STUDIES ON FUNGAL DISEASES OF PATCHOULI WITH SPECIAL REFERENCE TO WILT CAUSED BY *Fusarium solani* (Mart.) Sacc.

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1. INTRODUCTION

Mother Nature has created thousands of diversified species of plants, which synthesize and accumulate various primary and secondary metabolites in them. Among those innumerable plants, a group of plants are aromatic which produce odoriferous constituents i.e. essential oil in different parts. These essential oils play a major role in our daily life starting from birth to death. They have been extensively used in cosmetics, flavour, fragrance and pharmaceutical industries for diversified uses.

India has to play a dominant role in the production and processing of these natural extracts. Our country's biodiversity coupled with competent scientific force, make our country as the best choice to become a foremost leader in aroma business in the years to come (Varshney, 1999).

World's production of essential oil is estimated at about 1 lakh to 1,10,000 tonnes and India stands at third position with a share of 16 to 17 per cent. In terms of value, India's position is second and its share is 21 to 22 per cent due to the mint revolution in north India. (Varshney., 1999).

Patchouli (*Pogostimon patchouli* Pellet.), (Syn. *P. cablin* Benth.), belonging to family Lamiaceae or mint family, is the source of commercial patchouli oil. The word "patchouli" seems to have been derived from Sanskrit word "Pacholi", which means aromatic herb. It is one of the most important aromatic crops of the perfume industry, as yet there is no synthetic chemical to replace the oil of patchouli. It is native of the Phillipines and grows wild in Malaysia, Indonesia, Singapore, China and India. The cultivation of Patchouli is reported to have started first in Java in 1895 with planting material from Singapore, though the identity of the species was uncertain (Ahmed, 2002)

Patchouli is a perennial aromatic herb that yields fragrant leaves containing very sweet smelling oil, attains a height of about 1m – 1.2m. Patchoulene is the important component of essential oil of patchouli. It is propagated by stem cuttings. The commercial oil of patchouli is obtained by steam distillation of the shade dried herbage. The oil of patchouli has a strong fixative property, which helps to prevent the rapid evaporation of perfume and thereby promotes tenacity. The oil is generally blended with other essential oils . It is used in a wide range of toilet soaps, scents, body lotions, etc. At very low concentration, the oil is extensively used to add flavour to major food products including alcoholic and non alcoholic beverages, frozen dairy desserts, candy, meat and meat products (Ahmed, 2002). Dried patchouli leaves are used for scenting wardrobes to protect silk and other fabric from insects. It is helpful in relieving from all forms of depression, anxiety and stress related conditions. It is used as general tonic and a stimulant and helps the digestive system (Sarwar *et. al.*, 1982)

Among the countries growing patchouli, Indonesia is the leading one with an oil production of 600 tonnes (80% of the world production) and China about 30-40 tonnes of oil. Commercial cultivation of patchouli in India was first attempted by Tata oil mills in 1942. After the initial few attempts to grow the crop, its systematic cultivation started in 1962 by Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. Patchouli is cultivated in Assam, Madhya Pradesh, Tamil Nadu, Kerala and Karnataka. Currently, India is producing a small quantity of patchouli oil against its requirements of 40 tonnes of pure oil and about 70 tonnes of formulated oil. Hence, majority of these oils are being imported from Indonesia (Jadhav *et al.*, 2003)

Patchouli crop suffers from fungal and nematode diseases. Among the fungal diseases, wilt disease caused by *Fusarium solani*, has become very serious now a days. The disease is found to be severe during *kharif*. The infected plants are characterized by gradual yellowing and drying of leaves followed by premature death. The plants show discoloration of roots and collar regions of fully grown plants. Severe disintegration of secondary roots surface is also observed.

Although the disease has become very severe now-a-days, not much work is carried out on wilt disease of patchouli. In view of this, there is a need for systematic work which includes survey for knowing disease incidence. This will enable us to locate the hot spots for disease in Karnataka. In addition, it will help us to know their characteristic symptoms.

The present investigation gives us a clear picture of morphological, cultural, physiological and nutritional characters of fungal pathogen and to understand the variability observed in pathogen. It also helps us to develop integrated management practices by using biocontrol agents, botanicals and fungicides. Thus with the help of these strategies we can reduce the losses caused in patchouli due to wilt disease and grow patchouli successfully.

Keeping all these points in view, present investigation was carried out with the following objectives;

1. Survey and surveillance of diseases of patchouli in Karnataka
2. Isolation, identification of pathogens of patchouli and proving pathogenicity
3. Morphological, cultural, physiological, nutritional and host range studies of *Fusarium solani*
4. To study cultural, morphological and molecular variability in *Fusarium solani*
5. Management of Fusarium wilt of patchouli through plant extracts, biocontrol agents and fungicides

2. REVIEW OF LITERATURE

Patchouli is one of the important aromatic and medicinal plants. The crop is affected by various diseases, *viz*. wilt caused by *Fusarium solani* (Mart.) Sacc., leaf blight caused by *Alternaria alternata*(Fr.) Keissler, *Sclerotium* rot caused by *Sclerotium rolfsii* Sacc., and root knot caused by *Meloidogyne incognita* Kuhn. However, not much research work is being carried out and also not much information is available regarding these diseases in the literature .The work pertaining to the various aspects of pathogen(s) and disease(s) have been initiated. Review was made on the diseases and pathogens of patchouli and other related hosts which is presented hereunder.

2.1 SURVEY AND SURVEILLANCE FOR DISEASES OF PATCHOULI

Sudarshan Rao (1975) stated that survey and surveillance form the basis for any successful plant protection strategy. Successful plant protection depends upon early detection of the disease incidence followed by timely adoption and application of preventive measures.

Sheshadri (1970) was first to report specifically that patchouli raised over an area of 20 hectares by the Government Cinchona Department, Tamilnadu, at Annamalai was completely wiped out by *Meloidogyne.*

Pardede (1973) reported that patchouli crops are attacked by *Meloidogyne* and *Pratylenchus* sp in several parts of world.

Roopali Singh and Angadi (1990) reported wilt of patchouli during the months of April-May 1990 at the Farms of IIHR, Bangalore. Incidence of the disease gained momentum from June and continued throughout the year causing plant mortality in patchouli plants.

The survey for the prevalence of leaf blight of turmeric caused by *Alternaria alternata* was conducted in four districts of northern Karnataka, *viz*. Belgaum, Bijapur, Dharwad and Uttar Kannada during 1994-95. The local varieties and PCT series of turmeric recorded highest per cent incidence of disease (Mallikarjun, 1996).

2.1.1 Symptomatology

Sheshadri (1970) reported that *Meloidogyne incognita* Kuhn. causes large multiple galls on the roots of Patchouli which causes distortion of root system.

Agarwal and Kotasthane (1971) reported that *Sclerotium rolfsii* causes symptoms like pre-emergence as well as post emergence death of seedlings of potato. A sudden yellowing, browning and wilting of the entire plant is the first symptom. Leaves of infected plants turn brown, dry and often cling to the dead stem. The most characteristic sign of the disease is white, fan like mat of fungal mycelium that forms on stem bases, leaf debries and the soil surface. Numerous tan to brown, spherical sclerotia of mustard seed size are formed on infected plants

Sarwar and Khan (1972) found that the symptom of slow wilt and presence of fairly big galls caused by *M. incognita* on the apical root regions of patchouli.

Parameswaran *et al*. (1987) reported that the initial symptoms of leaf blight caused by *Alternaria alternata* in Patchouli were observed as a brown discoloration at the tip of mature leaves. Brown discolouration advanced towards the leaf margin. Gradually the leaf lamina presented a blighted appearance.

Grafted mango plants were reported to develop symptoms of epinasty and progressive foliar chlorosis, often accompanied by transient wilting, followed by necrosis and defoliation due to infection of *F. solani* (Saxena and Rawal, 1989)

Roopali Singh and Angadi (1990) reported that *Fusarium solani* wilted plants of Patchouli symptoms were characterized by blackening of roots at collar regions of fully grown plants. Severe disintegration of secondary roots surface was also observed.

Rathnamma (1994) described the symptoms of root rot of Garden rue (*Ruta graveolens* L.), a medicinal plant caused by *F. solani* as yellowing of the leaves which was the first external symptom, later advanced to middle and terminal leaves. The tap root of the plants showed dark brown to red discoloration, which covered entire tap root with longitudinal fissures along the main root.

Mallikarjun (1996) described the symptoms of leaf blight of turmeric caused by *A. alternata*. The symptoms noticed initially on lower set of leaves later blighting extended to top leaves. The spots were light brown in colour with a clear yellow halo, subcircular to irregular, measuring 0.5 mm to 10 mm wide. Under favourable conditions the plant exhibited a burnt appearance which could be very easily detected from a distance.

Raju (1997) recognized *F. solani,* the cause of wilt of Crossandra and the disease was characterized by loss of turgidity, drooping and wilting of leaves followed by brown to black discoloration of roots.

2.2 ISOLATION OF THE PATHOGENS

Rangaswamy (1972) isolated *Sclerotium rolfsii* from the infected part of stem or collar region of potato showing typical symptoms of the disease by following the standard tissue isolation techniques. The pure culture of the fungus was obtained by further growing the culture following hyphal tip culture technique under aseptic conditions.

Isolation of *Fusarium solani* from different plants by following the standard tissue isolation techniques has been reported by several workers (Satyaprasad and Rama Rao, 1981; Madan, 1983; Kore and Patil, 1985; Kore and Mashalkar, 1987). Kaewruang *et al.* (1987) isolated seven species of fungi from root rot of Gerbera including *F. solani* by host baiting technique and dilution plate method.

Zambolim *et al*. (1983) isolated three soybean root infecting pathogens including *F. solani* on selective medium by using dilution plate technique.

Nan *et al.* (1991) estimated the complex fungus infecting red clover roots by employing dilution plate technique on *Fusarium* selective media. Hassan *et al.* (1994) isolated root rot pathogen of Capsicum, *Fusarium* spp. using soil plating technique and tissue isolation.

Sunanda (2000) and Mallesh (2005) isolated *F. solani* and *R. solani* causing root rot of sage by standard tissue isolation method. *Fusarium solani* and *R. solani* causing root and stem rots of Lemon balm and Thyme were isolated from superficially infected roots and stem using artificial culture method (Machowicz *et al.,* 2004).

Mamatha (2004) isolated *Alternaria alternata* from infected leaves of turmeric following standard tissue isolation technique. Further, the pure culture of the fungus was obtained by single spore isolation method.

2.2.1 Pathogens

The genus *Fusarium* was identified by Link in 1809 and was accepted in an amended form by Fries (1849) for species with fusiform, septate spores described and clearly illustrated by Martius and Von (1842).

Fries (1832) had proposed the *Alternaria alternata* as *Torula alternata* Pers. The reasons why the specific epithet '*alternata*' should be used instead of the more commonly accepted one '*tenuis*' was clearly stated by Simmon (1967).

Sclerotium rolfsii Sacc., is a well known 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 *S. rolfsii*.

Siddaramaiah *et al.* (1982) reported *F. solani* on tulsi (*Ocimum sanctum*) for the first time from Dharwad. Kishore *et al.* (1985) observed damping off of seedling of opium poppy (*Papaver somniferum* L.) caused by *F. solani* reported from Balarampur of Karnataka.

Chung-Wonchuan *et al.* (1998) reported root rot disease of Periwinkle (*Carthamus roseus* L.) caused by *F. solani* from Taiwan. Root rot of Garden rue (*Ruta graveolens* L.) was found to be caused by *F. solani* in Bangalore, during 1992-93 (Rathnamma *et al.*1999).

Sunanda (2000) reported root rot of sage (*Salvia officinalis*) caused by *F. solani* and *R. solani*. Gaetan *et al.* (2004) reported the crown and root rot on medicinal plant St. John's wort (*Hypericum perforatum* L.) caused by *F. solani* from Buenos Aires, Argentina.

2.3 MORPHOLOGICAL STUDIES

Spore morphology was the major character in the identification of fusaria. Conidia produced were as simple or polyphialidic slime spores or as enteroblastic spores. Chlamydospores were formed as the resting spores. Conidia might occur as 0-1 septate, pyriform, fusoid to oval microconidia as straight or curved, 0-10 or more septate macroconidia and the size varied from 17-35 x 3.5µm to 135-200 x 12.5-20µm (Booth, 1971)

Satyaprasad and Rama Rao (1981) described *Fusarium solani* on Gaur (*Cyamopsis tetragonoloba* (L.) Taub. According to them the fungus produced extensive cottony mycelium on potato sucrose agar with light brown pigmentation, microconidia produced from long lateral phialides, cylindrical, 5-10 x 3-4µm; macroconidia upto 5 septate, 13-27.5 x 3-4.5µm and chlamydospores were globose to subglobose, smooth or rough walled, 6-9 x 5-8µm.

2.4 CULTURAL STUDIES

Major (1923) studied the cultural characteristics of *F. solani* isolated from lupins and asters on various natural and synthetic media and observed that the type of growth varied on different media from complete absence to a profuse production of aerial mycelium with varied colour.

Wardlaw (1931) described the cultural characters exhibited by five strains of *Fusarium cubense,* when grown under uniform conditions on standard media. Important differences were observed in vegetative growth, color production, formation of chlamydospores and sporulation and stated that two strains were not identical.

McCulloch (1944) observed that in cultures, the pathogen *F. oxysporum* f .sp. *gladioli* produced white to peach pale salmon or purple mycelium. Microconidia were abundant, hyaline, ovoid to ovate. Macroconidia were scarce, often lacking and variable, 3-septate. Chlamydospores were hyaline, usually vacuolate and spherical.

Subramaniam (1955) observed considerable variation in cultural characters of *Fusarium udum*. Venkataraman (1955) showed that culture of *Fusarium* wilt of muskmelon produced fluffy mycelium with sparse number of conidia differing with 'wild type' strain having abundant sporulation.

Sharma and Mathur (1971) showed that monoconidial lines of the linseed wilt pathogen *Fusarium oxysporum* f.sp*. lini* isolated from different linseed growing regions differed in their cultural and morphological characters with marked diversity in virulence.

Jhamaria (1972) reported that, potato dextrose agar, Richards's agar and Czapek's agar provided maximum growth and sporulation of *F. oxysporum.* f .sp*. niveum.*

Sowmya (1993) studied the growth of *Fusarium oxysporum* f. sp. *cubense* on different nutrient media and observed maximum growth and sporulation of the pathogen on Potato sucrose agar and Richards's agar, respectively.

2.5 SPORE GERMINATION STUDIES

Rathnamma (1994) studied spore germination of *Fusarium solani* in different media, *viz*. tap water, sterile distilled water, glucose (1%), sucrose (1%), lactose (1%) and garden rue leaf extract media. Maximum germination was observed in garden rue leaf extract medium (97.48%). Least germination was observed in sterile distilled water (67.9%).

Madhukar (2001) studied spore germination of *Fusarium solani* in sterile distilled water by Hanging drop method. The germination of spores was studied from 5 hrs onwards upto 24 hrs at an interval of two hrs. Complete germination of spores was recorded at 16 hrs of incubation.

2.6 PHYSIOLOGICAL STUDIES

2.6.1 Effect of Temperature

The behavior of *F. oxysporum* f. sp. *lycopersici* was tested by Clayton (1923) at different ranges of temperature. He found that minimum temperature for growth of pathogen was 9 – 10°C, the optimum was 28°C and maximum was 37°C.

Moore (1924) studied the effect of temperature on growth of two strains of *F. coeruleum* and found that optimum temperature for maximum growth was 30°C. Neal (1927) reported that *F. oxysporum* f .sp. *vasinfectum* Atk. grew slowly at temperature below 10°C, the optimum temperature for development being 28°C to 30°C.

Experiments carried out by Ward (1930) on different strains of *F. oxysporum f.sp. cubense* showed the most vigorous growth at 20^0C. At room temperature (28°C) growth was less vigorous, but better than 35°C. See Muller (1968) working with six isolates of *F. poae* determined its minimum growth at 2.5°C, optimum at 22.5–27.5°C and maximum at 32.5°C.

Joffee *et al.* (1974) studied the development of *F. poae* isolates on potato dextrose agar and maltose agar media and all of them showed best growth at 24- 25°C. Growth and sporulation of *Fusarium solani* was best at temperature of 22-28°C (Shukla and Bhargava, 1977). The fungus *F. solani* showed good growth and sporulation in Duggar's modified basal medium at 28-30^0C and very poor growth at 36°C (Madan, 1983).

Optimum temperature for growth of *F. oxysporum* f .sp. *cubense* isolates was found to be 25 – 30°C (Chuang, 1988). Sowmya (1993) noticed maximum growth of four isolates of *F. oxysporum* f .sp. *cubense* at 35°C. Desai *et al.* (1994) reported that maximum growth of all the four races of *F. oxysporum* f .sp. *ciceri* was recorded at 25°C.

2.6.2 Effect of pH

Moore (1924) working with two strains of *F. coeruleum* (Lib.) Sacc. reported that the fungus could tolerate pH range of 3.0 to 11.0.

Neal (1927) observed good growth of *F. oxysporum* f .sp. *vasinfectum* over a range of pH from 3.0 to 5.5. The fungus could tolerate strongly acidic and alkaline (pH 4 to 9.0) culture solutions.

Jhamaria (1972) reported that *F. oxysporum* f .sp. *niveum* could grow well on wide range of pH varying from 3.2 to 8.3 and the optimum was between pH 5.5 to 6.5. Marras *et al.* (1981) reported that the optimum pH for *F. roseum var avanaceum* was 7.0. Desai *et al.* (1994) reported that all the four races of *F. oxysporum* f .sp. *ciceri* recorded maximum growth at pH 6.0.

Profuse growth and sporulation of *F. oxysporum* f. sp *sesamum* was recorded at pH 6.6 to 7.5 (Raghuvanshi, 1995). Jadhav *et al.* (2000) reported that, the *fungus F. 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* sp.

2.7 NUTRITIONAL STUDIES

2.7.1 Effect of Carbon Source

Brannon (1923) observed that glucose and fructose were utilized equally well by *Fusarium* spp. for spore formation when grown on Czapek's modified solution.

Moore (1924) reported that weight of fungal mycelium varies with sugar. Moore and Chupp (1952) reported that, *F. oxysporum* f .sp. *lycopersici, F. conglutinans* and *F. niveum* were able to utilize a number of carbon sources and also hydrolysed starch.

Sebek (1952) obtained maximum quantity of mycelium of *F. oxysporum*. f .sp. *lycopersici* and *F. vasinfectum* in the presence of 10 per cent glucose and 2.5 to 5 per cent xylose, respectively. Kushwaha *et al.* (1974) reported that 80 strains of *F. oxysporum*. f .sp. *lentis* differed in their capacity for utilizing carbon from different sources. Fructose and sorbitol were the best sources of carbon.

Kesavan and Prasad (1975) reported that among the carbon sources tested, glucose and sucrose were found to be the best for the production of fusaric acid and sporulation by the muskmelon wilt pathogen while starch provided maximum growth.

Brayford and Bridge (1989) reported growth and pigmentation of 99 strains of *Fusarium* mainly *F. oxysporum* and *F. solani* on ammonium salts agar containing either manitol, sorbitol or xylitol as sole source of carbon. Patel (1991) demonstrated that, among carbon sources, maltose and mannitol were best utilized by *F. solani*.

2.7.2 Effect of Nitrogen source

Moore (1924) reported that potassium nitrate, asparagine and ammonium salts of certain organic and inorganic acids served as nitrogen sources for *Fusarium coeruleum* whereas oxalates and formates did not support its growth.

Sarojini and Yogeshwari (1947) observed optimum sporulation of *Fusarium udum* Butler in two per cent nitrate nitrogen in standard Horne and Milters liquid medium. Moore and Chupp (1952) reported that ammonium ion, urea, peptone, protease and asparagine were utilized as nitrogen sources by *F. oxysporum* f .sp. *lycopersici.*

Subramaniam and Shrinivas Pai (1953) reported good growth of *F. vasinfectum* on potassium nitrate, while ammonium sulphate was found to be poor source of nitrogen.

Chi and Hanson (1964) found variation among three isolates of *F. oxysporum* and two isolates of *F. solani* from red clover with respect to nitrogen and temperature requirements. Bhatnagar and Prasad (1968) showed good growth of *F. solani* in D- leucine and asparagine whereas asparagine and non-valine proved to be good for 11 isolates. However, sodium nitrate did not support any growth of both the isolates.

All the isolates of *F. oxysporum* f .sp. *niveum* preferred nitrate to ammonical nitrogen. Maximum growth was obtained on potassium nitrate, ammonium oxalate, ammonium sulphate and ammonium phosphate (Selvaraj, 1971).

Kushwaha *et al.* (1974) working with eight strains of *F. oxysporum* f .sp. *lentis* for nutritional requirement noticed variation with regard to utilization of nutrients. Kesavan and Prasad (1975) found that sodium nitrate and ammonium nitrate were the best nitrogen sources for the growth of *F. oxysporum* f .sp. *melonis.*

Mahendrapal and Grewal (1975) showed that ammonium salts in general supported growth of *F. oxysporum* f .sp. *ciceri*. Its growth was meager on calcium nitrate, whereas moderate to good growth of the fungus was recorded on monosodium dicarboxylic acids and amides.

2.8 HOST RANGE STUDIES

Leath and Kendall (1978) conducted experiments on host range of *F. solani* and showed the susceptibility of white clover and *Lotus corniculatus* to root rot pathogen. Cock foot and *Coronilla varia* were the most resistant while lucerne, red clover, subterranean clover and *Coronilla globosa* showed moderate reaction.

Nielson and Mayer (1979) used *F. solani* causing root rot of stored sweet potatoes for studying the reaction with other crops. The results revealed that roots of the Cv.Jewel were more susceptible than those of jersey orange, georgia red, continental, nugget, fruits of apple, cucumber, egg plant, squash, tomato and potato which were susceptible.

Moubasher *et al.* (1984) observed that *F. solani* and *F. oxysporum* were most common species infecting cotton, pea, wheat and tomato.

Out of 16 different hosts treated for determining the host range of *F. solani,* black gram, green gram, bengal gram, tomato, brinjal, ladies finger, potato and guava were found to be susceptible. However, maize, peanut, linseed, sunflower, niger, pigeonpea, soybean and safflower were free from infection (Kore and Patil, 1985).

2.9 VARIABILITY OF *F. solani*

2.9.1 Morphological and cultural variability

Prasad (1949) studied thirty strains of *F. solani*, which were found to differ from each other in culture type, rate of growth, pigmentation and size of macroconidia.

Shit and Sengupta (1978) reported that seven isolates of *F. oxysporum* f .sp. *udum* grown on different media showed variation in cultural characters like amount of aerial mycelium and texture. They also differed in their ability to sporulate.

Morphological studies of six isolates of *F. oxysporum* f .sp. *ciceri* revealed the variation in size of micro and macro conidia, growth pattern, sporulation and pigmentation of medium which varied from normal white to pale cream dark brown, crimson and middle buff (Gupta *et al*., 1986).

A virulent isolate of *F. oxysporum*. f .sp. *lupini* grew faster on potato dextrose agar than on Czapek's medium and spore production was also greatest on potato dextrose Agar (Saheb *et al*. 1987).

Patel (1991) reported considerable variation among 13 isolates of *F. solani*. Sharma and Agnihotri (1972) recorded morphological and pathogenic variation among three isolates of *Fusarium orthocerus* App. & Wr.

Krishnarao and Krishnappa (1997) reported that *Fusarium* sp. isolated from wilted chickpea plants collected from different locations of Karnataka differed in growth pattern, pigmentation, sporulation and pathogenicity.

2.9.2 Genetic variability

The information about molecular polymorphism is reviewed here with special emphasis on Random amplified polymorphic DNA (RAPD).

Random Amplified polymorphic DNA (RAPD)

The RAPD technique has quickly gained wide spread acceptance and application because it has provided a relatively simple tool of genetic in biological systems. However, RAPD is the best assay when the nucleotide sequence is not known. Unlike other PCR protocols, which utilize, two primers of defined sequence, RAPD detects nucleotide polymorphism using only one primer of an arbitrary nucleotide sequence.

Kim *et al*. (1993) reported that polymerase chain reaction (PCR) has been widely and successfully employed for the diversity analysis of the important plant pathogenic fungi including *Fusarium* sp.

In many cases, primers for these uses were based on DNA sequence polymorphisms existing within highly conserved regions of the nuclear ribosomal DNA, such as the internal transcribed spacers or the intergenic spacer region (Ward, 1994; Edel *et al*. 2000).

Sau Paulo (2004) reported that *Fusarium solani* is of relevance for agriculture. PCR based methods were used to investigate the molecular variability of 18 *F. solani* isolates from four Brazilian state, collected from different substrates. Genetic variability revealed the interspecific variability within *F. solani* isolate, without any correlation to their geographical region and substrate. Its polymorphism was observed even in the very conserved sequence of rDNA.

Bhim Pratap Singh (2006) opined that molecular biology has brought many powerful new tools to fungal taxonomists including the potential for rapid identification of isolates, methods for rapid determination of virulence or toxicity of strains, and the means to elucidate the relationships among fungal species. Molecular methods have also been used to distinguish between closely related species with few morphological differences and to distinguish strains within a species.

Restriction fragment length polymorphism (RFLP) analysis and random amplified polymorphic DNA (RAPD) techniques, with arbitrary 10 mer oligonucleotide primers, have been used to identify formae speciales of *Fusarium oxysporum* (Assigbetse *et al*., 1994; Manulis *et al*.,1994).

De Haan *et al*. (2000) tested 160 arbitrary 10-mer oligonucleotide primers on *F. oxysporum.* f .sp*. gladioli* by PCR to find RAPD specific marker. They found that RAPD primer G-12 amplified two discrimination DNA fragments.

2.10 MANAGEMENT OF *FUSARIUM* WILT OF PATCHOULI:

2.10.1 Botanicals

Essential oils of *Thymus vulgaris* L.*, Lavandula angutifolia, Mintha piperita*, Neem oil, *Allium sativum* were tested in the laboratory against *F. solani* . *T. vulgarisis* was effective from a minimum concentration of 50 ppm. Mint and Lavender oils were only effective at higher concentrations (400-800 ppm). In the field, neem oil gave good protection against *F. solani* (Aulerio and Zambonelli, 1997).

Shivapuri *et al.* (1997) investigated that fungitoxic properties of different plant extracts against *F. solani* and other pathogenic fungi. Ethanol extract of *Allium cepa* L., *Allium sativum, Lantana camara* L., *Polyalthia longifolia* Benth and Hook, *Tagetus erecta* L., *Vincarosea* L. and *Withania somnifera* showed fungitoxic effect against *F. solani*.

The inhibitory effects of essential oils extracted from the genus Eucalyptus, *O. basilicum, Prosopis cineraria* and *Pongamia pinnata* were evaluated against *F. solani.* Among these oils extracted from the Eucalyptus species markedly inhibited fungal growth (Rai *et al.* 1999).

The effects of aqueous leaf extract of 5 medicinal plants *(Strychnosnux – vomica, Calotropis procera, Azadiracta indica,* A. Juss, *Ocimum sanctum and Allium sativum)* on the spore germination of five species of *Fusarium* viz. *F. solani, F. moniliformae, Fequiseti, F. acuminatum* and *F. oxysporum* were examined. Spore germination of all the fungi tested was completely inhibited by 100% aqueous extract of *A. indica* while only 20% of germination of spores was observed with treatment of 100% aqueous extract of *A. sativum* (Tripathi *et al.* 1999).

Leaf extracts of *L. camara* followed by *A. indica,* and *Acalypha indica* were found to be equally effective in inhibiting the growth of *F. solani in vitro*. Leaf extracts of *L. camara* has been reported to exhibit maximum toxicity against spore germination of *F. solani* (Mamatha and Ravishankar Rai, 2004).

2.10.2 Bioagents

Sarhan (1989) observed that an isolate of *B. subtilis* antagonistic to *F. solani* in culture was found effective against root rot of Fabae beans in green house studies. Bacterium applied as a seed treatment significantly reduced seed colonization and root rot. *Bacillus subtilis* inhibited the growth of *F. solani* on potato dextrose agar and resulted in significant spore reduction due to production of toxic metabolites.

Patel (1991) found that *T. harzianum* initially showed two mm inhibition zone of *Fusarium* sp. and later it overgrew the colony of pathogen.

Fluorescent Pseudomonads (FPs) have received much attention in the last few years for their ability to suppress plant diseases especially those caused by soil-borne plant pathogens (Thomashow and Weller, 1990).

Fluorescent Pseudomonads with potential antagonistic activity and *Trichoderma harzianum* strain THA were found to be highly effective and inhibited the growth of *F. solani* in dual culture (Zapata *et al.,* 2001).

Twelve isolates of *Trichoderma* obtained during survey from soils in dry root-rot affected acid lime gardens of Andhra Pradesh were evaluated for their variation in phenotypic characters, growth rate and antagonistic potential against *F. solani.* Out of 12 isolates, only two isolates (T_2 and T_4) showed very fast growth rates and the antagonistic potential (Kavitha *et al.,* 2004).

Mishra *et al*. (2004) reported that an isolate of *Trichoderma virens* was antagonistic to *Fusarium oxysporum* f .sp*. gladioli*, a pathogen of gladiolus corm rot and wilt. The proliferation and population density of the pathogen in the soil was significantly reduced after the incorporation of *Trichoderma virens.*

2.10.3 Fungicides

Several techniques for evaluating fungicides have been described from time to time. Poisoned food technique is the most commonly practiced method for evaluating fungicides under laboratory conditions (Flack, 1907).

Six fungicides were evaluated in laboratory against mycelial growth and conidial germination of *Fusarium solani the* cause of decay in colacasia and yam. carbendazim was found to be the best fungicide among the tested, in disease control (Mishra and Rath, 1987)

Fusarium solani, the incitant of wilt disease in Kagzi lime, was completely inhibited under *in-vitro* condition by carbendazim (0.1%) (Dhrub Singh, 1988). Chauhan and Patil (1990) reported the maximum inhibition of *Fusarium solani* causing rhizome rot of zinger by Bordeaux mixture (3000 ppm).

Mishra and Rath (1988) reported that bavistin, benomyl, tribendazole, and pamogen-15 inhibited mycelial growth of *Fusarium solani,* at 15 mg per litre.

Kapoor and Kumar (1991) reported that carbendazim and benomyl (500 mg a.i. /ml) were found to be most toxic to *Fusarium solani*. Etebarian (1992) found that iprodione and carbendazim totally inhibited fungal growth of *F. solani* at 10 ppm and 100 ppm concentrations. Wahid *et al.* (1995) concluded that Derosol (carbendazim) and benlate (benomyl) at 10 ppm completely inhibited the growth of *F. solani* which was isolated from soybean seed. Topsin-M and vitavax gave 100 percent inhibition at 50 ppm.

Growth and sporulation of *Fusarium solani* causing root rot of garden rue was completely inhibited by methoxy ethyl mercury chloride (1000 ppm), thiram (1000 ppm) and carbendazim (50 ppm) (Rathnamma, 1994).

2.10.4 Management of the disease under the field condition

The strategy of chemical control of plant disease is based on the principal of immobilizing the pathogen so that it does not interact with the host to cause disease (Sinha *et al*. 1988).

Shatla *et al.* (1975) reported that root rot of Lentil caused by *F. solani* and could be effectively controlled by treating the soil with thiophonate methyl and quintogene. Seed treatment with carbendazim (2g/kg) followed by soil drenching of carbendazim and thiophoinate methyl (1g/lit) gave good control of root rot of cowpea incited by *F.solani* (Gangopadhyay and Grover, 1985).

The lowest rate of wilting and death of egg plants infected with *Fusarium solani* was recorded by following root dipping of seedlings in chlorothalonil (2g/l) at transplanting followed by soil drenching (2g/l) at 15 days interval (Kapoor and Sharma, 1988).

Integration of biological and chemical control seems to be a promising way of controlling many pathogens with minimum interference in the biological equilibrium in soil (Papavizas, 1973). Integration of chemicals and bioagents (*Trichoderma* Sp.) has been the subject of intense research during recent years (Chet, 1991).

Certain strains of *Trichoderma hamatum* and *T. pseudokoingii* are reported to be adapted to excess soil moisture, whereas *T. viride* and *T. polysporum* are restricted to low temperature conditions. *T. harzianum* is most commonly distributed in warm climate region, whereas *T. harmatum T. koningii* are reported to be distributed under diverse environmental conditions. Species of *Trichoderma* have been shown to be effective against wide range of plant pathogens, such as *Botrytis* sp., *Fusarium* sp., *R.solani*, *Scloritium rolfisii,* etc. Various mechanism of parasitism by *Trichoderma* sp. on different plant pathogenic fungi are reported to be varied such as inhibition by contact action, parasitism, hyphal interaction, coiling, penetration and lysis of hyphal cell, antibiosis and production of toxic metabolites etc. (Hriday, 1991). Hartman and Fletcher (1991) reported *trichoderma harzianum* to be efficient against *Fusarium* root rot of tomato.

3. MATERIAL AND METHODS

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

Present investigations were carried out during 2006-07. Laboratory experiments were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka. Survey for wilt disease on patchouli was undertaken in Shimoga, Haveri/Hangal, Belgaum and Dharwad districts of Karnataka.

GENERAL PROCEDURE

Cleaning of glassware

Borosil and Corning glassware were used for all the laboratory experimental studies. They were kept for a day in the cleaning solution containing 60 g potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in one litre of water. Then they were cleaned by washing with detergent solution followed by rinsing several times in tap water and finally in distilled water.

Sterilization

All the glassware used in the studies were sterilized in an autoclave at 1.1 kg/cm^2 pressure for 20 min and kept in hot air oven at 60°C for one hour. Both solid and liquid media were sterilized at 1.1 kg/cm^2 pressure for 15 minutes.

3.1 SURVEY AND SURVEILLANCE FOR DISEASES OF PATCHOULI

A fixed plot survey was conducted during 2006-07, to know the incidence of diseases, in patchouli growing districts such as Shimoga, Uttar kannada, Belgaum, Haveri and Dharwad. In Haveri survey was conducted in Adur. In Belgaum district survey was conducted in places like Arabhavi, Itagi, Mangenkoppa and Beedi. In Dharwad district survey was conducted in Saidapur and Tadas.

The disease incidence was assessed on Patchouli crop by recording the number of plants showing diseased symptoms for the total number of plants present. In each field rows were selected randomly and the number of plants showing typical symptoms and the total number of plants were recorded. Per cent disease incidence was calculated by using formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of plants showing wilting symptom}}{\text{Total no. of plants}} \times 100$$

During survey, characteristic symptoms of diseases were recorded and also samples were collected for isolation of pathogens.

3.2 ISOLATION OF THE PATHOGENS

The infected seedlings showing typical symptoms of the diseases were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts were surface sterilised with 1:1000 mercuric chloride ($HgCl_2$) solution for 60 seconds and washed serially in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilised Petriplates containing potato dextrose agar (PDA).

The Petriplates were incubated at room temperature (27±1°C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27±1°C for 15 days. Then such slants were used to study characters.

3.2.1 Hyphal tip isolation

This method was followed for maintaining pure culture, since *Fusarium solani* is known to be heterokaryotic in nature. Hyphal tip isolation was done on water agar plates. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Single spore was then marked under the microscopic field with ink on the glass surface of the plate and it was allowed to germinate. Such plates were incubated at 27±1℃ and periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 27±1℃ for 10 days. Later, mycelial bits of the fungus were placed in the center of Petriplates containing potato dextrose agar medium and incubated at 27±1°C for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such culture was used for further studies.

3.2.2 Maintenance of the culture

The fungal pathogen was subcultured on PDA slants and allowed to grow at 27±1℃ for ten days and such slants were preserved in a refrigerator at 5℃ and revived once in 30 days.

3.2.3 Identification of the pathogen

The culture obtained was compared with the original description of the fungus. It was also sent to Agharkar Research Institute, Pune for further confirmation.

3.2.4 Mass Multiplication of *Fusarium solani*

The multiplication of inoculum of the pathogen on large scale was achieved by inoculating the culture of the pathogen into the flasks containing sterilized sorghum seeds. The procedure is as follows:

Sorghum grains were soaked in water for overnight and excess of water was removed. Then about 200-250 g seeds were placed in each 1000 ml conical flask. These flasks were sterilized at 1.1 kg/cm^2 pressure for an hour. The contents of the flasks were shaken after sterilization to prevent clumping. A 5 mm disc of *F. solani* was aseptically transferred to the cooled flasks. The flasks were incubated at 27 ± 1□ for 15 days. To obtain uniform growth, the contents of the flasks were shaken periodically. After 15 days the giant cultures were ready.

3.2.5 Proving the pathogenicity

Patchouli seedlings were planted in a steam sterilized potting media consisting of soil:sand:farm yard manure in 3:1:1 ratio. Further sick soil was made by inoculating the giant culture of *Fusarium solani* to the sterile soil. A control treatment was maintained without adding the inoculum. Observations were made regularly for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected plants. The symptoms observed in inoculated plant and culture obtained were compared with the original symptoms and original culture for confirmation.

3.3 MORPHOLOGICAL STUDIES

Spore shape and size

Spores of *Fusarium solani* (Dharwad isolate) were taken from infected host tissue and mounted on a clean glass slide. Spores were mixed with lactophenol thoroughly in order to obtain a uniform spread, on which cover slip was placed. One hundred spores were measured under high power objective using Motic Images 2.0 Software. The average size of the spore was calculated. Microphotographs were taken to show the typical spore morphology of the pathogen.

3.4 CULTURAL STUDIES

3.4.1 Growth characters on solid media

The growth characters of *Fusarium solani* were studied on thirteen solid media *viz.,* Asthana and Hawker's agar, Czapek's Dox agar, Richards's agar, Sabouraud's agar, Tochinai's agar, carrot agar, corn meal agar, host leaf extract agar, malt extract agar, oat meal extract ,potato dextrose agar and V-8 juice agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carryout the study, 20 ml of each of the medium was poured in 90 mm Petriplates. Such Petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1℃. Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petriplate in any one of the media. The colony diameter was recorded. The fungus colony colour, margin and sporulation were also recorded. The data on radial growth was analyzed statistically. The composition of each medium used is furnished below.

1. Potato dextrose agar (Tuite, 1969)

Peeled and sliced potatoes	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

The peeled and sliced potatoes were boiled in 400 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was added to the mixture. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

2. Malt extract agar (Tuite, 1969)

Malt extract	25 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

Malt extract was dissolved in 400 ml of distilled water. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly and volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

3. Oat meal agar (Tuite, 1969)

Oat flakes	30 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

Oat flakes were boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

4. Corn meal agar

Corn meal	30 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

Corn meal was boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

5. Host leaf extract agar media

Patchouli leaves	200 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

Patchouli leaves were cut into small bits and boiled in 400 ml of distilled water for 20 min and extract was collected by filtering through a muslin cloth. Twenty grams of agar-agar was melted separately in 400 ml of distilled water and both solutions were mixed. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

6. Sabouraud's agar

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

Agar-agar was melted in 400 ml of distilled water. All other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. This was sterilized at 1.1 kg/cm^2 pressure for 15 min.

7. 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 off pieces were boiled in 800 ml of water and extract was filtered through muslin cloth. The dextrose was dissolved into the solution and agar-agar was added and boiled until it was properly dissolved. Further, volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

8. Richards's agar (Ainsworth, 1971)

Sucrose ($C_{12}H_{22}O_{11}$)	50 g
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 ($FeCl_3$. $6H_2O$)	0.02 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at 1.1 kg/cm^2 pressure for 15 min and subsequently mixed together.

9. Czapek's agar (Tuite, 1969)

Sucrose ($C_{12}H_{22}O_{11}$)	30 g
Sodium nitrate ($NaNO_3$)	2 g
Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Magnesium sulphate ($MgSO_4$. $7H_2O$)	0.5g
Potassium chloride (KCl)	0.5g

Ferrous sulphate ($FeSO_4$. $7H_2O$) 0.01g

Agar-agar 20 g

Distilled water (to make up) 1000 ml

Agar-agar was melted in 500 ml of distilled water. All the other ingredients were mixed in 400 ml of distilled water. The two solutions were mixed thoroughly. The volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

10. V8 juice agar

V8 juice (high media) 44.3 g

Distilled water (to make up) 1000 ml

44.3 g of V8 juice agar powder obtained from high media was suspended in 1000 ml distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

11. Asthana and Hawker's medium (Tuite, 1969)

Glucose ($C_6H_{12}O_6$) 5.0 g

Potassium nitrate (KNO_3) 3.5 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 Agar-agar was melted in 500 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm^2 pressure for 15 min.

12. Brown's medium

Glucose ($C_6H_{12}O_6$) 2.0 g

L-asparagine 2 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 15.0 g

Distilled water (to make up) 1000 ml

Agar-agar was melted in 400 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm^2 pressure for 15 min.

13. 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 of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the

volume was made upto 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm^2 pressure for 15 min.

3.4.2 Growth phase

The growth phase study was conducted on potato dextrose broth. Thirty ml of broth was added in each of the 100 ml conical flasks and sterilized at 1.1 kg/cm^2 pressure for 15 min. These flasks were allowed to cool and 5 mm disc of *Fusarium solani* was inoculated to each of the conical flasks. They were incubated at 27±1°C. Each treatment was replicated thrice. Culture was filtered through Whatman No. 42 filter paper disc of 12.15 cm diameter, which was dried to a constant weight at 60°C 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 20th day. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60°C, cooled in a dessicator and weighed immediately in an analytical electric balance and dry mycelial weight was calculated. Results were analyzed statistically.

3.4.3 Growth in liquid media

The liquid media used were same as that of solid media. The composition and preparations of different liquid media used, were the same as that of solid media except that the agar-agar was not added.

Thirty ml of the medium was added to each of 100 ml flask. All the flasks were sterilized at 1.1 kg/cm^2 pressure for 15 min. Inoculum disc of five mm size was inoculated to all flasks and incubated at 27±1°C for ten days. Each treatment was replicated thrice. The mycelial mat was harvested, dried and weighed as described above. The best synthetic medium was found out and used as a basal medium for further studies.

3.5 SPORE GERMINATION STUDIES

3.5.1 Germination of spores in different nutrient solutions

The germination of fungal spores (Dharwad isolate) was studied in tap water, distilled water, one per cent glucose, sucrose, lactose and dextrose solution. A drop of spore suspension from the respective medium was placed on clean slide. Slide was then placed in Petridishes lined with moistened blotting paper to provide high relative humidity and incubated at room temperature for 24 hours.

Observations were taken at regular intervals and the percentage of spore germination was calculated by taking the average number of spores germinated out of the total number of spores present in five microscopic fields.

3.5.2 Type of germination of spores

The spore suspension was prepared in sterile distilled water and a drop was placed on a clean slide. Slide was then placed in Petridishes lined with moistened blotting paper and kept at room temperature for 24 hours. The type of germination of spores was then studied under microscope from 4 hours onwards upto 24 hours at an interval of two hours.

3.6 PHYSIOLOGICAL STUDIES

3.6.1 Temperature requirement

Richards's liquid medium was used in this experiment. Conical flasks of 100 ml capacity containing 30 ml of liquid medium were inoculated with 5 mm mycelial disc and incubated at different temperature levels *viz.,* 5, 10, 15, 20, 25, 30, 35 and 40°C. In each case, three replications were maintained. The dry mycelial weight at each temperature level was recorded after incubating for ten days and the results were analyzed statistically.

3.6.2 Hydrogen ion concentration

Richards's liquid medium was used as a basal medium. pH of the liquid media was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). The reaction of the medium was adjusted to the desired pH by using di-hydrogen phosphate citric acid buffer according to

schedule of Vogel (1951). The pH of the medium used were 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. After sterilization there was slight change in pH, which was negligible. The culture was inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 27±1℃ for ten days. Three replications were maintained in each treatment. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

3.7 NUTRITIONAL STUDIES

3.7.1 Carbon utilization

The carbon requirements of the fungus was studied by replacing sucrose with different carbon compounds in Richards`s solution. Eight carbon sources *viz*., dextrose, fructose, glucose, mannitol, starch, maltose, sucrose, lactose were tried. 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 was of sucrose present in the basal medium. Each treatment was replicated thrice. All the sugars were dissolved properly. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. Ten days old culture was used for inoculation and incubated at 27°±1°C for ten days. Dry mycelial weight of the fungus was recorded. Results are analyzed statistically.

3.7.2 Nitrogen utilization

The utilization of eight different nitrogen sources viz., Ammonium chloride, Ammonium nitrate, Asparagine, Potassium nitrate, Sodium nitrate, Ammonium sulphate, Urea, Ammonium orthophosphate were studied in Richards`s medium. The quantity of each nitrogen compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of nitrogen as was of potassium nitrate present in the basal medium. All the nitrogen sources were dissolved properly. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. From ten days old culture of the fungus, 5 mm discs were cut and inoculated and incubated at 27°±1°C for ten days. Dry mycelial weight of the fungus was recorded. Results we analyzed statistically.

3.7.3 Light requirement

Potato dextrose broth and agar were used in this experiment. Conical flasks of 100 ml capacity and each containing 30 ml of liquid medium were inoculated and exposed to different lengths of light hours, *viz.* alternate cycles of twelve hours light and 12 hours darkness, continuous light and continuous darkness in an environmental chamber. Petriplates containing 30 ml media were inoculated with 5 mm mycelial discs taken from the periphery of ten days old pure culture and incubated at different light intensities. Each treatment was replicated seven times and incubated for eight days. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

To carryout the study on solid media, 20ml of Potato dextrose agar was poured in 90mm Petriplates. Such Petriplates were inoculated with 5mm disc cut from periphery of the actively growing culture and incubated at different light intensities. Each treatment was replicated seven times and incubated for ten days. Colony diameter and sporulation were recorded. The data on radial growth was analyzed statistically.

3.8 HOST RANGE of *Fusarium solani*

Host range experiment was undertaken with an objective of knowing whether the pathogen can infect any host other than Patchouli. Totally twelve different hosts were selected and details of plants are given below.

Sl. No	Common name	Botanical name	Family
1	Hoary Basil	*Ocimum canum* Sims.	Lamiaceae
2	Sacred Basil	*Ocimum sanctum* L.	Lamiaceae
3	Clocimum	*Ocimum gratissimum* L.	Lamiaceae
4	Pandanus	*Pandanus amaryllifolius* Roxb.	Pandanaceae

5	Lemon grass	*Cymbopogon flexuosus* (D.C) Stapf.	Graminae
6	Citronella	*Cymbopogon winterianus* Jowitt	Graminae
7	Brahmi	*Bacopa mannieri* (L.) Pennell	Scrophulariaceae
8	Mint	*Mentha spicata* L.	Lamiaceae
9	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae
10	Coleus	*Coleus forskohlii* Briq.	Lamiaceae
11	Indian borej	*Coleus ciamboini* L.	Lamiaceae
12	Stevia	*Stevia rebaudiana* Bertoni	Asteraceae

All the above plants were raised in earthern pots of 12″ size. Gaint culture of *Fusarium solani* multiplied on sorghum grains was added to the pots when the plants were established. The hosts planted in pots containing sterilized soil served as control and these were incubated in glass house at 27±1 and watered regularly. The early stage of symptoms development and type of symptoms developed on different hosts were recorded.

3.9 VARIABILITY IN *F. solani*

For variability studies, eight isolates of *Fusarium solani* were collected from different places, *viz.* Arabhavi (*Fs1*) , Belgaum (*Fs2*) , Mangenkoppa (*Fs3*), Adavisomapur (*Fs4*), Saidapur (*Fs5*) , Shimoga (*Fs 6*), Uttar Kannada (*Fs7*), Adur (*Fs 8*).

3.9.1 Cultural and morphological variability

Eight different isolates of *F. solani* collected from different places were grown on potato dextrose agar for variability studies. Growth of each isolate, *viz.* colony colour, colony diameter, sporulation and spore size were recorded.

3.9.2 Genetic variability

Random Amplified Polymorphic DNA (RAPD) analysis was used to detect the variation among the isolates of *F.solani*. Standard protocols were used for the isolation of DNA and RAPD analysis.

Requirements

Stock solutions

1. 65 ℃ lysis buffer
   50 mm Tris (pH 8) s
   50 mm EDTA
   3% SDS
   1% BME
2. 950 µl bufferd red phenol
3. Chloroform : isoamyl alcohol 1: 24:1
4. 50 µl of 7.5 Ammonium acetate.
5. T_{10} E_1:
   10mM Tris
   1mM EDTA, pH 8
6. 100 µm Random primers
7. 25ng μl^{-1} Template DNA
8. 3-0 U μl^{-1} Taq DNA polymerase

Procedure

I Isolation of template DNA of *F. solani*

Five days old fungal mycelial mat grown on potato dextrose broth was placed in pre cooled pestle and mortar.

- The pestle and mortar was filled with liquid nitrogen and was allowed to evaporate for 2 minutes. Immediately 500 µl of 65℃ lysis buffer was added and transferred to 1.5 ml microcentrifuge tube.
- The tube was vortexed and placed in 65℃ water bath for one hour by vortexing after 30 minutes and 60 minutes.
- About 500 µl phenol was added to extract and tube was spinned for 5 minutes at 8000 rpm to separate phases. And about 450 µl of aqueous phase was taken in a fresh tube.
- Again, 450 µl of buffered phenol was added and spinned for 5 min at 8000 rpm. The aqueous phase of 400 µl was placed in a fresh tube.
- Further 400 µl of chloroform: isoamyl alcohol (24:1) was added and spinned for 5 min at 8000 rpm. 350µl of aqueous phase was removed and placed in a fresh tube.
- About 50 µl of 7.5 Ammonium acetate was added and gently mixed. Immediately 880 µl of 95% ethanol was added and placed in 20℃ freezer for overnight.
- The DNA pellet obtained was spinned down for 20 min at 13,000 rpm and pellet was rinsed in 70% ethanol.
- The pellet obtained was allowed to dry and resuspended in 20 µl of T10 E1. Further, the DNA template was quantified by agarose gel electrophoresis.

II. Random primers: Commercial kit OPA and OPB of decamer DNA primers were obtained from M/s Integrated DNA technologies supplied by Sigma Industrial and laboratory Equipments Inc., Bangalore, India.

III dNTP'S :

The four individual dNTP's such as dATP, dGTP, dCTP and dTTP were obtained from M/s Bangalore Genei, Pvt. ltd, Bangalore.

IV. Taq DNA Polymerase

Taq DNA polymerase and 10x Taq buffer were obtained from M/s Bangalore Genei, Pvt, Ltd. Bangalore.

V. Thermo cycler

Corbett Research gradient PCR supplied by M/s. JH Bio Innovation Pvt. Ltd R.T. Nagar Bangalore was used for cyclic amplification of DNA.

The thermoprofile for PCR

The PCR amplification for RAPD analysis was performed according to Williams *et al*. (1990) with certain modifications. The optimum conditions for DNA amplifications used were as follows.

Sl. No.	Step	Temperature (℃)	Duration (min)	Number of cycles
1	Denaturation	94	4	1
2	Denaturation	94	1	40
3	Annealing	36	1	
4	Extension	72	2	
5	Final extension	72	5	1
6	Hold temperature	4	-	-

After the completion of the PCR , the products were stored at 4℃ until the gel electrophoresis was done.

A total of 20 random primers with the following sequences belonging to Operon A and B series were used in the study.

Sequences of random primers used in RAPD analysis for different isolates of *F. solani*

Primer	Sequence
OPA-01	5 CAG GCC CTT C-3
OPA-02	5 TGC CGA GCT G-3
OPA-03	5 AGT CAG CCA C-3
OPA-04	5 AAT CGG GCT G-3
OPA-05	5 AGG GGT CTT G-3
OPA-06	5 GGT CCC TGA C-3
OPA-07	5 GAA ACG GGT G-3
OPA-08	5 GTG ACG TAG G-3
OPA-09	5 GGG TAA CGC C-3
OPA-10	5 GTG ATC GCA G-3
OPA-11	5 CAA TCG CCG T-3
OPA-12	5 TCG GCG ATA G-3
OPA-13	5 CAG CAC CCA C-3
OPA-14	5 TCT GTG CTG G-3
OPA-15	5 TTC CGA ACC C-3
OPA-16	5 AGC CAG CGA A-3
OPB-02	5 TGA TCC CTG G-3
OPB-03	5 CAT CCC CCT G-3
OPB-04	5 GGA CTG GAG T-3
OPB-05	5 TGC GCC CTT C-3

Master mix for PCR

Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing following components. The total volume of each reaction mixture was 20µl. The following reaction mixture was found to be optimum for PCR amplification.

1. 10 x assay buffer with 15 mM $MgCl_2$ 2.50µl
2. dNTPs mix (2.5 mM each) s 1.0µl
3. Primer (5pM / µl) 1.0µl
4. Template DNA (25ng / µl) 1.0 µl
5. Sterile distilled water 14.30 µl
6. Taq DNA polymerase (3.0U μl^{-1}) 0.2 µl

Except template the master mix was distributed to PCR tubes (19 µl/ tube) and later 1 µl of template DNA from the respective isolates was added making the final volume to 20 µl.

Separation of amplified products by agarose gel electrophoresis

Requirements

1. Electrophoretic unit: Gel casting trough, gel combs, power pack, UV Transilluminator.
2. Agarose (1.2%)
3. Bromophenol blue
4. Ethidium bromide (0.5 µg ml^{-1})
5. 20 x TAE (stock): tris – base – 60 .5 g

   Glacial acetic acid – 14.25 ml

   0.5 M EDTA- 25 ml

   Make up the volume to 250 ml , pH 8.0
6. Working solution (1 x TAE): 20 ml of 50 x TAE was made upto 1000 ml by using distilled water.

Procedure

- Three grams of agarose was weighed and added to a conical flask containing 250 ml of 1 x TAE buffer.
- The agarose was melted by heating the solution in an oven and the solution was stirred to ensure even mixing and complete dissolution of agarose.
- The solution was then cooled to about 40 – 45°C.
- Two to three drops of ethidium bromide (0.5 µg ml^{-1}) was added.
- The solution was mixed and poured into the gel casting platform after inserting the comb in the trough. While pouring sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1 x TAE) so as to cover the wells completely.
- The amplified products (20µl) to be analysed were carefully loaded into the sample wells, after adding bromophenol blue with the help of micropipette.
- Electrophoresis was carried out at 60 volts, until the tracking dye migrated to the end of the gel.
- Ethidium bromide stained DNA bands were viewed under UV transilluminator and photographed for documentation.

Scoring the amplified fragments

The amplified profiles for all the primers were compared with each other and the bands of DNA fragment were scored as '1' for the presence and '0' for the absence of a band generating the '0' and '1' matrix and per cent polymorphism was calculated by using the formula.

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Analysis of the profile of the amplified fragments

Pair wise genetic similarities between isolates were estimated by DICE similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using Sequential agglomerative hierarical nested (SAHN) cluster analysis of NTSYS-PC version 2.0 (Rohlf, 1998).

3.10 MANAGEMENT STUDIES

3.10.1 *In vitro* evaluation of botanicals

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some plant extracts. The following plant extracts were selected.

Sl. No	Botanical name	Common name	Family	Parts used
1	*Azadirachta indica* A. Juss	Neem	Meliaceae	Seeds
2	*Allium sativum* L.	Garlic	Liliaceae	Bulbs
3	*Bougainvillea spectabilis* L	Bougain villea	Nyctaginaceae	leaves
4	*Cassia occidentalis* L.	Negro coffee	Caesalpiniaceae	leaves
5	*Clerodendron inerme* Gaerth	Kashmir bouquet	Verbenaceae	leaves
6	*Durantha repens* L	Durantha	Verbenaceae	leaves
7	*Eucalyptus globes* Labill	Eucalyptus	Mystoraceae	leaves
8	*Glyricidia maculata* L	Glyricidia	Leguminaceae	leaves
9	*Parthenium hysterophorus* L	Congress weed	Asteraceae	leaves
10	*Pongemia pinnata* L.	Honge	Simarubaceae	leaves
11	*Ocimum sanctum* L	Tulsi	Labiateae	leaves

Preparation of cold aqueous extract

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1973). Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten PDA medium, respectively so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of medium was poured into sterile Petriplates, mycelium of five mm size discs form periphery of actively growing culture (Dharwad isolate) were cut out by sterile cork borer and one such disc was placed on the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1℃ temperature and radial growth was taken when maximum growth occurred in the control plates.

The efficacy of plant products or botanicals were expressed as per cent of radial growth over the control which was calculated by using the following formula (Vincent ,1947)

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

3.10.2 *In vitro* evaluation of biocontrol agents

Six biocontrol agents such as *Trichoderma harzianum* Rifai, *Trichoderma koningii* Oudern, *Trichoderma virens* Miller, *Trichoderma viride,* Pers. Ex.S.F.Gray, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn Emend Pras were tested against *Fusarium solani.* Both biocontrol agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. The cultures of antagonistic microorganisms used in the present study were obtained from the Project Directorate of Biological Control (PDBC) Bangalore, Karnataka state.

Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile Petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus was inoculated at one end of the Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at middle of the Petriplates and mycelial discs of the fungus was placed at the centre. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The percent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

3.10.3 *In vitro* evaluation of fungicides

The efficacy of six systemic fungicides (at the concentration of 0.025, 0.05 and 0.1 per cent) and six non-systemic fungicides (at the concentrations of 0.1, 0.2 and 0.3 per cent) were assayed. The fungicides used are given here under.

Sl. No.	Common name	Chemical name	Trade name
	Systemic fungicides		
1	Carbendazim	2-(Methoxy-Carboryl)–benzimidazole	Bavistin 50% WP
2	Combi product (Carbendazim 12% + Mancozeb 63% WP)	Manganese zinc ethylene bisdithiocarbamate +2-(Methoxy carboryl) –benzimidazole	Saaf
3	Hexaconazole	(RS)-2-C2, 4-dichlorophe myl)-1-(14-1, 2, 4-triazole-1-41) hexan-2-Ol	Contaf 5% EC
4	Propiconazole	1-(-2-(2,4dichlorophenyl)-4-propyl-1, 3	Tilt 25% EC
5	Benomyl	Methyl-10(Butyl Carbomyl) 2-Benzimidazole carbomate	Benomyl 50%s WP
6	Combiproduct (Carboxin 37.5% + Thiram 37.5%)	5,6- dihydro-2-methyl-1,4-oxathine-3-carboxamide	Vitavax power

	Non systemic fungicides		
Sl. No.	Common name	Chemical name	Trade name
1	Captan	N (trichloromethylthio) Cyclohix-4 ere-1, 2 dicarbozimide	Captaf 50% WP
2	Mancozeb	Manganese ethylene bis dithiocarbonate + Zinc	Kavach 75% WP

3	Propineb	Zincpropylene bisdithiocarbamate	Antracol 70WP
4	Copper oxychloride	Copper oxychloride	Blitox 50% WP
5	Thiram	Tetramethyl thiuram disulphate (TMTD)	Thiram 75% WP
6	Zineb	Zinc ethylene bisdithiocarbomate	Indofil Z-78 75% WP

Required quantity of individual fungicide was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of fungicides. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated thrice. Then such plates were incubated at room temperature for eight days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947).

3.10.4 *In vivo* studies

A pot culture experiment was conducted in the glasshouse of University of Agricultural Sciences, Dharwad to find out the best treatment for control of wilt disease of patchouli caused by *Fusarium solani.* The Patchouli cuttings were grown in earthen pots containing sick soil. The effective fungicides, botanicals, and bioagents evaluated in *in vitro* studies were further evaluated under pot culture.

About six kg of sterile soil was filled in each earthern pots of 12″ size. Rooted patchouli cuttings of different entities were planted separately at the rate of three cuttings per pot. Each treatment was replicated thrice. Inoculum of *F. solani* was added after establishment of seedlings to prepare the sick soil. After proper establishment of the seedlings, fungicides, *viz.* carbendazim, carbendazim+ mancozeb, vitavax, mancozeb and propiconazole, bioagents viz., *Trichoderma harzianum*, and *T. viride* and botanicals, *viz*. neem seed kernel extract (10%), *Allium sativum* (10%) were drenched individually to respective sets of pots. Patchouli plants planted in the sick soil without treatment served as control.

The treatment are as follows:

T1 : Combi product carbendazim12% + mancozeb63%WP (Saaf 0.2%) @ 0.1%

T2 : Carbendazim @ 0.1%

T3 : Carboxin 37.5% + Thiram 37.5% @ 0.1%

T4 : Propiconazole (Tilt 25% EC) @ 0.1%

T5 : Mancozeb @ 0.2%

T6 : *Trichoderma harzianum* @ 0.6 %

T7 : *Trichoderma viride* @ 0.6 %

T8 : Neem seed kernal extract @ 10 %

T9 : *Allium sativum* (Garlic) @ 10 %

T10 : Control

Observations were taken on per cent disease incidence and plant height. Data were analysed statistically.

4. EXPERIMENTAL RESULTS

The present investigation on diseases of Patchouli with special reference to *Fusarium* wilt caused by *Fusarium solani* (Mart.) Sacc., was conducted in Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad during 2006-07. The results of the experiments conducted are presented hereunder.

4.1 SURVEY AND SURVEILLANCE FOR DISEASES OF PATCHOULI

Patchouli is a newly introduced crop to India. However, frequent incidences of wilt, leaf blight and plant parasitic nematodes have hindered its commercial cultivation.

A fixed plot survey was conducted during 2006–07 in different villages of Belgaum, Haveri, Uttar Kannada, Shimoga and Dharwad districts to know the incidence of these diseases. Results indicated that there was a higher incidence of wilt disease compared to leaf blight and nematode diseases in all the districts surveyed. The data pertaining to this study are given in Table 1, Fig 1 and Plate 1.

The results indicated that wilt incidence ranged from 20.00 to 61.25 per cent. Based on this data district means were calculated and found that the maximum disease incidence was noticed in Dharwad district (48.26%) followed by Shimoga (45.62%), Belgaum district (40.25%) and Haveri (38.63%). Least disease incidence was noticed in Uttar Kannada district (25.62%).

In Belgaum district a high disease incidence was observed in Itagi (56.25%) and low was in Arabhavi (35.00%). In Dharwad district, the maximum disease was noticed in Adavisomapur (61.25%) and minimum incidence in Saidapur (35.00%). In Uttar Kannada district, maximum per cent disease incidence was observed in Hulekal (31.25%) and lowest incidence in Arekoppa (20.00%). In Shimoga, the per cent disease incidence was 45.62 per cent and in Adur village of Haveri district the disease incidence was 38.63 per cent.

The incidence of leaf blight on the crop was found to be less in all the areas surveyed. Highest incidence of leaf blight was observed in Haveri district (23.75%) followed by Belgaum district (22.52%) wherein Beedi village recorded the highest incidence (33.33%). Dharwad district recorded 20.83 per cent incidence of leaf blight, highest incidence was observed in Adavisomapur (25.00%) and least in Saidapur (16.66%). In Uttar Kannada the per cent incidence of 16.18 was observed. Haveri/Hangal and Shimoga districts recorded the leaf blight incidence of 23.75 and 18.75 per cent respectively. *Sclerotium* wilt was observed in Dharwad (Saidapur) with 2 per cent disease incidence.

Root Knot nematode

The root knot disease caused by *Meloidogyne incognita* was maximum in Mangenkoppa village of Belgaum district with 89 per cent disease incidence.

4.1.1 Symptomatology

4.1.1.1 *Fusarium* wilt

Yellowing of the older leaves was the first external symptom which later advanced to middle and terminal leaves. Drying of green parts followed exactly as if they were suffering from drought, even though there was plenty of moisture in the soil. Brown to black discolouration of stem and roots was observed (Plate 1d & c) followed by dark brown to red discoloration of vascular tissue (Plate 1e). Severe disintegration of secondary roots surface was also observed. The infected plants wilted completely (Plate 1b) and could be pulled off easily from the soil. Ultimately, the whole plant dried and there was premature death of the plant.

4.1.1.2 Leaf Blight

The symptoms of leaf blight caused by *Alternaria alternata* (Fr.) Keissler appeared as brown discolouration which initiated from the tip of mature leaves. The blighting of the leaves extended towards the margin of the leaves resulting in marginal necrosis (Plate 2b). The

Table 1. Survey for diseases of Patchouli

District	Taluka	Place/ locality	Per cent disease incidence	
			Wilt	Leaf blight
Dharwad	Dharwad	Saidapur*	35.00	16.66
	Hubli	Adavisomapur	61.25	25.00
District Mean			48.26	20.83
Belgaum	Khanapur	Itagi	56.25	28.52
		Mangenkoppa**	40.90	16.66
		Beedi	43.88	33.33
	Gokak	Arabhavi	20.00	12.00
District Mean			40.25	22.62
Uttar Kannada	Sirsi	Arekoppa	20.00	11.53
		Hulekal	31.25	20.83
District Mean			25.62	16.18
Haveri	Hangal	Adur	38.63	23.75
Shimoga	Shimoga	Shimoga	45.62	18.75

* Sclerotium wilt 2 per cent disease incidence
** Root knot 89 per cent disease incidence

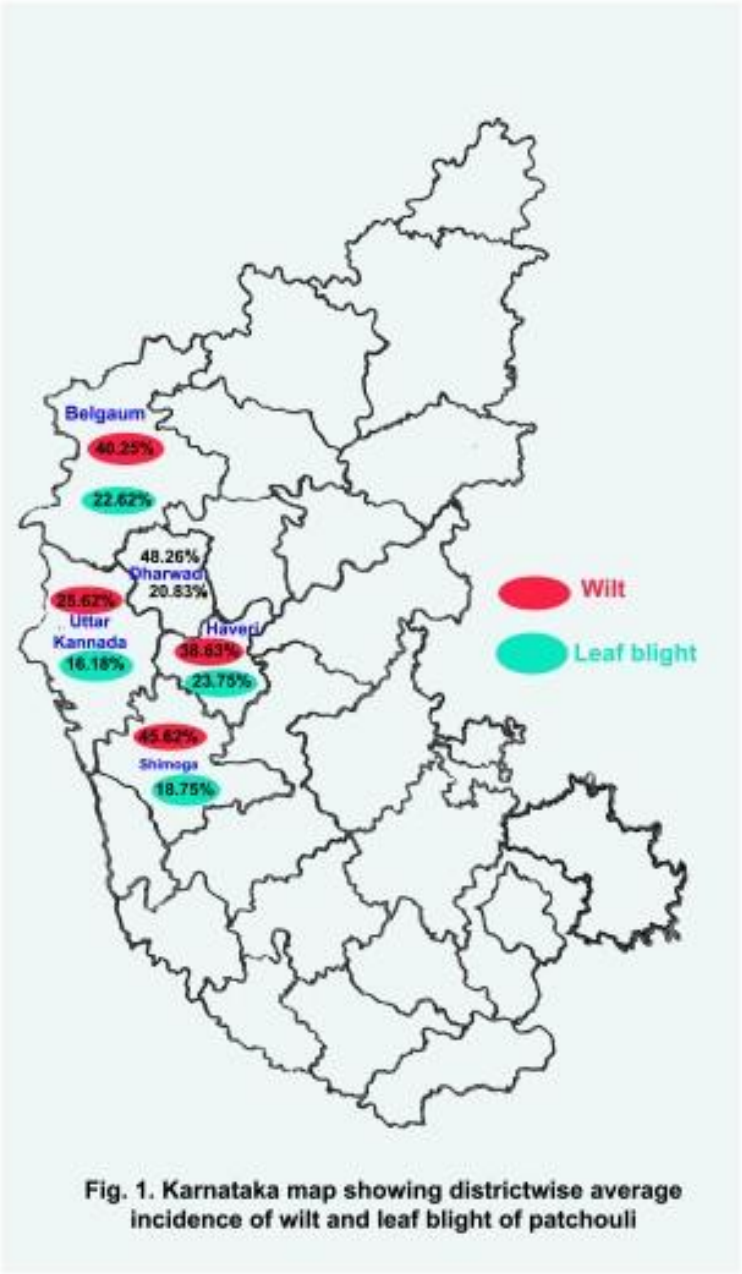


Fig. 1. Karnataka map showing districtwise average incidence of wilt and leaf blight of patchouli

Fig. 1. Karnataka map showing districtwise average incidence of wilt and leaf blight of patchouli

spots were light brown in colour, subcircular to irregular (Plate 2a). Under favourable conditions the plant exhibited a burnt appearance which could be easily detected from a distance.

4.1.1.3 *Sclerotium* wilt

Symptom started in the form of water soaked lesions near collar region followed by darkening of the tissue. 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 bodies were white initially, later turned brown with age. Finally the plant wilted and dried. Sclerotial bodies were also formed on the fallen leaves (Plate 3b).

4.1.1.4 Root knot

Infected patchouli plants were stunted in their growth with yellow colored leaves. When infected plants were uprooted, deformed roots with prominent multiple galls of varying size were noticed. (Plate 4) Microscopic examination of the galls revealed the presence of eggs, juveniles and females of *Meloidogyne incognita* in the vascular region of roots. On an average, 4-6 adult females were present along with immature stages in a single gall. The female was pyriform in shape while males were vermiform. The presence of egg masses outside the gall was a common phenomenon noticed in patchouli roots.

4.2 ISOLATION OF PATHOGENS

Standard tissue isolation techniques were followed to get culture of causal organisms from diseased parts of Patchouli as detailed in 'Material and Methods'. Pathogens isolated from infected plants were *Fusarium solani, Alternaria alternata* and *Sclerotium rolfsii*.

4.2.1 Identification of pathogen

On the basis of morphological and cultural characteristics the pathogens were identified as *Fusarium solani* (Mart.) Sacc., *Alternaria alternata* (Fr.) Keissler and *Sclerotium rolfsii* Sacc. Further, the identity of *Fusarium solani* was confirmed from Maharastra Association for cultivation of sciences, Agharkar Research Institute, Pune.

4.2.1.1 *Fusarium solani*

Repeated isolations from the infected plants yielded *Fusarium solani*. The culture was identified based on morphology, mycelial character, spore production and pigmentation on PDA. *Fusarium solani* on potato dextrose agar put forth moderately rapid growth covering the Petriplate in 7-10 days. The mycelium was sparse to dense, greyish white to light pinkish in colour.

The microscopic observation revealed the production of both micro and macro conidia. The microconidia were formed abundantly on the aerial mycelium from elongated phialides. They were hyaline, cylindrical to falcate. The size (LxB) of microconidia varied from 6.6 - 19.8 x 3.3 – 6.6 μm. The macro conidia developed in 6 days from well developed conidiophores. They were hyaline, cylindrical to falcate with 2 – 4 septa, measured 29.7 – 47.8 x 4.9 - 6.6 μm. Apex of macroconidia were pointed and beaked.

Chlamydospores were produced 12 – 14 days after incubation in cultures. They were globose to oval, single celled, smooth to rough walled, 8.25 - 11.5 x 6.60 - 9.90 μm., produced terminally or intercalary.

4.2.1.2 *Alternaria alternata*

The culture of *A. alternata* on potato dextrose agar was grey coloured with fluffy dense mycelium, smooth margin and distinct concentric rings.

4.2.1.3 *Sclerotium rolfsii*

The fungus produced white, dense, radiating mycelial growth on PDA. In the early stages the fungus produced silky white mycelium and gradually lost its luster and became dull in appearance. Initiation of sclerotial bodies were observed. In the beginning, they were white later turned to buff brown colour and then to chocolate brown.

a. General view of *Fusarium* wilt of patchouli

b. Wilted plants

c. Black discolouration of roots

d. Brown discolouration

e. Vascular discolouration

Plate 1. Symptoms of Fusarium wilt of patchouli

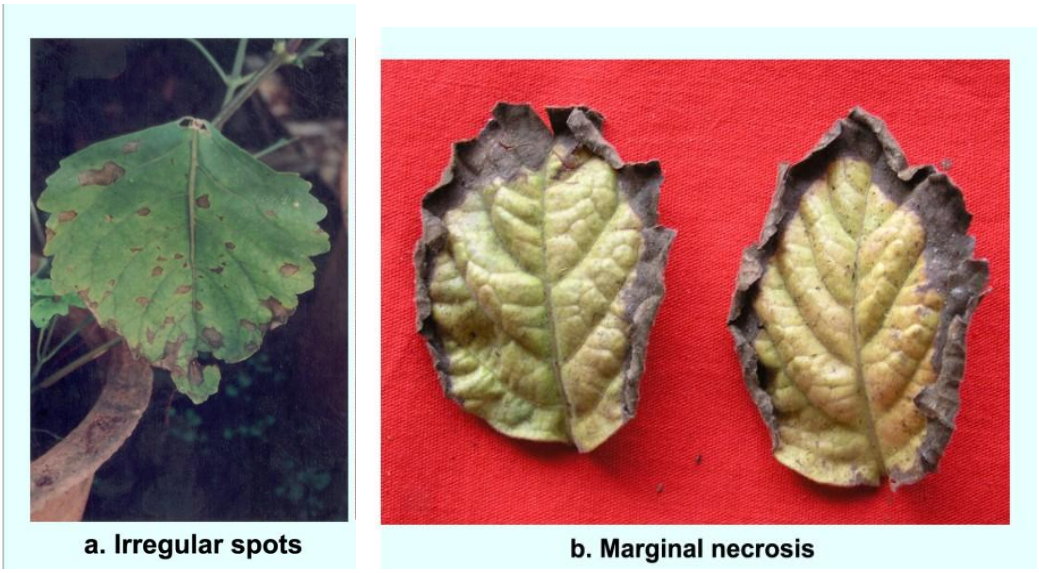


Plate 2. Alternaria leaf blight of patchouli

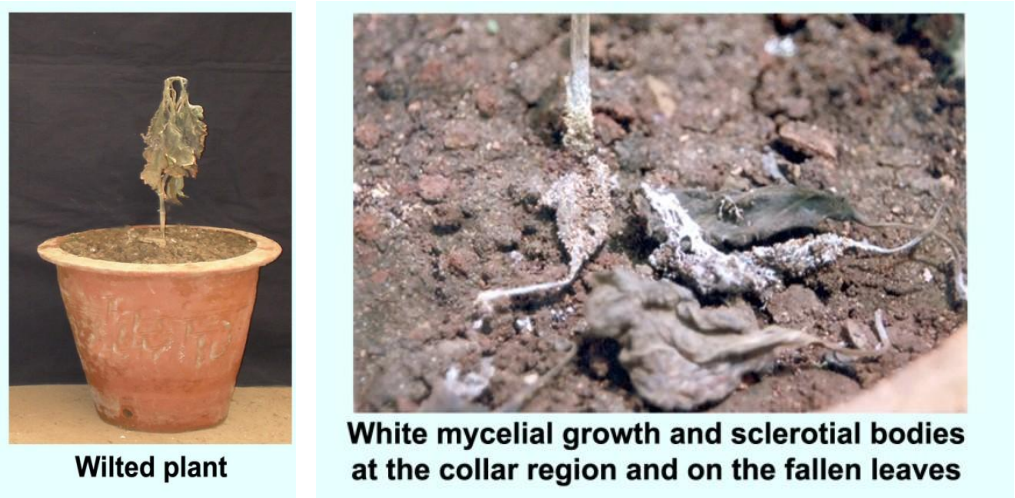
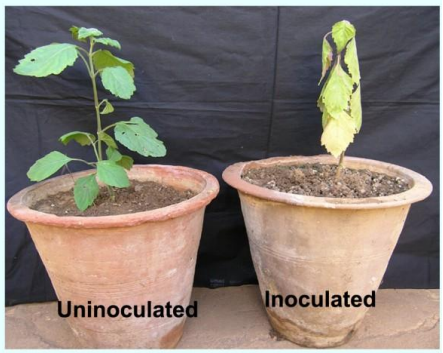


Plate 3. Sclerotium wilt of patchouli

Plate 4. Root knot of patchouli

Plate 5. Giant culture of *Fusarium solani*

Plate 5. Giant culture of Fusarium solani



25 days after inoculation

60 days after inoculation

Plate 6. Proving pathogenicity of Fusarium solani

Table 2. Morphological characters of *Fusarium solani*

Spore	Measurement	
	Range (µm)	Average (µm)
Microconidia	6.6-19.8 x 3.3 – 6.6	13.5 x 4.3
Macroconidia	29.7 - 47.85 x 4.95 - 6.60	39.0 x5.7
Chlamydospore	8.2 -11.5 x 6.60-9.90	9.78 x 8.11

4.2.1.4 Pathogenicity of *F. solani*

Artificial inoculation of patchouli seedling were carried out as explained in "Material and Methods", the symptoms developed were recorded. The characteristic symptoms started as yellowing of lower leaves 25 days after inoculation which extended upwards and whole leaves gradually turned brown coloured after 34 days of inoculation. The plant became stunted. The plant height was 18 cm in inoculated seedlings where as it was 95.6 cm in control at 60 DAS. Finally death of the plant was observed on 60th day (Plate 6). When the infected plant was uprooted, dark brown to red discolouration of vascular tissue was observed

4.3 MORPHOLOGICAL STUDIES

The spores of the pathogen were taken from pure culture and temporary slide mounts were prepared in Lactophenol. Then they were observed under high power (45x). One hundred spores of pathogen were observed under microscope and measured using Motic Images 2.0 Software. The morphological characters of *F. solani* are depicted in Table 2.

Microconidia : Microconidia were abundant, hyaline, continuous, or 1 – septate, ovoid and ovate and measured 6.6 - 19.8 x 3.3 – 6.6 µm (Average 13.5 x 4.3 µm) (Plate 7)

Macroconidia : Macroconidia were 3 – 4 septate measuring 29.7 –47.8 x 4.9-6.6 µm (Average 39.0 x5.7 µm) (Plate 7)

Chlamydospore : Chlamydospores were hyaline, usually vacuolated, spherical and single celled, produced either singly or in chain, terminal or intercalary, measuring 8.25-11.5 x 6.60-9.90 µm (Average 9.78 x 8.11 µm) (Plate 7).

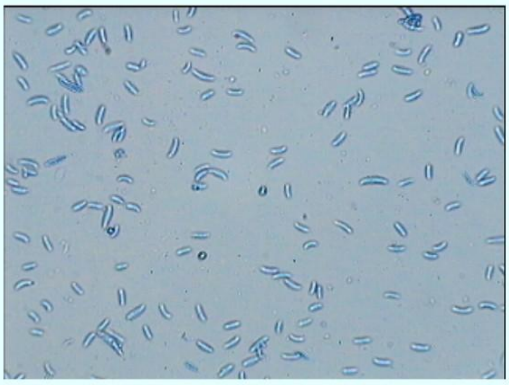
4.4 CULTURAL STUDIES

4.4.1 Growth characters on different solid media

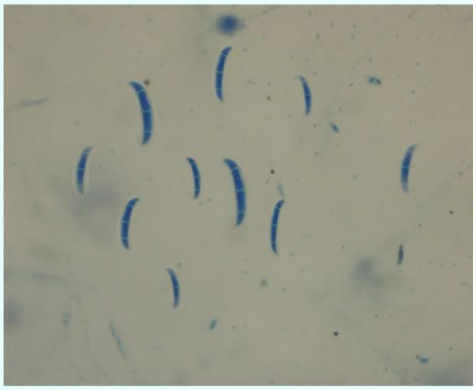
Cultural characters were studied on thirteen different solid media. The radial growth of *F. solani* was measured when the maximum growth was attained in any of the media tested. Observations on various cultural characters were recorded as described in ''Material and Methods''. The results are presented in Table 3, Fig 2 and Plate 8a.

The results of the cultural studies on solid media indicated that the radial growth of *F. solani* was maximum on Richards's agar (90 mm) and Potato dextrose agar (90 mm) followed by Oat meal agar (88.00 mm). These three were on par with each other and significantly

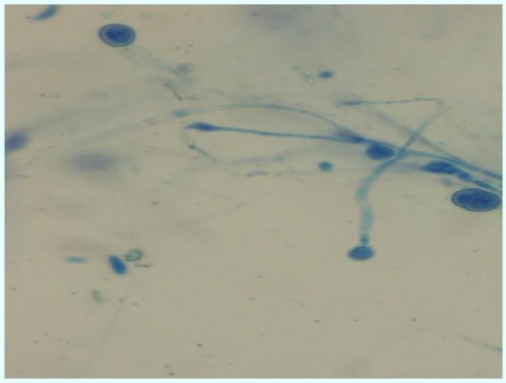
Culture



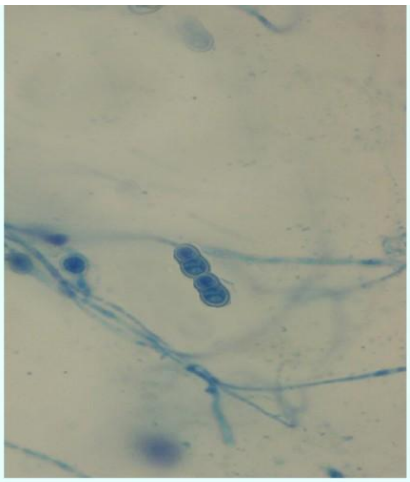
Conidia (40x)



Macroconidia (100x)



Terminal chlamydospores



Chlamydospores in chains

Intercalary chlamydospores

Plate 7. Morphological characters of Fusarium solani

Table 3. Growth of *Fusarium solani* on solid media

Media	Mean colony diameter (mm)
Asthana and Hawker's agar	73.00
Brown's medium	22.33
Carrot agar	82.33
Corn meal agar	81.16
Czapek's agar	70.23
Host extract agar	72.33
Malt extract agar	69.00
Oat meal agar	88.00
Potato dextrose agar	90.00
Richards's agar	90.00
Sabouraud's dextrose agar	76.00
Tochinai's agar	68.33
V-8 juice agar	75.00
S.Em±	1.07
CD at 1%	4.20

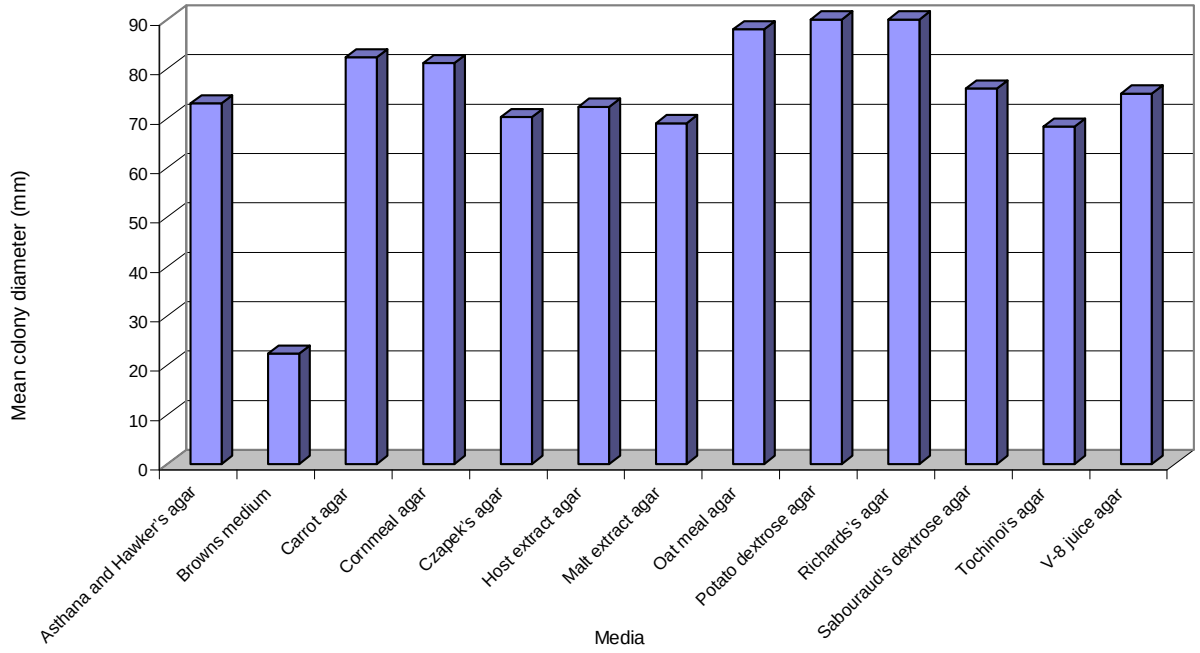


Fig. 2. Growth of *Fusarium solani* on solid media

Fig. 2. Growth of Fusarium solani on solid media

Table 4. Cultural characteristics of *Fusarium solani* on solid media

Medium	Growth characters	Sporulation
Asthana and Hawker's agar	White cottony growth	+
Brown's medium	White cottony growth with smooth margin	++
Carrot agar	White sparse growth	++
Corn meal agar	Pink cottony growth	++
Czapek's agar	White cottony growth with smooth margin	++
Host extract agar	White cottony mycelium with smooth margin	++
Malt extract agar	White cottony growth	++
Oat meal agar	White cottony growth	+++
Potato dextrose agar	Pink dense and pluffy growth, smooth margin	+++
Richards' agar	White cottony and pluffy growth, smooth margin	+++
Sabouraud's dextrose agar	Pink cottony growth	++
Tochinai's agar	White sparse growth	++
V-8 juice agar	White cottony and pluffy growth	++

+++ : Good sporulation >50 conidia/microscopic field

++ : Moderate sporulation 30-50 conidia/microscopic field

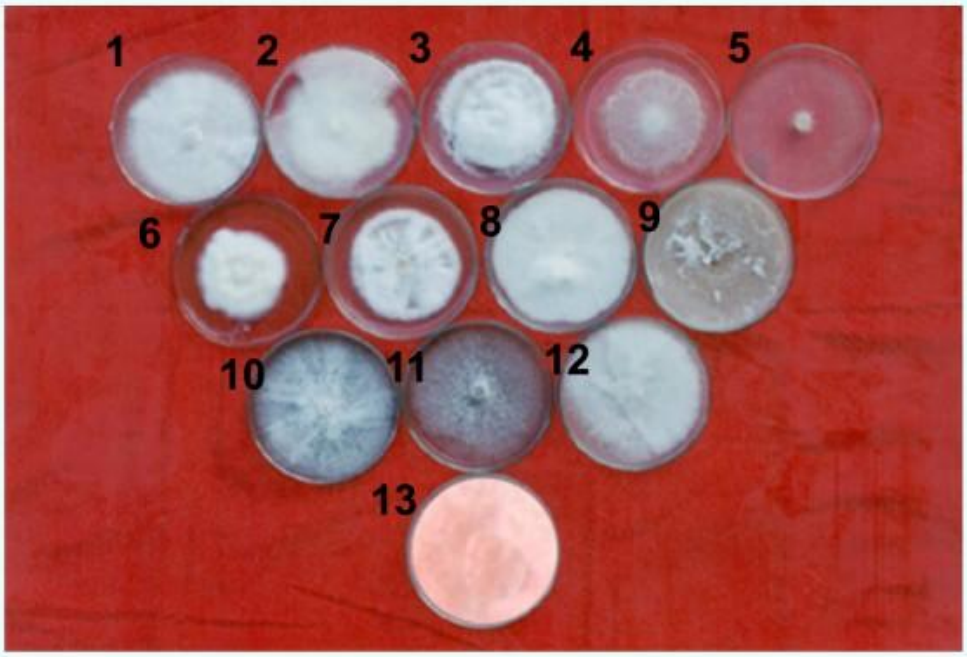
+ : Scanty sporulation <30 conidia/microscopic field

Plate 8a.

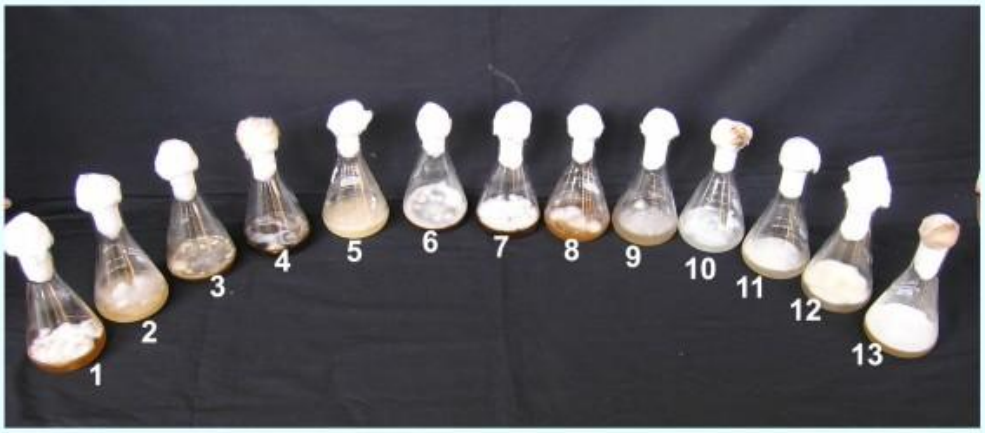
1. Oat meal agar
2. Czapek's agar
3. Tochinai's agar
4. Asthana and Hawker's agar
5. Browns media
6. Sabouraud's dextrose agar
7. Malt extract agar
8. Richards's agar
9. Corn meal agar
10. Host extract agar
11. Carrot agar
12. V-8 Juice agar
13. Potato dextrose agar

Plate 8b.

1. Carrot broth
2. Asthana and Hawker's broth
3. Brown's medium
4. V-8 Juice broth
5. Host extract broth
6. Sabouraud's dextrose broth
7. Czapek's broth
8. Oat meal broth
9. Malt extract broth
10. Tochinai's broth
11. Corn meal broth
12. Potato dextrose broth
13. Richards's broth



a. Growth of *Fusarium solani* on solid media



b. Growth of *Fusarium solani* on liquid media

Plate 8. Cultural Studies

superior over the rest of all other media tested. These were followed by carrot agar (82.33 mm) and corn meal agar (81.16 mm). Minimum radial growth was observed in Brown's agar (22.33 mm).

Sporulation was abundant in Richards's agar, Sabouraud's agar and potato dextrose agar. Good sporulation was observed in Czapek's Dox agar, Tochinai's agar, Brown's agar, carrot agar, host leaf extract agar and corn meal agar. The same was sparse in Asthana and Hawker's agar. The results are presented in Table 4.

4.4.2 Growth phase

The experiment was conducted as detailed in "Material and Methods" to ascertain the period when the maximum vegetative growth of pathogen could occur. The data are presented in Table 5 and Fig 3.

The data from table 5 indicated that dry mycelial weight of the *F. solani* was minimum on second day after inoculation and on subsequent harvest, it significantly increased and finally reached maximum on 10^{th} day. Later, the growth started decreasing. Maximum mycelial growth (365.66 mg) was observed on tenth day of incubation and was significantly superior to all other treatments. This period was used as maximum growth period for further studies.

4.4.3 Growth in different liquid media

Growth of the pathogen was studied in the liquid media to select the best liquid medium that would support maximum growth as explained in ''Material and Methods'' The average dry mycelial weight of fungus after 10 days of incubation is given in Table 6 and Fig 4 and Plate 8b.

The results indicated that maximum dry mycelial weight of fungus was obtained in Richards's broth (362.33 mg) which was significantly superior to all other media. This was followed by potato dextrose broth (352.33 mg) and Oat meal broth (315.66 mg) and Corn meal broth (311.33 mg). Least mycelial weight was obtained in Brown's medium (66.33 mg) and malt extract broth (66.66 mg).

4.5 SPORE GERMINATION STUDIES

4.5.1 Type of germination of spores

The type of germination of spores of *Fusarium solani* was studied as detailed in 'Material and Methods'.

The germination of conidia and chlamydospores started with the initiation of germ tubes four hours and six hours after incubation respectively. Germination was maximum at 12 hours of incubation. The germ tubes were produced form intermediary cells or from either one or both the polar cells. The type of germination of conidia and chlamydospores is illustrated in Plate 9.

4.5.2 Germination of spores in different nutrient solutions

The spore germination studies were conducted in different nutrient solutions, *viz* . tap water, sterile distilled water, glucose (1%), sucrose (1%), lactose (1%) and dextrose (1%). The germination count was taken after 12 hr of incubation and the results are presented Table 7 and Fig 5.

Conidial germination differed significantly in different solutions. Maximum conidial germination was observed in sucrose (98.07%) which was significantly superior to all other treatments. This was followed by dextrose (95.72%) and glucose (90.09%). Least conidial germination was recorded in sterile distilled water (68.18%).

4.6 PHYSIOLOGICAL STUDIES

4.6.1 Effect of temperature

The effect of temperature on the growth of *F.solani* was studied as explained in "Material and Methods" and results are presented in Table 8 and Fig 6.

Table 5. Dry mycelial weight of *Fusarium solani* in Potato dextrose broth

Days after seeding	Dry mycelial weight (mg)
2	65.22
4	130.11
6	200.66
8	254.51
10	365.66
12	330.48
14	310.66
16	289.51
18	250.25
20	237.48
S. Em±	0.35
CD at 1%	1.42

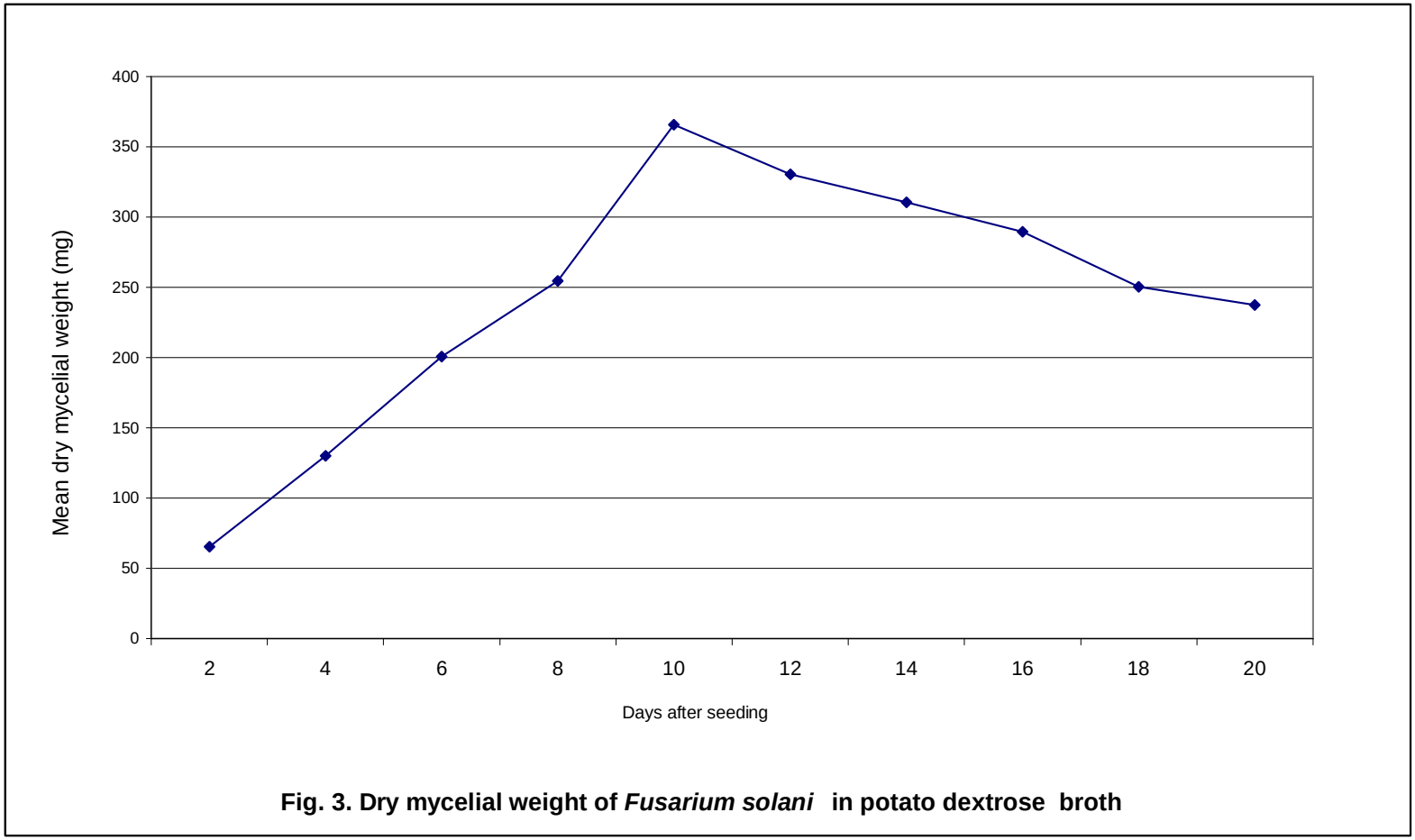


Fig. 3. Dry mycelial weight of *Fusarium solani* in potato dextrose broth

Fig. 3. Dry mycelial weight of Fusarium Solani in Potato dextrose broth

Table 6. Effect of liquid media on mycelial growth of *Fusarium solani*

Media / Broth	**Dry mycelial weight (mg)**
Asthana and Hawker's broth	289.66
Brown's medium	66.33
Carrot broth	288.33
Corn meal broth	311.33
Czepeck's broth	287.66
Host extract broth	259.00
Malt extract broth	66.66
Oat meal broth	315.66
Potato dextrose broth	352.33
Richards's broth	362.33
Sabouraud's dextrose broth	199.00
Tochinai's broth	77.66
V-8 juice broth	191.33
S. Em±	1.40
CD at 1%	5.52

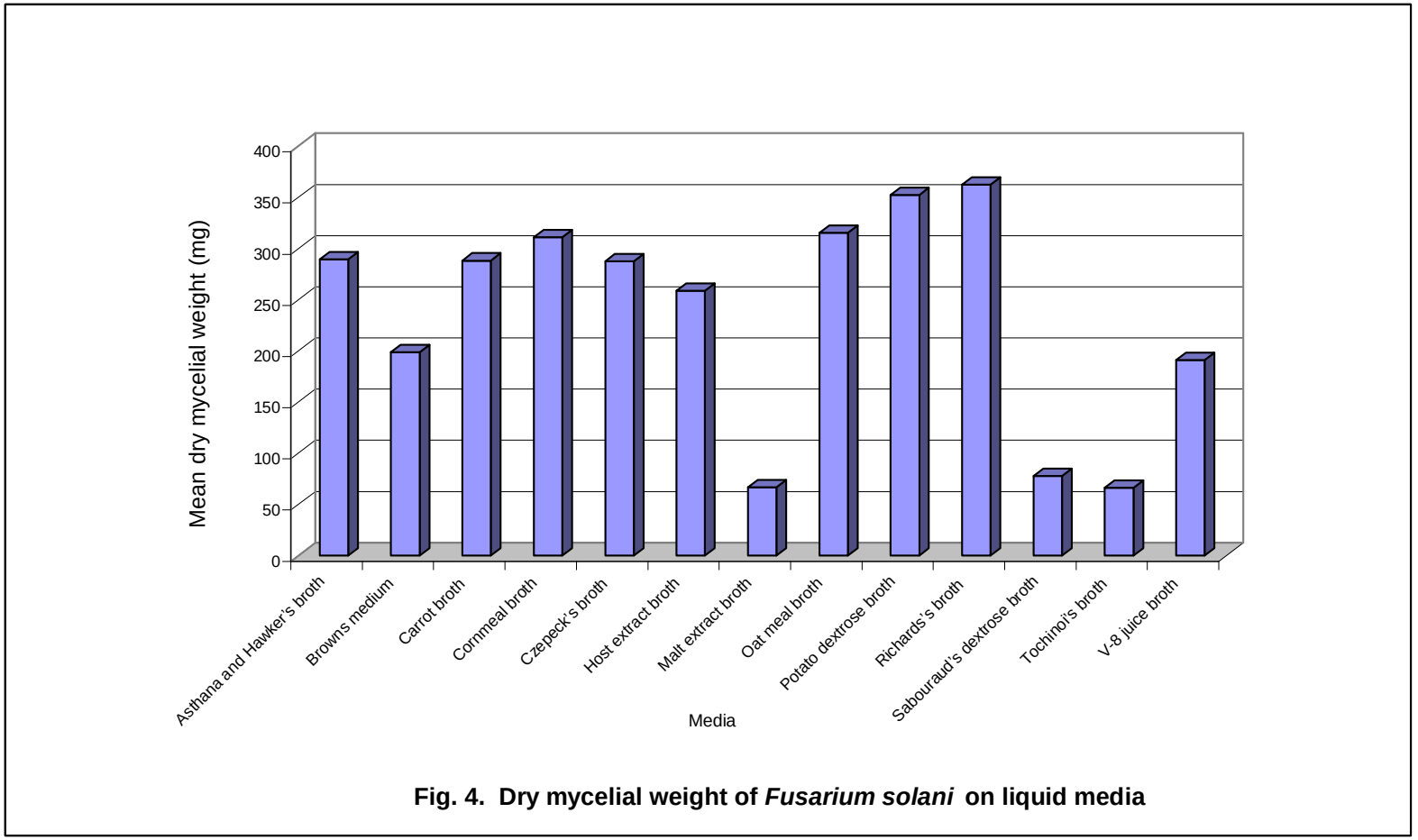


Fig. 4. Dry mycelial weight of *Fusarium solani* on liquid media

Fig. 4. Dry mycelial weight of Fusarium solani on liquid media

Table 7. Germination of conidia of *Fusarium solani* in different solutions

Media	Germination (%)
Distilled water	68.18 (55. 69)*
Tap water	84.24 (66.65)
Lactose (1%)	87.25 (69.12)
Glucose (1%)	90.09 (71.69)
Dextrose (1%)	95.72 (78.12)
Sucrose (1%)	98.07 (82.13)
Mean	87.26 (70.57)
S. Em± CD at 1%	0.38 1.57

* Figures in parenthesis indicate arc sin transformed values

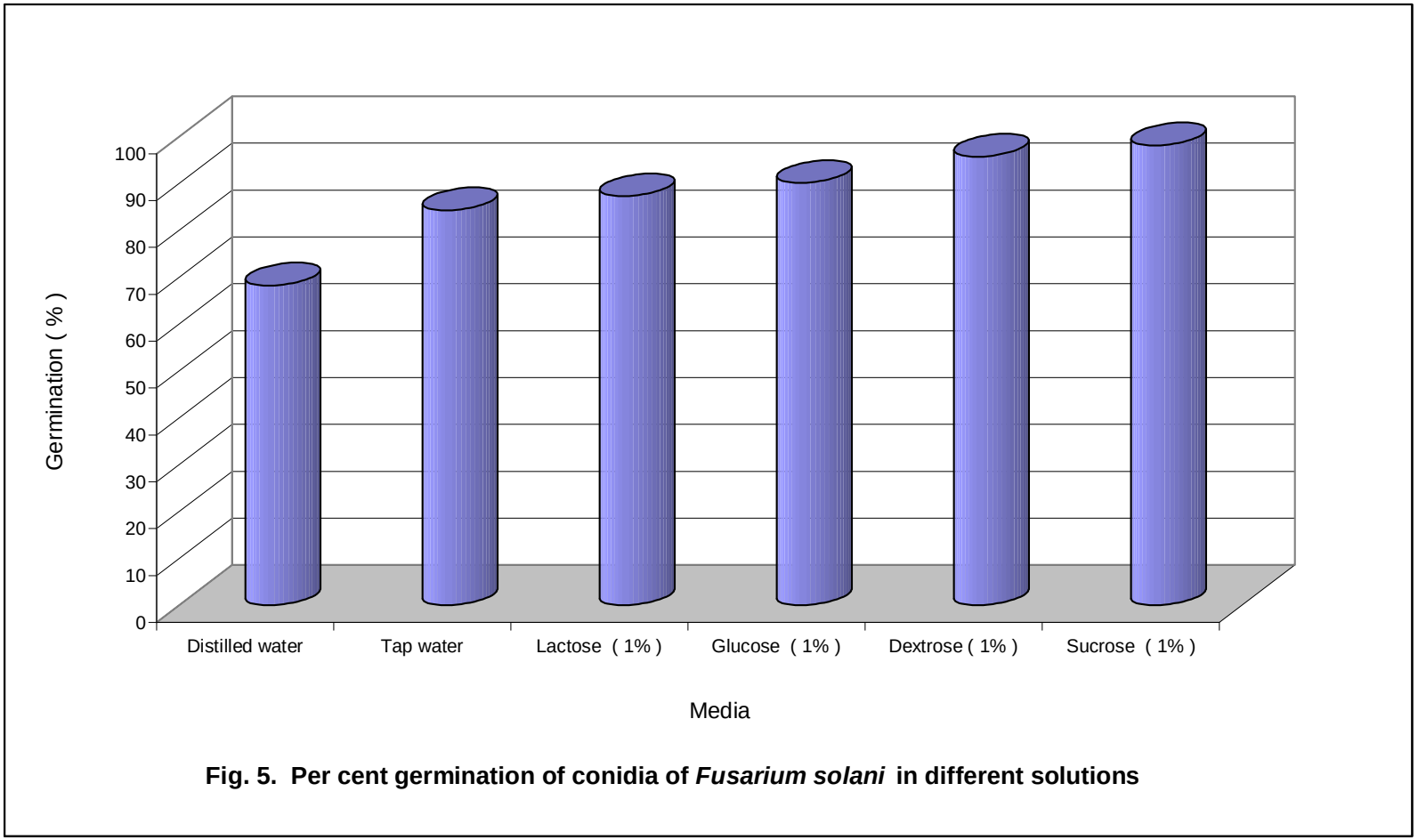
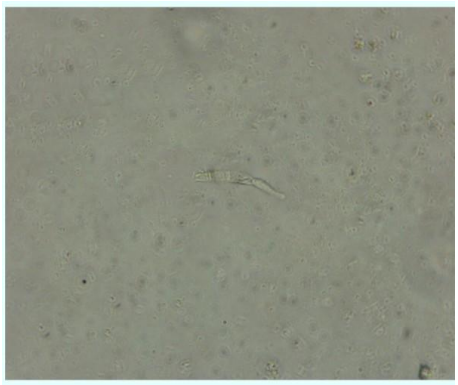
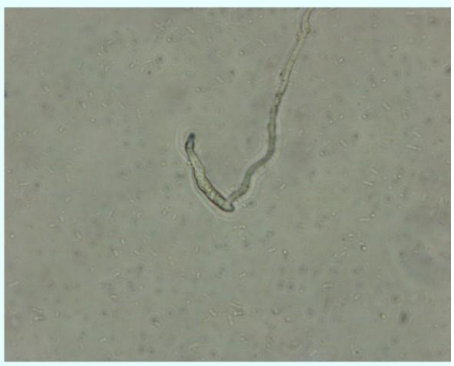


Fig. 5. Per cent germination of conidia of *Fusarium solani* in different solutions

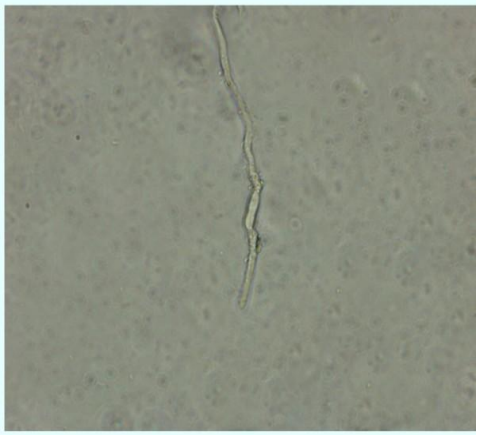
Fig. 5. Per cent germination of conidia of Fusarium solani in different soluations



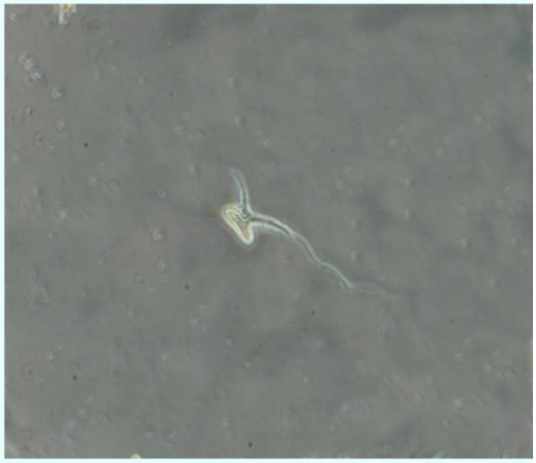
Initial germination of macroconidia



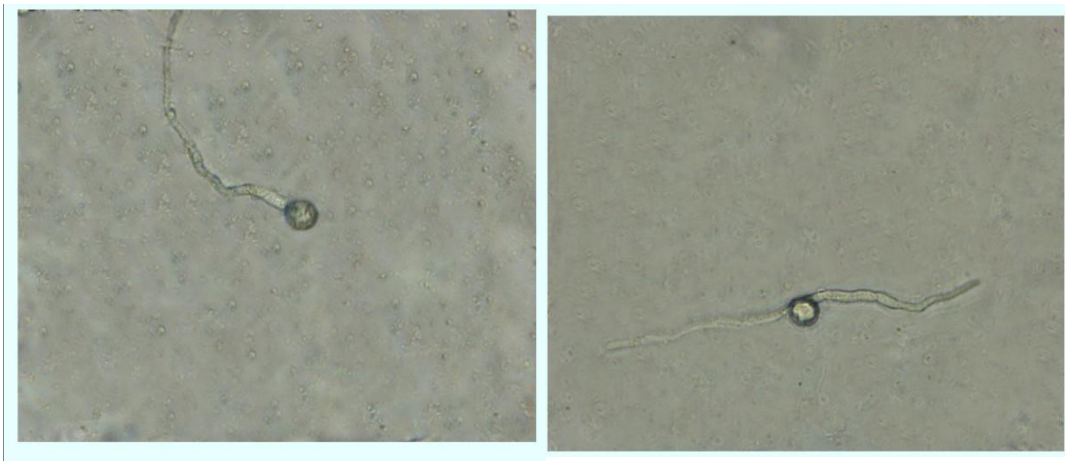
Germination from single polar cell



Germination from both polar cells



Germination from middle cell



Chlamydospores germination

Plate 9. Germination of macroconidia and chlamydospores of Fusarium solani

Table 8. Effect of temperature on mycelial growth of *Fusarium solani*

Temperature (ºC)	Dry mycelial weight (mg)
5	41.66
10	62.14
15	254.66
20	281.33
25	351.66
30	362.33
35	303.33
40	113.33
S. Em±	1.49
CD at 1%	6.23

Table 9 .Effect of pH on mycelial growth of *Fusarium solani*

pH	Dry mycelial weight (mg)
4.0	52.44
4.5	96.81
5.0	158.33
5.5	215.70
6.0	325.62
6.5	364.77
7.0	239.59
7.5	142.92
8.0	89.92
S. Em±	0.42
CD at 1%	1.73

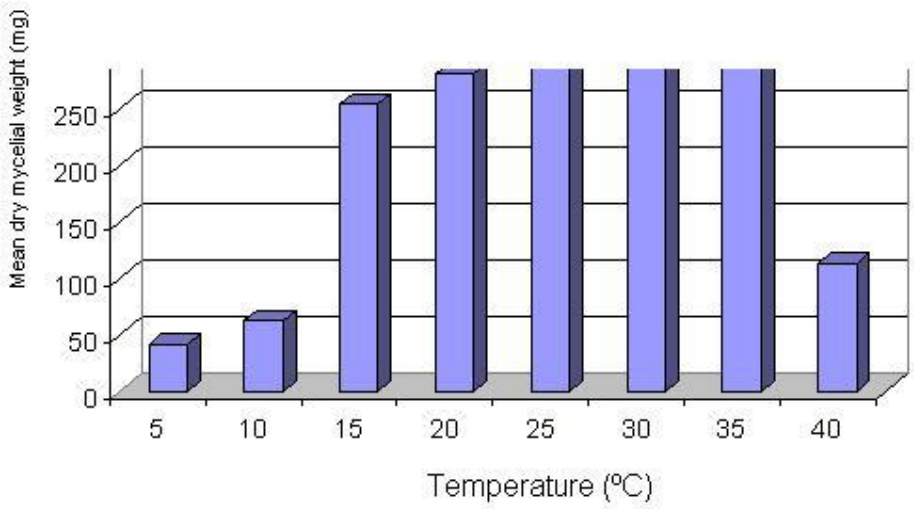


Fig.6. Dry mycelial weight of Fusarium solani at different temperature levels

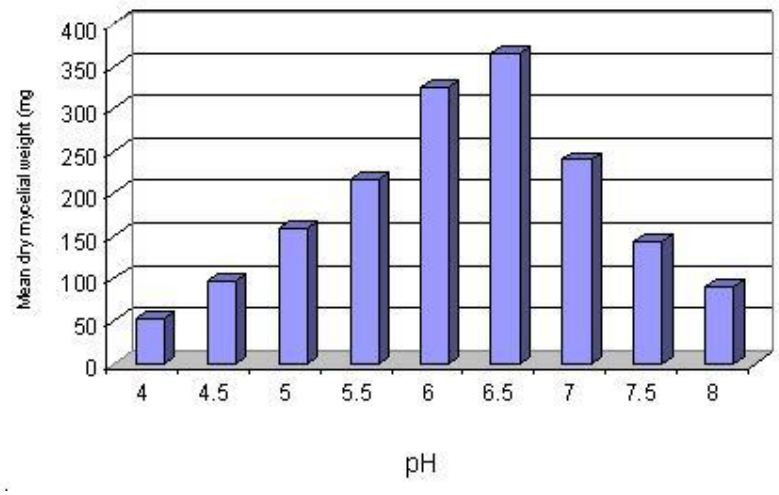


Fig. 7. Dry mycelial weight of Fusarium solani at different pH levels

The effect of different temperature levels on the growth of the fungus was significant. The maximum dry mycelial weight of fungus was observed at a temperature of 30^0C (362.33 mg) which was significantly superior to all other temperature levels tested. This was followed by 25^0c (351.66 mg), 35^0C (303.33 mg) and 20^0C (281.33 mg) which were in decreasing order and differed significantly. However, growth was poor at 5^0C (41.66 mg).

4.6.2 Effect of hydrogen ion concentration

The experiment was carried out to know the effect of pH on the growth of *F. solani*. The growth of the fungus was studied in various pH levels as detailed in "Material and Methods" and the results are presented in Table 9 and Fig 7.

The fungus growth increased with the increase in pH from 4.0 to 6.5 and then onwards there was a decline in the growth. The maximum dry mycelial weight of the fungus was noticed at pH level of 6.5 (364.77 mg) which was significantly superior to rest of the pH levels tested. This was followed by the pH 6.0 (325.62 mg) and 7.0 (239.59 mg). The least growth was observed at pH 4.0 (52.44 mg).

4.7 NUTRITIONAL STUDIES

4.7.1 Carbon utilization

The experiment was carried out to study the utilization of eight carbon sources by the fungus as described in "Material and Methods". The data are presented in Table 10 and Fig 8.

The effect of different carbon sources on the mycelial growth was significant. Maximum dry mycelial weight of 363.93 mg was obtained when sucrose was used as a carbon source. This was significantly superior to the rest of the sources tested and was followed by fructose (341.30 mg), dextrose (270.26 mg), lactose (266.73 mg), and glucose (250.71 mg), which were on par with each other. The least dry mycelial weight of the fungus was observed in starch (117.96 mg) and maltose (174.00 mg).

4.7.2 Nitrogen utilization

In the present investigation, the utilization of eight nitrogen sources by the fungus was tested as detailed in the 'Material and Methods'. The results of the experiment are presented in Table 11 and Fig 9.

The effect of different nitrogen sources on the growth of the fungus was significant. Maximum growth of 364.33 mg was recorded when potassium nitrate was used as a source of nitrogen. Growth supported by sodium nitrate (346.33 mg), L-asparagine (279.33 mg), ammonium nitrate (247.66 mg), urea (209.33 mg) and ammonium sulphate (200.66 mg) were significantly different amongst each other. Minimum growth was observed in ammonium orthophosphate (74.66 mg) and ammonium chloride (81.33 mg).

4.7.3 Effect of light

4.7.3.1 On dry mycelial weight

The exposure of the fungus to alternate cycles of 12 h light and 12 h darkness for ten days resulted in the maximum (363.30 mg) which was significantly superior to other two treatments tested. The dry mycelial weight of fungus exposed to continuous light resulted in moderate growth (319.72 mg) and continuous darkness resulted in minimum growth (134.07 mg) Table 12 and Plate 10b .

4.7.3.2 On radial growth

The exposure of the fungus to alternate cycles of 12 hrs light and 12 hrs darkness for ten days resulted in maximum radial growth of *F. solani* i.e., 89.41 mm. The sporulation was also good, which was significantly superior to other two treatments tested. The radial growth of fungus exposed to continuous light resulted in 81.07 mm and sporulation was moderate. The continuous darkness resulted in 67.38 mm and sporulation was poor Table 12 and Plate 10a.

Table 10. Effect of carbon sources on mycelial growth of *Fusarium solani*

Carbon sources	Dry mycelial weight (mg)
Dextrose	270.26
Fructose	341.30
Glucose	250.71
Lactose	266.73
Maltose	174.00
Mannitol	144.50
Starch	117.96
Sucrose	363.93
S. Em±	0.70
CD at 1%	2.93

Table 11. Effect of nitrogen sources on mycelial growth of *Fusarium solani*

Nitrogen sources	Dry mycelial weight (mg)
Ammonium chloride	81.33
Ammonium nitrate	247.66
Ammonium orthophosphate	74.66
Ammonium sulphate	200.66
Aspargine	279.33
Potassium nitrate	364.33
Sodium nitrate	346.33
Urea	209.33
S. Em±	1.82
CD at 1%	7.62

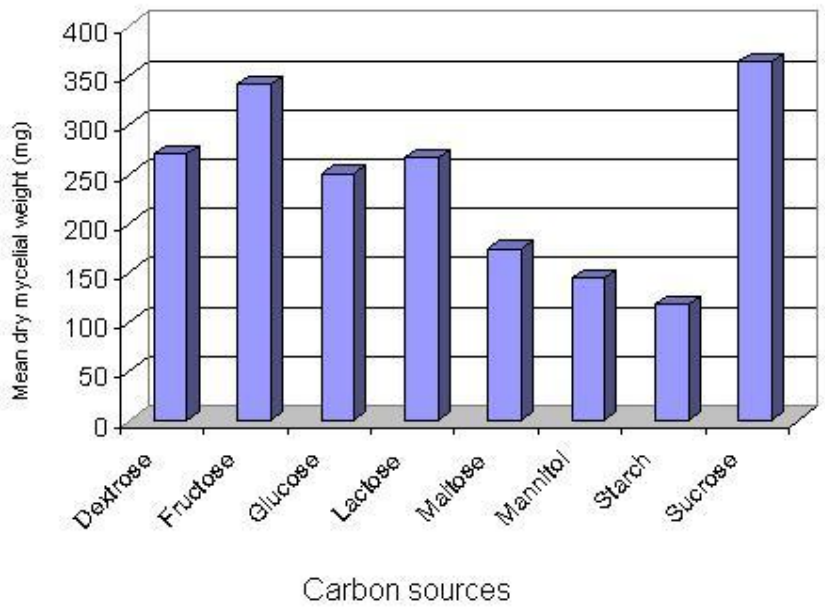


Fig. 8. Dry mycelial weight of Fusarium solani on different carbon sources

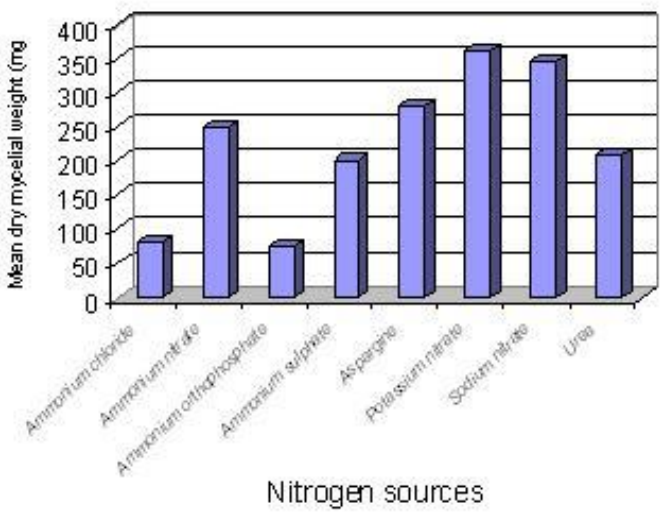


Fig. 9. Dry mycelial weight of Fusarium solani on different nitrogen sournces

Table 12. Effect of different light intensities on growth and sporulation of *Fusarium solani*

Treatment	Mean dry mycelial weight (mg)	Mean colony diameter (mm)	Sporuation
Continuous light	319.72	81.07	++
Continuous dark	134.07	67.38	+
Alternate cycle of 12 hour light and 12 hour darkness	363.30	89.41	+++
S. Em±	0.38	0.36	
CD at 1%	1.64	1.55	

+++ : Good sporulation >50 conidia/microscopic field

++ : Moderate sporulation 30-50 conidia/microscopic field

+ : Scanty sporulation 10-30 conidia/microscopic field

4.8 HOST RANGE of *Fusarium solani*

Twelve different hosts were artificially inoculated with gaint culture of *F. solani* in earthen pots under glass house condition as described in 'Material and Methods'. The results are furnished in Table 13 and illustrated in Plate 11.

Nine plants *viz*., *Ocimum canum, Ocimum sanctum ,O.gratissimum, , Mentha spicata*, *Cymbopogon flexuosus, Cymbopogon winterianus, Rosmarinus officinalis, Pandanus amaryllifolius, Bacopa mannieri* were infected by *Fusarium solani* isolated from Patchouli. The symptoms of the disease observed were similar to that of patchouli. However, there were some differences in the symptoms expression among the hosts. *Coleus ciamboini, C. forskohlii,* and *Stevia rebaudiana* did not show any infection by *Fusarium solani.*

Symptoms were first expressed on brahmi and mint in just four and five days respectively. The plants dried and wilted completely. In *Ocimum canum and Ocimum sanctum,* dark brown discoloration of roots, drying and withering of leaves was recorded eight days after inoculation. While, *O. gratissimum* showed discoloration of roots, drooping and drying of leaves ten days after inoculation. On *Pandanus amaryllifolius* water soaked lesions at the collar region, yellowing was observed seven days after inoculation. *Cymbopogon winterianus* and *Cymbopogon flexuosus,* showed similar type of symptoms of yellowing and drying of leaves six days after inoculation. These plants completely lost their vigour. In *Rosmarinus officinalis,* yellowing followed by blackening and narrowing of leaves was observed fifteen days after inoculation.

4.9 VARIABILITY IN *Fusarium solani*

4.9.1 Cultural and morphological

Studies on morphological and cultural characters of eight isolates were carried out on potato dextrose agar. The cultural characters were taken into account for assessing the existence of variations in isolates. The observations recorded were colony diameter, colony character, sporulation, spore size of *F. solani* and are presented in Table 14 and Plate 12.

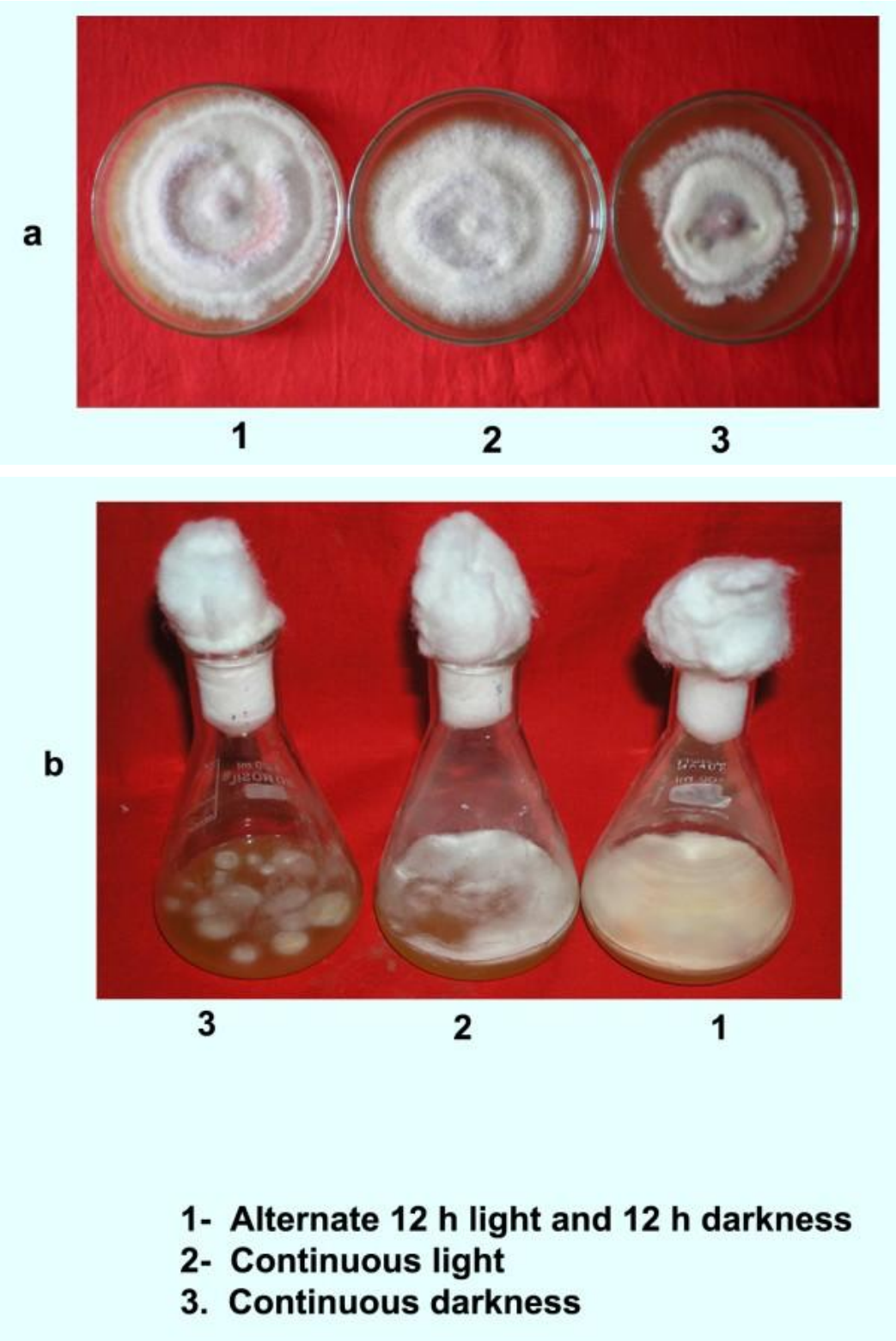


Plate. 10. Effect of light intensity on growth of Fusarium solani

Table 13. Host Range of *Fusarium solani*

Common name	Botanical name	Days taken for symptom expression	Symptoms
Hoary Basil	*Ocimum canum*	8	Yellowing, dryimg of leaves, dark brown discoloration of roots
Sacred Basil	*Ocimum sanctum*	8	Yellowing, dryimg, withering and dark brown discoloration of roots
Clocimum	*Ocimum gratissimum*	10	Drooping and drying of leaves, discoloration of roots
Pandanus	*Pandanus amaryllifolius*	7	Water soaked lesions at the collar region, yellowing and browning of leaves
Lemon grass	*Cymbopogon flexuosus*	6	Yellowing and drying of leaves, loss of vigour
Citronella	*Cymbopogon winterianus*	6	Yellowing and drying of leaves, loss of vigour
Brahmi	*Bacopa mannieri*	4	Drying of leaves, loss of vigour
Mint	*Mentha spicata*	5	Drying of leaves, loss of vigour
Rosemerry	*Rosmarinus officinalis*	15	Yellowing, blackening and narrowing of leaves
Coleus	*Coleus forskohlii*	-	No infection
Indian borej	*Coleus ciamboini*	-	No infection
Stevia	*Stevia rebaudiana*	-	No infection

1. *Ocimum cannum* (Hoary basil)

2. *Ocimum gratissimum* (Clocimum)

3. *Ocimum sanctum* (Sacred basil)

4. *Stevia rebaudiana* (Stevia)

5. *Cymbopogon flexuosus* (Lemon grass)

6. *Cymbopogon winterianus* (Citronella)

7. *Mentha spicata* (Mentha)

8. *Bacopa mannieri* (Brahmi)

9. *Pandanus amaryllifolius* (Pandanus)

10. *Coleus ciamboini* (Indian borej)

11. *Coleus forskohlii* (Coleus)

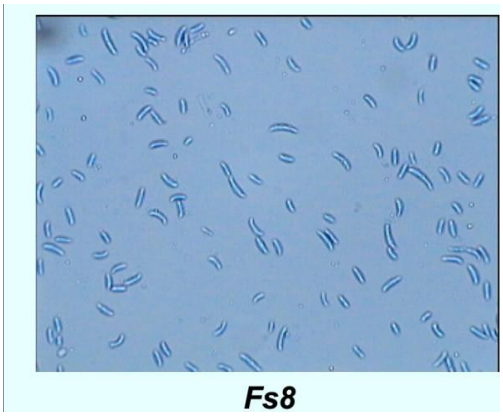
12. *Rosmarinus officinalis* (Rosemerry)

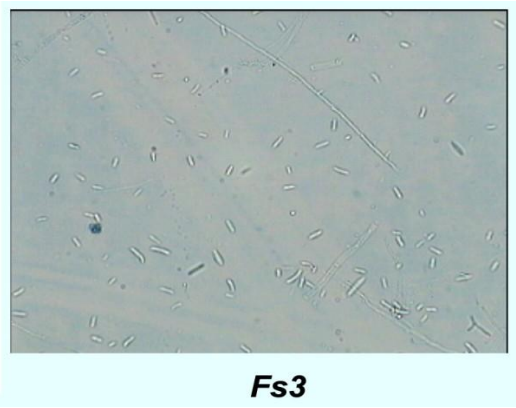
Plate 11. Host range of Fusarium solani

Table 14. Cultural and morphological variability in isolates of *Fusarium solani* on potato dextrose agar

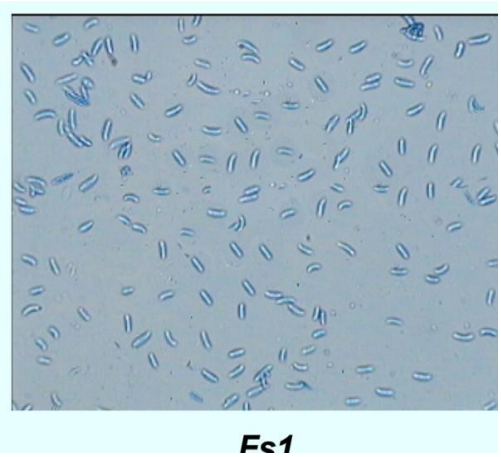
Isolates	Colony diameter (mm)	Growth characters	Sporulation	Size (µm)		
				Macroconidia	Microconidia	Chlamydospores
Fs1	90.00	White colour cottony growth with smooth margin	++++	27.3-33.5x6.9-8.8	13.0-18.1x7.8x8.4	14
Fs2	88.20	Pink coloured pluffy colony	++++	21.5-26.3x7.0-9.1	14.2-15.7x7.1-7.9	22
Fs3	89.00	Light pink cottony colony	++	21.2-31.8x6.7-8.5	11.4-13.8x5.0-6.5	18
Fs4	87.75	Light pink coloured colony with yellowish pigmentation	++++	21.9-25.76x8.5x9.7	10.2-11.8x6.3-7.1	20
Fs5	85.60	Yellowish pluffy colony	+++	24.4-31.7x7.6-8.9	11.4-13.4x4.5x6.4	16
Fs6	89.75	Light pink cottony growth with irregular margin	+	23.5-26.9x6.0-9.0	11.4-14.6x5.4-6.9	18
Fs7	84.00	Pink coloured pluffy growth with irregular margin	++	26.9-32.8x6.7-9.0	13.0-14.7x5.0x7.0	20
Fs8	89.25	Light pink coloured colony with orange pigmentation	+++	30.4-32.9x5.4-9.4	16.6-17.2x5.8x7.5	24

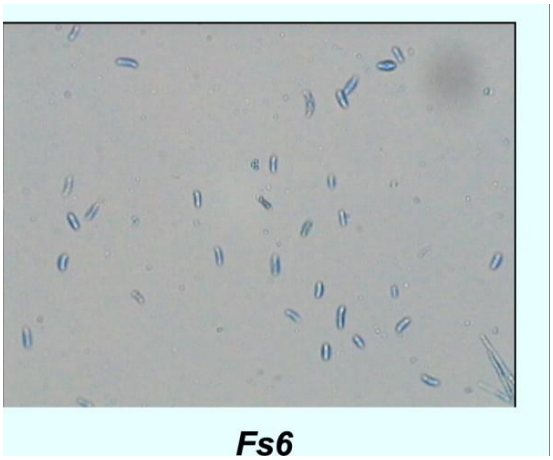
++++ = Abundant sporulation >80 number of conidia / microscopic field ++ =Moderate sporulation 25-50 number of conidia / microscopic field
+++ = Good sporulation 50-80 number of conidia / microscopic field + =Sparse sporulation <25 number of conidia / microscopic field





Size of Conidia





Number of conidia

Plate. 12. Cultural variability in Fusarium solani on potato dextrose agar

Cultural variation was noticed among eight isolates of *F. solani* on potato dextrose agar. Colony diameter ranged from 84.00 mm (*Fs7*) to 90 mm (*Fs1*). The maximum colony diameter (90mm) was observed in isolates *Fs1 and Fs6* followed by isolates *Fs8* (89.25mm) and *Fs3* (89mm) whereas minimum colony diameter was observed in isolate *Fs7* (84.00mm). All the isolates showed excellent mycelial growth. Isolates like *Fs2, Fs3 Fs6,* Fs7 showed pink coloured colony growth. *Fs4 and Fs8* showed light pink coloured colony with yellowish and orange pigmentation respectively at the border and *Fs5* showed grayish yellow pluffy growth and *Fs1* showed white coloured cottony growth.

Sporulation was abundant in isolates *Fs1, Fs2* and *Fs4,* good in *Fs5* and *Fs8*, moderate in *Fs3 and Fs7.* Whereas, it was sparse in isolate *Fs6.* The size of macroconidia was maximum in isolate *Fs8* (30.4 - 32.9 x 5.4 -9.4 µm) whereas, it was minimum in isolate *Fs3* (21.2 – 31.8 x 6.7 – 8.5µm). Microconidial size was maximum in isolate *Fs8* (16.6 – 17.2 x 5.8 – 7.9µm). and was minimum in isolate *Fs4* (10.8 – 11.8 x 6.3 – 7.1µm). Size of chlamydospores ranged from 14µm (*Fs1*) to 24µm (*Fs8*).

4.9.2 Genetic variability

It is difficult to distinguish these isolates using traditional morphological differences. The suitability of random amplified polymorphic DNA (RAPD) was used to detect the variations among the isolates of *F. solani.* OPA and OPB series primers were used to determine genetic distance between isolates and to construct a dendrogram. Banding profile of different primers for eight isolates of *F. solani* is given in Table 15 and Plate 13.

Of the 20 primers used for amplification OPA3, OPA4, OPA7, OPA10, OPA11, OPA15, OPB4 showed 100 per cent polymorphism. A total of 175 amplicon levels resulted from 20 primers and were available for analysis. Information on banding pattern for all the primers was used to determine genetic distance between isolates and to construct a dendrogram.

Based on simple matching coefficient a genetic similarity matrix was constructed to assess the genetic relatedness among the isolates of *F. solani.* Genetic similarity coefficient of eight isolates of *F. solani* based on RAPD analysis is given in the Table 16 . Similarity coefficient ranged from 0.63 to 0.92. The maximum genetic similarity of 92 was between *Fs*2 and *Fs1*. Whereas, least genetic similarity was observed between *Fs6* and *Fs2* isolates.

Further, the dendrogram constructed from the pooled data clearly showed two major clusters namely A and B at similarity coefficient of 0.71 (Fig 10). Cluster A was classified upto sub sub cluster A6. Cluster B was classified upto sub sub cluster B4. Cluster A was sub grouped into A1 and A2. A1 was comprised only one isolate *i.e*. *Fs6* (Shimoga isolate). A2 was sub grouped into A3 and A4. A3 comprised only one isolate *i.e*., *Fs7* (Uttar Kannada isolate). Sub cluster A4 comprised A5 and A6. A5 comprising of one isolate *viz.*, *Fs8* (Adur isolate). A6 comprised *Fs4* (Adavisomapur isolate).

Sub cluster B1, comprised only one isolate *viz*., *Fs5* (Saidapur isolate). B2 was sub grouped into B3 and B4. B3 comprised *Fs3* (Mangenkoppa isolate) and B4 comprised *Fs1*and *Fs2* (Arabhavi and Belgaum isolates, respectively).

4.10 MANAGEMENT STUDIES

4.10.1 *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. solani*. This was carried out by adopting the poisoned food technique as described in ''Material and Methods''.

Results relating to the effects of plant extracts on per cent inhibition of *F. solani* are presented in Table 17, Fig 11 and Plate 14.

Eleven botanicals were evaluated against *F. solani.* The results revealed that the effect of plant extracts on the fungal growth was significant. Neem seed kernel extract (NSKE) (68.43%) was found effective in inhibiting mycelial growth. Eucalyptus (56.93%) and Garlic (50.68%) were next best followed by Parthenium (47.81%), Tulsi (39.30%), Clerodendron (39.15%), Pongemia (37.92%), Bougainvillea (35.57) and Durantha (31.28%). Least inhibition was observed with Glyricidia (19.97%) followed by Cassia (26.18%).

Table 15. Banding profile of different primers for different isolates of *Fusarium solani*

Primers	Total bands	Polymorphic bands	Per cent polymorphism
OPA-01	7	5	71.4
OPA-02	7	6	85.7
OPA-03	9	9	100
OPA-04	8	8	100
OPA-05	8	7	87.5
OPA-06	7	6	85.7
OPA-07	8	6	100
OPA-08	9	8	88.8
OPA-09	9	7	77.7
OPA-10	12	12	100
OPA-11	13	13	100
OPA-12	12	12	75.0
OPA-13	11	9	81.8
OPA-14	10	9	90.0
OPA-15	11	11	100
OPA-16	7	6	85.7
OPB-02	7	4	57.1
OPB-03	2	1	50
OPB-04	10	10	100
OPB-05	8	7	87.5

Table 16. Similarity co-efficient of eight isolates of *Fusarium solani*

Isolates	*Fs1*	*Fs2*	*Fs3*	*Fs4*	*Fs5*	*Fs6*	*Fs7*	*Fs8*
Fs1	1.00							
Fs2	0.92	1.00						
Fs3	0.91	0.86	1.00					
Fs4	0.72	0.73	0.70	1.00				
Fs5	0.86	0.82	0.87	0.70	1.00			
Fs6	0.65	0.63	0.64	0.72	0.66	1.00		
Fs7	0.72	0.72	0.72	0.83	0.70	0.74	1.00	
Fs8	0.78	0.78	0.76	0.86	0.74	0.76	0.85	1.00

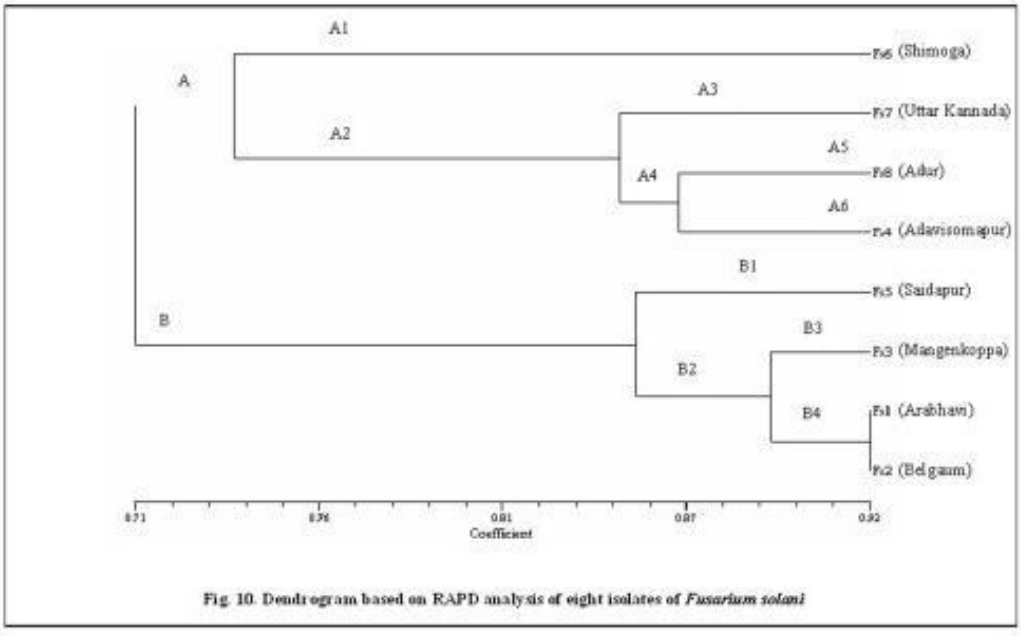
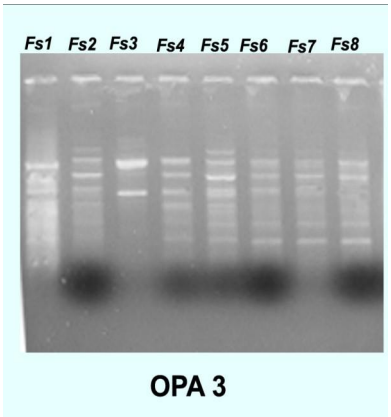
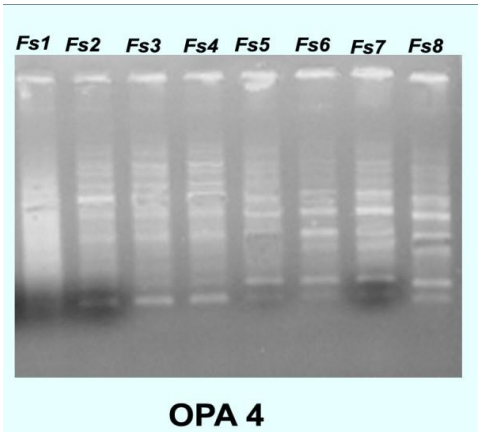
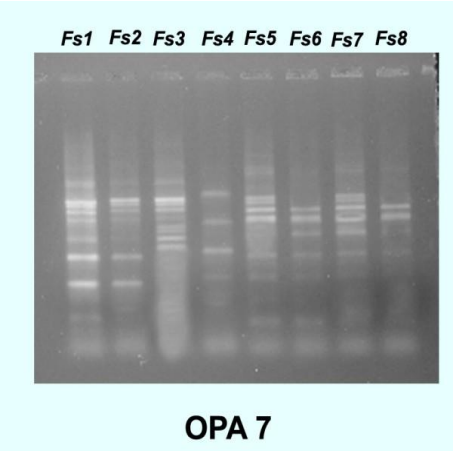
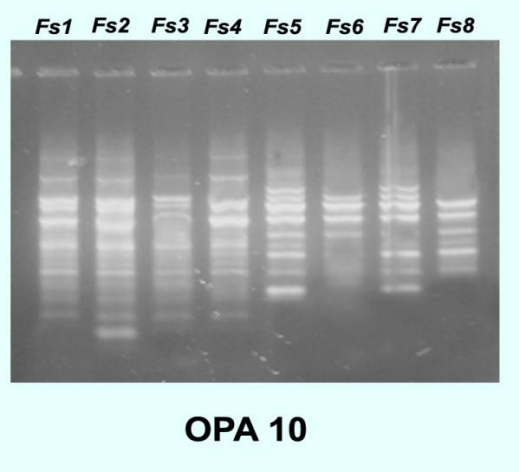


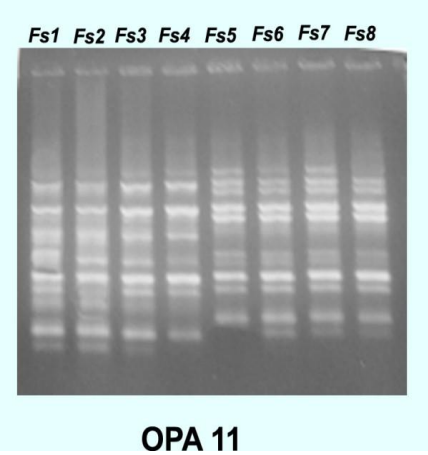
Fig. 10. Dendrogram based on RAPD analysis of eight isolates of Fusarium solani

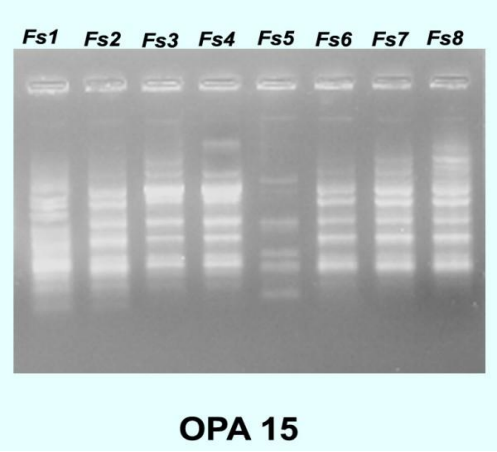












Fs1. arabhavi
Fs2. Belgaum
Fs3. Mangenkoppa
Fs4. Adavisomapur
Fs5. Saidapur
Fs6. Shimoga
Fs7. Uttar Kannada
Fs8. Adur

Plate 13. Genetic variability in eight patchouli isolates of Fusarium solani by RAPD method

Table 17. Effect of plant extracts on inhibition of mycelial growth of *Fusarium solani*

Plant extracts	Per cent inhibition of mycelial growth		
	Concentration (%)		Mean
	5	10	
Bougainvillea	31.96 (34.44)*	39.16 (38.76)	35.57 (36.60)
Cassia	19.16 (25.98)	20.77 (27.12)	19.97 (26.54)
Clerodendron	32.16 (34.56)	46.13 (42.80)	39.15 (38.66)
Durantha	28.46 (35.26)	34.10 (35.74)	31.28 (34.00)
Eucalyptus	56.50 (48.76)	57.36 (49.26)	56.93 (49.02)
Garlic	48.50 (44.17)	52.86 (46.66)	50.68 (45.42)
Glyricidia	20.43 (26.88)	31.93 (34.43)	26.18 (30.65)
Neem seed kernel	62.00 (51.97)	74.86 (59.94)	68.43 (55.95)
Parthenium	43.00 (40.99)	52.73 (46.59)	47.81 (43.79)
Pongamia	30.40 (33.47)	45.43 (42.40)	37.92 (37.93)
Tulsi	35.06 (36.33)	43.53 (41.30)	39.30 (38.81)
Mean	37.06 (34.21)	45.35 (40.47)	
	Botanicals (B)	Concentration (C)	B x C
S. Em±	0.14	0.05	0.19
CD at 1%	0.53	0.19	0.72

* Figures in parenthesis indicate arc sin transformed values

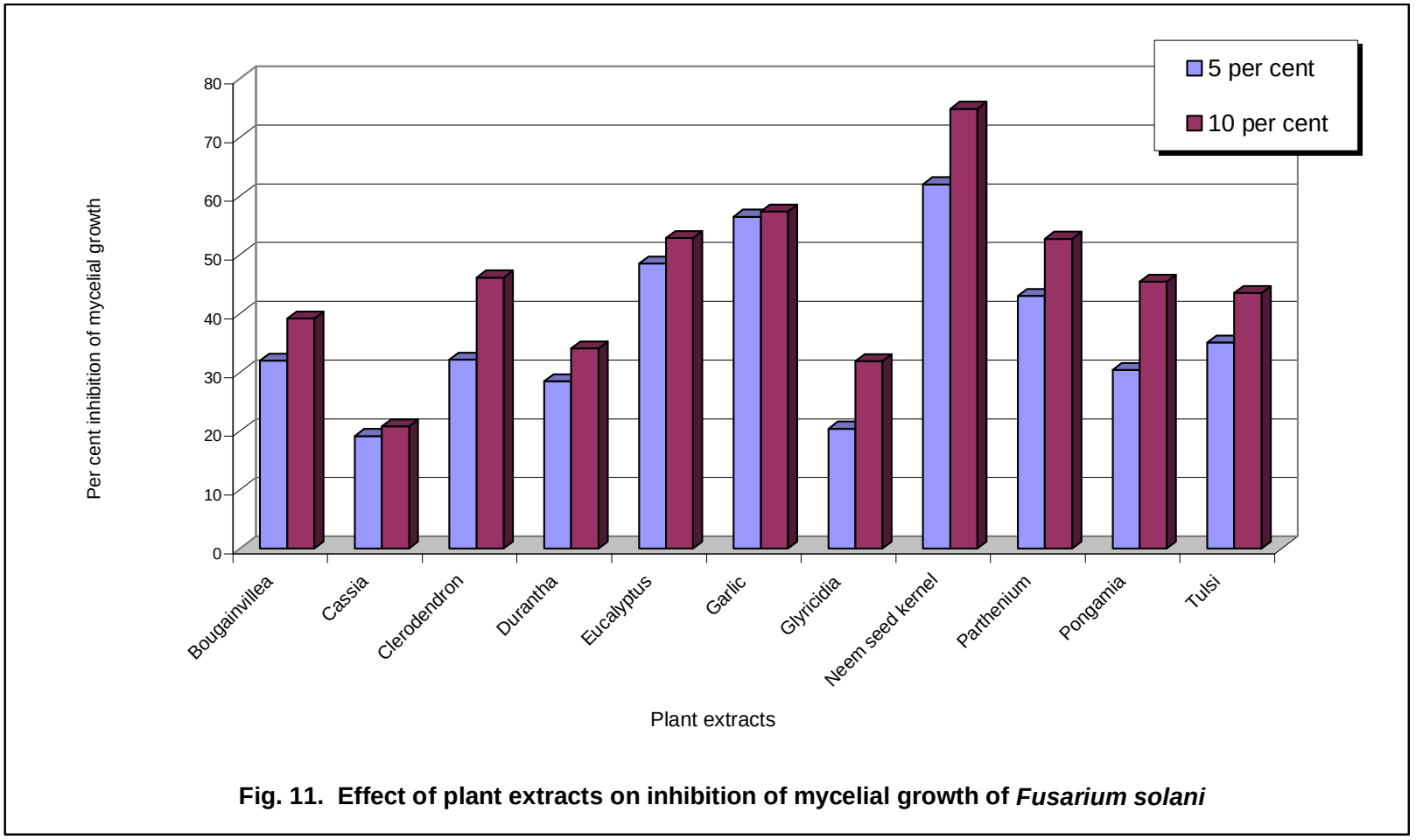


Fig. 11. Effect of plant extracts on inhibition of mycelial growth of *Fusarium solani*

Fig. 11. Effect of plant extracts on inhibition of mycelial growth of Fusarium solani

Table 18. Effect of biocontrol agents on inhibition mycelial growth of *Fusarium solani*

Biocontrol agents	**Per cent inhibition of mycelial growth**
Bacillus subtilis Cohn Emend Pers.	57.02 (49.06)*
Pseudomonas fluorescens Migula	65.10 (53.82)
Trichoderma harzianum Rifai (Dharwad isolate)	78.11 (62.13)
Trichoderma koningii Oudern	75.63 (60.45)
Trichoderma virens Miller	70.16 (56.92)
Trichoderma viride Pers.	74.92 (59.83)
S. Em±	0.28
CD at 1%	1.16

* Figures in parenthesis indicate arc sin transformed values

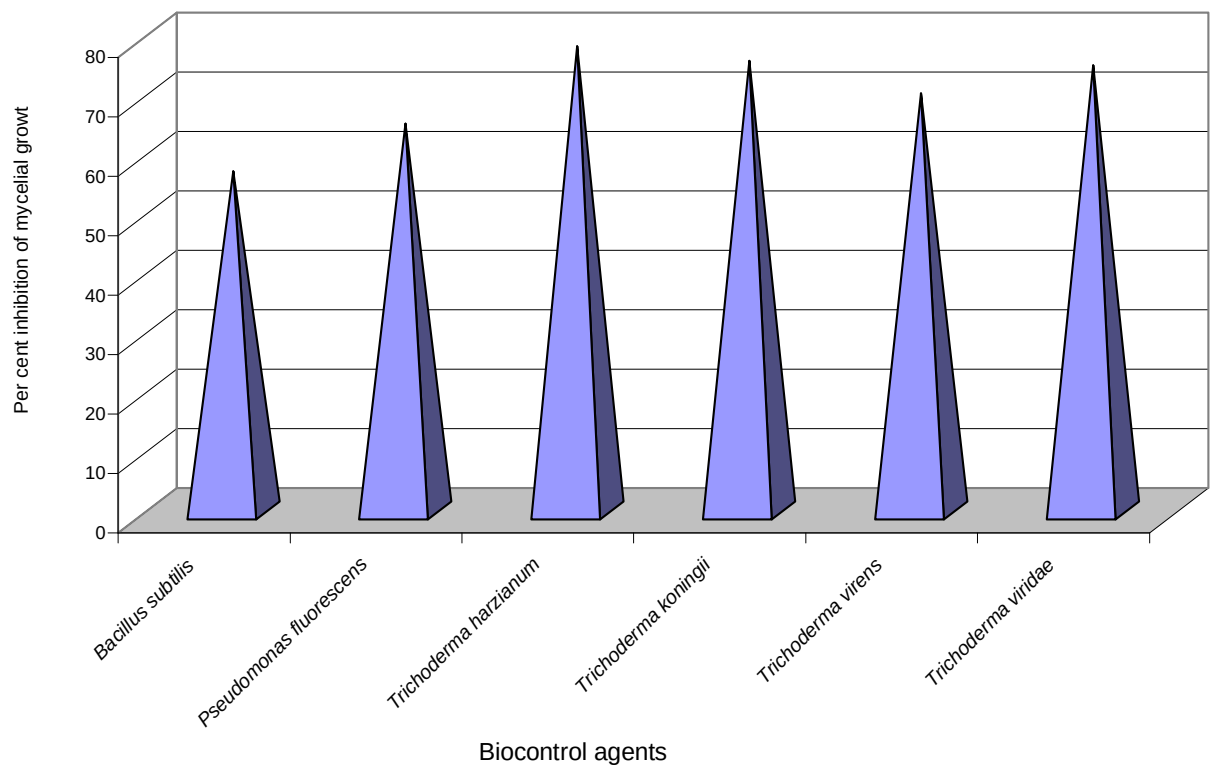


Fig. 12. Effect of bioagents on inhibition of mycelial growth of *Fusarium solani*

Fig. 12. Effect of bioagents on inhibition of mycelial growth of Fusarium solani

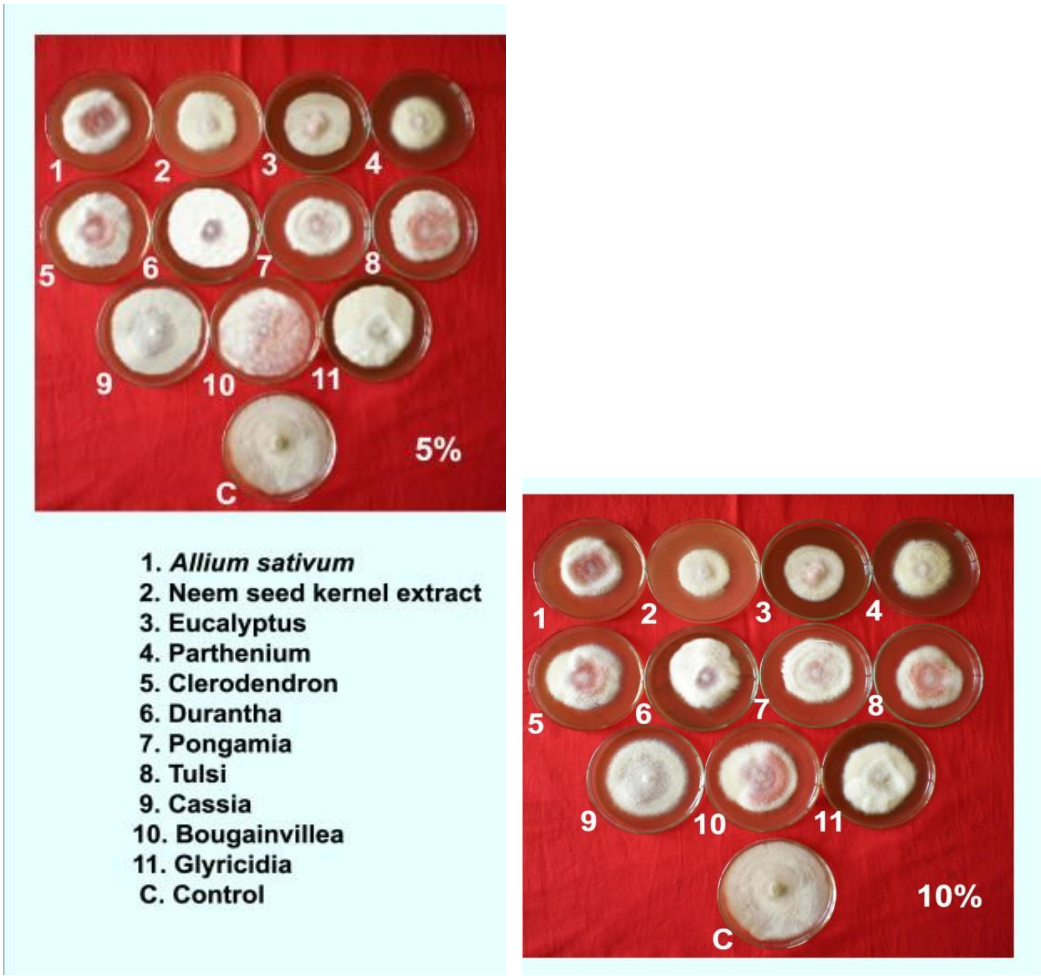


Plate. 14. In vitro evaluation of botanicals against Fusarium solani

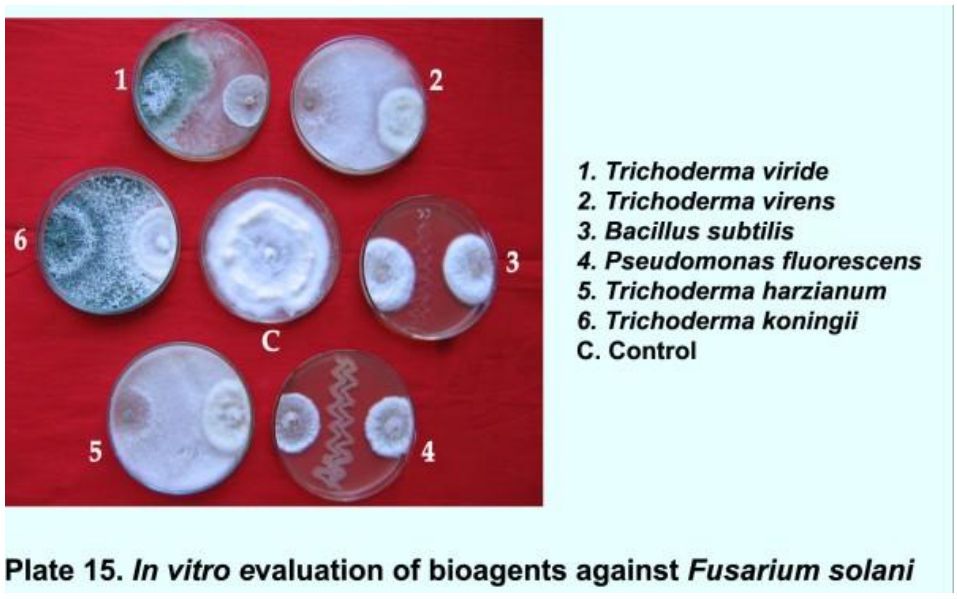


Plate. 15. In vitro evaluation of bioagents against Fusarium solani

The plant extracts at 10 per cent was significantly superior over 5 per cent. NSKE (74.86%) at 10 per cent was the best and significantly superior to all other plant extracts. Next best were Eucalyptus (57.36%) and Garlic (52.86%). Glyricidia at five per cent (19.46%) and Cassia at five per cent (20.43) were least effective in inhibiting the mycelial growth of *F. solani*.

4.10.2 *In vitro* evaluation of biocontrol agents

Six biocontrol agents, *viz*. *Bacillus subtilis, Pseudomonas fluorescens*, *Trichoderma koningii, T. harzianum*, *T. viride*, and *T. virens* were evaluated against *F. solani* and the results are presented in Table 18, Fig 12 and Plate 15.

The results revealed that the antagonists significantly reduced the growth of *F. solani* either by over growing or by exhibiting inhibition zones.

After measuring the colony diameter of *F. solani,* it was noticed that maximum reduction in colony growth was observed in *T. harzianum* (78.11%) which was significantly superior to all other bioagents tested. Next best was T. *Koningii* (75.63%) followed by *T. viride* (74.92%) and *T. virens* (70.16%). Least inhibition was noticed in *B. subtilis* (57.02%) followed by *Pseudomonas fluorescens* (65.10%).

4.10.3 *In vitro* evaluation of fungicides :

Efficacy of six systemic and six non systemic fungicides was tested at three concentrations in the laboratory against *F.solani.* Poisoned food technique was followed as detailed in ''Material and Methods''. The per cent inhibition over control was worked out based on the fungal growth in control plate. The data are presented in the Table 19, Fig 13 and Plate 16a.

The results indicated that there was a significant difference among the systemic fungicides in inhibiting the growth of *F.* solani. Among the six systemic fungicides evaluated, carbendazim (100%) and carbendazim + mancozeb(100%) were superior over other treatments, followed by propiconazole (99.08%) and vitavax (93.39n%). Least inhibition was observed in hexaconazole (67.35%).

Systemic fungicides like carbendazim and carbedazim + mancozeb were successful in completely (100.00%) inhibiting the growth of *F. solani* at all three concentrations (0.025, 0.05 and 0.1%). Benomyl at concentration of 0.1 per cent completely (100.00%) inhibited the growth of *F. solani.* Least inhibition of mycelial growth of pathogen was observed in hexaconazole at 0.1 per cent (88.30%).

Among the different concentrations tested, 0.1 per cent (98.04%) was very effective followed by 0.05 per cent (93.39%). Least inhibition was observed in 0.025 per cent concentration (88.75%).

Significant differences were recorded in the per cent inhibition of mycelial growth of *F. solani* with non systemic fungicides. Mancozeb (99.66%) gave maximum inhibition of mycelial growth, which was on par with the Propineb (98.72%). Least inhibition of mycelial growth was observed in Zineb (56.93%) (Table 20, Fig 14 and Plate 16b).

Among the different non systemic fungicides tested, mancozeb at 0.2 and 0.3 per cent and propineb at 0.3 per cent concentration completely (100%) inhibited the growth of *F. solani.* Least inhibition of mycelial growth was recorded in Zineb at 0.1 per cent (49.33%).

Among the different concentrations tested, 0.3 per cent concentration (81.44%) of non-systemic fungicide was effective and least inhibition of mycelial growth was observed in 0.1 per cent concentration (69.66%).

4.10.4 *In vivo* studies

A pot culture experiment was conducted as explained in the “Material and Methods”. Observations were recorded on per cent disease incidence and plant height. Results are depicted in Table 21, Fig 15 and Plate 17.

Among the various treatments tested, fungicides have given the good results. Disease was completely absent in carbendazim, carbedazim + mancozeb, carboxin + thiram, propiconazole and mancozeb. *Trichoderma harzianum, T. viride,* neem seed kernel extract

Table 19. Effect of systemic fungicides on inhibition of mycelial growth of *Fusarium solani*

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.025	0.05	0.1	
Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Hexaconazole	48.55 (44.19)	65.21 (53.88)	88.30 (70.02)	67.35 (56.03)
Propiconazole	97.24 (80.53)	100.00 (90.00)	100.00 (90.00)	99.08 (86.87)
Carbendazim 12% + mancozeb 3%	100.00 (90.04)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Benomyl	88.52 (70.23)	95.08 (77.27)	100.00 (90.00)	94.53 (79.18)
Carboxin 37.5 + Thiram 37.5%	88.75 (76.23)	93.39 (81.89)	98.04 (86.71)	93.39 (81.89)
Mean	88.75 (76.23)	93.39 (81.89)	98.04 86.71	
	Fungicides (F)	Concentration (C)		F x C
S. Em±	0.17	0.12		0.30
CD at 1%	0.70	0.49		1.24

* Figures in parenthesis indicate arc sin transformed values

Table 20. Effect of non-systemic fungicides on inhibition of mycelial growth of *Fusarium solani*

Fungicides	**Per cent inhibition of mycelial growth**			
	Concentration (%)			**Mean**
	0.1	**0.2**	**0.3**	
Captan	66.96 (54.94)*	76.21 (60.84)	78.07 (62.11)	73.75 (59.29)
Copper oxy-chloride	49.50 (44.73)	55.17 (47.99)	67.28 (55.13)	57.31 (49.28)
Thiram	55.62 (48.25)	64.33 (53.35)	77.24 (61.54)	65.73 (54.38)
Zineb	49.33 (44.64)	55.36 (48.10)	66.10 (54.42)	56.93 (49.05)
Mancozeb	99.00 (85.42)	100.00 (90.00)	100.00 (90.00)	99.66 (88.05)
Propineb	97.59 (81.14)	98.59 (83.29)	100.00 (90.00)	98.72 (84.83)
Mean	69.66 (59.85)	74.94 (63.85)	81.44 (68.88)	
	Fungicides (F)	Concentration (C)		F x C
S. Em±	0.35	0.25		0.60
CD at 1%	1.35	0.96		2.30

* Figures in parenthesis indicate arc sin transformed values

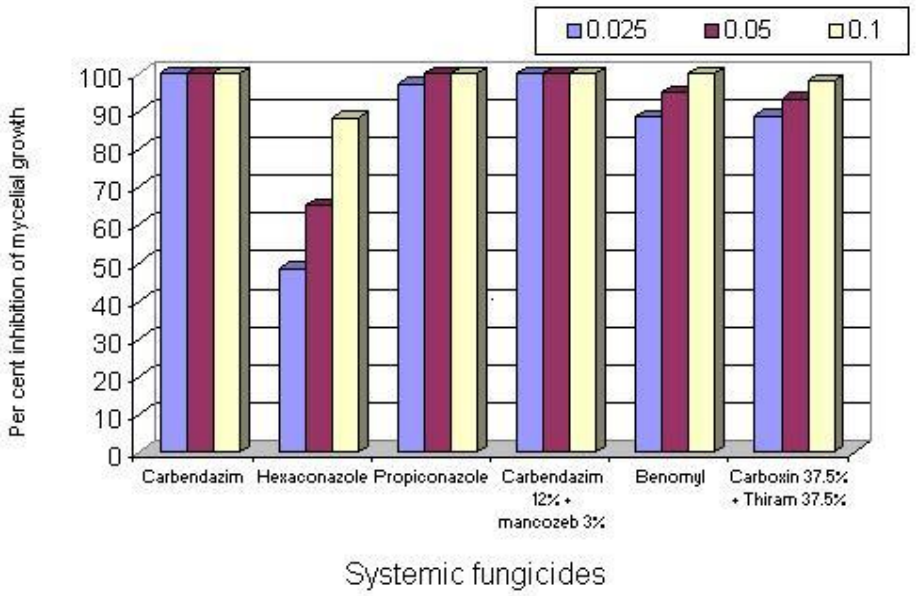


Fig. 13. Effect of systemic fungicides on inhibition of mycelial growth of *Fusarium solani*

Fig. 13. Effect of systemic fungicides on inhibition of mycelial growth of Fusarium solani

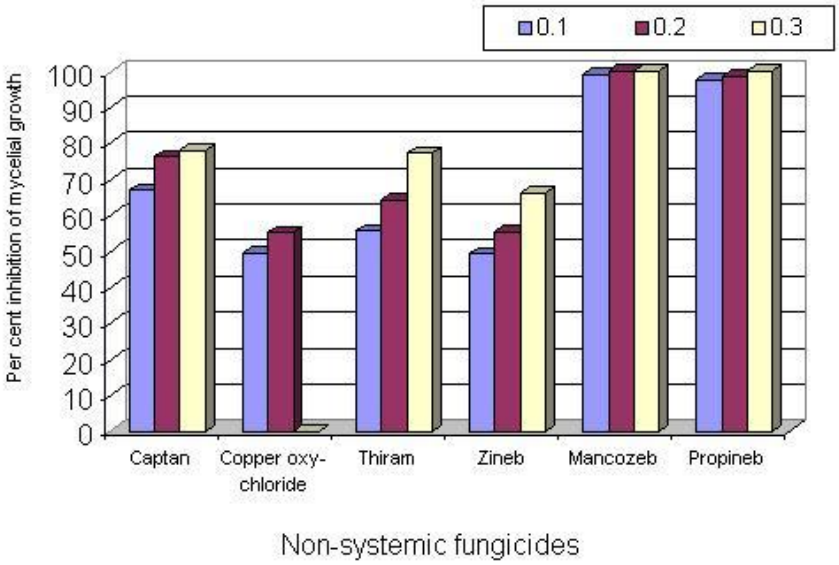


Fig. 14. Effect of non-systemic fungicides on inhibition of mycelial growth of *Fusarium solani*

Fig. 14. Effect of non-systemic fungicides on inhibition of mycelial growth of Fusarium solani

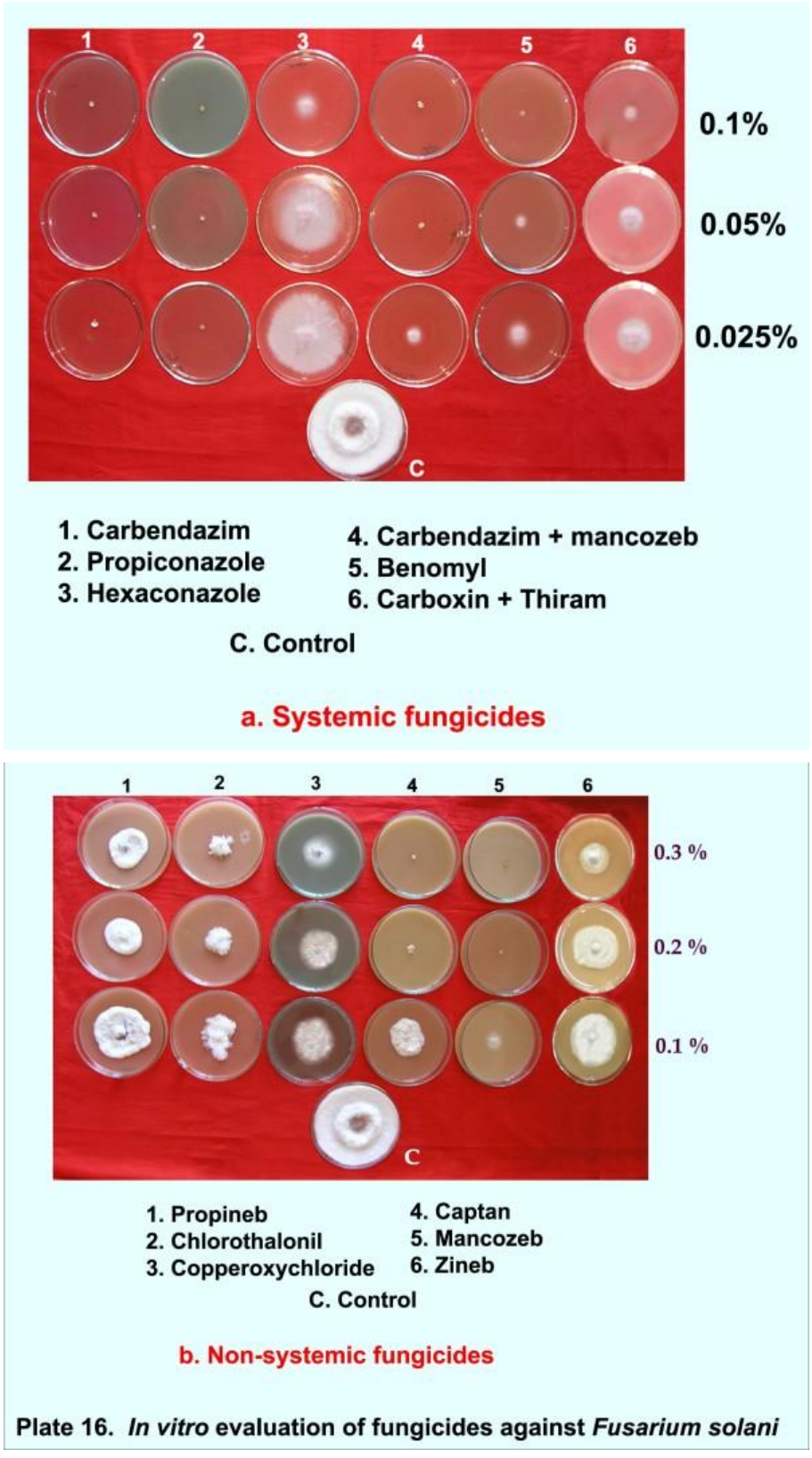


Plate 16. In vitro evaluation of fungicides against Fusarium solani

Table 21. Effect of fungicides, botanicals and biocontrol agents on wilt incidence and plant height of Patchouli

Treatments	Per cent disease incidence	Plant height (cm) (90 DAP)
Carbendazim 12% + Mancozeb 63% WP @ 0.1%	0.00 (1.00)*	86.3
Carbendazim @ 0.1%	0.00 (1.00)	93.6
Carboxin 37.5% + Thiram 37.5% @ 0.1%	0.00 (1.00)	81.6
Propiconazole @ 0.1%	0.00 (1.00)	87.0
Mancozeb @ 0.2%	0.00 (1.00)	84.0
Trichoderma harzianum @ 0.6%	11.11 (3.48)	80.6
Trichoderma viride @ 0.6%	33.33 (5.86)	74.0
Neem seed kernel extract @ 10%	22.22 (4.23)	81.0
Allium sativum @ 10%	33.33 (5.86)	78.3
Control	100.00 (10.05)	20.3
S. Em±	1.9	0.88
CD at 1%	4.6	3.57

*Figures in the parenthesis are $\sqrt{x+1}$ transformed values.
DAP – Days After Planting.

and *Allium sativum* were less effective compared to the fungicides. However, they were significantly superior over the control. The highest disease incidence was noticed in untreated control (100%).

Plant height was significantly superior in all treatments as compared to untreated control.

The plant height (90 DAP) was maximum in Carbendazim (93.66cm) which was significantly superior to all other treatments. This was followed by propiconazole (87.00cm) which was on par with carbendazim + mancozeb (86.33 cm), mancozeb (84.0 cm). In *Trichoderma viride* and *Allium sativum* plant height of (78.3cm) and (74.0cm) was recorded.

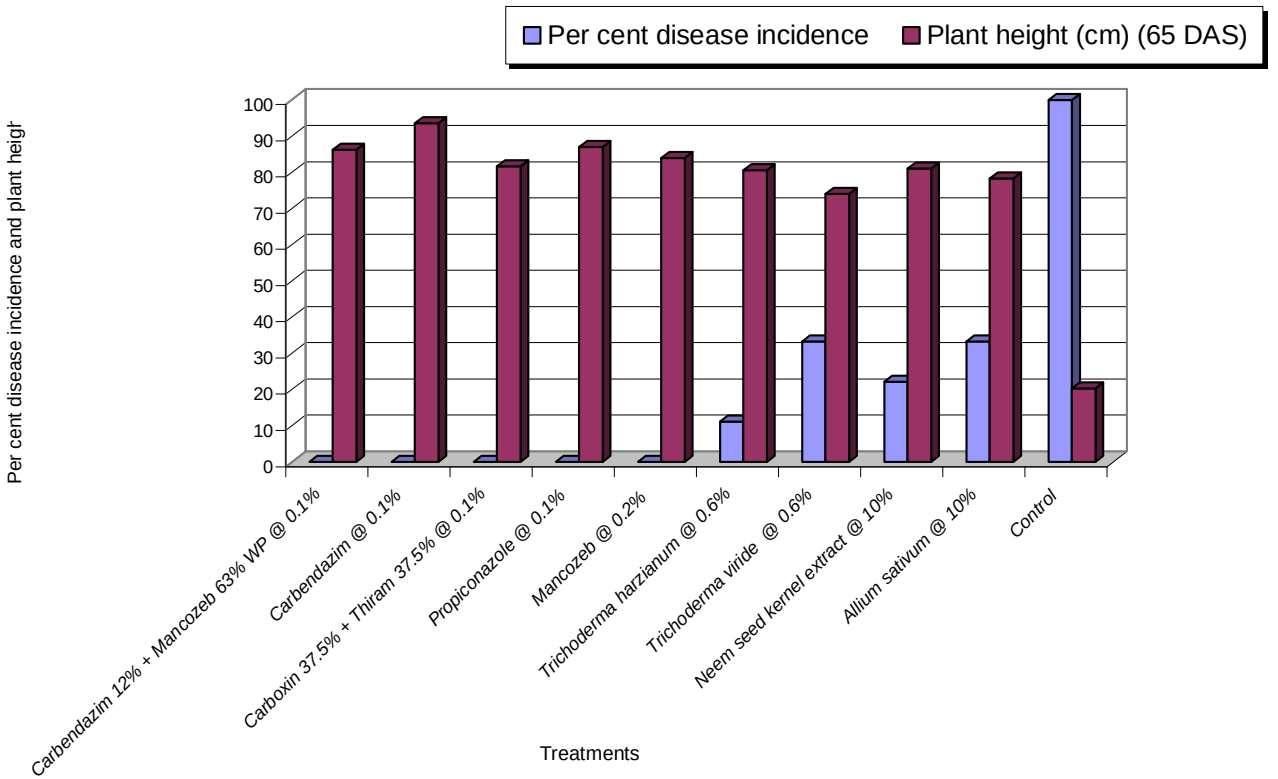


Fig. 15. Effect of fungicides, biocontrol agents and plant extracts on wilt incidence and plant height of patchouli

Fig. 15. Effect of fungicides, biocontrol agents and plant extracts on wilt incidence and plant height of patchouli

1. Mancozeb @ 0.2%
2. Carboxin + Thiram @ 0.1%
3. Propiconazole @ 0.1%
4. Carbendazim @ 0.1%
5. *Allium sativum* @ 10%
6. Carbendazim + mancozeb @ 0.1%
7. *Trichoderma viride* @ 0.6%
8. *Trichoderma harzianum* @ 0.6%
9. Neem seed kernel extract @ 10%
C. Control

Plate. 17. In vivo evaluation of botanicals, bioagents and fungicides against Fusarium solani

5. DISCUSSION

Patchouli (*Pogostimon patchouli* Pellet.) is a perennial aromatic herbaceous plant, belonging to family Lamiaceae. The essential oil is one of the best fixatives for heavy perfumes, which imparts strength, character, alluring notes and lasting qualities. The oil blends well with other essential oils like sandalwood, rose, jasmine, vetiver, lavender, geranium, etc. Because of this reason, patchouli enjoys importance as a crop and its essential oil as aromatic oil. Infact, it is perfume by itself and is highly valued in perfumes, soaps, cosmetics and flavour industries. Dried patchouli leaves are used for scenting wardrobes to protect silk and other fabric from insects. It is helpful for all forms of depression, anxiety and stress related conditions Tenacity of odour is one of the virtues of patchouli and is one of the reasons for its versatile use (Ahmed, 2002).

Patchouli is commonly propagated by stem cuttings. It is affected by fungal diseases, *viz*. Fusarium wilt, leaf blight, Sclerotium wilt and several nematode diseases mainly root knot caused by *Meloidogyne incognita*. Among all these diseases *Fusarium solani* (Mart.) Sacc., which causes a vascular disease called *Fusarium* wilt is one of the limiting factors in commercial cultivation of patchouli (Roopali singh and Angadi,1990). The information available on these diseases and the pathogens is very scanty. Therefore, in the present investigation, different aspects of wilt disease and the pathogen have been investigated, *viz*. survey and surveillance for identification of pathogens. morphological, cultural, physiological, nutritional studies, variability, host range and management were studied.

5.1 SURVEY AND SURVEILLANCE FOR DISEASES OF PATCHOULI

An intensive survey was carried out in five districts, *viz*. Dharwad, Belgaum Haveri, Uttar Kannada and Shimoga districts of Karnataka during 2006-07 to know the incidence of diseases of patchouli. The data on the survey revealed that the incidence and severity of the disease varied from locality to locality. Incidence of wilt and leaf blight was observed in all the districts surveyed, whereas root knot disease was recorded in Mangenkoppa village (Belgaum) with a highest incidence of 89 per cent. *Sclerotium* wilt was observed in Dharwad (Saidapur) and this is the first report of *S. rolfsii* on Patchouli. Highest incidence of *F. solani* was observed in Dharwad (48.26%). This was followed by Shimoga (45.62%), Belgaum district (40.25%) and Haveri (38.63%). Least disease incidence was noticed in Uttar Kannada district (25.62%). The incidence of leaf blight on Patchouli was found to be less in all the areas surveyed. Highest incidence of leaf blight was observed in Haveri district (23.75%) followed by Belgaum district (22.52%) Dharwad (20.83%) and Shimoga district (18.75%). Least disease incidence was noticed Uttar Kannada district (16.18%). Roopali Singh and Angadi (1990) reported about wilt of Patchouli from IIHR, Bangalore.

5.1.1 Symptomatology

F. solani is one of the most important fungal pathogens which cause yellowing of leaves, stunting and wilting of plants. The infected plants lost vigour. Blackening of portions of the stem starting from collar to fine branches was observed followed by dark brown to red discolouration of vascular tissue. Severe disintegration of secondary roots was also observed. It gradually resulted in drying of leaves, stems, fine branches and finally death of whole plant. The infected plants could be easily pulled off from the soil. Similar symptoms were also reported on crossandra and garden rue infected with *F. solani* (Raju, 1997; Rathnamma, 1994)

The symptoms of leaf blight caused by *Alternaria alternata* (Fr.) Keissler appeared as brown discolouration which initiated from the tip. The blighting of the leaves extended from the margin of the leaves. The spots were light brown in colour, subcircular to irregular. Under favourable conditions the plant exhibited a burnt appearance which could be easily detected from a distance. Similar symptoms were also reported on patchouli and tumeric infected with *Alternaria alternata* (Parameswaran *et. al.* 1987; Mamatha, 2004)

The earliest symptom caused by *Sclerotium rolfsii* was discolouration 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 first, later turned brown with age. Finally, the plant wilted and dried. Similar symptoms were also reported on potato and coleus infected with *Sclerotium rolfsii* (Agarwal and Kotasthane, 1971; Ramprasad, 2005).

Meloidogyne sp. infected patchouli plants were stunted in their growth, showed yellow colored leaves. When infected plants were uprooted, deformed roots with prominent multiple galls of varying size were noticed. The presence of egg masses outside the gall was a common phenomenon noticed in patchouli roots. Similar symptoms were also reported on patchouli and coleus infected with *Meloidogyne incognita* by Sarwar and Khan (1972) and Ramprasad (2005).

5.2 ISOLATION OF PATHOGENS

The causal organisms *viz*., *Fusarium solani, Alternaria alternata, Sclerotium rolfsii* were isolated from diseased parts of patchouli by following standard tissue isolation method. The pure culture of *F. solani* was obtained by hyphal tip method and that of *A. alternata* was obtained by single spore isolation. Sachidananda (2005), Shyla (1998) Boby and Bhagyaraj (2003), Sumitra (2006) isolated *Fusarium* by hyphal tip method. Mamatha (2004) isolated *A. alternata* and Kamalakannan *et al.* (2003) isolated *Sclerotium* from collar region of coleus following standard tissue isolation technique.

5.2.1 Identification of the pathogens

The pathogen was identified as *Fusarium solani* based on their morphological characters as described by Booth (1971). It was also confirmed by Maharashtra Association for the Cultivation Sciences (MACS), Agharkar Research Institute, Pune (M.S.). *Altenaria alternata* was also identified based on morphological and cultural characters. The culture of *A. alternata* was grey coloured with fluffy dense mycelium, smooth margin and distinct concentric rings. There was no sporulation of *A. alternata* on potato dextrose agar. *S. rolfsii* was identified based on 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 were noticed.

5.2.2 Pathogenicity of *F. solani*

Pathogenicity was proved by inoculating the giant culture of *F. solani* to sterile soil and control was maintained without inoculum. Yellowing was observed 30 DAP followed by wilting of the plants at the end. Death of the plant was observed on $6O^{th}$ DAP.

5.3 MORPHOLOGICAL STUDIES

The pathogen *F. solani* put forth moderately rapid growth, covering the agar plate within 6-10 days. The colony was sparse to dense, greyish white to pinkish in colour. The pathogen produced three kinds of spores *viz*., macroconidia, microconidia and chlamydospores. Microconidia were abundant, hyaline, cylindrical, single or two celled and measures 6.60-19.80 x 3.30 – 6.60 µm. Macroconidia were 3-4 septate and measured 29.70 –47.85 x 4.95-6.60 µm. Chlamydosproes were hyaline, spherical and 1-celled and measured 8.25-11.5 x 6.60-9.90µm. They were produced singly or sometimes in chains. The description of the pathogen is in agreement with that of *F. solani* (Mart.) Sacc. Emend Snyder and Hansen, given by Commonwealth Mycological Institute, Kew, Surrey, England, Booth (1971) and Rathnamma (1994).

5.4 CULTURAL STUDIES

Every living being requires food for its growth and reproduction, fungi are not exception to it. Fungi secure food from the substrate upon which they live in. In order to culture the fungus in the laboratory, it is necessary to furnish the essential elements and compounds in the medium, for their growth and other life processes. All media are not equally good for all fungi, nor there is a universal substrate or artificial medium, upon which all fungi can grow. So, different media including both synthetic and non synthetic were tried for *F. solani* in the present experiment. The amount of vegetative growth was estimated by measuring diameter of the colony on solid media and by weighing the dry mycelial weight in liquid media.

5.4.1 Growth characters on different solid media

The fungus grown on thirteen different solid media indicated that the maximum radial growth of *F. solani* was observed on potato dextrose agar (90 mm) and Richards's agar (90 mm) followed by oat meal agar (88 mm). Minimum growth of fungus was observed in Browns agar (22.33 mm) and Asthana and Hawker's agar (68.33mm).The results are in confirmity with the findings of Ramprasad (2005) in case of *Fusarum chlamydosporum*. Colony was of pink colour in potato dextrose agar, and Sabouraud's agar and in all other cases colony was white. Margin was irregular in potato dextrose agar and Sabouraud's agar and smooth margin was observed in all other media. Pinkish, profuse cottony growth of *Fusarium* sp. was observed by Adiver (1996). Sporulation of fungus was found to be abundant on Richards's agar, Sabouraud's agar and potato dextrose agar. Good sporulation was observed in Czepeck's Dox agar, Tochinai's agar, Brown's agar, carrot agar, host leaf extract agar and corn meal agar. Sporulation was sparse in Asthana and Hawker's agar. Similar result was reported by Ramprasad (2005) in case of *Fusarum chlamydosporum*.

5.4.2 Growth phase

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. solani* attained maximum vegetative growth on the 10^{th} day of incubation (365.66 mg) this was indicative of optimum growth period beyond which, autolysis occurred. Barnett and Hunter (1951) have reported that cellular enzymes begin to digest the various cell constituents. This is in confirmity with the findings of Adiver (1996) in case of *Fusarium* sp. and Sataraddi (1998) in *Fusarium udum*.

5.4.3 Growth in different liquid media:

Fungi posses an ability to utilize a wide range of nutrients as a source of energy. Among the liquid media used for growth of *F. solani,* Richards's broth supported maximum growth (362.33 mg) and this was followed by potato dextrose broth (352.33 mg). In the radial measurements, it is not possible to consider the amount of submerged mycelium. Hence, Cochrane (1958) has opined the determination of dry mycelial weight as the best method for precise work. The ability of fungus to grow in Richards's medium indicated the requirement of certain nutrients and vitamins which may be present in the medium.

5.5 SPORE GERMINATION STUDIES

5.5.1 Type of germination of spores

Cochrane (1958) has opined that any consideration of ecology for spread of economically important fungi must take spore germination into account, since spore of *F. solani* have important role in both initiation and spread of the disease and subsequent infection. It is necessary to take up studies on spore germination of the fungus. The germination of conidia and chlamydospores started with the initiation of germ tubes four hours and six hours after incubation respectively. Germination was maximum at 12 hours of incubation. The germ tubes were produced from intermediary cells or from either one or both the polar cells. Similar observations were recorded by Madhukar (2001) in *Fusarium solani*.

5.5.2 Germination of spores in different nutrient solutions

The extent of spore germination depends greatly on the nature of media used. In the present study, maximum spore germination was obtained in sucrose (98.07%) followed by dextrose (95.72%) and glucose (90.09%). This could be attributed to the presence of certain stimulating factors in these nutrient media. Least conidial germination was recorded in sterile distilled water (68.18%). Similarly Rathnamma (1994) has recorded maximum spore germination of *F. solani* in sucrose (95 %) and least germination in distilled water (67.35%).

5.6 PHYSIOLOGICAL STUDIES

5.6.1 Effect of temperature

Temperature plays an important role, among the external factors which influence the growth and reproduction of fungi. All the fungi have minimum temperature, below which they

cannot grow and above which they are inactivated or killed. Each fungus has its temperature range for the growth and sporulation. In the present study, maximum growth of *F. solani* was obtained at 30℃ (362.33 mg), whereas optimum temperature range was 20-30℃. Similarly Sataraddi (1998) reported 25-30℃ as optimum temperature range for *Fusarium udum*. *In vitro* studies conducted by Chi and Hanson (1964) indicated that *F. solani* isolates grew well at higher temperature of 28℃. Since, *Fusarium* is mesophytic, perhaps, it requires higher temperature and low moisture for its better growth, spore formation, spore germination, mycelial growth and infection. All these processes are favoured at a temperature of 25-30℃. Dhingra *et al*. (1974) also observed maximum growth of *F. oxysporum.* f. sp. *lintis* at 25℃ .

5.6.2 Effect of hydrogen ion concentration

pH of the medium has profound effect the rate and the amount of growth and many other life processes (Lilly and Barnett, 1951). The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This shows importance of hydrogen ion concentration for the better fungal growth. The results indicated that maximum growth of fungus was observed at pH 6.5 (364.77 mg). The present findings are in agreement with the reports of Moore (1924), Neal (1927). Yogeshwari (1948), Singh and Chaube (1975), Sowmya (1993), Sataraddi (1998), Ramprasad (2005), Sachidananda (2005) and Sumitra (2006) on *Fusarium* spp.

5.7 NUTRITIONAL STUDIES

5.7.1 Carbon utilization

Carbon is an important element required, by all the living organisms for metabolic activities. Almost half of the dry mycelial weight of the fungal cell consists of carbon which is the main structural element (Lilly and Barnett, 1951). In the present study, among the carbon sources tested, sucrose supported maximum mycelial growth of *F. solani*, next best sources were fructose and dextrose. The utilization of various carbon compounds may depend on 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 source tested for *Fusarium* sp. 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 Patel (1991) and Ramprasad (2005) and Sachidananda (2005) in case of *Fusarium* sp.

5.7.2 Nitrogen utilization

Nitrogen is an important element for protein synthesis and like carbon it is used by fungi for functional as well as structural purpose. In the present study, among the nitrogen sources tested, potassium nitrate (364.33 mg) was found superior over others and it was followed by sodium nitrate. Minimum growth of fungus was observed in ammonium orthophosphate (74.66 mg).

Fusarium sp. utilize potassium nitrate more efficiently and it is a better nitrogen source than any other nitrogen source. The nitrate compounds are excellent nitrogen sources for imperfect fungi and also ascomycetes. Similar findings were made by Moore (1924), Subramanian and Srinivas (1953), Agarwal (1958), Ramprasad (2005) and Sachidananda (2005) in case of *Fusarium* spp. The next best nitrogen sources were L-asparagine and sodium nitrate. (Sowmya , 1993; Ramprasad, 2005 and Sachidananda, 2005).

5.7.3 Effect of light

5.7.3.1 On dry mycelial weight

When the fungus was exposed to alternate cycle of light and darkness maximum dry mycelial weight of 363.30 mg was recorded. This was followed by continuous light (319.72 mg). Similar result was recorded by Sumitra (2006) in *Fusarium oxysporum*.

5.7.3.2 On radial growth

Light has a profound effect on growth of fungi. The preliminary studies carried out in the present investigation with *F.solani* indicated that maximum growth (89.41mm) was seen

when exposed to alternate cycle of light and darkness. This was followed by continuous light (81.07 mm). This is in agreement with the results of Sumitra (2006) in case of *Fusarium oxysporum*.

5.8 HOST RANGE of *Fusarium solani*

Host range studies were conducted to know the ability of the test pathogen to infect other hosts. Nine hosts were susceptible to *Fusarium solani* and can be considered as collateral hosts. *Ocimum canum and Ocimum sanctum* ,recorded similar type of symptoms like yellowing, drying and withering of leaves and dark brown discoloration of roots eight days after inoculation. *O. gratissimum* showed drooping and drying of leaves ten days after inoculation followed by dark brown discoloration of roots at later stages. Water soaked lesions and yellowing was observed on *Pandanus amaryllifolius,* seven days after inoculation. *Cymbopogon winterianus* and *Cymbopogon flexuosus* showed symptoms of yellowing and drying of leaves six days after inoculation. In *Bacopa mannieri* and *Mentha spicata* drying and wilting was recorded four and five days after inoculation respectively. Yellowing was the first symptom observed in *Rosmarinus officinalis* followed by drying and narrowing of leaves fifteen days after inoculation. Similarly, Kore and Patil (1985), Moubasher *et al.* (1984) and Rathnamma (1994) have studied the host range of *Fusarium solani.*

5.9 VARIABILITY IN *Fusarium solani*

5.9.1 Cultural and morphological

Studies on cultural and morphological features of eight isolates of *F. solani* was carried on potato dextrose agar. Little variation was noticed among the isolates. On potato dextrose agar the colony diameter ranged from 84.0 mm (*Fs7*) to 90 mm (*Fs* 1). Isolates *Fs1, Fs2,* and *Fs4* showed good mycelial growth and abundant sporulation. Whereas *Fs5* and *Fs8* showed good growth and good sporulation. Sporulation was sparse in *Fs3* isolate. Isolate Fs6 though registered good growth but sporulation was sparse.

According to Van der Plaats- Niterink (1981), due to intraspecific variation in morphology, many isolates cannot be identified unambiguously. Chauhan (1962) and Sarojani and Yogeshwari (1947) noticed variation in *Fusarium* affecting chick pea. Gupta *et al.,*(1986) categorized isolates of *F. oxysporum.* f .sp. *ciceri* into six groups on the basis of cultural and morphological characters. Patel (1991) categorized 19 chickpea wilt isolates into six groups on the basis of morphology and cultural characters. However, in present investigation, morphological and cultural variation could not help to group the isolates of *F. solani*. To understand existence of variation among the isolates of *F. solani*, PCR based technique *ie*., RAPD was used in the present investigation.

5.9.2 Genetic variability

The suitability of random amplified polymorphic DNA (RAPD) was used to detect the variations among the isolates of *F. solani*. In the present investigation, OPA and OPB series primers were used to determine genetic distance between isolates and to construct dendrogram. Of the 20 primers used for amplification OPA3, OPA4, OPA7, OPA10, OPB11, OPA15, OPB4 showed 100 per cent polymorphism. This information helps us to identify or diagnose the pathogen directly from the infected seedlings, but needs to be conducted several times to get repetitive results.

Information on banding pattern for all primers was used to determine genetic distance between isolates and to construct dendrogram. Similarity coefficient ranged from 0.63 to 0.92.The maximum genetic similarity of 92 was observed between *Fs2* and *Fs1*. Whereas, least genetic similarity was observed between *Fs6* and *Fs2* isolates.

The dendrogram by RAPD study revealed that eight isolates were differentiated into two major clusters A and B. Cluster A was classified upto sub sub cluster A6, whereas cluster B was classified upto sub sub cluster B4. Major cluster A composed of isolates belonging to Shimoga, Uttar Kannada, Adur and Adavisomapur. Major cluster B composed of isolates belonging to Belgaum, Saidapur, Mangenkoppa and Arabhavi. In the present investigation, the results revealed that isolates of same geographical locations were closely related. So results obtained from the cluster analysis revealed that sub cluster group was composed of isolates belonging to same geographical locations with very less variability.

Similar work was carried out by De Haan (2000) who tested 160 arbitrary 10- mer oligonucliotide primers on *F. oxysporum.* f .sp. *gladioli* by PCR to find RAPD specific marker. He found that RAPD primer G-12 amplified two discriminating DNA fragments.

5.10 MANAGEMENT STUDIES

Continuous use of chemicals or fungicides in the management of disease also brought new problems with them. More alarming amongst them are air, water, soil pollution, 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, readily biodegradable, relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management. Hence, screening of plant products for its 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).

5.10.1 *In vitro* evaluation of botanicals

In the present investigation, eleven plant extracts were evaluated under *in vitro* condition against *F. solani* to know the fungitoxic nature. Though complete inhibition of the pathogen was not observed in any of the plant extracts tested but considerable amount of inhibition was noticed in some of them.

Among eleven plant extracts tested against *F. solani,* neem seed kernel extract at 10 per cent (74.86%) was significantly superior to all other plant extracts. Next best was eucalyptus at 10 per cent (57.36%) followed by garlic at 10 per cent (52.86%), parthenium at 10 per cent (52.73%), pongemia at 10 per cent (45.43%) and neem seed kernel extract at 5 per cent (62.00%). Cassia and glyricidia were least effective in inhibiting the growth of the pathogen. In the present investigation, the mycelial growth of fungus was inhibited to a greater extent by neem seed kernel extract, which is said to have insecticidal property. The fungicidal spectrum of *Azadirachta indica* has been attributed to azadirachtin which belongs to C_{25} terpenoides (Subramaniam, 1993). Similar results were observed by Ramaprasad (2005) in *Fusarium chlamydosporum*.

5.10.2 *In vitro* evaluation of biocontrol agents

In the light of present day constraints in plant disease control practices especially those on the use of pesticides, biological control is increasingly occupying the minds of scientists all over the world as they are eco-friendly and cost effective. In recent years, the use of *Trichoderma* has gained more importance. These antagonistic organisms act on the pathogen by different mechanisms *viz.,* competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1999). Formulations of antagonistic organisms are available at cheaper rate and these organisms once introduced into the soil survive for a longer period. There is also circumstantial report that native antagonists are more efficient than introduced antagonists (Kulkarni and Sagar, 2006).

In the present study, maximum reduction in colony growth was observed in *T. harzianum* which was significantly superior to all the bioagents tested. Next best were *T. koningii* and *T. viride*. There was minimum inhibition of *F. solani* by *P. fluorescence* and *B. subtilis.* Species of *Trichoderma, viz*. *T. harzianum*, *T. viride*, *T. koningii* and *T. virens* showed more mycelial inhibition of organism compared to bacterial antagonists. This can be attributed to higher competitive ability of these *Trichoderma* spp. Similar results were reported by Sivan and Chet (1986), Kempf and Walf (1989), Thomashow and Weller (1990), Kavitha *et. al* (2004) and Mishra *et al.,* (2004).

5.10.3 *In vitro* evaluation of fungicides

In vitro evaluation of fungicides provides preliminary information regarding its efficacy against a pathogen within a shortest period of time and therefore, serves as a guide for further field testing. Among systemic fungicides, carbendazim and carbendazim 12% + mancozeb 63% at 0.025, 0.05 and 0.1 per cent completely inhibited the growth of *F. solani*.

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

In the present investigation, among the non-systemic fungicides tested, mancozeb at 0.2 and 0.3 per cent was found very effective among all the non-systemic chemicals followed by propineb at 0.3 per cent. Least inhibition of fungal growth was recorded in zineb (49.33%) at 0.1 per cent.

5.10.4 *In vivo* studies

Among the different chemicals, botanicals and bioagents tested, chemicals have given good results. They were significantly superior to all other treatments tested. Carbendazim was very effective and it completely inhibited the disease with maximum plant height (93.6 cm) at 90 DAP followed by carbedazim + mancozeb, propiconazole and mancozeb, *T. harzianum and* NSKE but plant height was less compared to carbendazim. These results are comparable with findings of Gangopadhyay and Grover (1985), Lakshmi and Jayarajan (1987). Rathnamma (1994) reported that carbendazim effectively checked the growth of *F. solani* under *in vivo* condition.

5.11 FUTURE LINE OF WORK

There is no full stop to gain insight into scientific knowledge. Any amount of work does not satisfy the hunger of scientists as problems crept in, new ideas will continue to flow and this work is not an exception. The present investigation has opened up new information and given rise to new ideas on diseases of patchouli, *viz*. wilt caused by *Fusarium solani*, leaf blight caused by *Alternaria alternata*, *Sclerotium* rot caused by *Sclerotium rolfsii*, root knot caused *Meloidogyne incognita*. Hence, the following future lines of work are being suggested.

1) There is a need to undertake an intensive survey for diseases of patchouli in all patchouli growing areas of Karnataka and quantify the loss caused by pathogens and the effect of diseases on quality of the crop and oil

2) Detailed studies on pathogens of patchouli such as *Alternaria alternata*, *Slerotium rolfsii*, plant parasitic nematodes associated with patchouli are required

3) Detailed investigations on interaction between fungal and nematode diseases are essential

4) Detailed investigation on the epidemiology on the wilt and other diseases needs immediate action

5) Integrated management strategies have to be developed to manage diseases of patchouli

6. SUMMARY AND CONCLUSIONS

Patchouli (*Pogostimon patchouli* Pellet.) is an important perennial commercial aromatic crop grown mainly for its essential oil. Patchoulene is the important component of essential oil of patchouli. Patchouli is affected by diseases like *Fusarium* wilt, leaf blight, *Sclerotium* wilt and root knot. Among the different diseases affecting patchouli, wilt caused by *Fusarium solani* is observed in severe form. The information available on these diseases as well as pathogens are very scanty. Hence, it demanded an early investigation into various aspects of the pathogen and also the disease.

The present investigation include different studies, *viz.* survey for the incidence of diseases of patchouli, isolation, proving pathogenicity, identification, symptomatology, morphological, cultural, physiological, nutritional studies, variability of pathogen, host range and its management by using botanicals, biocontrol agents and fungicides.

- A survey was conducted in 5 districts of Karnataka during 2006-07 to know the incidence of diseases of the crop. Higher incidence of wilt was observed in Dharwad district (48.26%) followed by Belgaum district (40.25%) and Haveri (38.63%). Least incidence was noticed in in Uttar Kannada district (25.62%). Highest incidence of leaf blight was observed in Haveri (23.75%) followed by Belgaum district (22.52%) and Dharwad (20.83%). Least disease incidence was noticed in Uttar Kannada district (16.18%). Root Knot was severe in Mangenkoppa village (Belgaum) with a incidence of 89 per cent.
- It was observed that *F. solani* is one of the most important fungal pathogens which caused yellowing of leaves, stunting and wilting of plants. The infected plants lost vigour. Dark brown to red discoloration of vascular tissue was observed, while in case of blight caused by *Alternaria alternata,* the infection started as brown discolouration of the leaves which began from the tip. The blighting of the leaves extended towards the margin of the leaves. The plant exhibited a burnt appearance. In case of *Sclerotium* wilt caused by *S. rolfsii,* infected plants exhibited darkening of the stem at collar region. The leaves became flaccid and dropped off. Sclerotial bodies were observed on fallen leaves. This is the first report of *S. rolfsii* on Patchouli. Root-knot infected plants showed stunted growth, marginal yellowing of leaves. When plants were uprooted, deformed roots with prominent galls of varying size were noticed.
- The pathogens were isolated from infected parts of patchouli by following standard tissue isolation method. The pure cultures of *F. solani* and *S. rolfsii* were obtained by hyphal tip method while pure culture of *A. alternata* was obtained by single spore isolation. On the basis of morphological and cultural studies, the pathogens were identified as *Alternaria alternata* (Fr.) Keissler and *Sclerotium rolfsii* Sacc.and *Fusarium solani* (Mart.) Sacc. The identity of *Fusarium solani* (Mart.) Sacc., was also confirmed by MACS, Pune.
- *Fusarium solani* produced three kinds of spores *viz.,* microconidia, macroconidia and chlamydospores. Microconidia were abundant, hyaline, continuous or 1 septate, cylindrical. Macroconidia were 3- 4 septate. Chlamydospores were globose to oval, single celled, smooth to rough walled. Both intercalary and terminal chlamydospores were noticed.
- Among thirteen solid media tested, maximum radial growth (90 mm) was observed on Richards's agar and potato dextrose agar (90 mm) followed by oat meal agar (88 mm). The least mycelial growth was observed is Brown's agar (22.33 mm). Colony was pink in potato dextrose agar and Sabouraud's agar and it was whitish in all other media. Sporulation was abundant in Richards's agar, Sabouraud's agar, host leaf extract agar and corn meal agar. The same was sparse in Asthana and Hawker's agar.
- Among thirteen liquid media tested for the growth of *F. solani* Richards's broth (362.33 mg) supported maximum mycelial growth followed by potato dextrose broth (352.33 mg) and oat meal broth (315.66 mg). Least mycelial weight was obtained in Tochinoi's broth (66.33mg).
- Spore germination studies revealed that germination of conidia was maximum at 12 hours of incubation. The germ tubes were produced from intermediary cells or from either one or both the polar cells. The extent of spore germination depended greatly on the

nature of solution used. In the present study, maximum spore germination was obtained in sucrose (98.07%) followed by dextrose (95.72%) and glucose (90.09%).

- The temperature studies revealed that maximum mycelial weight of fungus was observed at temperature of 30℃ (362.33 mg), which was followed by 25℃ (351.66 mg) and 35℃ (303.33mg). Least mycelial growth was observed at 5℃(41.66 mg). The optimum temperature range for *F. solani* was 25-35℃.
- *F. solani* grew at different pH levels tested, however maximum growth of fungus was obtained at pH 6.5 (364.77mg) followed by 6.0 (325.62mg). While, the least growth was observed in pH 4.0 (215.70mg). The optimum pH range was found to be between 6.0 to 7.0.
- Nutritional studies were carried out to study the utilization of carbon and nitrogen sources by the fungus. Among the carbon sources tested sucrose supported maximum dry mycelial weight (363.93 mg) followed by fructose (341.30 mg). Minimum growth of fungus was supported by mannitol (144.50mg) and starch (117.96 mg)
- In the present investigation, eight nitrogen sources were tested, among these, potassium nitrate was found to be the best nitrogen source for the growth of fungus (364.33mg) followed by sodium nitrate (346.33 mg). Minimum growth of fungus was observed in ammonium orthophosphate (74.66 mg) and ammonium chloride (81.33 mg).
- Alternate cycles of 12h light and 12h darkness resulted in maximum dry mycelial weight (363.30mg) and maximum radial growth (89.41 mm).
- The results of host range revealed that among the twelve hosts tested, nine hosts, *viz*. *Ocimum canum, Ocimum sanctum, O*. *gratissimum, Rosmarinus officinalis, Pandanus amaryllifolius, Cymbopogon winterianus, Cymbopogon flexuosus, Bacopa mannieri, Mentha spicata* were susceptible to *F. solani* and can be considered as collateral hosts.The remaining three hosts, *viz*. *Coleus ciamboini, C. forskohlii,* and *Stevia rebaudiana* did not show any symptoms.
- Variability studies on morphological and cultural characteristics of eight isolates of *F. solani* carried on potato dextrose agar indicated that there was not much variation noticed among isolates. Similarity coefficient ranged from 0.63 to 0.92. RAPD data distinguished the eight isolates into two major clusters A and B. Cluster A was classified upto sub-sub cluster A6, whereas cluster B was classified upto sub-sub cluster B6. Major cluster A composed of isolates belonging to Shimoga, Uttar Kannada, Adur and Adavisomapur. Major cluster B composed of isolates belonging to Belgaum, Saidapur, Mangenkoppa and Arabhavi. In the present investigation, the results revealed that isolates from geographically nearby locations were closely related.
- Among eleven botanicals tested *in vitro* against *F. solani*, neem seed kernel extract at 10 per cent (74.86%) was found superior.This was followed by garlic clove extract at 10 per cent (57.36%), eucalyptus at 10 per cent (52.86%) and parthenium at 10 per cent (52.73%). Least growth inhibition of *F. solani* was obtained in Cassia at 5 per cent (19.46%) and glyricidia at 5 per cent (20.43%).
- The results of dual culture technique revealed that fungal bioagents were better than bacterial bioagents in inhibiting the growth of *F. solani*. Maximum reduction in colony growth was observed in *T. harzianum* (Dwd) which was very effective when compared to all other bioagents tested.
- Twelve fungicides were tested *in vitro* against *F. solani*. Among systemic fungicides, carbendazim and carbendazim 12% + mancozeb 63% were highly effective in inhibiting the growth of *F. solani* in all the three concentrations (0.025%, 0.05% and 0.1%). Among the non systemic fungicides mancozeb at 0.2 and 0.3 per cent was found significantly superior to other fungicides in inhibiting *F. solani*.
- *In vivo* studies conducted in Department of Plant Pathology, Agricultural College, Dharwad revealed that among the various treatments, disease was completely absent in carbendazim, carbendazim + mancozeb, carboxin + thiram, propiconazole, and mancozeb. *T. harzianum, T. viride*, neem seed kernel extract and *Allium sativum* were less effective but were significantly superior over control. The highest disease incidence

was noticed in untreated control (100%). Plant height was also more in all the treatments compared to control.

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* Originals not seen

STUDIES ON FUNGAL DISEASES OF PATCHOULI WITH SPECIAL REFERENCE TO WILT CAUSED BY *Fusarium solani* (Mart.) Sacc.

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ABSTRACT

The major constraint for cultivation of patchouli is Fusarium wilt caused by *Fusarium solani* (Mart.) Sacc. Fusarium wilt was noticed in all the locations surveyed with the range of 20.00 to 61.25 %. The maximum disease incidence was noticed in Dharwad district (48.26%) and least in Uttar Kannada district (25.62%). Other diseases observed were leaf blight caused by *Alternaria alternata* (Fr.) Keisser., Sclerotium wilt caused by *Sclerotium rolfsii* Sacc. Leaf blight incidence was more in Belgaum district (22.52%). Sporadic incidence of Sclerotium wilt was observed in Saidapur and this is the first report of Sclerotium wilt of Patchouli.

F.solani produced microconidia, macroconidia and chlamydospores. Potato dextrose agar and Richards' agar supported maximum radial growth and dry mycelial weight on 10th day of incubation. The temperature of 30°C, pH 6.5 and 12 h light and 12 h darkness were found to be best for fungal growth. Sucrose and potassium nitrate were found to be best carbon and nitrogen sources for the growth of fungus, respectively. Among twelve hosts tested for host range of *F. solani*, nine hosts were susceptible which can be considered as collateral hosts.

The results of morphological and cultural studies indicated that there was slight variation among the isolates of *Fusarium solani.* RAPD data distinguished eight isolates into two clusters A and B, which is in accordance with their geographical locations. Under *in vitro* conditions, neem seed kernel extract at 10% and *Trichoderma harzianum* were able to reduce the growth of pathogen to a remarkable extent. Among fungicides, carbendazim and carbendazim + mancozeb at 0.025, 0.05 and 0.1 and mancozeb at 0.2 and 0.3% were found effective against the pathogen. Under *in vivo* studies, carbendazim, carbendazim + mancozeb, carboxin + thiram, propiconazole @ 0.1 and mancozeb @ 0.2% were very effective in inhibiting the disease completely.