neutrality. Maximum growth of these fungi i.e., *F. chlamydosporum* (188.47 mg), *R. bataticola* (218.13 mg) and *S. rolfsii* (505.00 mg) was obtained at pH 7.0, 6.5 and 3.5 respectively.

Similar results of maximum growth of *Fusarium* spp. at acidic to neutral pH were recorded by Moore (1924); Neal (1927); Sowmya (1993) and Sachidananda (2005).

Similar results of maximum growth of *R. bataticola* were observed at pH 6.5. These findings are in accordance with Uppal (1936); Muthukrishnan *et al.* (1995); Kulkarni (2001); Lokesha (2002) and Sachidananda (2005).

Similar results of maximum growth of *S. rolfsii* was observed at pH 3.5. These findings are in accordance with the reports of Lingaraju (1977) and Hari *et al.* (1988).

At reduced pH, the cell membrane becomes studded with H^+ ions which limit the passage of cations. The reverse condition could be obtained when the medium is alkaline and the accumulated H^+ ions prevent the passage of essential anions. Enzyme activity is also conditioned by the reaction of the medium, as a result, the reduced growth of fungi was observed at extremities. The pH of the culture filtrate in majority of the cases decreased towards acidic side showing preference for the cations in the media.

Besides chemical control, biological control is an effective, eco-friendly and alternative approach for any disease management practice.

In the present study, *T. harzianum* produced maximum inhibition of mycelial growth (56.17%), this was followed by *T. viride* (52.20%), *T. virens* (48.90%) and *T. koningii* (43.33%). These results are in accordance with Yahia *et al.,* (1985); Elias *et al.* (1993); Beltaief (1995); Mathew and Gupta (1998) and Sachidananda (2005).

The results of dual culture technique on *R. bataticola* revealed that *T. harzianum* (78.80%), *T. viride* (64.80%) and *T. koningii* (56.23%) were found best in inhibiting the colony growth. All the species of *Trichoderma* showed more hyphal inhibition compared to bacterial antagonists. This can be attributed to higher competitive ability of *Trichoderma* spp. similar trend was observed by Guffar (1968); Ayers and Adam (1981); Kowalik (1997); Indra *et al.* (2003) and Sachidananda (2005).

The results indicated that *T. viride* (52.90%), *T. harzianum* (50.60%) and *T. virens* (48.80%) were found best in inhibiting the colony growth. Similar results were observed by Singh and Singh (1994); Iqbal *et al*. (1995) and Bagwat (1997).

Continuous use of chemicals/fungicides in the management of disease also brought new problems with them. More alarming amongst them are pollution of air, water, soil, residual toxicity, development of resistance in pathogens against chemicals and harmful effects on non-target organisms. Consequently, there has been alarming development of harmful environment for human beings. Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally non pollutive, renewable, inexhaustible, indigenously available, easily accessible, largely non phytotoxic, systemic, ephemeral, thus readily biodegradable, relatively cost effective and hence constitute a suitable component of plant protection in the strategy of integrated disease management. Hence, screening of plant products for their effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the component in the integrated disease management (Khadar, 1999 and Nagesh, 2000).

Among 12 leaf extracts tested against *F. chlamydosporum, P. hysterophorus* leaf extract, *A. indica* leaf extract and *E. globulus* leaf extract were effective in inhibiting the fungus. Several workers have reported antifungal properties of above botanicals, while working with *Fusarium* spp. (Gohil and Vala, 1996; Pokhar Rawal *et al.,* 2003 and Sachidananda, 2005).

The present study revealed that *P. hysterophorus* leaf extract, *A. indica* leaf extract and *C. inerme* leaf extract were effective in inhibiting mycelial growth of *R. bataticola*. These results are in conformity with the results of earlier workers, who have reported the antifungal properties of botanicals on *R. bataticola,* such as neem and garlic clove extract (Datar, 1999; Sindhan *et al.* 1999; Ahamad, 2000, Lokesha, 2002 and Sachidananda, 2005).

The present study revealed that *E. globulus* leaf extract, *C. inerme* leaf extract and *P. hysterophorus* leaf extract were found to be effective in inhibiting the *S. rolfsii*. These results are in conformity with Singh and Dwivedi (1987).

In vitro evaluation of fungicides provide useful and preliminary information regarding efficacy of fungicides against pathogens within a shortest period of time and therefore, serve as a guide for field testing.

Among systemic fungicides *viz.,* carbendazim and propiconazole were successful in completely (100%) inhibiting the growth of *F. chlamydosporum* at both the

concentrations (0.05% and 0.1%). Among non-systemic fungicides chlorothalonil (94.16%) at 0.2% and mancozeb (92.83%) at 0.2 per cent were effective.

Carbendazim being benzimidazole group fungicide, interfere with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1973). Further, they also reported the effectiveness of triazoles, which inhibit sterol biosynthetic pathway in fungi. According to Davidse (1986) carbendazim induced nuclear instability by disturbing the mitosis and meiosis.

Similar results of efficacy of carbendazim on *Fusarium* spp. were reported by Dwivedi and Pathak (1981); Gangopadhyay and Grover (1985); Mishra and Rath (1988); Shyla (1998) and Sachidananda (2005). Whereas, Kapoor and Sharma (1988) noticed chlorothalonil applied as root dip at transplanting and as a soil drench reduced the infection by *F. solani*.

In the present investigation, among the ten fungicides tested, carbendazim and propiconazole at both concentrations (0.05% and 0.1%) and among non-systemic fungicides, captan at 0.2 per cent were found to be most effective in inhibiting cent per cent growth of the *R. bataticola*. Among non-systemic fungicides. Similar results were reported by Sobti and Sharma (1988); Pal *et al.* (1991) Peshney *et al.* (1992) and Sachidananada (2005).

The present study revealed that, among systemic fungicides carbendazim and propiconazole completely (100%) inhibited the fungus at both the concentrations whereas among non-systemic fungicides mancozeb and captan completely (100%) inhibited the fungus growth at 0.2 per cent concentration. Similar results were obtained by Waterfield and Sisler, (1985); Hagan *et al.* (1992) and Virupaksha Prabhu (1994).

Nematodes and fungi cause severe damage to coleus. However, the effect on plant growth and wilt incidence increased when both pathogens were present together. Increased wilt incidence in the presence of nematode on coleus is not reported earlier but reported on other crops (Marimuthu (1991) on betle vine; Krishna Rao and Krishnappa (1994)).

Sequential inoculation of *M. incognita* seven days prior inoculation of combination of all the fungal pathogens simultaneously (*F. chlamydosporum* + *R. bataticola* + *S. rolfsii*) resulted in the earliest and highest wilt incidence as compared to other treatments. This is in conformity with the findings of Jan and Khan (2002). High incidence in the presence of nematode might be due to the physiological changes in the host induced by the nematode infection and resulting in accumulation of carbohydrates and amino acids. Such an environment would be favourable to fungal growth (Kleineke – Borchers and Wyss, 1981). The experiment also brought out the fact that the presence of nematode resulted in not only increasing wilt incidence but also shortening the incubation period for disease expression.

Similar results were obtained on other crops like chickpea, cotton and tomato (Maiti *et al.,* 1998; Yang *et al.,* 1976; Liburd and Mai, 1977; Jan and Khan, 2002; Ramnath and Dwivedi, 1981). The data obtained in the present investigation clearly indicated that *M. incognita* played a significant role in increasing wilt incidence as a predisposing factor.

Plants receiving sequential inoculations of *M. incognita* followed by any of the three fungi recorded significantly lowest shoot and root lengths, fresh and dry shoot weights, fresh and dry root weights compared to healthy plants. However, the treatment receiving the *M. incognita* seven days prior to inoculation of all the three fungal pathogens, showed the highest reduction in plant growth parameters as compared to other treatments. These findings are in conformity with those recorded by Mani and Sethi (1987) who suggested that the nematodes provide a congineal, atmosphere either by facilitating the entry of fungi, into roots through the openings or modifying the substarate or by producing stimulants in the form of secretions which help the multiplication of fungi. Kumar and Vadivelu (1997) also recorded similar reduction in growth parameters of brinjal when *M. incognita, R. reniformis* and *R. solani* were present together. Similar results were obtained by Jan and Khan (2002).

Root knot index and final nematode population in soil were maximum in the treatment with nematode alone, but were significantly less in other treatments receiving fungi. However, the treatment receiving sequential inoculation of all the three fungal pathogens seven days prior to inoculation of *M. incognita* recorded significantly lowest root knot index and nematode population over other treatments.

The reduction in galling and nematode population, in the present investigation, could be possibly attributed to deleterious effects of metabolites of *S. rolfsii, R. bataticola* and *F. chlamydosporum* on the juveniles of root knot nematode. This is further supported by greater reduction when fungi and nematodes were inoculated simultaneously. Species of Fusarium have also been observed to parasitise the eggs and females of root knot nematode and also the giant cells formed by it (Fattah and Webster, 1981). *In vitro* studies have established presence of toxic chemicals in fungal culture media, which kill or immobilize the juveniles of

root-knot nematode and also reduce hatching of nematode eggs (Shukla and Swarup, 1971; Alam *et al.,* 1973; Sakhuja *et al.,* 1978; Mani and Sethi, 1984 and Jan and Khan, 2002).

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 methods 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).

Using the best management strategies and tactics for the disease complex at hand, constitutes the integrated management systems. Except for Brodie (1970); and Khan and Parvatha Reddy (1993), there is hardly any comprehensive effort to highlight the significance of multipathogenic scenario vis-à-vis their integrated management.

The results in the present study clearly indicated that the wilt incidence, nematode population, number of galls and colony forming units of *F. chlamydosporum* and *R. bataticola* were significantly minimum in the plots where *T. viride* (10 ml/plant spore suspension) combined with neemto (500 g/5 m^2) were applied compared to other treatments.

Similar results were obtained by Spiegel and Chet (1998). They reported the suppression of root-knot nematodes with the 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. and *R. bataticola* on many crops (Mishra and Ghosh, 1978; Tarabeih and Attarackehi, 1979 and Pal and Chowdhary, 1983).

However treatment receiving *T. viride* and Neemto significantly controlled the disease complex compared to individual application of either chemicals or bioagents.

Future line of work

During the present investigation certain thrust areas have been identified which could be suggested 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 ecological 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 integration of several other methods of management under field conditions and exploring the indigenous biocontrol agents.

VI. SUMMARY

Coleus forskohlii is an important medicinal plant. Forskolin content of dry tubers varies from 0.07 to 0.59 per cent. Among the different diseases affecting coleus, collar rot complex caused by *F. chlamydosporum, R. bataticola, S. rolfsii* and *M. incognita* were observed in severe form. The information available on this disease as well as pathogens involved are very scanty. 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 in northern Karnataka, isolation, proving the pathogenicity, identification, symptomatology, cultural, nutritional and physiological studies, interaction studies of the pathogens involved, *in vitro* evaluation of the bioagents, botanicals and fungicides and management of the disease.

Survey on the occurrence of root-knot disease in Raichur, Bellary, Belgaum, Bagalkot, Dharwad and Bidar districts revealed 0.0 to 72.00 per cent root knot disease incidence. High density of root knot and other plant parasitic nematodes was noticed in Hitnal (Bellary dist.) and Bidar. The present survey also indicated the association of *Meloidogyne* with fungi namely, *F. chlamydosporum, R. bataticola* and *S. rolfsii* in most of the locations surveyed, with a high frequency of occurrence of both the groups of these pathogens (nematode and either of the three fungi) from soil and root samples collected from Bellary district.

The pathogens were isolated from infected roots of the coleus on the basis of cultural characters on artificial media, the fungi were identified as *F. chlamydosporum* (Frag. and Cif.) Booth, *R. bataticola* (Taub.) Butler and *Sclerotium rolfsii* Sacc. It was confirmed by the cultural characters given by different authors.

The fungus *F. chlamydosporum* produced microconidia which were fusiform to clavate with rounded apex. Macroconidia were curved, three to five septate. Chlamydospores were usually rough walled. Fungus produced whitish to pink colonies, with woolly and abundant mycelium on potato dextrose agar. Growth rate was rapid. The fungus, *R. bataticola* grew rapidly and profusely on potato dextrose agar. Hyphae were hyaline at first and gradually turned grey to brownish black. As the culture grew old, abundant tiny black sclerotia were produced and the culture turned completely black. Mycelium of the fungus was septate and was branched at right angles. The sclerotia were spherical to oblong in shape and were of dark brown to black colour. The fungus *S. rolfsii* also grew rapidly and profusely on potato dextrose agar. Mycelium produced was silky white and gradually lost its lustre and became dull in appearance. Sclerotial bodies were initiated on fifth day after inoculation. In the beginning sclerotial bodies were white but gradually turned to buff brown then to chocolate brown at maturity.

The typical symptoms were produced by respective organisms by artificial inoculation. The fungus *F. chlamydosporum* showed typical root rot symptoms. The affected plants showed yellowing of foliage, gradual drying, wilting, discolouration of roots, shredding and Sloughing off of the bark, while in fungus *R. bataticola* the infection started at the collar region as water soaked areas and the affected tissues soon turned into a soft, black, watery mass. The infection spread to the roots and caused decay which ultimately toppled and collapsed the plants. Whereas, *S. rolfsii* affected plants exhibited darkening of the stem at the collar region. The leaves became flaccid and dropped off. Finally the plant wilted and dried. Root-knot nematode infected plants showed stunted growth marginal yellowing of leaves. When infected plants were uprooted, deformed roots with prominent galls of varying size were noticed.

Among the solid media tested, maximum and minimum growth of fungi *F. chlamydosporum, R. bataticola* and *S. rolfsii* was supported by potato dextrose agar and host leaf extract respectively. In growth phase studies, maximum growth was reached on 16th day of incubation in *F. chlamydosporum,* on 12th day in case of *R. bataticola* and in *S. rolfsii* it was on 10th day of incubation. This was taken as maximum peak period for further studies.

Among the liquid media tested, the fungi *F. chlamydosporum, R. bataticola* and *S. rolfsii* produced the maximum dry mycelial weight on Richard's broth and minimum dry mycelial weight on host leaf extract broth.

Among the carbon sources tested, sucrose was found to be the best for the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii* and the least mycelial growth was supported by citric acid as Carbon source for *F. chlamydosporum, R. bataticola* and *S. rolfsii.* Among the nitrogen sources tested, potassium nitrate was found to be the best for growth of *F. chlamydosproum, R. bataticola* and *S. rolfsii,* while least growth was observed when ammonium chloride was used.

Temperature studies revealed that maximum growth of the fungi *F. chlamydoporum, R. bataticola* and *S. rolfsii* was observed at 30^0C and least at 10^0C. However, 25 to 30^0C, 25 to 35^0C and 30 to 35^0C were found to be the optimum temperature range for the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii* respectively.

F. chlamydosporum, R. bataticola and *S. rolfsii* grew at all pH levels tested. However, the maximum growth of these fungi were obtained at pH 7.0, 6.5 and 3.5 respectively. The optimum pH range was found to be between 5.5 and 7.0 for *F. chlamydosporum* and *R. bataticola* and 3.5 to 5.0 for *S. rolfsii.*

In the interaction studies, individually, *S. rolfsii* was the most aggressive pathogen followed by *R. bataticola, F. chlamydosporum* and *M. incognita.* In sequential inoculation of nematode followed by any one of the fungi, *M. incognita* followed by *S. rolfsii* caused more damage than the *M. incognita* followed by any of the other two fungi. However, in case of sequential inoculation of any one of the fungi followed by *M. incognita, S. rolfsii* followed by root-knot nematode caused more reduction in plant growth. Whereas, sequential inoculation of root knot nematode followed by all the three fungi caused early wilting symptoms but considerable reduction in plant growth was observed in inoculation of all the three fungi followed by root knot nematode.

The results of the dual culture technique revealed that the fungal bioagents were better than the bacterial bioagents in inhibiting the growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.* Stronger antagonistic activities were noticed incase of *T. viride* and *T. harzianum.*

Out of twelve botanicals evaluated *in vitro, P. hysterophorus* leaf extract was found to be most effective in inhibiting the mycelial growth against *F. chlamydosporum* and *R. bataticola. E. globulus* leaf extract at 10 per cent was found to be effective in inhibiting the growth of *S. rolfsii.* The least per cent inhibition was recorded in *C. occidentalis* leaf extract at five per cent against *F. chlamydosporum* and *Prosopis juliflora* leaf extract at five per cent against *R. bataticola* and *S. rolfsii.*

In vitro evaluation of chemicals revealed that out of ten fungicides tested against *F. chlamydosporum, R. bataticola* and *S. rolfsii,* both carbendazim and propiconazole were found to be the most effective at both 0.05% and 0.1% in inhibiting the mycelial growth upto 100 per cent. Similarly, captan at 0.2% completely (100%) inhibited all the three fungi tested. Whereas, mancozeb at 0.2% completely inhibited the *S. rolfsii.*

In disease management study conducted in a naturally sick plot, it was found that a combined application of an organic amendment (Neemto) with a biocontrol agent (*Trichoderma viride*) was found to be effective in reducing the wilt incidence, nematode population, number of galls and colony forming units of *F. chlamydosporum* and *R. bataticola.*

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STUDIES ON COLLAR ROT COMPLEX OF *Coleus forskohlii* (Wild.) Briq.

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ABSTRACT

The *Coleus forskohlii* is subjected to attack by several diseases among which collar rot complex caused by *Fusarium chlamydosporum, Rhizoctonia bataticola, Sclerotium rolfsii* and *Meloidogyne incognita* are the most important.

A survey on the occurrence of root knot disease in northern districts of Karnataka revealed 0.0 to 72.00 per cent root knot disease incidence. The present survey also indicated the association of *Meloidogyne* spp. with fungi namely, *Sclerotium rolfsii, Rhizoctonia bataticola* and *Fusarium chlamydosporum* in most of the locations surveyed. Pathogenicity tests confirmed that *M. incognita, S. rolfsii. R. bataticola* and *F. chlamydosporum* were pathogenic to *Coleus.*

Among the solid and liquid media tested, maximum growth of *S. rolfsii, F. chlamydosporum* and *R. bataticola* the fungi was supported by PDA and Richard's broth. Among carbon and nitrogen sources tested: sucrose and potassium nitrate supported maximum growth in all the three fungi. The temperature studies revealed the maximum growth of the fungi *F. chlamydosporum, R. bataticola* and *S. rolfsii* were observed at 30^0C. Maximum growth of these fungi was obtained at pH 7.0, 6.5 and 4.0 respectively. *T. harzianum* was found to be the best antagonist in inhibiting the growth of *F. chlamydosporum, S. rolfsii* and *T. viride* was found best in inhibiting *R. bataticola.* The parthenium leaf extract at 10 per cent was found to be the most effective in inhibiting the mycelial growth of *F. chlamydosporum, R. bataticola* and *S. rolfsii.*

The effect of simultaneous inoculations of *M. incognita* with either *S. rolfsii* or *R. bataticola* or *F. chlamydosporum* on growth of *Coleus* was additive in nature. However, when *M. incognita* was inoculated with all the three fungi the resultant effect was more than simple additive effect.

A reduction in root knot index and final nematode population was observed in various combinations of nematode and fungi inoculations. Management studies in field revealed that *T. viride* + Neemto treatment recorded lowest per cent wilt incidence, lowest population of root knot nematode and also lowest number of galls.