DISEASES OF MILKY MUSHROOM (*Calocybe indica* P & C) AND THEIR MANAGEMENT

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By

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THESIS

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2007

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I, hereby declare that this thesis entitled **"Diseases of milky mushroom (*Calocybe indica* P&C) and their management"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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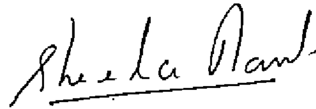

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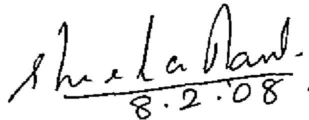

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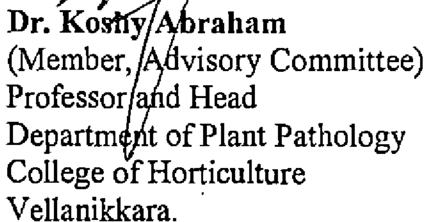

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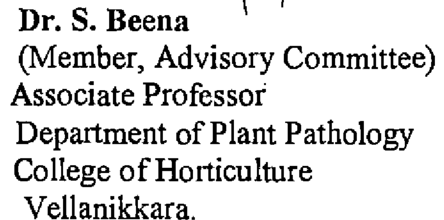

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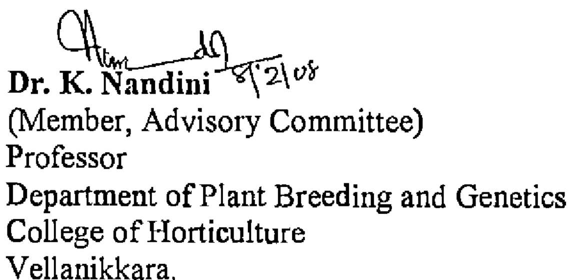

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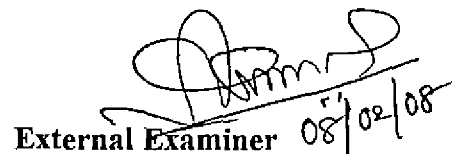

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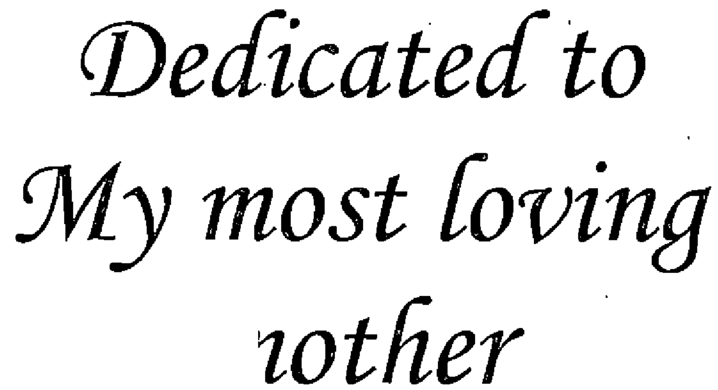
Dedicated to
My most loving
ıother

CONTENTS

CHAPTER	TITLE	PAGE. NO
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-24
3	MATERIALS AND METHODS	25-48
4	RESULTS	49-95
5	DISCUSSION	96-113
6	SUMMARY	114-118
	REFERENCES	
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page no.
1	Details of survey conducted in milky mushroom farms	50
2	Major diseases observed in Agrowe mushroom farm at Eravimangalam	51
3	Details of pathogens / weed fungi isolated from milky mushroom farms	53
4	Colony characters of bacterial isolates	61
5	Morphological characters	62
6	Effect of pH on the growth of bacterial isolates	63
7	Effect of temperature on the growth of bacterial isolates	64
8	Biochemical test	65
9	Utilization of carbohydrates	67
10	Effect of temperature on the growth of pathogenic / weed fungi	71
11	Effect of pH on the growth of pathogenic / weed fungi	71
12	Interaction between bacterial pathogens and *C. indica*	73
13	Interaction between pathogenic/ weed fungi and *C. indica*	75
14	Effect of culture filtrate of weed fungi on the growth of *C. indica*	76
15	Antagonistic effect of weed fungi on linear growth of *C.indica*	77
16 a	Influence of seasonal variations on the incidence of weed fungi	79
16 b	Weed moulds observed in milky mushroom beds from August 2006- July 2007	80
17	Effect of weed fungi on growth and yield of *C. indica*	81

18	Effect of different methods of sterilization of substrate on linear growth of *C. indica*	83
19	Effect of different methods of sterilization of substrate on growth and yield of *C. indica*	84
20	*In vitro* effect of phytoextracts against bacterial pathogens	86
21	*In vitro* effect of phytoextracts against pathogenic /weed fungi	88
22	*In vivo* effect of selected phytoextracts on growth and yield of *C. indica*	91
23	Effect of phytoextracts on incidence and severity of bacterial diseases	93
24	Biochemical constituents in bacteria infected mushroom	95

LIST OF FIGURES

Figure No.	Title	Page no.
1	Effect of temperature on the growth of pathogenic/ weed fungi	71-72
2	Effect of pH on the growth of pathogenic/ weed fungi	71-72
3	Influence of seasonal variations on the incidence of weed fungi	79-80
4a	Effect of weed fungi on mycelial growth of *C. indica*	82-83
4b	Effect of weed fungi on yield of *C. indica*	82-83
5a	Effect of different methods of sterilization of substrate on per cent mycelial growth	84-85
5b	Effect of different methods of sterilization of substrate on yield	84-85
6a	Effect of phytoextracts on bacterial disease incidence	94-95
6b	Effect of phytoextracts on bacterial disease severity	94-95

LIST OF PLATES

Sl.No.	Title	Between pages
1	Milky mushroom farms visited	26-27
2	Symptoms of abiotic stress	56-57
3	Symptoms of fungal infection	60-61
4	Identification of pathogenic / weed fungi	69-70
5	Interaction between bacterial pathogens and *Calocybe indica*	74-75
6	Interaction between pathogenic / weed fungi and *Calocybe indica*	75-76

Introduction

1. INTRODUCTION

Mushroom, the god given natural health food for humanity, is growing in almost all parts of the world. It is a good source of quality proteins, minerals, vitamins and having excellent medicinal properties. Mushrooms in general are highly preferred table delicacies and occupy an important place in the food habits of people around the world because of their nutritive value, innate flavour and taste.

In an under developed and over populated country like India, the problem of malnutrition is quite obvious. To fight against protein malnutrition the cultivation of mushroom need to be popularized. At present mushroom cultivation is the only economically feasible and ecofriendly process for the bioconversion of agricultural wastes into high protein foods. The group of cultivated mushroom is heterogeneous with different shape, size and colour. White button mushroom (*Agaricus* sp.), oyster mushroom (*Pleurotus* sp.) and paddy straw mushroom (*Volvariella* sp.) are the common species cultivating in India.

Milky mushroom, *Calocybe indica* (P&C) is a new introduction to the world of cultivated mushrooms. Purkayastha and Chandra first reported this mushroom from India in 1974. Krishnamoorthy *et al.* (1998) have evolved the commercial production techniques for milky mushroom. Eventhough it is a new introduction to the edible mushroom world, milky mushroom has got a wide acceptance among the mushroom growers due to its simple production technique, sustainable yield, long shelf life, milky white colour, robust nature and high biological efficiency. Being a tropical mushroom it prefers higher temperature of 30-35^0 C and high humidity, more than 85 per cent (Krishnamoorthy and Muthusamy, 1997). It contains 32.3 per cent crude protein, 0.67 per cent fat, 41 per cent crude fibre and 9.85 per cent carbohydrate (Krishnamoorthy, 2004). Milky mushroom cultivation is now popular in South India including Kerala.

Since, mushrooms are indoor crops grown on specific substrate, their productivity and quality are adversely affected by large number of biotic and abiotic factors. Slight variation in the optimum conditions adversely affects the yield of mushroom. As any other living organisms, mushrooms are vulnerable to the attack of several organisms, which includes fungi, bacteria, virus, nematodes and insects. These factors significantly affect the production and the interest of the growers on the enterprise. In the absence of proper infrastructure and hygienic measures, mushroom cultivation is greatly threatened by air and substrate borne contaminants. Recently, many milky mushroom growers abandon mushroom growing after two to three crops due to the occurrence of several pathogens and weed fungi. Complete failure of the crop by cobweb disease in milky mushroom was reported from India in 2003 (Pandey *et al.,* 2003). Competitor moulds like, *Trichoderma* spp., *Aspergillus* spp., *Rhizopus* spp. and *Coprinus* spp. hamper the mycelial growth of milky mushroom (Pathak *et al.,* 1998). They also noticed the yellowing of stipe tissue and killing of buttons due to bacterial infection.

Pesticides are not preferred in mushroom culture because of their residual effect as well as due to environmental pollution. Consequently research efforts have been made to find out an alternative to chemical fungicides. Fungicidal property of botanicals can be exploited as an effective way out. Many workers have reported the use of plant extracts for the control of fungal diseases.

The present study is aimed to identify the major diseases of milky mushroom in Kerala and to evolve suitable ecofriendly management practices against them. Keeping this in view, the current study entitled "Diseases of milky mushroom (*Calocybe indica* P&C) and their management" was taken up with the following objectives.

1. Survey on the occurrence of various diseases of milky mushroom in selected mushroom farms
2. To study etiology and symptomatology of diseases of milky mushroom.
3. To study seasonal effect on the incidence of disease
4. To evolve ecofriendly management of diseases using different substrate sterilization methods and by using phytoextracts

Review of Literature

2. REVIEW OF LITERATURE

Milky mushroom, an indigenous tropical mushroom was first reported from India by Purkayastha and Chandra (1974). Krishnamoorthy (1995) identified a new edible species of *Calocybe* from the sugarcane fields of Coimbatore, which was later released as a superior variety APK 2 for commercial cultivation. Gogoi *et al.* (2000) reported the occurrence of *Calocybe* sp. in the moist shade tree bases in Assam. Two species of milky mushroom namely *C. indica* and *C. gambosa* were reported from Kerala by Balakrishnan and Das (2001). Kalpana *et al.* (2004) reported six different wild isolates of *C. indica* from Coimbatore and Erode district of Tamil Nadu. Milky mushroom was a new introduction among the commercially domesticated mushrooms. A perusal of literature revealed that few studies were carried out for identification and control of diseases of milky mushroom.

2.1 Bacterial diseases

Bacterial diseases have been reported from all over the world on cultivated mushrooms. But only one report is available on bacterial diseases in milky mushroom. Pathak *et al.* (1998) reported yellowing of stipe tissue and killing of young buttons in milky mushroom due to bacterial infection.

Bacterial blotch is one of the most common and serious disease of *Agaricus bisporus* and is responsible for considerable losses. In India bacterial blotch on white button mushroom caused by *Pseudomonas tolaasii* was first reported by Guleria (1976). Initial symptom of the disease appeared as pale yellow lesions on mushroom tissue later become golden yellow. Typical spotting is observed at or near the edge of mushroom cap. Severely affected mushroom may be distorted and caps may split where the blotch symptom occur.

Poppe *et al.* (1985) reported the incidence of fist shaped fruiting bodies in white button mushroom caused by *P. fluorescence*. Femour (1986) reported *P. tolaasii* causing brown blotch diseases in oyster mushroom. Bacterial blotch diseases in *A. bisporus* caused by *P. tolaasii* and *P.fluorescence* was also reported by Fletcher *et al.* (1986), which had a worldwide occurrence. Bacterial soft rot of *A. bitorquis* was reported to be caused by *P. gladioli pv. agaricicola* (Atkey *et al.*, 1991). Raina (1992) identified the organism responsible for bacterial blotch as *P. tolaasii* and the blotch bacterium can induce symptoms on *Agaricus bitorquis* and also resulted rotting of *Pleurotus sajor-caju.* A brown discolouration of mushroom caused by *P. agarici* caused considerable yield loss in *Agaricus* sp. (Geels *et al.,* 1994). The genus *Bacillus* was reported as a cellulolytic bacteria from mushroom compost by Shirkot *et al.* (1994).

Jandaik and Jandaik (2003) reported that bacterial blotch of white button mushroom was characterized by brown spots or blotches on the pilei and in more severe cases on the stipe. Brown and slightly concaved spots appeared on the surface of diseased fruiting bodies. According to them high relative humidity and continuous persistence of water film on the surface of pilei enhances bacterial multiplication. Growth of *P. tolaasii* on the cap surface of *A. bisporus* was reported by Varszegi (2003). Rinker and Castle (2007) reported diversity of organisms responsible for blotch disease of cultivated mushrooms and it includes *P. fluorescence* biotype III and *P. putida.*

Biswas *et al.* (1983) reported *P. alcaligens* causing rot of oyster mushroom. The first symptoms of the disease are water soaked areas and yellow brown discolouration of young sporophores. In advanced stage, the rotting of fruiting body starts from centre towards periphery. Besette *et al.* (1985) reported yellow blotch diseases in *Pleurotus ostreatus*. Jandaik *et al.* (1993) reported that yellow blotch disease of *P. sajor-caju* was caused by *P. agarici.* The first symptom of yellow blotch disease was the appearance of yellow hazel brown or orange coloured blotches of varying sizes on pilei. In later stages, the infected

fruit bodies rot and emit foul smell. Suyama and Fuji (1993) reported *Pseudomonas* sp causing brown to dark brown spots and rot symptoms on cap and stem of oyster mushroom. According to Almeda and Mignucci (1998) *Burkholderia cepacia* was the causal agent of bacterial blotch of oyster mushroom. Pathak *et al.* (1998) reported reduction of mycelial growth by *Bacillus* sp. in *Lentinula* edodes. Another report of bacterial soft rot and blotches on the stipe and pilei of *Flammulina velutipes* and *P. ostreatus* as caused by *Erwinia carotovora* sub sp *carotovora* was made by Okamato *et al.*, 1999. Desrumaux *et al.* (2005) reported a bacterium of *Pseudomonas* group caused small, deep bacterial pits on fruit bodies of oyster mushroom and it spreads over the whole surface of fruiting body with larger slimy spots.

2.2 Fungal disease

There are mainly three fungal diseases *viz.,* dry bubble, wet bubble and cobweb disease reported from cultivated mushrooms. In milky mushroom Pandey *et al.,* 2003 first reported the incidence of cobweb disease. The first symptom appeared as patch of whitish mycelium on the casing soil, which rapidly grows into white fluffy mycelial growth covering the entire casing soil. The infected mushrooms showed browning and softening of the stipe, which gradually progressed towards the pileus. The older mycelium on casing soil and the infected tissues become pink in colour as disease progressed. Buvaneswari (2004) also reported the incidence of cobweb disease in milky mushroom caused by *Dactylium dendroides*.

First report of incidence of dry bubble disease on white button mushroom in India was by Seth *et al.* (1973). In early stages of infection typical onion shaped mushrooms and at later stages of infection, crooked deformed mushroom were produced. In 1977, Seth reported the incidence of cobweb disease on *A. bisporus* from Shimla and Chail. Symptoms of the disease seen as small circular, white patches of the mycelium on the surface of casing. As the disease progress, fluffy

white mould grows over the mushroom and the infected mushrooms become brown, begin to rot and die-off. In severe cases, the colour of white mould changes to pink or even red with age. The disease was caused by *D. dendroides*.

In India, Kaul *et al.* (1978) first recorded the incidence of wet bubble disease on button mushroom from Jammu and Kashmir, caused by *Mycogone perniciosa*. The initial symptom of the disease was development of monstrous shaped mushrooms. In advanced stages, the infected area become creamish brown and brown coloured drops are exuded from the bubble and emit an unpleasant odour. According to Dar (1985) the pathogen *Cladobotryum dendroides* found to attack all the developmental stages of the white button mushroom and the attacked mushrooms looked like cottony balls. He also reported that inoculation 12 day after casing recorded highest incidence of cobweb disease and the mean incidence of disease varied between 5.5 to 22.14 per cent.

Thapa (1985) reported that besides the *Agaricus brunnescens*, the pathogen *Verticillium fungicola* could infect *A. bitorquis* and was able to grow and sporulate on fruit bodies of different *Pleurotus* species with out expressing the typical symptoms of dry bubble disease. Jandaik (2002) recorded a higher per cent incidence of *V. fungicola* and *C. dendroides* compared to other fungal pathogens during white button mushroom cultivation. Bhatt and Singh (2003) reported that pathogen *C. dendroides* covered the stipe and edges of the curved pileus, converting the fruit bodies of *A. bisporus* into soft cottony balls. They also reported that inoculation of compost with different concentrations of *Hypomyces rosellus* showed a higher loss in yield (76.2 per cent) than when the pathogen inoculated on casing surface. Bayer *et al.* (n.d) reported infection of secondary invaders like *Penicillium* sp. on basidiocarp of white button mushroom and resulted brown discolouration.

The pathogen *C. verticellatum* causing cobweb disease was reported by Sharma and Jandaik (1994a) on *Pleurotus* sp. Rana and Singh (2002) reported

Cladobotryum sp. causing soft rot of oyster mushroom *P. sajor-caju* and early inoculation of the pathogen resulted in higher disease incidence and more reduction in yield than later inoculation. Sharma *et al.* (2006) described the symptomatology of cobweb disease in detail. The pathogen appeared as white fluffy mycelia over the beds of oyster mushroom. After creeping over the surface of sporophore it completely engulfed the young sporophore and mycelium showed conspicuous white cottony wefts resembling cobweb, the white mycelia turned yellowish pink on age. In severe cases a dense white mould develops over mushroom and fluffy cobweb change to dense mat of mycelium.

2.3 Competitor moulds/ weed moulds

Purkayastha (1984) observed green mould covering the entire surface of the casing soil with in two to three days of casing in milky mushroom. Pathak *et al.* (1998) reported incidence of *Trichoderma* spp., *Aspergillus* spp., *Rhizopus* spp. and *Coprinus* spp. in milky mushroom beds. Pandey and Tewari (2002) described green mould, *Chaetomium globosum* and *Coprinus* sp. as the major problematic weed moulds in mushroom cultivation. Pandey *et al.* (2003) reported simultaneous inoculation of paddy straw with *C. indica* and *T. harzianum* resulted in 80 percent inhibition of spawn growth. Krishnamoorthy (2004) and Gogoi *et al.* (2006) reported incidence of indicator mould *Coprinus* sp. in milky mushroom beds. The isolates of *T. harzianum* from the beds of *C. indica, P. sajor-caju* and *A. bisporus* have good activity of all the extra cellular lignocellulolytic enzymes (Sharma *et al.*, 2005b). Heera (2006) reported incidence of *Trichoderma* sp. during cultivation of *C. indica*.

Gupta *et al.* (1975) reported occurrence of olive green mould *Chaetomium olevaceum* as weed fungi in *A. bisporus* beds. In India Thapa and Seth (1977) first recorded 2-5 per cent crop loss due to *T. viride* during white button mushroom cultivation. Thapa *et al.* (1979) reported incidence of *C. globosum* during cultivation of *A. bisporus*. The earliest symptoms of the fungus consist of

greyish white fine mycelium in the compost ten days after spawning and initial spawn growth was delayed. Later many perithecia of olive green colour were formed with in two weeks of spawning. Seaby (1989) reported the green moulds appear in the substrate or in the casing layer. Surface of casing soil covered with mushrooms are severely spotted, often distorted. Sharma *et al.* (1991) reported 45 per cent yield loss by *T. viride* and two per cent loss by *Coprinus* incidence in *A. bitorquis* cultivation. Of the undesirable fungi *viz., T. harzianum, T. longibrachiatum, C. globosum* and *Epicoccum nigrum* on *A. bisporus* bed, maximum yield loss of 50 per cent was due to *Trichoderma* sp. (Tewari and Singh, 1991). Goyal *et al.* (1994b) reported that inoculation of bits with suspension of weed fungi *C. globosum, T. viride, Myceliophthora lutea, Papulospora byssina* and *Trichotheceum roseum* resulted in significant reduction in yield of white button mushroom.

Several moulds *viz., Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Chaetomium* spp. and *Coprinus* spp. were found to infest button mushroom and loss in yield in different *Pleurotus* spp. by the competitors was about 70 per cent (Sharma, 1995). He describes in detail the symptoms due to infection by *Trichoderma* sp. on *A. bisporus*. Fungal mycelium grows as cottony weft of greyish mycelium over the casing surface also cover whole of mushrooms with cottony web causing wet rot. Later green clusters of spore heads develop on affected tissues of mushroom. The parasitic forms of Trichoderma produced deep, sunken, brown lesions on pileus.

Sharma and Vijay (1995) reported the incidence of *Coprinus fimetarius* in *A. bisporus*. In infected beds mushroom spawn run was very poor and the bags were almost black with the slimy ink mass of cap and gills of Coprinus. Spawn run was patchy and the pinheads appeared very late. On autolysis *Coprinus* sporocarps produce slimy ink mass, which interfere with spawn run. A study conducted by Sharma and Vijay (1996a) revealed the incidence of brown plaster mould (90 per cent), false truffle (10-90 per cent), green mould, yellow mould and

wet bubble in white button mushroom. They also observed that inoculation of *T. viride* and *C. dendroides* on compost at the time of spawning resulted in loss in yield of 80.85 per cent and 66.66 per cent respectively.

Jandaik and Guleria (1999) reported that spawn run and yield of *A. bisporus* was reduced significantly, when *Trichoderma* sp. was inoculated at five days after spawn run. They also reported decrease in yield of *A. bisporus* corresponding to increase in level of inoculum. Raina (2005) reported that the yield loss varied between 7 to 42 per cent with *T. viride* and 18 to 62 per cent with *T. harzianum* when inoculated in compost at spawning. Inoculation of *T. viride* and *T. harzianum* in casing mixture resulted yield loss in number and yield of cut mushroom by 2.8-2.9 to 2.7-36.7 per cent respectively. Dayaram *et al.* (2007) reported the incidence of *T. viride* on *A. bisporus* as green spots. During spawn run period or after casing, small greenish spots appeared on the bed surface, it destroyed or lysed the mushroom mycelium and such beds failed to produce the sporocarps. They also reported the occurrence of *Aspergillus terreus* during cultivation of *A. bitorquis*. The initial symptoms appeared as small creamy white spots on the cap, which later increased in size and covered the larger area of cap. Finally, the entire fruiting body turned light pinkish in colour with rotting symptoms.

The growth rate of *T. viride* and *Rhizopus* sp. seems to be much higher than that of *A. bisporus* and *P. sajor-caju* (Sharma and Jandaik, 1980). In oyster mushroom *C. globosum* and *T. harzianum* act as strong competitors and inhibited the growth by 73 per cent (Pandey and Tewari, 1988). Pandey and Tewari (1989) reported *A. niger, A. terreus, Cladosporium oxysporum, Fusarium equiseti, F. moniliforme, Mucor* sp, *Nigrospora spaerica, Penicillium citrinum* and *T. harzianum* from various stages of oyster mushroom cultivation. Pandey and Tewari (1990) observed maximum inhibition of *P. sajor-caju* by *C. globosum* (73.07 per cent) followed by *Acremonium strictum* (44.23 per cent), *A. versicolor*

(42.30 per cent) and *T. harzianum* (40.1 per cent). According to Rajarathnam *et al.* (1992) growth rate of *Sclerotium rolfsii* was greater than that of *P. flabellatus*.

Das *et al.* (1993) described in detail the symptoms due to infection by *Trichoderma* on oyster mushroom beds. The growth was reported to start on beds 5- 10 days after spawning. A dark green patch spread over the entire substrate as white fluffy growth and suppressed spawn run. Balakrishnan (1994) described the occurrence of *Coprinus* sp. as a weed mould in oyster mushroom beds where other competitor moulds had already established. Rao *et al.* (1994) reported the presence of *Aspergillus* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp., *Mucor* sp., *Coprinus* sp. and *Chaetomium* sp. as contaminant in *Pleurotus* spp. Chitale and Singh (1995) reported that *Fusarium* sp, *Aspergillus* sp., *Trichoderma* sp., *Sclerotium* sp., *Coprinus* sp. etc are common weed fungi and competitor moulds during cultivation of *P. sajor-caju.*

Sharma and Vijay (1996b) reported the extend of yield loss caused by *T. viride* in *P. sajor- caju* and *P. ostreatus* on steam pasteurized substrate varied from 16-74 per cent and 18-90 per cent respectively and of *P. ostreatus* on chemically sterilized substrate from 5-45 per cent. They also reported that maximum reduction in yield was obtained when *T. viride* was inoculated at the time of spawning. In studies conducted by inoculating the contaminants the yield reduction of *P. eous* by *T. harzianum, A. flavus* and *A. niger* ranged between 17.23 – 77.27 per cent, 8.5- 70.91 and 11.77- 74.04 per cent respectively (Anandh *et al.,* 1999).

Pani (2000) noted the incidence of *Trichoderma* sp., *Sclerotium rolfsii* and *Coprinus* sp. during cultivation of paddy straw mushroom. He also noted that other weed fungi like *Curvularia* sp., *Alternaria* sp., *Gliocladium* sp., *Drechslera* sp. and *Rhizoctonia solani* showed significantly lower presence in beds of paddy straw mushroom compared to other weed moulds. Deepthi (2003) reported incidence of *T. viride, T. harzianum, A. niger* and *Coprinus* sp. during oyster

mushroom cultivation. Chaudhury and Dayaram (2006) identified *Coprinus* spp., *T. harzianum, T. viride, Penicillium* spp., *A. niger, A. flavus, Alternaria alternata, Rhizopus* sp., *Mucor* sp. and *Helminthosporium* sp. from naturally infected *P. sajor-caju*.

Fungi occurring in substrate and competing with mushroom mycelium for space and nutrition include *Alternaria solani, A. alternata, Arthrobotrys* sp., *Aspergillus niger, A. flavus, A. fumigatus, Cephalosporium* sp., *Coprinus* sp., *Cochliobolus* sp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Doratomyces stremonitis., Geotrichum* sp., *Gliocladium* sp., *Fusarium* sp., *Memnoniella echinata, Mucor* sp., *Penicillium* sp., *Paecilomyces varioti, Phialosphora* sp., *Rhizopus* sp, *Stachybotrys* sp., *Stilbum* sp., *Stysanus medius, S. rolfsii, Sordardia fumicola, Oedocephalum* sp., *T. viride, T. harzianum, Trichcurus* sp. and *Verticillium* sp. (Sharma and Jandaik,1980,1981a,1981b; Doshi and Singh, 1985; Singh and Saxena, 1987; Vijay and Sohi, 1987).

2.4 Characterization of pathogens

2.4.1 Bacterial pathogens

Paine (1919) identified *Pseudomonas tolaasii* as the causal organism of bacterial blotch disease in white button mushroom. Detailed biochemical study conducted by several workers revealed that *P. tolaasii* could best to be placed in biotypes (Biovar V) of *P. fluorescens* (Goor *et al.*, 1986 and Devos *et al.*, 1985). Krieg and Holt (1984) reported that *P. fluorescens* Biovar V showed positive oxidase, arginine dihydrolase, catalase, and gelatin liquefaction and negative levan formation, lipase, starch hydrolysis and nitrate reduction. According to Bradhury (1986) *P. tolaasii* was fluorescent pseudomonad, positive for oxidase, arginine dihydrolase, gelatin liquefaction and lipase. They showed negative reaction to levan, nitrate reduction and denitrification.

Bessette *et al.* (1985) decribed biochemical characters of *P. agarici* in detail. Oxidase, catalase, vogues proskauer reaction were positive and nitrate reduction, starch hydrolysis negative. Gluconate, benzonate and citrate were utilized for growth. According to Bradhury (1986) *P. agarici* produced fluorescent pigment on King's B medium, catalase, oxidase, citrate were positive and levan, potato soft rot, gelatin liquefaction, arginine dihydrolysis and nitrate reduction were negative. It was able to produce acid from arabinose, fructose, glucose, galactose, manitol, ribose and not from cellobiose, maltose, lactose, sucrose, mannose and inositol.

According to Krieg and Holt (1984) *Pseudomonas* spp. were gram negative, straight or curved rods, motile, strict aerobic and with polar flagella. They showed positive catalase and oxidase reaction. Nitrate can be used as an alternate electron acceptor. Bradhury (1986) reported that *Pseudomonas* spp. are straight or slightly curved gram negative rods, occur singly or in chains with polar flagella and few species can denitrify using nitrate. All are strict aerobes. They showed positive response to catalase and oxidase and some species were capable of producing pigments in King' B medium.

Smith *et al.* (1952) reported that *Bacillus* spp. produced small irregular shaped colonies on NA medium. According to Harrigan and Mc cane (1966) the species of *Bacillus* are rods capable of producing endospores and grew aerobically. Bradshaw (1973) described that *Bacillus* spp. are gram positive with rod shaped cells, spore forming and motile, generally proteolytic and are good fermenters. According to Bergey's manual of systematic bacteriology Bacillus are rod shaped gram positive, endospore producing motile bacteria. They are facultative aerobes, catalase and starch hydrolysis positive. According to Schaad (1992) *Bacillus* spp. are rod shaped, catalase positive, non acid fast and endospore forming bacteria.

2.4.2 Characterization of pathogenic / weed fungi

2.4.2.1 Cladobotryum dendroides/ Dactylium dendroides

It is an imperfect stage of *Hypomyces rosellus* with verticilloid conidiophore bearing cylindrical, oblong, obtuse, one to three septate. Conidia measuring 25-35 x 10-13 µm (Petch, 1938). Storey (1965) noticed the production of two celled conidia measuring 9-16 x 6-9 µm on a verticilliate conidiophore while observing the fungi *D. dendroides*. Bhatt and Singh (2003) described the morphological characters of *D. dendroides* as septate, hyaline hyphae, with 3-4 pointed and oppositely placed branches. The conidiophores erect, simple and branched in many whorls. Conidia were single elongated, pointed at the base, two celled, slightly constricted at the septa and measured 15-32 x 8-14 µm. Buvaneswari (2004) observed that the pathogen produced highly branched hyaline hyphae with pinkish tinge and verticilliate conidiophores bearing two to three celled conidia measuring 19.2-26.7x 9.3-11.3µm. Sharma *et al.* (2006) described the characters of *C. dendroides* as conidiophores slender, erect, septate and each branched into usually three pointed branchlets, conidia elongate, pointed at the base, with three septa 26- 32 x 10-14 µm size.

2.4.2.2 Penicillium sp.

Several workers reported *Penicillium* sp. as weed fungi in mushroom beds. According to Gilman (1957) *Penicillium* sp. produce creeping, septate, vegetative hyphae, conidiophores erect usually unbranched at the apex with a whorl of erect primary branches. Each primary branches produce metulae with phialides. Conidia borne in chains, which typically form a brush like head, globose, ovate or elliptical. Size of conidia 3.5 x 2 µm. Pal (2000) reported that *Penicillium* sp. usually produce bright coloured ascogonia, asci small, globose and often formed in chains. Ascospores hyaline usually bivavate and often ornamented.

*2.4.2.3 **Trichoderma*** **sp.**

Gilman (1957) enlisted the characters of *Trichoderma* sp. in detail. They produce sterile septate creeping hyphae. Conidiphores erect, branched, bearing conidial heads terminally. Conidia small, mostly globose, bright coloured or hyaline. Subramanian (1971) described the characters of *Trichoderma* sp. Conidiophores indefinite, consisting of an unbranched or branched hypha, bearing phialides laterally and terminally, phialides surmounted by heads, rarely in short chains. Spores hyaline or bright coloured and one celled. Saifuneesa (2001) reported that colonies of *Trichoderma* sp. attained nine cm diameter in seven days. Hyphae 2.81- 4.13 µm, conidiophore produced side branches, phialides meausure 1.5-11.25 x 1.88 µm, phialophores subglobose to oblong, pale green, 2.63-3.75x1.88 µm in size.

*2.4.2.4 **Coprinus*** **sp.**

Singer (1975) enumerated the characters of the genus *Coprinus* with the type species being *Coprinus comatus* as follows. Pileus usually conical or companulate initially and expand when old. Stipe central and more or less straight. Spore and spore print black..It decay and form a blackish slimy mass due to liquefaction or autodigestion. Geetha (1982) described in detail the characters of *Coprinus lagopus* (Fr.) Fr. As pileus usually cylindrical or oval turning to pointed and stipe with length 1 to 5 cm at first,.later to 10 to15 cm and spore size 12.5 x 5.5- 7µm. Purkayastha and Chandra (1985) described *C. comatus* as follows, sporophore produced singly, scattered or in clumps. Pileus 6-15 cm long, 2.5-5 cm wide cylindrical or oblong when young and tan or purplish when old. Stipe centrally placed, tapering at top, 3.5-8 cm long, 0.6- 1.5 cm thick, whitish smooth and hollow. Spores black, smooth, elliptical, 12-17 x 6-7 µm and black spore print.

2.4.2.5 Aspergillus sp.

According to Gilman (1957) *Aspergillus* sp. produced septate branched, colourless hyphae. Conidial apparatus developed as stalks and heads from foot cells. Conidiophores non septate or septate, enlarging upward and broadening in to globose or hemispherical vesicles bearing phialides. Conidia varying greatly in colour, size and shape. Subramanian (1971) enumerated the characters of *Aspergillus* as septate, branching, hyphae, colourless or bright coloured. Conidiophores distinct, arising from specialized, large, thick walled foot cells and terminating in globose or hemispherical or elliptical vesicle bearing numerous phailides. Conidia in chains, globose, oval or elliptical, one celled. Lal, (2000) described characters of *Aspergillus flavus* Link. in detail. Colonies fast growing heavily sporulating, conidial heads yellow to green. Conidiophores 10-12 µm, conidia sub globose to globose 3.5- 4.5 µm.

2.4.2.6 Chaetomium sp.

According to Gilman (1957) *Chaetomium indicum* produced black small, globose to verruciform perithecia, 180-160 µm firmly attached to the substratum by dark olive brown to black rhizoids. Lateral hairs rigid, septate and tapering to a blunt point, hyaline at tip and dark brown to black at base. Asci club shaped, eight spored 30 x 9.4 µm. spores hyaline when young and when mature dark brown, ovate to lemon shaped, 5.5 x 4.5 µm in size. Thapa *et al.* (1979) described the morphological characters of *Chaetomium globosum* Kunze ex steudel in detail. Colony thin, hairy, white to slight brownish in colour. Perithecia ovate or ellipsoid and evenly clothed with slender flexous hairs. Hairs were 3-4µm thick and 6-7 µm long. Asci oblong, evanescent and ascospores were dark 8-9.5 x 6-8 µm in size.

2.4.2.7 Curvularia **sp.**

Gilman (1957) described the characters of *Curvularia* sp. in detail. According to him mycelium branched, septate, sub hyaline or brown. Conidiophores brown, thread like and septate. Conidia acrogenous, olivaceous or brown, ellipsoidal or cylindrical, curved or bent and 3-4 septate. One of the central cells being larger and darker than terminal cells and measure 18 -29 x 10-18µm. Subramanian (1971) reported the characters of *Curvularia* Boedijn as follows. Hyphae branched, septate and subhyaline to brown. Conidiophore erect or decumbent, straight, bent or flexuous, septate, brown and geniculate toward the apex. Conidia produced singly and acrogenously at the tip, 3-4 septate. One of the central cells distinctly larger and darker than the terminal cells.

2.4.2.8 Sclerotium **sp.**

Mycelium densely floccose, bearing numerous dark coloured, black, hard, globose sclerotia 0.8- 2.5 mm in diameter (Gilman, 1957). Subramanian (1971) reported that *Sclerotium* sp. produced variously, elongate or globose or flattened sclerotia. Usually hard and dark coloured. According to Ou (1972), the fungus has many strains and variable morphology and grows luxuriantly on culture media, producing white aerial mycelium and abundant sclerotia.

2.4.2.9 Rhizoctonia sp.

Gilman (1957) reported that *Rhizoctonia* sp. produce sclerotia with out definite form, often grown together, with thinner undifferentiated edges, frequently embedded in the mycelium and bound together by mycelial strands. Subramanian (1971) enumerated the characters of *R. solani*. Mycelium highly variable in appearance. Fungus produce colourless hypahe, later become yellowish and then deep brown in colour. Sclerotia irregular in shape, closely adpressed to the host surface and varying in size.

2.5 Interaction of pathogenic or weed fungi and mushroom mycelium

Many workers tested antagonistic effect of pathogenic fungi on mushroom. Thapa *et al.* (1979) reported that in dual culture *C. globosum* spread at faster rate and dominated the mycelial growth of *A. bisporus.* Sharma and Jandaik (1980) reported that growth rate of *Rhizoctonia* sp. and *T. viride* were much more than that of oyster and button mushroom mycelia. These fungi grew on the mushroom mycelium and produced their spores on mycelial web of mushrooms. Doshi and Singh (1983) reported that *Rhizopus stolonifer, Mucor* sp, *Fusarium semitectum, F. moniliformae var ferbglutinans* and *Sclerotium rolfsii* overlapped the colonies of *P. sajor-caju..*

Pandey and Tewari (1990) recorded a faster growth rate of *T. harzianum* compared to *Pleurotus* mycelium and it overgrew the mushroom mycelium with no zone of inhibition. They also observed an inhibition against mushroom mycelium from 0.1 per cent to 53.7 per cent by *C. globosum.* Dar (1997) reported that contact between *C. dendroides* and *A. bisporus* in bixenic culture was started on third day after inoculation and the pathogen over grews the mushroom mycelium after contact.

Bhatt and Singh (2003) reported 14.06 per cent inhibition in the growth of *A. bisporus* by *Hypomyces roselles.* The rate of growth of *T. harzianum* was much faster than that of *C. indica* and it over grew the mushroom mycelium without any toxicity or zone of inhibition (Pandey *et al.,* 2003).

2.6 Effect of culture filtrate of weed fungi on the growth of mushroom mycelium

Culture filtrate of *Rhizopus* sp., *Stysanus medius, T. viride* and *Memmoniella echinata* reduced the growth of *A. bisporus and P. sajor-caju* (Sharma and Jandaik, 1980). Vijay and Sohi (1989) reported that the culture

filtrate of *Alternaria alternata, Cephalosporium asperum, Cochliobolus* spi*cifer, Drechslera bicolor, Phialo*sp*hora* sp., *Aspergillus niger* and *Mucor* sp. reduced the growth of *Pleurotus* sp by 10 to 100 per cent. They also reported that *Penicillium oxalicum* and *C. globosum* recorded 20-25 per cent inhibition and *Sclerotium rolfsii* gave 1- 8.7 per cent inhibition against *Pleurotus* sp. Pandey and Tewari (1990) reported that the volatile antibiotic produced by *T. harzianum* inhibited the growth of *P. sajor- caju* by 9.03 per cent after 144 h of inoculation.

Goyal *et al.* (1994a) reported a significant inhibition in the vegetative growth of *Agaricus bisporus* by the metabolites of *T. viride*, *C. globosum*, *Trichothecium roseum* and *Myceliophthora lutea*. Seshagiri and Eswaran (2002) reported the effect of cultural filtrates of the contaminants on the growth of *C. indica* and the effect was proportional to the concentration of culture filtrates. Raina (2004a) reported that culture filtrates of *T. harzianum* and *T. viride* reduced the mycelial growth of *Agaricus bisporus* by 72.29 and 69.23 per cent respectively. Incorporation of culture filtrate of *Trichoderma* sp. in compost at spawning delayed the process of spawn run by 5 to 7 days and pinning by 10 to 14 days in *A. bisporus* (Raina, 2005).

2.7 Seasonal occurrence of contaminants

Moorthy and Mohanan (1996) conducted a study on seasonal variation in contaminations in oyster mushroom farm for three years from 1984 to 1987. The study revealed that during 1984 to 85 and 1986-87 incidence of contaminants was highest in monsoon season. But during 1985-86, the contamination was highest in pre monsoon season. They also reported that contamination of mushroom bags was influenced not only by the seasonal variation but also by the capacity of utilization of mushroom farm.

Thakur *et al.* (2001) reported that maximum incidence of fungal competitors was observed in January and March. Siddique *et al.* (2004b) recorded

contaminants in mushroom beds through out the year and high frequency of contaminants during May to August with maximum incidence in June. Incidence of contaminants was less during September to March and least in January. Chaudhury and Dayaram (2006) reported that November was the best month for cultivation of *Pleurotus sajor-caju* and the maximum incidence of contaminants was observed in September and least incidence in December to January due to low temperature.

2.8 Methods of substrate sterilization

Soaking straw in clean water at 63-80^{0}C for three hours has been recommended for cultivation of *C. indica* (Purkayastha, 1984). Trivedi *et al.* (1991) suggested chemical sterilization of substrate as an effective method of maximizing the yield of *Calocybe* and reducing contaminants. Sterilizing the substrate with carbendazim (50, 100, 200 ppm) and dichlorovos (250 and 500 ppm) helps to check insect and mould infestation. Boiling of presoaked paddy straw of 3-5 cm length for 30-50 minutes was recommended for milky mushroom cultivation (Krishnamoorthy, 1995). Theradimani *et al.* (2001) reported presoaking of paddy straw in cold water for four hours followed by boiling for 30-40 minutes as a method for substrate sterilization in *Calocybe.*

Pandey and Tewari (2002) suggested pasteurization of straw by hot water or steam for one hour to avoid contamination by microorganism in milky mushroom. Pandey and Tewari (2003) devised solar pasteurization as an effective sterilization technique in the cultivation of *C. indica* and *Tricholoma giganteum*. Study conducted by Sharma *et al.* (2005a) revealed that *C. indica, A. polytricha* and *A. aegerita* were most sensitive to formaldehyde and failed to exhibit any mycelial growth even at 0.1 per cent concentration of formaldehyde.

Heera (2006) reported that solarisation of substrate resulted in higher number of sporophore and maximum yield of 602 g / Kg of substrate in milky

mushroom. She also reported that chemical sterilization and boiling of paddy straw produced 203g and 145g yield in milky mushroom. Sharma *et al.* (2006) reported that for the cultivation of milky mushroom pasteurization method was found to be the best followed by hot water treatment and autoclaving. Verma *et al.* (2007) reported that wheat straw supplemented with wheat bran (5 per cent) and sterilization with chemicals formalin 200ml + Bavistin 12g + Nuvan 100 ml per 100 liters of water gave significantly higher yield of 1398g/ 1.5 Kg substrate and biological efficiency of 93 percent during *Calocybe* cultivation.

Vijay and Sohi (1987) standardized method of chemical sterilization of wheat straw for cultivation of *Pleurotus* sp. in which wheat straw was treated with carbendazim (75 ppm) and formaldehyde (500 ppm) for 18 hours. Formalin was effective against some common fungal moulds (*Sepedonium chrysospermum, Papulospora byssina, V. fungicola*) occurring in mushroom but *T. viride* was completely resistant to it (Sharma *et al.*, 1997). Upamanya and Rathaiah (2000) reported that pasteurization of straw in boiling water recorded higher yield than steam pasteurization. Treatment of sugarcane trash with 2 per cent sodium hydroxide for cultivation of *P. sojor-caju* resulted in biological efficiency of 83 per cent (Chandrashekar *et al.*, 2001).

Addition of lime into soaking water during chemical sterilization of paddy straw resulted in good spawn run and higher yield in oyster mushroom (Deepthi, 2003). Mishra *et al.* (2004) reported that sugarcane trashs treated with sodium hydroxide (2 per cent) recorded maximum sporophore yield and 86.5 percent biological efficiency in *P. flabellatus*. Ram and Thakur (2005) reported substrate fumigated with formalin for 12 hours yielded 254 g and 72.57 per cent biological efficiency for *P. florida*.

2.9 Effect of phytoextracts against various pathogens

Pradeepa *et al.* (1990) reported that mycelial growth of *Pleurotus ostreatus* on PDA was considerably enhanced by extracts from *Cassia occidentalis, Lantana camera* and *Thithonia diversifolia*. Extract of *L. camera* suppressed the competitive weed moulds *Aspergillus* sp. and *Penicillium* sp. Raina and Jandaik (1994) reported toxicity of extracts of some commonly available plants against the growth of *Pseudomonas tolaasii*. Among different extracts used neem cake was found to be significantly superior to all others except garlic extract. Sharma and Jandaik (1994b) observed extracts from leaves of *Azadiracta indica, Eucalyptus treticonnis, E. crassipes* and garlic cloves inhibited mycelial growth of *T. viride in vitro* and all amendments increased mushroom yields when incorporated in compost that was inoculated with various moulds before spawning of *A. bisporus.*

Pani and Patra (1997) studied about the utilization of phytoextracts of some commonly available plants *viz., A. indica, Psidium guajava, Lantana camera, Sopindus trifoliata, Rawolfia serpentina, Lowsonia innermis, Aegle marmelos, Cynadon dactylon, Tamarindus indica, Eichornia crassipes, Adhatoda vasica, Moringa olifera, Pongamia glabra* and *Tagetes erecta* against major weed mould *Sclerotium rolfsii* in paddy straw mushroom. Among the phytoextracts leaf extracts of *T. indica* followed by extracts of *S. trifoliata* and root extract of *M. olifera* appeared promising in suppressing the growth of *Sclerotium rolfsii* with least interference of mycelial run of the mushroom in laboratory as well as in field condition.

Singh (1999) tested the efficacy of leaf extracts against fungal contaminants mainly *Aspergillus flavus, A.niger* and *Penicillium citrinum.* Among the different plants tested extracts of *Hyptis suaveolens* gave the best fungal control followed by extracts from *Adenocalymma allecea* and *Aegle marmelos.* Kumar (2001) reported that the spraying of phytoextracts on sterilized substrate

against competitor moulds in oyster mushroom beds. Raina *et al.* (2003) stated that plant extracts of *A. indica. Chrysanthemum* sp, *Tagetes erecta* and *E. hybrida* were at par with bavistin 50 WP in reducing the losses caused by *Trichoderma* sp. Mishra and Singh (2003) tested 27 botanicals against *Mycogone perniciosa* causing dry bubble disease of *Agaricus bisporus*. Among them water diluted extract of *A. marmelos*, *Cleome viscosa* and sesamum cake at 5 per cent concentration reduced the radial growth of the pathogen by 32 per cent and glycerol diluted extract of *A. marmelos* by 96 per cent.

Bhanwar and Thakur (2004) reported that leaf extracts of *Bixa orellana*, *Terminalia arjuna* and *Rawolfia serpentina* had severely affected the mycelial growth of *Pleurotus* spp and weed fungi. Among the weed moulds, *Aspergillus* sp was most sensitive to phytoextracts compared to *S. rolfsii* and *Coprinus* sp. Raina (2004b) reported that the essential oil of *Citrus sinensis* showed strong antifungal activity against *Alternaria alternata, Chaetomium globosum, Curvularia lunata, Fusarium oxysporum, F. solani, Absidia ramosa, A. niger, A. flavus; A. fumigatus, Verticillium fumicola* and *Helminthosporium oryze* and proved non toxic to *A. bisporus* mycelium. Shinde and Patel (2004) reported that garlic extract at 10 per cent concentration showed a complete inhibition of growth of *R. solani* and extract of ocimum leaf gave 11.8 per cent inhibition, no inhibition was recorded with neem leaf extract.

Siddique *et al.* (2004a) evaluated phytoextracts of some commonly available plants *viz., A. marmelos, Allium cepa, Alternanthera sessaelis, A. indica, Datura stramonium, Chromolaena odorata, Eichornia crassipes, Mentha* sp, *Murayya koenigii, Ocimum sanctum, Polygonum hydropiper, Psidium guajava* and *Wedelia chinensis* against contaminants of oyster mushroom mainly *T. harzianum and Pencillium notatum*. Among 20 extracts tested against *T. harzianum*, best treatment was onion followed by *A. marmelos and Wedelia chinensis*. Minimum mycelial growth of *Pencillium notatum* was recorded by extracts of guava, onion and mint.

Tewari *et al.* (2004) tested the efficacy of medicinal plants against *Xanthomonas campestris pv campestris* the black rot pathogen of cabbage. Extracts of *Adhatoda vasica, Achyranthus aspera, Withania somnifera, Alpinia galanga, Colocasia* etc, were found to inhibit the complete growth of pathogen at 5 per cent concentration. Neem leaf extracts at 10 per cent concentration was most effective in inhibiting the mycelial growth of *Sepedonium chrysospermum* (yellow mould) and was safe to the host fungus *A. bisporus* (Sharma and Kumar, 2005). They also reported that neem cake amended compost resulted in highest yield followed by neem leaves.

2.10 Biochemical changes in diseased mushroom

Purkayastha and Chandra (1976) reported protein content of *Calocybe* mycelium as 19.81 per cent. The accumulation of phenols may act as an active resistant factor in defense mechanism (Sharma *et al.*, 1983). Pathak *et al.* (1998) reported dried sporocarp of milky mushroom contains about 17.69 per cent protein and 3.4 per cent fibre. Buvaneswari (2004) reported an increase in phenol and protein content in *D. dendroides* infected milky mushroom compared to healthy one. According to Krishnamoorthy (2004) APK 2 variety of milky mushroom contains about 32.3 per cent crude protien and 9.85 per cent total carbohydrate. Ram (2004) reported dried milky mushroom contain 32.3 per cent protein, 4.5 per cent fat, 41 per cent crude fibre and 64.4 per cent carbohydrate. Heera (2006) reported crude fibre content in healthy milky mushroom isolates ranged between 16.02 to 24.1 per cent.

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Diseases of milky mushroom *Calocybe indica* (P&C) and their management" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period 2005 to 2007. The details of the materials used and the technique adopted for the investigation are described below.

3.1 Isolation and pure culturing of *Calocybe indica*

Isolation and maintenance of *C. indica* was carried out by standard tissue culture technique. For that a fresh mushroom was first surface sterilized using ethanol in the laminar flow chamber, then the mushroom was split in to two equal halves from pileus to stipe and a small piece of the tissue was scooped out with a sterile scalpel from the centre of the junction of pileus and stipe. The tissue was transferred into Petri dish plated with PDA medium under aseptic condition. The dishes were incubated at room temperature for seven days and observed for fungal growth. The mycelial growth was sub cultured periodically and maintained as pure culture.

3.2 Preparation of spawn

Spawn was prepared as per the method described by Sinden (1934). Sorghum grains were used for the preparation of spawn. The grains were cooked for 30 min in boiling water. The excess water was drained off and the grains were spread on a clean surface and mixed with calcium carbonate @ 40 g / kg of seed. The treated grains were filled in glucose drip bottles up to 2/3rd capacity and plugged with cotton. The filled bottles were then sterilized at 1.05 kg/cm^2 for 2 1/2 h in an autoclave. After cooling, these bottles were inoculated aseptically with mycelial growth from seven day old actively growing pure culture of *C. indica* and were incubated at room temperature for complete colonization of grains. The

spawn thus obtained as mother spawn was used for further spawn production and also to raise beds.

3.3 Survey and collection of diseased samples of milky mushroom

Survey was conducted in few farms each at Eravimangalm, Kodungallur and Kattilapoovam area in Thrissur district to study the occurrence of major diseases in milky mushroom cultivation (Plate 1). Based on the survey Agrowe farm at Ervimangalam that was regularly cultivating milky mushroom was selected. The selected farm was visited once in a month and the incidence of different diseases was recorded. The diseased mushroom samples were collected for further experimental studies. Disease incidence was expressed on the basis of per cent sporophore infected.

Incidence of diseases in milky mushroom beds laid in the mushroom production unit of Department of Plant Pathology was examined regularly. Presence of pathogens or weed moulds associated with milky mushroom cultivation was recorded.

3.4 Isolation of pathogens

From the naturally infected milky mushrooms collected from the four farms; both bacterial and fungal pathogens were isolated.

3.4.1 Pathogens associated with diseased sporophores

The bacterial and fungal pathogens associated with naturally infected milky mushroom were isolated on NA and PDA medium respectively. For that mushrooms showing different types of symptoms were collected separately from the mushroom farm. For the isolation of bacterial pathogen small pieces of infected sporophores were surface sterilized by using ethyl alcohol and were

Plate1: Milky mushroom farms visited

a. Eravimangalam

b. Vellanikkara

c. Kodungalloor

d. Kattilapoovum

thoroughly rinsed in sterile distilled water. Then the pieces were placed on a sterile slide with a few drops of sterile distilled water and cut into small pieces by using a sterile blade. From this, a loopful of suspension was taken and streaked on NA medium and incubated for 48 h at room temperature (Bessette *et al.*, 1985).

The fungal pathogens were isolated after surface sterilizing the diseased sporophore with ethyl alcohol and then cut into small pieces and transferred in to Petri dishes containing PDA medium and dishes were incubated under room temperature.

3.4.2 Weed fungi from contaminated beds

The mushroom beds were selected at random from the cropping house of selected mushroom farms. Right from spawning the substrate, the straw beds were examined from time to time for locating the weed moulds. Small pieces of straw colonized by weed fungal growth were surface sterilized with one per cent sodium hypochlorite solution and washed three times with sterile water. Then the pieces were transferred to PDA medium and the Petri dishes were incubated at room temperature (Pani, 2000).

3.4.3 Purification of pathogens

After incubation at room temperature for 24 to 48 h slimy fluid bacterial colonies were selected, then purified by repeated streaking on NA medium. Permanent stock cultures of the bacterial pathogens were maintained in sterile water taken in vials by inoculating loopful of pure culture of bacteria. The suspensions were stored under refrigeration and also under room temperature.

The fungal pathogens on the PDA medium were purified by hyphal tip method and sub cultured and maintained on PDA slants. The cultures were stored under refrigeration and also under room temperature.

3.5 PATHOGENICITY

3.5.1 Bacterial pathogens

Pathogenicity of the bacterial isolates was proved by using 24 to 48 h old culture. Inoculation of bacterial pathogens was done with bacterial suspension prepared in sterile distilled water having a dilution of 10^8 cfu/ml.

Inoculation was done by swabbing a thick suspension of bacterium on the surface of healthy sporophore disinfected with ethyl alcohol. Bacterial suspensions were also spray inoculated using an atomizer on healthy sporophore after disinfection (Besette *et al.*, 1985). Inoculated sporophores were kept under humid chamber and observed daily for the appearance of the symptom.

3.5.2 Fungal pathogens

Mycelial discs of seven day old pathogens grown on PDA medium were used for inoculating the casing soil of healthy bed. Ten mycelial disc were inoculated per bed. After inoculation the beds were incubated for a period of ten days and observed for the symptom expression on bed and sporophore (Buvaneswari, 2004).

3.5.3 Weed fungi

Colonization by weed fungi on substrate was tested by the method described by Pandey and Tewari (1990). Wet and sterilized (121^0C and 1.05 kg/cm^2 for 1 hr) paddy straw pieces with one cm length were used for filling test tubes of size 32 x 200mm @ 50g/tube. The tubes were filled with sterilized straw in three layers alternating with spawn. These tubes were inoculated separately with isolates of weed fungi, one mycelial disc in each layer. The tube inoculated only with spawn was kept as control.

3.6 SYMPTOMATOLOGY

The symptoms produced by different bacterial and fungal pathogens were studied in detail both under natural and artificial conditions. Damage caused by weed fungi was also studied.

3.7 IDENTIFICATION OF PATHOGENS

The pathogens associated with the diseases were identified based on cultural, morphological and biochemical characters.

3.7.1 Characterization of bacterial isolates

Characterization and identification of bacterial pathogens was done according to the methods recommended in Laboratory Methods in Microbiology (Harrigan and Mc Cane, 1966). Before each test a loopful of bacterial suspension from stock culture was transferred to nutrient agar plates and incubated under room temperature for 24 to 48 h. The resulting bacterial growth was used for each study.

3.7.1.1 Morphological characters

3.7.1.1.1 Colony Characteristics of bacterial isolates

Colony characteristics of the bacterial isolates were studied in NA medium. Dilute suspension of bacterial isolates was streaked on the medium and colony characters like size, shape, colour, were studied after 24 to 48 h of incubation.

3.7.1.1.2 Gram staining

Smears of 24 h old bacterial isolates were prepared on a clean glass slide and heat fixed by passing over the flame. The smear was then flooded with crystal violet solution for one minute and then washed in a gentle stream of running tap water. It was then flooded with Gram's iodine solution for one minute and again washed with water. Later the smear was decolorized with 95 per cent ethyl alcohol. After washing, the smear was stained with counter stain saffranin for one minute and the excess stain was washed off in tap water. The smear was then dried and examined under oil immersion objective of light microscope for Gram's reaction (Hucker and Conn, 1923).

3.7.1.1.3 Solubility in 3 per cent KOH

A loopful of each bacterial isolate was taken on a clean glass slide. One drop of three per cent potassium hydroxide was added over it and thoroughly mixed with the help of a needle. After eight seconds the loop was alternately raised and lowered off the slide surface to detect the presence or absence of viscous strand formation. Presence of viscous strand indicates Gram negative bacteria (Suslow *et al.*, 1982)

3.7.1.1.4 Endospore staining

Smears of 24 h old bacterial isolates were prepared on clean glass slide and heat fixed, and then a few drops of 1.5 per cent amidoblack was added and allowed to stay for 70 sec. Then the slide was washed under a gentle stream of running water and stained for 20 sec with 1 per cent carbol fuchsin and again washed thoroughly under tap water. The slide was blot dried and observed under microscope for endospores.

3.7.1.1.5 Pigment production

The bacterial cultures were streaked on King's A and King's B medium (Appendix 1) and incubated for one day. The dishes were observed under ultraviolet light for fluorescent pigment production around the colonies.

3.7.1.2 Physiological characters

3.7.1.2.1 Growth of bacterial isolates at different pH levels.

Nutrient broth with varying pH *viz.*, 5, 6, 7 and 8 was used for the study. The isolates were inoculated into the broths adjusted to above mentioned pH levels using 0.1N NaOH and 0.1N HCl. The growth of each isolate was measured after 48 h using a Bausch and Lomb spectronic 20 colorimeter adjusted to a wavelength of 660 nm. Uninoculated broth served as control.

3.7.1.2.2 Growth of bacterial isolates at different temperature

Growth of bacterial isolates was studied at varying temperature of 25, 30, and 35^{0} C. A loopful of each of 24 h old bacterial culture was inoculated into the nutrient broth and incubated at different temperatures mentioned above. The growth of each isolates was measured as described in 3.7.3.1.Uninoculated broth served as control.

3.7.1.2.3 Oxygen requirement

To determine whether the bacteria is aerobic or anaerobic, nutrient agar columns containing bromocresol purple in test tubes were inoculated by stabbing with 48 h old bacterial culture using a straight inoculation needle. To create an anaerobic condition, the agar surface in one of the tubes was sealed with molten plain agar to a depth of one cm. All the tubes were incubated at room temperature and observation was recorded.

3.7.1.3 Biochemical characterization

3.7.1.3.1 Catalase test

Smears of 24 h old bacterial isolates were prepared on clean glass slide and covered with a few drops of three per cent hydrogen peroxide. Effervescence indicates the presence of catalase in the culture (Capucino and Sherman, 1992)

3.7.1.3.2 Starch hydrolysis

Nutrient agar medium containing 0.2 per cent soluble starch (Appendix 1) was used for this test. The bacterial isolate was spot inoculated on the plated medium and kept for incubation. Starch hydrolysis was tested after four days of incubation, by flooding the agar surface with Lugol's iodine solution. A colourless zone around the bacterial growth in contrast to the blue background indicated positive starch hydrolysis.

3.7.1.3.3 Arginine dihydrolase reaction

For this test Thornley semisolid medium (Appendix 1) was used. About 5ml quantities of the medium was dispensed in test tubes and sterilized in autoclave at 121^0 C. The medium was inoculated by stabbing with a loopful of bacterial isolate. The surface of the medium was sealed with mineral oil. The tubes were incubated for seven days at room temperature. Any colour change from violet to pink or red was recorded at regular intervals for a period of seven days (Thornley, 1960).

3.7.1.3.4 Production of levan

Peptone beef extract medium (Appendix 1) containing five per cent sucrose was used for this test. Dilute suspension of the bacterial isolates was streaked over the plated medium and the growth characters were observed after 48

h. Presence of large, white domed and mucoid colonies characterized the production of levan from sucrose (Hayward, 1964).

3.7.1.3.5 Lipase test

Bacterial cultures were streaked on the Sierra's medium (Appendix 1) containing 10 per cent Tween 80 and incubated for 10 days. Dishes were examined daily for the presence of a dense precipitate around the bacterial growth which indicate lipid hydrolysis (Sierra, 1957).

3.7.1.3.6 Citrate utilization test

One day old bacterial cultures were streaked on agar slants of Simmon citrate medium (Appendix 1). The slants were incubated under room temperature. The colour change of slants from green to blue indicates utilization of citrate (Schaad, 1992).

3.7.1.3.7 Nitrate reduction test

The Nitrate reduction medium (Appendix 1) was dispensed in tubes, autoclaved and inoculated with 24 h old culture of bacterial isolates. The test tubes were then incubated at room temperature and tested for the reduction of nitrate at regular intervals up to 15 days. The test was performed by adding few drops of Griess Llosvay's reagent consisting of sulphanilic acid (0.8% in 5 M acetic acid) and dimethyl alpha-naphthyl amine (0.5% in 5 M acetic acid) to the bacterial culture. If no pink or red colour developed, it indicated that nitrate was present as such or reduced to ammonia and free nitrogen.

3.7.1.3.8 Oxidase test

Ready to use oxidase disc from HI MEDIA, Mumbai were used for the test. A disc was placed at the centre of glass slide. A loopful of inoculum was taken and rubbed on the disc. Time taken for the development of colour, if any, was noted.

3.7.1.3.9 Lecithinase test

Lecithinase test was done by spot inoculating bacterial cultures in Petri dishes containing NA medium with egg yolk (1 egg /200 ml). Then inoculated plates were incubated under room temperature from 24 to 48 h. After 48 h incubation plates were examined for the development of opaque zone around the bacterial growth.

3.7.1.3.10 Utilization of carbon sources

The basal medium used for the study was Haywards semisolid medium (Hayward, 1964). Nine ml of Haywards semisolid medium (Appendix 1) was dispensed in tubes and sterilized by autoclaving. Glucose, dextrose, mannose, lactose, maltose, fructose, sucrose, cellobiose, dulcitol, sorbitol, inositol and manitol were used as carbon sources. One gram of each carbohydrate was dissolved in 10 ml sterile distilled water and filter sterilized. One ml of the solution was added to 9 ml basal medium and mixed well. A control was maintained for each isolate with out any carbohydrate.

Bacterial suspension prepared by dispensing single colony in sterile distilled water, adjusted to 10^8 cfu /ml was used for stab inoculation. The tubes were incubated at room temperature. Observations on change in colour of medium were recorded periodically for one month.

3.7.2 Cultural and morphological characters of pathogenic / weed fungi

Cultural characters of pathogenic / weed fungi such as rate of growth, colour and formation of fruiting bodies in PDA medium were studied in detail.

Morphological characters of pathogenic / weed fungi like type colour and width of the hyphae, conidiophore, and conidia were recorded by slide culture technique (Riddle, 1950). Photomicrographs of the organisms were also taken.

3.7.2.1 Physiological characters

3.7.2.1.1 Growth of pathogenic / weed fungi at different temperature

Pathogenic / weed fungi inoculated in paddy straw agar (Appendix 1) medium were used for the study. After inoculation the dishes were incubated at different temperature *viz.*, 25, 30 and 35^0 C. Number of days taken for full growth was recorded (Ram and Pant, 2001).

3.7.2.1.2 Growth of pathogenic / weed fungi at different pH levels

Paddy straw agar medium with varying pH *viz.,* 5, 6, 7 and 8 was used for the study. The pH of the medium was adjusted using 0.1 N NaOH and 0.1 N HCl. The dishes were inoculated with 1cm agar disc of each fungus and incubated under room temperature. Number of days taken for full growth was recorded (Ram and Pant, 2001).

3.8 Interaction between pathogens / weed fungi and *C. indica*

3.8.1 Interaction between bacterial pathogens and *C. indica*

A mycelial disc of *C. indica,* having 1cm size was inoculated at the centre of Petri dish with PDA medium. After 48 h the bacterial isolates were inoculated

as a line of streak on either sides of *C. indica* leaving 2.25 cm from the edge of the Petri dish.

The Petri dishes were incubated at room temperature and observations on growth of milky mushroom were recorded till the growth in the control plates fully covered the medium. The per cent inhibition of growth over control was calculated by the formula suggested by Vincent (1947).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C- Growth of fungus in control (cm)

T- Growth of fungus in treatment (cm)

3.8.2 Interaction between pathogenic / weed fungi and *C. indica*

The antagonistic effect of pathogenic/ weed fungi was carried out by dual culture technique (Skidmore and Dickinson, 1976). Agar discs of *C. indica* (1 cm) were inoculated aseptically on one side of Petri dish and incubated at room temperature for 48 h. After this, mycelial disc of pathogen was inoculated in the same plate, 4cm away from the mushroom fungal disc. Three replications were maintained for each fungus. Monoculture of *C. indica* and pathogen served as control. Growth measurements were taken at regular intervals, after 24 h of inoculation of pathogen. The per cent inhibition of mycelial growth of milky mushroom over control was calculated as described in 3.8.1.

3.9 Effect of culture filtrate of weed fungi on *C. indica*.

The various weed moulds were grown in conical flasks containing 100 ml liquid malt medium. After sterilization the flasks were uniformly inoculated by 1 cm discs of 10 day old culture of weed fungi. These were incubated under room temperature for 10 days and then filtered. The filtrate was diluted with equal

quantity of malt extract medium (Appendix1). It was then autoclaved and poured in sterile Petri dishes. These were then inoculated with *C. indica* and incubated under room temperature. Malt extract medium with out culture filtrate served as control (Vijay and Sohi, 1989).

Observations on growth of *C. indica* were taken at an interval of 48 h till the growth in control plates fully covered the medium. The per cent inhibition by the filtrate was calculated as described in 3.8.1.

3.10 Antagonistic effect of weed fungi on linear mycelial growth of *C. indica*

Inhibitory effect of weed fungi on linear mycelial growth of milky mushroom was done by the method described in 3.5.3. Linear growth of *C. indica* between two spawned layers was measured at 5, 10 and 15 days after incubation and per cent inhibition over control was calculated.

3.11 Influence of seasonal variations on the incidence of weed fungi

3.11.1 Lay out of mushroom beds

Mushroom beds were prepared by following the standard compact poly bag method of mushroom cultivation (Baskaran *et al.,* 1978) using the paddy straw as substrate. The substrate was chemically sterilized using mixture of carbendazim (75ppm) and formaldehyde (500 ppm) (Vijay and Sohi, 1987).

Chopped paddy straw of 5 -10 cm length was soaked in water containing the above chemicals for 18 h. The excess water was drained off and spread over a clean area for drying. The moisture content in straw was reduced to optimum level and was used for bed preparation. For the preparation of mushroom beds poly bag of size 30 x 60 cm with 150-200 gauges was used. Thirty holes with 0.5mm size were made on each polythene bag for air circulation and the bottom was tied with twine. The perforated polythene bag was filled up to five cm height with

processed straw and pressed with hand for making it even. Then 20-25 g spawn was sprinkled over the filled straw around peripheral region. A second layer of processed straw was filled and spawned as above. The process was repeated four times. Finally the bag was closed tightly with twine. For filling one bed 125 g. spawn was used. The spawned bags were then transferred to incubation room for spawn run.

After completion of spawn run the beds were horizontally cut in to equal halves and cased with soil: sand: cow dung mixture prepared in 1:1:1 ratio. The pH of the casing mixture was adjusted to 8-8.4 with calcium carbonate. Over each half bed, casing soil was applied to a thickness of two cm from the surface of mycelial growth. Beds after casing kept in cropping chamber. Roof of cropping chamber was made of U V stabilized high density polythene film. Orchid shade net was placed above the U V film in order to reduce the light intensity. The size of cropping chamber was 8 x 2.5 x 2 m.

Ten numbers of beds were made at fortnightly intervals by the above method. The beds were examined regularly for the occurrence of weed moulds from spawning to casing. Weather parameters like mean monthly temperature, mean relative humidity in and outside the farm and monthly rainfall were recorded. Total number of beds damaged was noted and per cent weed mould incidence was calculated.

Per cent incidence of weed fungi =

$$\frac{\text{Total number of beds damaged with weed fungi}}{\text{Total number of beds observed}} \times 100$$

3.12 Effect of weed fungi on growth and yield of milky mushroom

Effect of weed fungi on growth and yield of milky mushroom was studied by inoculating mycelial disc of weed fungi into milky mushroom beds. Mushroom

beds were prepared by following the compact poly bag method using sterilized paddy straw as substrate. Mycelial disc of (1 cm) weed fungi were inoculated over filled paddy straw around peripheral region at various points in two lower layers. Layer spawning was done and polybags were made compact, tied at the top. The spawned bags were then transferred to an incubation room for spawn run. After spawn run, the beds were cased with casing mixture and transferred to cropping room.

Area covered by mycelial growth of *C. indica* was taken using a graph paper and expressed in per cent, number of days taken from spawn run to harvest, number of sporophore and average yield per bed were recoded.

3.13 *In vitro* evaluation of different methods of substrate sterilization on the linear growth of *C. indica*

This experiment was conducted to study the nature of mycelial growth of milky mushroom due to different sterilization methods. Chopped paddy straw (1cm) was sterilized by different methods and filled in test tubes, were used for the study. Different methods of sterilization used were chemical sterilization, over night soaking, boiling, chemical sterilization along with 0.2 per cent calcium carbonate, chemical sterilization along with 0.02 per cent NaOH and pasteurization. Three replications were maintained for each treatment.

3.13.1 Chemical sterilization

The straw pieces were soaked in water containing carbendazim (75 ppm) and formaldehyde (500ppm) for 18 h. Excess water was drained off and spread over a clean area for drying.

3.13.2 Over night soaking

In this treatment, paddy straw pieces were soaked in water for 18 h and excess water was drained off and spread over a clean area for drying.

3.13.3 Boiling

In boiling, paddy straw pieces (1cm) were pre soaked for 12 h in water. After dewatering fresh water was added and boiled for 30 min. Dewatered, cooled and spread over a clean surface under shade for few hours to attain the correct moisture level.

3.13.4 Chemical sterilization along with 0.2 per cent calcium carbonate

Chopped paddy straw (1cm) was soaked in water containing carbendazim (75ppm) and formaldehyde (500 ppm) and 0.2 per cent calcium carbonate for 18 h. Excess water was drained off and spread over a clean area for drying.

3.13.5 Chemical sterilization along with 0.02 per cent NaOH

Substrate was soaked in water containing carbendazim (75ppm), formaldehyde (500 ppm) and 0.02 per cent sodium hydroxide for 18 h. Then the excess water was drained off and further moisture content was reduced by shade drying.

3.13.6 Pasteurization

The straw pieces were soaked in water for 18 h. Then the pre wetted straw was heated at 65-75^0 C for 6-7 h.

After shade drying the processed paddy straw were filled in test tubes of size 38 x 200 mm @50 g per tube and inoculated with spawn of *C. indica* @ of 2g per tube. The tubes were filled with treated straw in three layers alternating with spawn and plugged with cotton. Then the tubes were incubated under room temperature and observations on linear growth of *C. indica* between spawned layers were recorded after 5, 10 and 15 days of incubation (Sharma *et al.*, 2006).

3.14 On farm evaluation of different methods of substrate sterilization in milky mushroom cultivation

The effect of different methods of sterilization on growth and yield of milky mushroom were studied. Different methods of sterilization used were chemical sterilization, chemical sterilization along with 0.2 per cent calcium carbonate, boiling, chemical sterilization along with 0.02 per cent NaOH and boiling along with 0.2 per cent calcium carbonate. For each treatment chopped paddy straw pieces of 5-10 cm length were used. The substrate was sterilized by the methods described in 3.13.

After the sterilization, moisture in the substrate was reduced by shade drying. Then the substrate was used for bed preparation. Four replications were maintained for each treatment. All the treated beds were incubated under identical conditions for spawn run. After spawn running the beds were cased with casing mixture and the beds were transferred to cropping chamber. Observations on nature of mycelial growth, number of days taken from casing to harvest, number of sporophore produced per bed, average sporophore yield per bed and natural incidence of weed moulds / diseases were recorded.

3.15 *In vitro* effect of phytoextracts on mycelial growth of milky mushroom and selected pathogens

3.15.1 Preparation of phytoextracts

Fresh leaves of selected plants *viz.*, neem (*Azadirachta indica)*, thulasi (*Ocimum sanctum*), marigold (*Tagetes erecta)* garlic bulbs (*Allium sativum)*and turmeric rhizomes(*Curcuma longa*) were collected separately and washed thoroughly in running tap water. The plant parts were surface sterilized using one per cent sodium hypochlorite solution and washed in three changes of sterile water. Extract was prepared by macerating the plant parts using sterile pestle and mortar with equal quantity of sterile water (w/v) under aseptic condition and was filtered through clean, sterile muslin cloth. This extract was used as crude plant extract (100 per cent) solution (Siddique *et al.,* 2004a).

3.15.2 Screening of phytoextracts against bacterial pathogens

The inhibitory effects of phytoextracts against four bacterial pathogens were studied. To achieve five per cent concentration 5 ml of phytoextracts were dispensed in 95 ml molten PDA medium at temperature of 40^0C. Similarly other concentrations (10 and 15 per cent) of plant extracts were prepared in PDA medium. One ml of bacterial suspension having 10^8 cfu/ml was transferred to sterile Petri dishes, to that medium phytoextracts of required concentrations was poured and swirled thoroughly to get uniform distribution. After solidification the dishes were incubated at room temperature. Petri dishes containing bacterial suspension mixed with medium alone served as control. After 48 h of incubation number of bacterial colonies in treatment and control was recorded (Tiwari *et al.*, 2004).

3.15.3 Screening of phytoextracts against pathogenic /weed fungi

Poison food technique was used for screening the phyto extracts against pathogenic/ weed fungi. Three different concentrations of the extracts (5, 10, and 15 per cent) were tested. For that, five ml of crude extract (100 per cent) was added to 95 ml of molten PDA medium (40^0C) and mixed thoroughly. Similarly the other concentrations of plant extracts were prepared in PDA medium and transferred 15 ml of the medium in to each Petri dish. An agar disc of one cm diameter from10 day old culture of test fungi were inoculated at the centre of the dish. Three replications were maintained for each treatment. The basal medium with out extract served as control. All inoculated Petri dishes were incubated at room temperature. Growth of the test fungi was measured till the mycelial growth touched the periphery in control plate.

3.16 *In vivo* evaluation of selected phytoextracts

Based on the *in vitro* study the best concentrations for each plant extracts were selected. The selected extracts were prepared by grinding the plant parts with equal amount of water using mixer grinder. The prepared extracts were sprayed on sterilized paddy straw. For spraying of 5, 10 and 15 per cent concentrations on 1 kg sterilized paddy straw, 50, 100 and 150 ml of crude extract (100 per cent) was used respectively. The best sterilization methods selected for *in vivo* study includes chemical sterilization along with calcium carbonate and sterilization by boiling. Substrates sprayed with extracts were used for bed preparation and substrate treated with distilled water was used as control. All the treated substrates were shade dried to correct moisture level. The beds were prepared by the treated substrate following the compact poly bag method.

Treatments: 12

Replications: 3

Design : CRD

T1 : Chemical sterilization + $CaCO_3$ and 5 per cent Garlic extract

T2 : Chemical sterilization + $CaCO_3$ and 5 per cent Ocimum leaf extract

T3 : Chemical sterilization + $CaCO_3$ and 10 per cent Turmeric extract

T4 : Chemical sterilization + $CaCO_3$ and 15 per cent Neem leaf extract

T5 : Chemical sterilization + $CaCO_3$ and 15 per cent Marigold leaf extract

T6 : Chemical sterilization + $CaCO_3$ and Distilled water

T7 : Sterilization by boiling and 5 per cent Garlic extract

T8 : Sterilization by boiling and 5 per cent Ocimum leaf extract

T9 : Sterilization by boiling and 10 per cent Turmeric extract

T10 : Sterilization by boiling and 15 per cent Neem leaf extract

T11 : Sterilization by boiling and 15 per cent Marigold leaf extract

T12 : Sterilization by boiling and distilled water

The spawned beds were incubated under identical condition. After completion of spawn running the beds were horizontally cut into two halves and cased with soil: sand: cow dung (1:1:1) mixture (pH 8.4) and transferred to cropping chamber. After the first pinhead emergence natural incidence of bacterial diseases was recorded. During the subsequent pinhead emergence, two days after emergence treatments were sprayed on sporophore. One spray each was given before the second and third harvest. Observations on nature of mycelial growth, incidence of weed fungi, number of days from casing to harvest, average sporophore yield per bed, incidence of bacterial disease (before and after spraying) and severity of disease were recorded.

3.16.1 Assessment of disease incidence and severity of bacterial diseases

Sporophores produced in each bed were observed for disease incidence

$$\text{Per cent disease incidence} = \frac{\text{Total number of Sporophore infected}}{\text{Total number of sporophore produced}} \times 100$$

Score chart for bacterial diseases

Grade	Description
0	No symptom
1	< 10 per cent infected sporophore
2	10-25 per cent infected sporophore
3	25-50 per cent infected sporophore
4	50-75 per cent infected sporophore
5	>75 per cent infected sporophore

Per cent disease severity was calculated by using the formula suggested by Wheeler (1969)

Per cent disease index =

$$\frac{\text{Sum of all disease ratings} \times 100}{\text{Total number of sporophores observed} \times \text{Maximum disease grade}}$$

3.17 Biochemical changes in milky mushrooms infected with bacteria

Five days old sporophores were selected for biochemical analysis. Both healthy and diseased sporophores were analyzed for the following parameters.

3.17.1 Crude protein

The per cent of nitrogen in the sample was determined by microkjeldahl method and the crude protein was determined by multiplying per cent of nitrogen by 6.25.

Both healthy and diseased mushrooms were dried and powdered. Digest 0.5 g of the sample with 10 ml of concentrated sulphuric acid and with pinch of digestion mixture (Sodium sulphate and Copper sulphate 20:1) for about six hours till the solution become clear. The digested material was made up to 100 ml. Ten ml of digested material was transferred to the distillation apparatus. A 100 ml conical flask with 5 ml of 4 per cent boric acid solution and 2-4 drops of methyl red and bromocresol green mixed indicator was placed at the condenser tip. Then eight to ten ml of NaOH $Na_2S_2O_3$ solution was added to the distillation flask. About 15 ml distillate was collected and it was titrated against 0.02N HCl. The end point was indicated by appearance of a violet colour.

$$\text{Per cent of nitrogen} = \text{Titre value- Blank value} \times \frac{0.02}{0.5} \times \frac{100}{10} \times 0.014$$

3.17.2 Phenol

One gram of fresh sporophore from both healthy and diseased was homogenized using 10 ml of 80 per cent ethanol separately. The homogenate was centrifuged at 10000 rpm for 20 min. The supernatant was evaporated to dryness and the residue was dissolved in 5 ml of distilled water. To this 0.25 ml of Folin - Ciocalteau reagent was added. After three minute one ml of 20 per cent sodium carbonate was added and mixed thoroughly. Then the tubes were placed in boiling water for one minute and cooled. The absorbance was measured at 650 nm against a reagent blank. The phenol content was expressed in μg of catechol per gram of mushroom (Zieslin and Zaken, 1993).

3.17.3 Crude fibre

Two mg of dried sample of healthy and diseased mushroom was boiled separately with 100 ml of concentrated sulphuric acid (1.25 per cent) for 30 min, by adding bumping chips. The digested sample was filtered through a muslin cloth and washed with boiling water until the washings were no longer acidic. The sample was then boiled with 100 ml sodium hydroxide (1.25 per cent) for 30 minute. The sample was then filtered through a muslin cloth and washed with boiling water until the washings were not alkaline. The sample was washed with 25 ml of 1.25 per cent boiling sulphuric acid, 50 ml of boiling water and 25 ml of alcohol. The residue was removed and transferred to pre weighed Petri dish. The residue was dried till a constant weight was obtained (Sadasivan and Manickan, 1992).

$$\text{Per cent crude fibre} = \frac{\text{Dry weight of fibre}}{\text{Weight of sample (2g)}} \times 100$$

3.17.4 Sugars

Sugar content was estimated by anthrone method (Hedge and Hofreiter, 1962).

Weigh out 100 mg of fresh healthy and diseased mushrooms and transferred to separate test tubes. It was hydrolyzed by keeping it in boiling water bath for three hours with 5 ml of 2.5 N Hydrochloric acid. Then the tubes were cooled to room temperature and neutralized with sodium carbonate until the effervescence was ceased. The tissue was ground and the volume made up to 100 ml and then centrifuged at 5000 rpm for 15 min. The supernatant collected was used as aliquot for analysis. From the supernatant 0.5 ml and 1 ml of aliquot were drawn for analysis, volume was made up to one ml using distilled water and added four ml of anthrone reagent. Heated for 8 min in boiling water bath and then rapidly cooled to room temperature. The absorbance was measured at 630 nm

against a reagent blank and the sugar content was expressed in µg/g of mushroom tissue.

3.18 Statistical analysis

Analysis of variance was performed on the data collected in the experiments using statistical package of MSTAT (Freed, 1986). Multiple comparisons of the means were done using DMRT. Correlation study also conducted, to find out the influence of weather parameters on incidence of weed fungi.

Results

4. RESULTS

Investigations on different diseases of milky mushroom (*Calocybe indica*) and various aspects of disease such as etiology, symptomatology, seasonal occurrence and development of ecofriendly management strategy were carried out and the results are presented below.

4.1 Isolation and purification of *C. indica*

The isolation of *C. indica* was done as per the standard tissue culture technique. Visible white mycelial growth was observed from the second day onwards. Initially the growth was very feeble. It took 14 days to reach full growth in 9cm Petri dish. The culture was maintained on PDA slants by periodical sub culturing.

4.2 Preparation of spawn

Spawn was prepared in sorghum grains as described under 3.2 and was used for the preparation of milky mushroom beds.

4.3 Survey on the occurrence of diseases of milky mushroom

Survey was conducted on the occurrence of diseases in milky mushroom in private farms, one each at Eravimangalam, Kodungalloor, Kattilapoovum in Thrissur district and in the mushroom production unit, Department of Plant Pathology, College of Horticulture, Vellanikkara. The major diseases observed in the above mentioned farms are presented in Table 1.

Table 1: Details of survey conducted in milky mushroom farms

Sl.No	Farms	Diseases
1	Eravimangalam	Bacterial blotch and malformation of sporophore Weed mould incidence
2	Kattilapoovum	Cobweb disease
3	Kodungalloor	Fungal infection and bacterial blotch on sporophore Weed mould incidence
4	Vellanikkara	Bacterial blotch on sporophore and weed mould incidence

Bacterial blotch on sporophore was the common problem in all farms except at Kattilapoovum where severe incidence of cobweb disease was noticed. At Kodungalloor fungal infection on very young sporophore was also noticed. Occurrence of weed fungi during spawn running stage was observed at Eravimangalam, Kodungalloor and Vellanikkara. Malformation of sporophore was also noticed at Eravimangalam.

Among the local private farms Agrowe mushroom farm at Eravimangalam, which was continuously cultivating milky mushroom was selected for visiting once in a month for one year. Other farms have temporarily stopped mushroom cultivation due to various diseases. Monthly observations on diseases and percent disease incidence are presented in Table 2.

During the survey both biotic and abiotic problems were observed. Bacterial blotch was the major biotic problem observed in almost all months in more or less severe form. It was severe in April with 66.7 per cent incidence. Lowest disease incidence was recorded in December (18.6 per cent). Abiotic problems like, malformations, scaling and splitting of the mushroom stipe were observed during June, August and September, which recorded 52.6, 41.5 and 90 per cent incidence respectively. Malformed sporophores were distorted with

Table 2: Major diseases observed in Agrowe mushroom farm, Eravimangalam

Sl.No	Period 2006-2007	Diseases	Per cent disease incidence
1	April	Bacterial blotch on sporophore	66.7
		Malformation of sporophore	16.6
2	May	Bacterial blotch on sporophore	46.6
		Malformation of sporophore	29.3
3	June	Malformation of sporophore, scaling and spliting of stipe	52.6
4	July	Bacterial blotch on sporophore	36
5	August	Malformation of sporophore, scaling and splitting of stipe	41.5
		Weed mould incidence	35
6	September	Malformations, scaling and splitting of stipe	90
7	October	Weed mould incidence	54
8	November	No production	
9	December	Bacterial blotch on sporophore	18.6
10	January	Bacterial blotch on sporophore	25.4
11	February	Bacterial blotch on sporophore	32.5
12	March	Bacterial blotch on sporophore	45.3
		Weed mould growth in mushroom beds	8

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sunken centre, bent and twisted stipe and scaling and splitting of stipe were also noticed (Plate 2a). During the spawn running stage patchy growth of mushroom mycelium and weed mould growth were recorded during August, October and March.

4.4 Isolation of pathogens associated with diseased sporophore

Diseased sporophores were collected from the three mushroom farms *viz.,* Eravimangalam, Kodungalloor, Kattilapoovum and also from the mushroom production unit, Department of Plant Pathology, College of Horticulture, Vellanikkara, for the isolation of pathogens. The details of the pathogens isolated from the diseased sporophores are presented in the Table 3.

4.4.1 Bacterial pathogens

Sporophores showing bacterial blotch, discolourations and rotting were collected from the various farms.

Pathogenic bacteria were isolated from infected mushrooms in NA medium and yielded six different isolates *viz.,* B_1, B_2, B_3, B_4, B_5 and B_6 .Colonies were identified based on uniform shape, size and colour. The bacterial isolates B_1 B_3 and B_4 were isolated from Agrowe farm at Eravimangalam. Isolates B_2 and B_5 were obtained from the farm at Kodungalloor and B_6 from Vellanikkara. The cultures were purified by repeated cycles of streaking. Single colonies were selected and stored in sterile distilled water under refrigerated condition and also under room temperature.

Table 3: Details of pathogens / weed fungi isolated from milky mushroom farms

Sl. No	Location	Stage of growth	Pathogens / Weed fungi
1	Eravimangalam	Sporophore	Bacterial isolates B_1, B_3, and B_4
		Spawn running	*Rhizoctonia* sp. *Coprinus* sp. *Curvularia* sp. *Chaetomium* sp. *Sclerotium* sp.
2	Kodungalloor	Sporophore	Bacterial isolates B_2 and B_5
			Penicillium sp.
		Spawn running	*Aspergillus* sp. *Trichoderma* sp. *Sclerotium* sp.
3	Kattilapoovum	Sporophore	*Dactylium* sp.
4	Vellanikkara	Sporophore	Bacterial isolate B_6
		Spawn running	*Coprinus* sp. *Rhizoctonia* sp. *Sclerotium* sp. *Curvularia* sp. *Chaetomium* sp. *Aspergillus* sp. *Trichoderma* sp.

4.4.2 Fungal pathogens

Small diseased mushroom pinheads with greyish green fungal growth were collected from the Kodungalloor farm and upon isolation fungal species belonging to *Penicillium* sp. was obtained.

Sporophores with fluffy white mycelial growth and brown or pink discolourations were collected from milky mushroom farm at Kattilapoovum. From this specimen pathogen belonging to *Dactylium* sp. was isolated. The cultures were purified by hyphal tip method and maintained on PDA slants.

4.4.3 Isolation of weed fungi from contaminated mushroom bed

Various types of weed fungi were isolated from mushroom beds in mushroom production unit and also from local private mushroom farms. The isolation yielded seven types of fungal colonies belonging to seven different genera, namely *Rhizoctonia* sp., *Chaetomium* sp., *Aspergillus* sp., *Curvularia* sp., *Trichoderma* sp., *Coprinus* sp. *and Sclerotium* sp. Weed fungi isolated were purified by hyphal tip method and maintained on PDA slants.

4.5 Pathogenicity

4.5.1 Bacterial isolates from sporophore

The bacterial isolates obtained from three locations were inoculated separately on healthy sporophores by swabbing with cotton soaked in bacterial suspension. The sporophores inoculated showed infection with in three to four days. The bacterial isolates were re-isolated from the infected sporophores and colonies showed similarity with previous cultural characters on NA medium.

4.5.2 Fungal isolates from sporophore

Pathogenicity of the two fungal isolates was tested by inoculating in casing soil. Inoculation of *Dactylium* sp. showed the typical symptoms of cobweb disease as white mycelial patches on the surface of casing soil after nine to ten days of incubation. Gradually it attacked the sporophore and lead to the rotting of mushroom. The pathogen was re-isolated from the inoculated sporophore.

Growth of *Penicillium* sp. was observed on the surface of casing soil after five days of inoculation and it prevented the development of pinheads of mushroom. The pathogen re-isolated from the infected beds showed same type of colony characters as that of the original culture.

4.5.3 Weed fungi

Substrate colonization by the seven weed fungi isolated from mushroom bed was proved by simultaneous inoculation of mycelial disc of test fungi and milky mushroom spawn into test tubes containing sterilized paddy straw. All the weed fungi grew rapidly over the paddy straw. One week after inoculation *Rhizoctonia* sp., *Sclerotium* sp., and *Trichoderma* sp. showed inhibitory activity against *C. indica* by over growth. Other weed fungi like *Chaetomium* sp., *Aspergillus* sp., *Coprinus* sp., and *Curvularia* sp. took ten days for same inhibitory effect.

4.6 Symptomatology

Symptomatology of various diseases caused by the different pathogens collected from four farms was studied under natural and artificial condition. Types of symptoms varied depending on pathogens, but no variation in symptom was noticed among the locations.

4.6.1 Symptomatology of bacterial disease

Natural incidence of bacterial infection on sporophore was noticed both on pileus and stipe. At first the bacterial isolates produce a yellow to light brown discolouration on sporophore, which is superficial in nature. Later with in 2-3 days underlying tissues were damaged and produced typical water soaked lesions and slimy bacterial growth was observed on the infected areas. If the favourable conditions persist the lesions deepened and were filled with slimy bacterial growth. Finally the lesions widened and deepened resulting in rotting of whole sporophore and emitted foul smell (Plate 2b).

Under artificial inoculation all the six bacterial isolates produced similar symptoms. Initially yellow to light brown discolouration appeared which later developed into water soaked lesions. Later the adjacent tissues become water soaked and slimy bacterial growth was observed. Four to five days after inoculation whole sporophore become rotten and emitted foul smell.

4.6.2 Symptomatology of fungal diseases

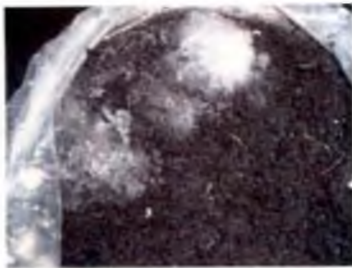
4.6.2.1 Cobweb

Natural incidence of cobweb disease was observed at any stage after casing. Symptoms of the disease started as small, circular white patches of mycelial growth on the surface of casing soil. As the disease progressed, mycelial growth spread over the casing soil and a fluffy white fungal growth developed on basal portions of the stipe. The fluffy growth spread fast on sporophores, which later discoloured, softened and finally rotten. Young sporophores were discoloured and deformed and rotten earlier than mature ones. The fluffy mat of mycelium turn to pink or even red with age (Plate 3a).

Under artificial condition, nine to ten days after inoculation the symptom appeared as white mycelial patches on the surface of casing soil. Later it

Plate 3: Symptoms of fungal infection

a. Cobweb disease



b. Penicillium infection on sporophore

c. Nature of damage by weed fungi

Rhizoctonia solani | *Chaetomium indicum* | *Sclerotium* sp.

progressed towards the sporophore and attacked the stipe producing fluffy mycelial growth. Finally entire sporophore was decayed.

4.6.2.2 Penicillium infection

Infection of *Penicillium* sp. was noticed on pinheads as brown discolouration. Later greyish green growth of fungus was observed on pinheads and the casing soil surface (Plate 3b). The growth of fungus on casing surface prevented further pinhead emergence. The infected pinheads were small and suppressed and failed to complete its development. Upon artificial inoculation the fungal growth was covered over the entire casing surface with in 7-10 days and prevented the production of pinheads.

4.6.3 Nature of damage caused by weed fungi

Numerous weed fungi were noticed growing in the mushroom beds during spawn running stage. The nature of damage by weed fungi was identical. The attack of weed fungi was initiated at early stage of spawn run, but the damage become visible only six to seven days after spawning.

4.6.3.1 Rhizoctonia sp.

Incidence of *Rhizoctonia* sp. was observed through out the year. Only patchy growth of mushroom mycelium was observed in infected beds. Later in infected area signs of weed fungus was seen as orange coloured soft irregular structures of varying size (Plate 3c).

Artificial inoculation of weed fungus was conducted in test tubes. One day after inoculation white coloured mycelial growth of fungus was spreading over the straw bits. Three to four days after inoculation colour of the mycelium was changed to light orange. After seven days, orange coloured irregular structures

were produced. Hence the affected areas appeared patchy with out mushroom mycelium. The fungal growth was faster than milky mushroom over the substrate and inhibited its growth.

4.6.3.2 *Trichoderma* sp.

The growth of *Trichoderma* sp. was noticed on five to six days after spawn run as green powdery growth on bed. As the organism grew faster than mushroom mycelium, during spawn running patchy area appeared with the growth of *Trichoderma* sp. In certain beds *Trichoderma* sp. completely covered the substrate and resulted in 100 per cent yield loss. The weed fungus can also be seen colonizing the mycelium of mushroom under favourable conditions.

Under artificial inoculation the growth of *Trichoderma* sp. started as white fluffy growth similar to the growth of milky mushroom. Hence detection in early stages was difficult. Three to four days after inoculation as the fungus produced spores the colonized area became green in colour. The growth rate of *Trichoderma* sp. was faster than that of *C. indica*, and spread rapidly over the substrate reducing the spawn run. The fungus over grew the *C. indica* on five days after inoculation.

4.6.3.3 Aspergillus sp.

Colonization by *Aspergillus* sp. was noticed as light green patches on the bed surface during spawn run. In artificial inoculated tubes yellowish green growth of fungus was noticed three to four days after inoculation, when it sporulates. The distribution of the weed mould was uneven. The fungus spores rapidly spread inside the tubes and inhibited the growth of *C. indica.*

4.6.3.4 Coprinus **sp.**

The incidence of Coprinus was observed from spawn run to harvest. The mycelial growth of *Coprinus* sp. was unnoticed in mushroom beds and became evident when the fungus started producing fruiting bodies. The fruiting body of the Coprinus was emerged as 4 mm sized pinheads and remained like that for two more days. Later it developed into tiny button with stipe and at maturity the pileus expanded and turned black due to sporulation. The entire structure collapsed and converted to a black inky fluid with in a day. Pinheads of *Coprinus* sp. was also noticed on casing surface, five to six days after casing.

The mycelial growth of Coprinus was similar to the growth of *C. indica*, as evidenced by artificial inoculation. The growth rate of fungus was faster than milky mushroom. After 12-15 days small fruiting bodies were produced in tubes and release black coloured spores.

4.6.3.5 Curvularia **sp.**

The growth of *Curvularia* sp. was found associated with sorghum grains. The fungal growth appeared as black coloured mycelial growth with in four to five days after spawning. The spawn run was considerably reduced when the growth of *Curvularia* sp. initiated from spawn grains.

Two days after artificial inoculation black coloured mycelial growth of fungi was radiating from the inoculated fungal disc. Hence early stage detection of the fungus became easy. The growth rate of *Curvularia* sp. was slow compared to other weed fungi and it starts over growth 10-12 days after inoculation.

4.6.3.6 Chaetomium sp.

The sign of Chaetomium growth was noticed one week after spawn run as black or olivaceous green coloured fruiting structures. The perithecia were observed as small green round structures clustered along the whole length of straw pieces. In damaged beds isolated areas with these fruiting structures can be seen. The Chaetomium colonized beds emit a musty odour (Plate 3c).

On artificial inoculation the growth of fungus was seen as greyish white mycelium spreading over the straw three days after inoculation. After one week black or olivaceous green perithecia were produced. The perithecia were seen along the straw pieces as small black dots and Chaetomium prevents the spawn run on the substrate.

4.6.3.7 Sclerotium sp.

Growth of fungus started five to six days after spawning as white thick mycelium. Mycelial growth of the fungus was similar to the growth of milky mushroom, hence detection in the early stages become difficult. Later by 10-15 days after spawning small round or irregular dark brown coloured sclerotia were formed. Growth of fungus was much faster than *C. indica* and covers the entire bed resulting in 100 per cent reduction in spawn run. In damaged beds the entire substrate was covered with dark brown coloured sclerotia (Plate 3c).

Under artificial inoculation mycelial growth of *Sclerotium* started one day after inoculation. The fungal mycelium appeared as white, thick strands and grew faster than milky mushroom. After five days, the mycelium started over growing the mushroom mycelium and the whole substrate was covered with weed fungus which later developed into dark brown coloured sclerotia.

Plate2a: Symptoms of abiotic stress

Scaling Splitting Malformation

Plate 2b: Different symptoms of bacterial blotch

4.7 Identification of pathogens

4.7.1 Characterization of bacterial isolates

Cultural, morphological, physiological and biochemical characters of the six bacterial isolates were studied.

4.7.1.1 Colony characters of bacterial isolates

Colony characters of the bacterial isolates on NA medium were studied 24 h after inoculation. The isolates showed slight variation in colony characters (Table 4).

Table 4: Colony characters of bacterial isolates

Isolates	Size	Shape	Elevation	Colour	Margin	Opacity
B_1	Punctiform	Circular	Convex	Cream	Entire	Translucent
B_2	Punctiform	Circular	Flat	Cream	Entire	Translucent
B_3	Punctiform	Circular	Flat	Yellow	Entire	Translucent
B_4	Punctiform	Irregular	Flat	Cream	Lobate	Translucent
B_5	Punctiform	Circular	Convex	Cream	Entire	Translucent
B_6	Punctiform	Circular	Convex	Cream	Entire	Translucent

Among the six isolates B_1, B_5 and B_6 produced circular shaped colonies with punctiform size (pin point). Colonies of B_1, B_5 and B_6 isolate has convex elevation and entire margin, whereas colonies of B_2 and B_3 isolates were circular in shape with punctiform size and produced flat colonies with entire margin. The B_4 isolate produced colonies of irregular shape, punctiform size, flat elevation and lobate margin. All isolates produced cream coloured colonies except B_3, which produced yellow coloured colonies. Colonies of all isolates were translucent in nature.

4.7.1.2 Morphological characters

4.7.1.2.1 Gram staining

All isolates except B_4 were gram negative and all were found to be rod shaped (Table 5).

Table 5: Morphological characters

Isolates	Gram's staining	Endospore staining	KOH test	Pigment production	Shape
B_1	-	-	+	-	Rod
B_2	-	-	+	+	Rod
B_3	-	-	+	-	Rod
B_4	+	+	-	-	Rod
B_5	-	-	+	-	Rod
B_6	-	-	+	-	Rod

4.7.1.2.2 Solubility in 3 per cent KOH

All the bacterial isolates except B_4 showed positive reaction by forming thick threads upon raising the loop. This observation further confirmed the gram negative reaction of these isolates.

4.7.1.2.3 Endospore staining

Only B_4 isolate showed positive endospore staining reaction.

4.7.1.2.4 Pigment production

Pigment production of bacterial isolates were studied by streaking them on King's A and King's B medium. None of the isolates produced pigmentation on King's A medium, where as B_2 isolate produced diffusible pigments on King's B medium.

4.7.1.3 Physiological characters

4.7.1.3.1 Effect of pH on the growth of bacterial isolates

Growth of the six bacterial isolates in nutrient broth adjusted to different pH were recorded by measuring optical density of broth and results are presented in Table 6.

Table 6: Effect of pH on the growth of bacterial isolates

Ph	O.D value of broth 24 h after inoculation					
	Isolates					
	B_1	B_2	B_3	B_4	B_5	B_6
5	0.551	0.322	0.845	0.193	0.598	0.480
6	0.426	0.274	0.798	0.193	0.542	0.325
7	0.348	0.256	0.494	0.143	0.486	0.233
8	0.284	0.249	0.269	0.104	0.296	0.222

All the bacterial isolates recorded maximum growth at pH 5 and minimum growth at pH 8. There was a gradual decrease in OD value from pH 5 to 8. The isolate B_4 recorded same OD value at pH 5 and 6. Among the six isolates B_3 recorded maximum OD value of 0.845 at pH 5. Minimum growth was recorded by B_4 at pH 8 (0.104).

4.7.1.3.2 Effect of temperature on the growth of bacterial isolates

Growth of the bacterial isolates at different temperature was studied and the results are presented in Table 7. The bacterial isolates B_3, B_4 and B_5 recorded maximum growth at 30^0C where as the isolates B_1, B_2 and B_6 recorded maximum growth at 35^0C. At 25^0C all the isolates recorded poor growth.

Table 7: Effect of temperature on the growth of bacterial isolates

Temperature (^{0}C)	O.D value of broth 24 h after inoculation					
	Isolates					
	B_1	B_2	B_3	B_4	B_5	B_6
25	0.168	0.005	0.077	0.073	0.051	0.023
30	0.303	0.228	0.472	0.161	0.319	0.262
35	0.313	0.306	0.456	0.110	0.261	0.288

4.7.1.3.3 Oxygen requirement

The isolate B_4 grew and changed the colour of the nutrient dextrose medium from purple to yellow in tubes with and without molten agar seal indicating that it can grow both under aerobic and anerobic condition. All other isolate grew only in tubes without molten agar seal indicating that they are aerobic in nature.

4.7.1.4 Biochemical characterization

Biochemical characters of the six bacterial isolates were studied and presented in Table 8.

All the bacterial isolates produced effervescence when hydrogen peroxide was added to smears of bacteria, which indicated the production of catalase.

4.7.1.4.2 Citrate utilization

Simmon's citrate agar in the test tube was stabbed with the bacterial isolates and observed for the colour change. The colour of the medium changed from green to blue in all the tubes except in control. This indicated that all the isolates could utilize citrate.

4.7.1.4.3 Denitrification

All the bacterial isolates except B_3 showed the ability to reduce nitrate

Table 8: Biochemical test

Tests	Bacterial isolates					
	B_1	B_2	B_3	B_4	B_5	B_6
Catalase test	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+
Denitrification	+	+	-	+	+	+
Arginine dihydrolase	+	+	+	+	+	+
Levan Production from sucrose	-	-	-	-	-	-
Starch hydrolysis	-	-	-	+	-	-
Lipase test	-	-	+	-	-	-
Lecithinase test	+	+	+	-	+	+
Oxidase test	+	+	+	+	+	+

4.7.1.4.4 Arginine dihydrolase test

All the isolates gave positive result by giving a pink colouration to the medium, showed their ability to hydrolyse arginine.

4.7.1.4.5 Levan production from sucrose

None of the isolates produced white domed and mucoid colonies, indicating negative reaction for levan production.

4.7.1.4.6 Starch hydrolysis

Only B_4 isolate showed the ability to hydrolyse starch which was evidenced from the appearance of a colourless zone in contrast to the blue background of the medium around the bacterial growth on addition of iodine solution.

4.7.1.4.7 Lipase test

The isolate B_3 showed lipase activity by the formation of dense precipitate around the bacterial growth after 24 h of incubation. All other isolates showed negative lipase activity.

4.7.1.4.8 Lecithinase test

All the isolate except B_4 produced opaque zone around the bacterial growth indicating positive reaction for lecithinase test.

4.7.1.4.9 Oxidase test

A loopful of inoculum was taken and rubbed on oxidase disc. There was purple colour development for all the isolates, proving that the isolates were positive for oxidase test.

4.7.1.4.10 Utilization of carbohydrates

Of the twelve carbon compounds tested the isolates B_1, B_5 and B_6 produced acid from glucose, dextrose, fructose, lactose, mannose, sorbitol, dulcitol, manitol, inositol and cellobiose and failed to utilize sucrose and maltose. The results were given in the Table 9. The isolate B_2 utilized all carbon sources except sucrose and lactose and the B_3 isolate utilized all the twelve carbon

compounds. The B_4 isolate failed to utilize all the carbon compounds tested except mannose and manitol. Utilization of carbohydrate was indicated by the change in colour of the medium from green to yellow.

Among the six bacterial isolates B_1, B_5 and B_6 showed same result for all the tests, hence the three isolates were considered as single isolate B_1. Based on the cultural, morphological, physiological and biochemical characters the bacterial isolates were tentatively identified into two groups. All the isolates except B_4 belongs to the genus *Pseudomonas* sp. and B_4 belongs to the genus *Bacillus* sp.

Table 9: Utilization of carbohydrates

Sugars	**Bacterial isolates**					
	B_1	**B_2**	**B_3**	**B_4**	**B_5**	**B_6**
Glucose	+	+	+	-	+	+
Dextrose	+	+	+	-	+	+
Sucrose	-	-	+	-	-	-
Fructose	+	+	+	-	+	+
Lactose	+	-	+	-	+	+
Mannose	+	+	+	-	+	+
Maltose	-	+	+	+	-	-
Sorbitol	+	+	+	-	+	+
Dulcitol	+	+	+	-	+	+
Manitol	+	+	+	+	+	+
Inositol	+	+	+	-	+	+
Cellobiose	+	+	+	-	+	+

4.7.2 Cultural and morphological characters of the pathogenic / weed fungi

Cultural and morphological characters of pathogenic / weed fungi in PDA medium were studied in detail and based the characters they were identified.

4.7.2.1 Dactylium sp.

Colonies are white to light yellow coloured on PDA medium. Fast growing, completely covered 9 cm Petri dish in three days. Later the colour of

media changed to pink or deep red. Hyphae hyaline, branched and septate. Conidiophores are erect verticilliately branched. Conidia single, elongate, 3-4 septate, and measuring 15.8- 23.7 x 7.9-10.3 µm (Plate 4). Based on the characters studied and the characters described by Buvaneswari (2004) pathogen was identified as *Dactylium dendroides*.

4.7.2.2 ***Penicillium*** **sp.**

Colonies on PDA medium grows rapidly with radial wrinkles, 0.4 mm to 0.9 mm diameter, greyish green, velvety and heavily sporing. Colony on the reverse side light yellow. It took five to six days to reach full growth in 9 cm Petri dish. Conidiophores terminating in a verticel of metulae bearing conidia in chains. Conidia globose, smooth, 1.88-2.6 µm diameter and appearing faintly green in mass (Plate 4). Based on these characters the organism was identified as *Penicillium* sp. Link.

4.7.2.3 ***Chaetomium*** **sp.**

Colonies greyish white, thin, raised hairy mycelium. Sporulation was observed six days after incubation. Perithecia opaque, globose, with apical tuft of dark bristle or setae. Ascospores dark brown ovoid and measuring 11.85 x 10.5 µm (Plate 4). Based on these characters the fungus was identified as *Chaetomium* sp. It was further confirmed from "National centre of fungal taxonomy, New Delhi" as *Chetomium indicum* (NCFT - 1311.07).

4.7.2.4 ***Curvularia*** **sp.**

Colonies black, fluffy, moderately fast growing on PDA medium. Completely covered the 9 cm Petri dish in 6-7 days. Hyphae branched, colourless to pale brown. Width of hyphae 3.95-5.26 µm. Conidiophores arising singly or in groups terminally or laterally on the hyphae. Conidia 3-4 septate, dark brown. The

third cell from the base always curved, darkest and larger than others. Conidia 19.75- 27.6 µm long and 11.85-19.75 µm wide (Plate 4). Based on these characters the weed fungus was identified as *Curvularia* sp. Boedijn (Subramanian, 1971).

4.7.2.5 ***Trichoderma*** **sp.**

Colonies smooth with rapid growth. Initially white gradually turned to light green to bright green. Mycelium septate, hyaline and smooth walled. Conidiophores with numerous side branches. Phialides arise in group, skittle shaped, short, and narrow at the base. Philaospores subglobose, short obovoid, smooth surface and pale green and measure 3 X2.2 µm. Based on these characters the organism was identified as *Trichoderma* sp. (Persoon) Harz (Plate 4).

4.7.2.6 ***Coprinus*** **sp.**

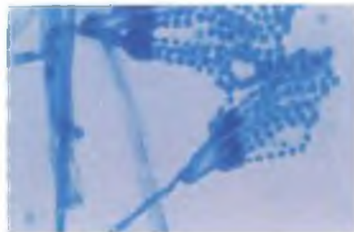
Pileus first cylindrical or conical expanded when fully grown. Initially cover with dirty white woolly scales and measure 2.5-3 cm diameter. As the cap expands margin splits and turn to umbrella shape. Gills free first and have 3-4 gills/mm. White when young and turn black on liquefying. Stipe centrally placed, 3.5-8 cm long and 1mm thick, white hollow, slightly hairy and fragile. Spores are black and produced black spore print (Plate 4). Based on the characters studied and the characters described by Singer (1975) the weed fungus was identified as *Coprinus* sp.

4.7.2.7 ***Sclerotium*** **sp.**

Colony smooth, white, with rapid growth completely cover 9 cm dish in two to three days. Hyphae hyaline produced white small sclerotia in six days after inoculation. Later the colour of the sclerotia changed to dark brown (Plate 4).

Plate 4: Identification of pathogenic / weed fungi

Dactylium dendroides
(400X)

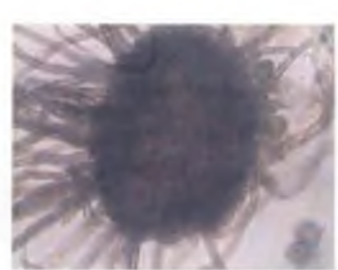


Penicillium sp.
(400X)

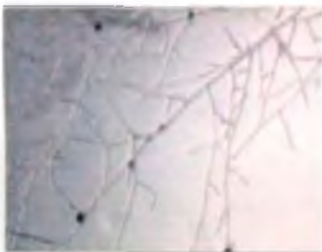
Curvularia sp.
(400X)

Aspergillus sp.
(400X)

Rhizoctonia solani
(400X)



Chaetomium indicum
(100X)



Sclerotium sp.
(400X)

Trichoderma sp.
(400X)

Coprinus sp.

Based on the characters the organism was identified as *Sclerotium* sp. Tode ex Fries. (Subramanian, 1971)

4.7.2.8 Rhizoctonia sp.

Colony light orange coloured, with rapid growth and completely covered 9 cm Petri dish within three days. Hyphae hyaline, septate with a width of 3.95 µm. Six days after inoculation orange coloured soft irregular shaped bodies of varying size were produced in medium(Plate 4). The organism was identified as *Rhizoctonia solani* from "National centre of fungal taxonomy, New Delhi" with identification number NCFT-1310.07.

4.7.2.9 Aspergillus sp.

Yellowish green colonies appear on PDA plates, attained 9 cm growth in five days. Hyphae hyaline, conidial heads in yellow to green shade radiating on long conidiophores. Vesicle globose to subglobose 39.5- 47.4µm diameter Conidia globose, minutely echinulate and measure 3.95µm diameter. Based on these characters the organism was identified as *Aspergillus* sp. Micheli (Plate 4).

4.7.3 Effect of temperature on the growth of pathogenic / weed fungi

Growth of pathogenic / weed fungi and milky mushroom at different temperature was studied. Number of days taken for full growth in 9 cm Petri dish was recorded and the data are presented in the Table 10 and Fig.1.

All the fungal pathogens and weed fungi showed a faster growth rate at 30^0C except *Penicillium* sp. and *D. denroides*. At 35^0 C *Penicillium* sp. attained 9 cm growth in six days of incubation and at 30^0C it took seven days to reach the full growth. All other fungi except *Aspergillus* sp., *Coprinus* sp. and *Penicillium* sp. recorded faster growth at 25^0C than at 35^0C. Same trend was observed in the

Table 10: Effect of temperature on the growth of pathogenic / weed fungi

Temperature °C	Days to complete 9 cm mycelial growth									
	D.dendroides	*Penicillium* sp.	*Sclerotium* sp.	*Aspergillus* sp.	*C.indicum*	*Curvularia* sp.	*Coprinus* sp.	*R.solani*	*Trichoderma* sp.	*C.indica*
25	3	8	4	7	8	5	7	2	4	14
30	6	7	3	5	6	4	3	2	3	13
35	20	6	5	6	9	6	4	4	5	17

Table 11: Effect of pH on the growth of pathogenic / weed fungi

pH	Days to complete 9 cm mycelial growth									
	D.dendroides	*Penicillium* sp.	*Sclerotium* sp.	*Aspergillus* sp.	*C.indicum*	*Curvularia* sp.	*Coprinus* sp.	*R.solani*	*Trichoderma* sp.	*C. indica*
5	10	7	2	6	3	9	2	3	3	15
6	6	7	2	6	3	8	2	3	3	14
7	4	6	2	5	2	8	3	2	2	14
8	8	5	2	5	2	5	4	2	3	12

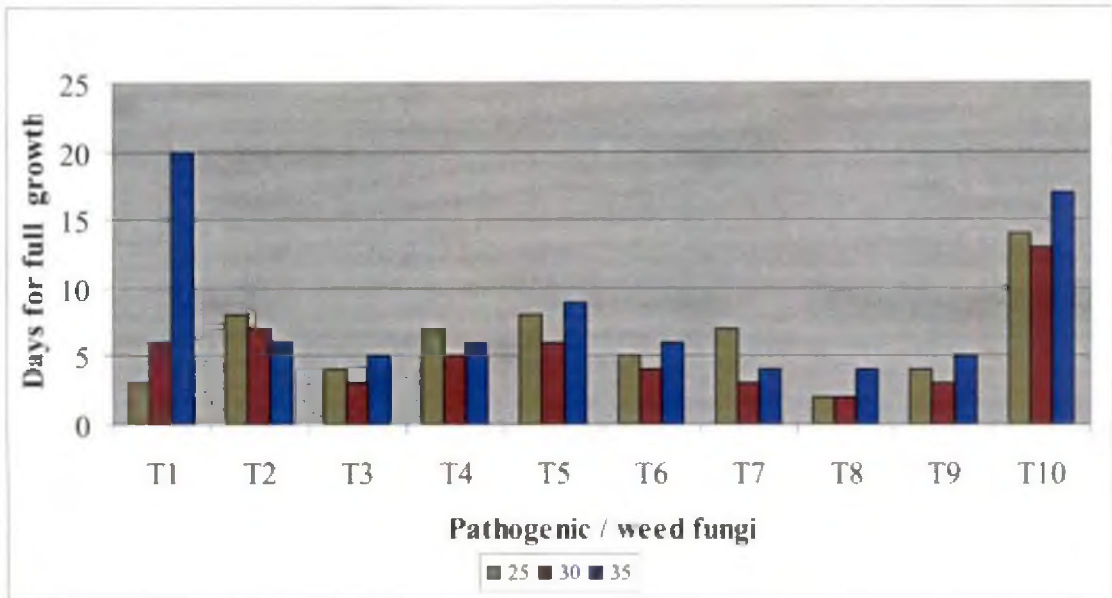


Fig. 1. Effect of temperature on the growth of pathogenic/ weed fungi

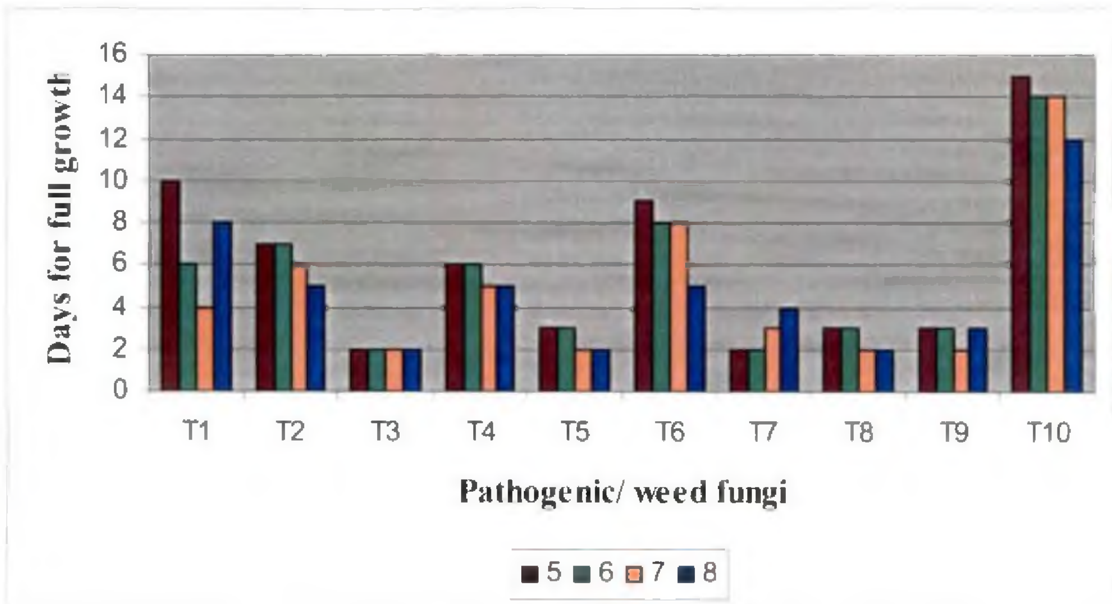


Fig. 2.Effect of pH on the growth of pathogenic/ weed fungi

T_1- *D. dendroides*
T_2-*Penicillium* sp.
T_3-*Sclerotium* sp.
T_4-*Aspergillus* sp.
T_5-*C. indicum*
T_6-*Curvularia* sp.
T_7-*Coprinus* sp.
T_8-*R.solani*
T_9-*Trichoderma* sp.
T_{10}-*C. indica*

case of milky mushroom. At low temperature (25^0C) *D. dendroides* recorded maximum growth and it took only three days to reach full growth in Petri dish. At higher temperatures (30^0C and 35^0C) the growth rate was low and it attained full growth in 9 cm Petri dish after 6 and 20 days of incubation respectively. At 30 ^{0}C *C. indica* took only 13 days, while at 25^0C and 35^0C it took 14 and 17 days respectively to reach full growth in Petri dish.

4.7.4 Effect of pH on the growth of pathogenic / weed fungi

Data on the effect of pH on the growth of pathogenic / weed fungi and milky mushroom are presented in the Table 11 and Fig. 2.

Among the fungi *Sclerotium* sp. recorded faster growth and it took only two days for 9 cm growth in all pH levels. At neutral pH all fungal pathogens except *Penicillium* sp., *Coprinus* sp., and *Curvularia* sp. recorded maximum growth. The *Coprinus* sp. showed faster growth in acidic pH and took only two days for complete growth in 9 cm dish. At pH 8 *Curvularia* sp. *and Penicillium* sp. attained full growth after five days of incubation. Weed fungi like *Aspergillus* sp., *R. solani,* and *C. indicum* recorded uniform growth at pH 7 and 8. Milky mushroom took only 12 days for 9 cm growth at pH 8. At pH 5 *C. indica* and *D.dendroides* recorded slow growth rate.

4.8 Interaction between pathogens/ weed fungi and *C. indica*

In vitro interaction between the pathogens and *C. indica* was carried out and the results of the study are presented in the Table 12 and 13.

4.8.1 Interaction between bacterial pathogens and *C. indica*

Bacterial pathogens were tested for their inhibitory effect on milky mushroom by dual culture technique. The results of the study are presented in the

Table12: Interaction between bacterial pathogens and *C. indica*

Sl. No.	Isolates	Days after incubation (Growth in cm)*														
		1		Per cent inhibition over control	3		Per cent inhibition over control	5		Per cent inhibition over control	7		Per cent inhibition over control	14		Per cent inhibition over control
		M	D		M	D		M	D		M	D		M	D	
1	B_1	1.7	1.4	17.6	3.1	2.3	25.8	4.3	2.9	32.6	4.8	2.9	32.6	9.0	2.9	67.8
2	B_2	1.7	1.5	11.7	3.1	2.9	12.9	4.3	2.9	32.6	4.8	2.9	32.6	9.0	2.9	67.8
3	B_3	1.7	1.3	23.5	3.1	2.0	35.5	4.3	2.3	46.5	4.8	2.3	46.5	9.0	2.3	74.4
4	B_4	1.7	1.4	17.6	3.1	1.9	38.7	4.3	2.4	44.2	4.8	2.4	44.2	9.0	2.4	73.3

* Mean of three replications

Bacterial isolates were inoculated 2days after inoculation of *C. indica*

M- Mono culture of *C. indica*
D- Dual culture of *C indica* and bacterial isolates

Table 12. In general, in dual culture the growth of *C. indica* was inhibited from fifth day onwards where as in monoculture it reached 9cm growth after 14 days of incubation.

One day after inoculation isolate B_3 showed maximum inhibition (23.5 per cent) against *C. indica* followed by the B_1 and B_4, which recorded 17.6 per cent inhibition. Three day after incubation B_4 isolate recorded maximum inhibition (38.7 per cent) followed by B_3 (35.5 per cent) and lowest inhibition (12.9 per cent) was recorded by B_2 isolate. But after five days of incubation isolate B_3 showed maximum inhibition of 46.5 per cent followed by B_4 (44.2 per cent). Isolates B_1 and B_2 recorded same rate of inhibition (32.6 per cent).

After 14 days of incubation, among the four isolates B_3 showed the maximum inhibition of 74.4 per cent against *C. indica*, which was followed by B_4 (73.3 per cent). Isolate B_1 and B_2 recorded 67.8 per cent inhibition over control (Plate 5).

4.8.2 Interaction between pathogenic / weed fungi and *C. indica*

Pathogenic / weed fungi isolated from milky mushroom/ mushroom bed were tested for their interaction with *C. indica*. All pathogenic/ weed fungi showed faster growth rate compared to that of milky mushroom, which was clearly evident from first day after incubation (Table 13). When the growth rate of different fungi was compared, *Sclerotium* sp. recorded maximum growth (4.5 cm) one day after incubation and followed by *R. solani*, *Trichoderma* sp. and *D. dendroides*. Slowest growth was recorded by *Penicillium* sp. The same trend was noticed upon further incubation.

Among the pathogenic /weed fungi *Sclerotium* sp. recorded maximum growth rate. On the second day of incubation it recorded 7.4 cm growth against 1.6 cm growth of *C. indica* and started over growing the mushroom fungus. The

Plate 5: Interaction between bacterial pathogens and *Calocybe indica*

B_1 x *C. indica*

B_2 x *C. indica*

Calocybe indica

B_3 x *C. indica*

B_4 x *C. indica*

Table 13: Interaction between pathogenic/ weed fungi and *C. indica*

Sl. No	Pathogenic/ weed fungi	Days after incubation (growth in cm)*																Type of reaction
		1		2		3		4		5		6		7		8		
		P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	
1	*D.dendroides*	2.5	1.9	5.7	2.3	7.6	1.4	9.0	0									Over growth
2	*Penicillium* sp.	0	1.7	3.2	2.2	3.9	2.7	5.7	2.9	6.1	2.9	6.8	2.2	7.4	1.6	9.0	0	Over growth
3	*Sclerotium* sp.	4.5	1.7	7.4	1.6	9.0	0											Over growth
4	*Aspergillus* sp.	1.6	1.7	3.8	2.4	4.4	3.0	5.7	3.2	6.8	2.1	8.2	0.8	9.0	0			Over growth
5	*C. indicun*	1.6	1.7	2.4	2.5	3.4	2.8	4.5	3.5	5.3	3.7	6.6	2.4	7.4	1.6	9.0	0	Over growth
6	*Curvularia* sp.	1.4	1.7	2.6	2.6	3.6	3.1	4.6	3.7	5.8	3.0	7.4	1.4	9.0	0			Over growth
7	*Coprinus* sp.	2.0	1.5	3.9	1.4	4.8	2.3	5.5	2.6	6.2	2.8	6.9	2.1	7.4	1.6	9.0	0	Over growth
8	*R.solani*	2.8	1.7	5.9	2.2	8.2	0.7	9.0	0									Over growth
9	*Trichoderma* sp.	2.6	1.7	5.3	2.1	7.2	1.7	8.1	0.9	9.0	0							Over growth
	Monoculture																	
10	*C. indica*		1.7		2.6		3.1		3.7		4.3		4.8		5.5		6.1	

* Mean of three replications

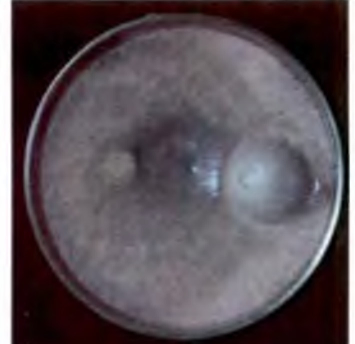
- : Pathogenic/ weed fungi were inoculated 2 days after inoculation *Calocybe indica*

P- Pathogenic/ weed fungi
M- Milky mushroom

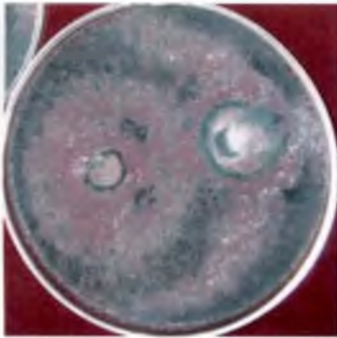
Plate 6: Interaction between pathogenic / weed fungi and *Calocybe indica*

Sclerotium sp. x *C. indica*

D. dendroides x *C. indica*



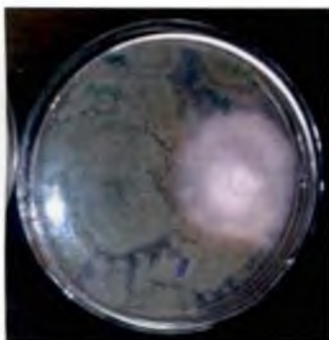
R. solani x *C. indica*



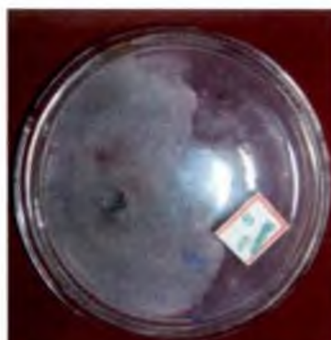
Trichoderma sp. x *C. indica*

Curvularia sp. x *C. indica*

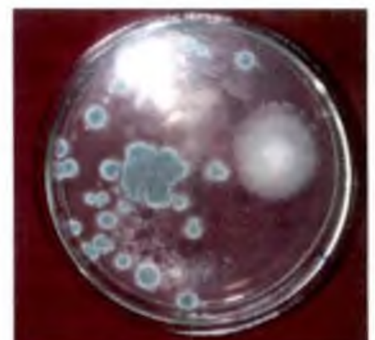
Coprinus sp. x *C. indica*



Aspergillus sp. x *C. indica*



C. indicum x *C. indica*



Penicillium sp. x *C. indica*

mushroom fungus was completely over grown by *Sclerotium* sp. on third day of incubation. Among other fungi *R. solani, Trichoderma* sp. and *D. dendroides* started over growth on third day onwards and recorded 100 per cent inhibition after four to five days of incubation (Plate 6).

Compared to other weed fungi *Aspergillus* sp., *Curvularia* sp., *C. indicum*, *Penicillium* sp., and *Coprinus* sp. showed a slower growth rate and started over growth on *C. indica* after five to six days of incubation. After seven to eight days of incubation these fungi also recorded 100 per cent inhibition of mushroom fungus. The monoculture of *C. indica* took 14 days to attain full growth in 9 cm Petri dish.

4.9 Effect of culture filtrate of weed fungi on the growth of *C. indica*

The effect of culture filtrate of weed fungi was tested on the growth of milky mushroom. It was observed that culture filtrate of *C. indicum* was most inhibitory to the growth of milky mushroom and it completely inhibited the mycelial growth (Table 14). Culture filtrate of *Curvularia* sp. recorded 53.3 per cent inhibition followed by *Trichoderma* sp. and *Aspergillus* sp.

Table 14: Effect of culture filtrate of weed fungi on the growth of *C. indica*

Sl. No	Weed fungi	Days after incubation (growth of *C. indica in cm**)							Per cent inhibition over control
		2	4	6	8	10	12	15	
1	*Sclerotium* sp.	1.3	2.5	3.5	4.4	5.5	6.6	8.3	7.7
2	*Aspergillus* sp.	0	1.4	2.2	2.7	3.5	4.2	5.3	41.1
3	*C. indicum*	0	0	0	0	0	0	0	100
4	*Curvularia* sp.	0	1.4	2.1	2.5	3.1	3.6	4.2	53.3
5	*Coprinus* sp.	1.2	2.1	3.5	4.6	5.6	6.5	8.5	5.5
6	*R.solani*	1.2	2.4	3.5	4.3	5.2	6.4	8.2	8.8
7	*Trichoderma* sp.	0	1.2	2.4	2.9	3.3	4.1	4.8	46.6
8	Control	1.3	2.6	3.7	4.8	5.8	6.9	9.0	

* mean of three replications

The culture filtrates of two fast growing organisms *Sclerotium* sp and *R. solani* recorded inhibition of 7.7 and 8.8 per cent respectively. In this case on 15 day after incubation *C. indica* recorded 8.2-8.3 cm mycelial growth against 9cm growth in control. Lowest inhibition (5.5 per cent) was recorded by *Coprinus* sp. In control milky mushroom recorded 9 cm growth after 15 days of incubation.

4.10 Antagonistic effect of weed fungi on linear growth of *C. indica* on substrate

The study was conducted to observe the inhibitory effect of weed fungi on milky mushroom during its growth on paddy straw. The observation was measured at 5, 10 and 15 days after spawning (Table 15).

Table 15: Antagonistic effect of weed fungi on linear growth of *C.indica*

Sl.No	Weed fungi	Days after incubation (Linear growth of *C. indica* in cm*)			Per cent inhibition over control
		5	10	15	
1	*Sclerotium* sp.	1.9	1.9	0	100
2	*Aspergillus* sp.	2.8	3.7	3.5	36.4
3	*C.indicum*	2.4	3.8	3.8	30.9
4	*Curvularia* sp.	2.7	4.1	4.0	27.2
5	*Coprinus* sp.	3.1	3.9	3.9	27.8
6	*R.solani*	2.2	3.9	3.0	45.5
7	*Trichoderma* sp.	1.9	0.85	0	100
8	Control	3.7	4.9	5.5	

* mean of three replications

From the data it is evident that *Trichoderma* sp. and *Sclerotium* sp. showed 100 per cent inhibition on growth of *C. indica*. These fungi started over growth on *C. indica* from fifth day onwards and on tenth day they recorded 0.85cm and 1.9 cm mycelial growth of milky mushroom respectively compared to 4.9cm growth in control. Fifteen days after incubation complete inhibition of *C. indica* by over

growth was observed. On straw the growth of *Trichoderma* sp. was most inhibitory to *C. indica* compared to other weed fungi.

All other weed fungi started over growth on mushroom mycelium by tenth day of spawn running. After 15 days of incubation *R. solani* recorded 45.5 per cent inhibition, which was followed by *Aspergillus* sp. and *C. indicum*. Least inhibition of 27.2 and 27.8 per cent was recorded by *Curvularia* sp. and *Coprinus* sp. respectively.

4. 11 Influence of seasonal variations on the incidence of weed fungi

Incidence of weed fungi depends on season. The effect of weather parameters on incidence of weed moulds was studied in the beds laid out in mushroom production unit for a period of one year (Table 16a).

The weed mould incidence was maximum in the month of June (100 per cent) and it was minimum (20 per cent) during the month of April. A correlation study between weather parameters and incidence was conducted. The study revealed a negative correlation between the incidence of weed fungi and minimum temperature (Fig. 3).

The condition inside farm during spawn running stage influences the weed fungi incidence especially the maximum temperature and relative humidity. The condition inside the spawn running room was influenced by external weather parameters. In April average maximum temperature inside the room was 34.9^{0}C and average relative humidity was 88.8 per cent with minimum incidence of weed fungi (20 per cent). The per cent incidence was maximum (100 per cent) in June with average relative humidity of 99 per cent and the average temperature 28-30.9^{0}C. From the table it was evident that monthly rainfall had no effect on weed fungi incidence. In periods with no rainfall also weed fungi incidence was noticed

Table 16a: Influence of seasonal variations on the incidence of weed fungi

Period (2006-2007)	Monthly mean temperature (out side ^{0} C)		Monthly mean RH per cent (out side)	Monthly Rain fall (out side)	Monthly mean temperature (inside^0 C)		Mean RH per cent (inside)	Per cent incidence of weed fungi
	Maximum	Minimum			Forenoon	Afternoon		
August	29.8	23.1	83	550.6	32.0	33.0	97.2	50
September	29.6	23	84	522.2	28.7	30.9	90.3	32.5
October	31	23	79	323.7	30.0	31.8	90.8	33.3
November	31.7	23.7	72	79.5	29.9	30.9	85.9	40
December	31.5	23.6	57	0.0	30.6	32.6	70.9	35
January	32.5	22	54	0.0	26.9	31	88.9	60
February	34	22.2	55	0.0	27.5	31	91.6	80
March	36	24.4	63	0.0	31.8	34.1	90.1	33.5
April	35.1	25	69	61	32	34.9	88.8	20
May	32.8	24.6	76	240.5	31.9	33.1	92.6	27.7
June	30.1	23.5	84	826.5	28	30.9	99	100
July	28.4	22.9	88	1131.9	26.5	29.7	98.7	50

RH – Relative humidity

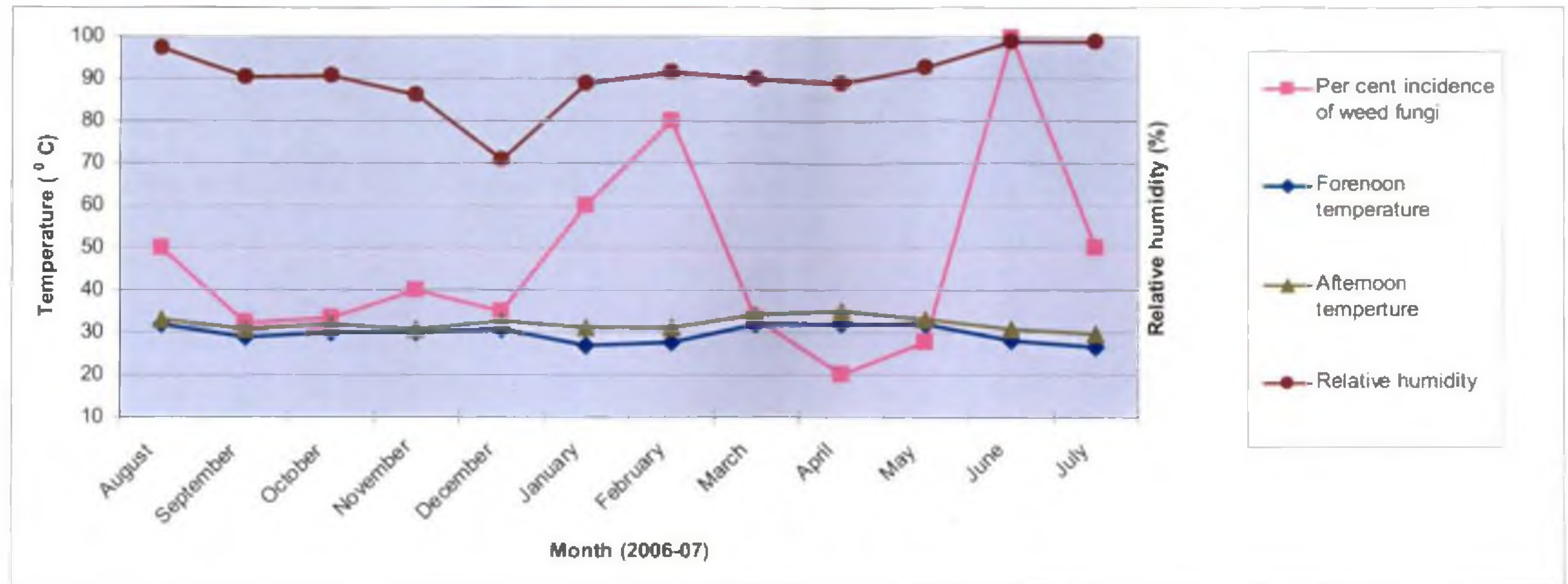


Fig.3. Influence of seasonal variations on the incidence of weed fungi

* Same scale of measurement has been applied along Y axis for all the parameters as all of them varied between 0-100

but the monthly rainfall can have an influence in increasing the relative humidity inside the spawn running room.

During the period of study *R. solani* was identified as the major weed fungus (Table 16 b). Incidence of *R. solani* was noticed through out the year. Other weed fungi like *C. indicum*, *Sclerotium* sp., *Aspergillus* sp., *Coprinus* sp. and *Trichoderma* sp. were also noticed as important contaminants affecting the spawn run during the study. In addition to the above fungi *Rhizopus* sp. and *Penicillium* sp were also observed in the month of June.

Table 16 b: Weed moulds observed in milky mushroom beds from August 2006- July 2007

Sl.No	Period (2006-2007)	Weed moulds observed
1	August	*R.solani* ,*Aspergillus* sp.,*Curvularia* sp. and *Coprinus* sp.
2	September	*R.solani, Curvularia* sp.,*Coprinus* sp. and *C.indicum*
3	October	*R.solani, Curvularia* sp., *C.indicum* and *Sclerotium* sp.
4	November	*R.solani, Curvularia* sp.,*Coprinus* sp. and *C.indicum*
5	December	*R.solani, Sclerotium* sp. and *C.indicum*
6	January	*R.solani* and*Sclerotium* sp.
7	February	*R.solani, C.indicum, Coprinus* sp. and *Sclerotium* sp.
8	March	*R.solani, Curvularia* sp. and*Sclerotium* sp.
9	April	*R.solani, Sclerotium* sp., *Aspergillus* sp. and *Coprinus* sp.
10	May	*R. solani, Aspergillus* sp. ,*Coprinus* sp. and *Sclerotium* sp.
11	June	*R.solani, Rhizopus* sp., *Curvularia* sp., *Aspergillus* sp., *C. indicum, Penicillium* sp., *Coprinus* sp. and *Trichoderma* sp.
12	July	*R. solani, Curvularia* sp., *Coprinus* sp. and *Aspergillus* sp.

4.12 Effect of weed fungi on growth and yield of milky mushroom

The effect of weed fungi on the growth and yield of milky mushroom was studied by simultaneous inoculation of the mycelial disc of weed fungi and milky mushroom spawn. The results of the study are presented in Table 17.

Table 17: Effect of weed fungi on growth and yield of *C. indica*

Sl. No.	Treatments	Per cent mycelial growth	Per cent reduction over control	Pinhead emergence (days after casing)	Number of Sporophore	Yield (g)/ 500 g substrate
1	*Sclertotium* sp.	9.3^{e} (3.1)	86.9^{a} (9.4)	0^{c} (0.7)	0^{d} (0.7)	0^{c} (0.7)
2	*Aspergillus* sp.	43.4^{cd} (6.6)	39.2^{cd} (6.3)	16.5^{ab} (4.1)	3.5^{bc} (2.0)	79.7^{b} (8.9)
3	*C. indicum*	16.9^{e} (4.2)	76.4^{b} (8.8)	22.0^{a} (4.7)	2.5^{cd} (1.7)	30.9^{c} (5.6)
4	*Curvularia* sp.	55.5^{bc} (7.5)	22.3^{e} (4.8)	19.5^{ab} (4.4)	5.5^{abc} (2.4)	104.8^{b} (10.3)
5	*Coprinus* sp.	66.0^{ab} (8.2)	7.5^{f} (2.8)	14.5^{b} (3.9)	8.5^{a} (3.0)	172.3^{a} (13.1)
6	*R.solani*	42.1^{d} (6.5)	41.1^{c} (6.5)	15.0^{ab} (3.9)	5.5^{abc} (2.4)	88.8^{b} (9.4)
7	*Trichoderma* sp.	48.9^{cd} (7.0)	31.4^{d} (5.6)	18.0^{ab} (4.3)	6.0^{ab} (2.5)	96.5^{b} (9.8)
8	Control	71.4^{a} (8.5)		17.5^{ab} (4.2)	8.0^{a} (2.9)	210.2^{a} (14.5)

Figures in parenthesis are square root transformed values

In each column figures followed by same letter do not differ significantly according to DMRT

Among the weed fungi tested *Sclerotium* sp. and *C. indicum* were giving maximum reduction in mycelial growth of *C. indica* over control, 86.9 and 76.4 per cent respectively. Mycelial colonization was less than 50 per cent in *R. solani*, *Aspergillus* sp. and *Trichoderma* sp. inoculated beds and was significantly inferior to that of control (71.4 per cent). Competition was minimum by *Coprinus* sp. and recorded 66 per cent colonization by the *C. indica* mycelium (Fig. 4a).

In *Coprinus* inoculated beds an early pinhead emergence was noticed. In all other fungi except *C. indicum* and *Sclerotium* sp. the *Calocybe* initials were produced between 15-19 days after casing. In treatment *C. indicum* the production of sporophore was noticed only after 22 days of casing.

In *Sclerotium* inoculated beds 100 per cent reduction in sporophore production was observed. Same trend as in case of mycelial growth was noted in the production of sporophore also. Maximum number of sporophore (8.5 and 8) was produced in *Coprinus* sp. and control. Least number of sporophore (2.5) was produced in *C. indicum* inoculated beds.

Maximum yield of 210.2 g was recorded in control, which was on par with *Coprinus* sp. In *Sclerotium* sp. 100 per cent reduction in yield was recorded. Lowest yield of 30.85g was recorded by *C. indicum*. Among all other treatments beds inoculated with *Curvularia* sp. contributed maximum (Fig.4b).

4.13 *In vitro* evaluation of different methods of substrate sterilization on the linear growth of *C. indica*.

The study was conducted in test tubes to test the effect of sterilization methods on the extent of growth of milky mushroom. Mycelial growth of *C. indica* after 5, 10 and 15 days of spawning was shown in the Table 18.

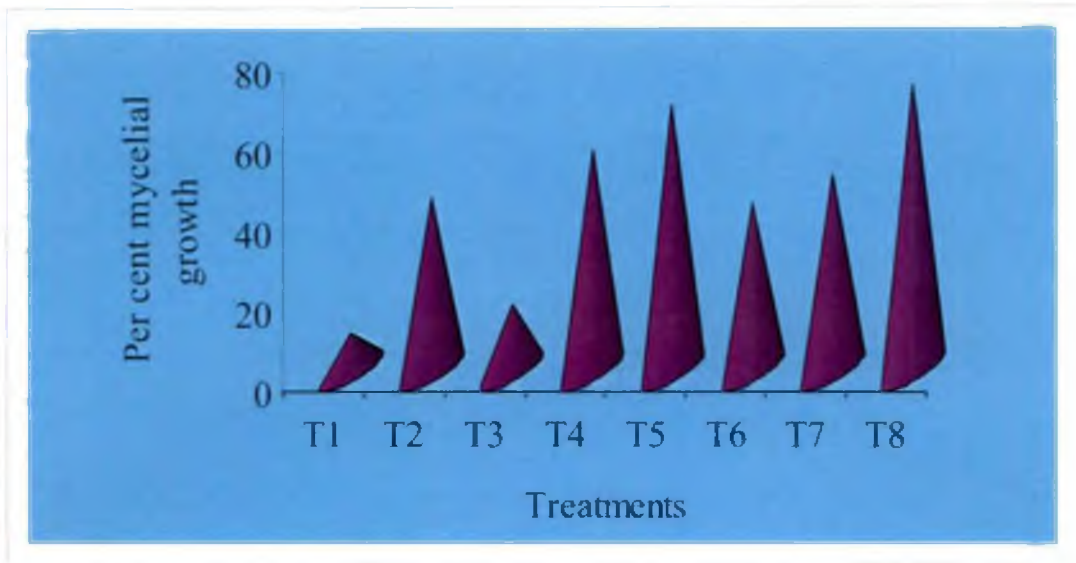


Fig. 4a. Effect of weed fungi on mycelial growth of *C. indica*

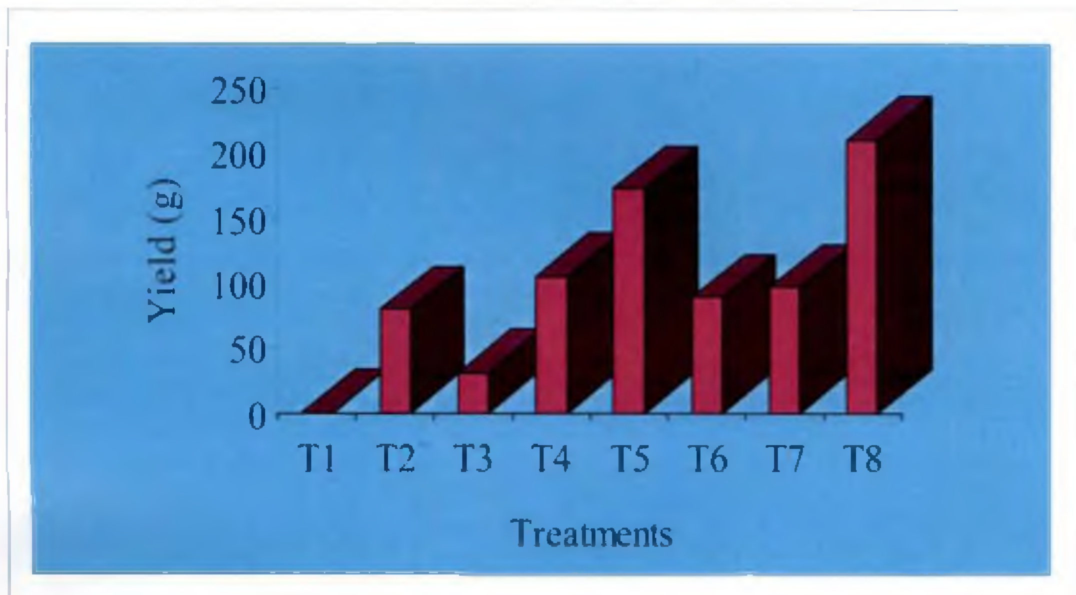


Fig. 4b. Effect of weed fungi on yield of *C. indica*

T_1 -*Sclertotium* sp
T_2- *Aspergillus* sp.
T_3- *C. indicum*
T_4- *Curvularia* sp.
T_5- *Coprinus* sp.
T_6- *R.solani*
T_7- *Trichoderma* sp.
T_8- Control

Table 18: Effect of different methods of sterilization of substrate on linear growth of *C. indica*

Sl.No	Linear growth of *C. indica* (cm *)			
	Treatments	**5 DAI**	**10 DAI**	**15 DAI**
1	Overnight soaking	2.9[d]	4.0[d]	4.0[d]
2	Chemical sterilization + NaOH	3.6[c]	4.8[c]	4.8[c]
3	Boiling	3.9[ab]	5.2[ab]	5.2[ab]
4	Pasteurization	4.2[a]	5.2[ab]	5.2[ab]
5	Chemical sterilization + $CaCO_3$	4.2[a]	5.9[a]	5.9[a]
6	Chemical sterilization (Control)	4.0[ab]	5.2[ab]	5.2[ab]

*mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

DAI- Days after incubation

In all treatments growth rate of mycelim was maximum during the first five days after spawning and there after the rate of mycelial growth was decreased. Five days after spawning the maximum mycelial growth of 4.2 cm was observed in substrate sterilized by pasteurization and chemical sterilization along with $CaCO_3$, which were on par. Ten days after spawning maximum mycelial growth of 5.9 cm was recorded in chemical sterilization along with $CaCO_3$. There after no further increase in mycelial growth after 10 days of spawning. In chemical sterilization, boiling and pasteurization 5.2 cm growth was recorded, which were on par with each other. Lowest mycelial growth (2.9 cm) was recorded in overnight soaked paddy straw.

4.14 On farm evaluation of different methods of sterilization of substrate

Effect of different methods of substrate sterilization on the growth and yield of *C. indica* was studied. The results of the study presented in the Table 19. The data on per cent mycelial growth of *C. indica* revealed a significant difference

among the treatments. Chemical sterilization along with $CaCO_3$ was significantly superior to others and recorded 91.3 per cent mycelial growth, this was on par with boiling treatment (80.3 per cent). The per cent mycelial growth was low in chemical sterilization + NaOH, which was on par with boiling+ $CaCO_3$ (Fig. 5a).

Table 19: Effect of different methods of sterilization of substrate on growth and yield of *C. indica*

Sl. No	Treatments	Mycelial growth (%)	Pinhead emergence (days after casing)	Number of Sporophore	Yield (g)/ 500g substrate
1	Boiling+ $CaCO_3$	35.5 [c] (5.9)	13.8[ab] (3.8)	4.0[c] (2.1)	88.0[c] (9.4)
2	Chemical sterilization +NaOH	33.9[c] (5.9)	14.8[a] (3.9)	4.5[bc] (2.2)	105.0[c] (10.3)
3	Boiling	80.3[ab] (8.9)	12.5[ab] (3.6)	7.3[ab] (2.8)	280.0[b] (16.7)
4	Chemical sterilization+$CaCO_3$	91.3 [a] (9.6)	10.5[b] (3.3)	8.5[a] (3.0)	415.0[a] (20.4)
5	Chemical sterilization (Control)	75.7 [b] (8.7)	12.0[ab] (3.5)	7.5[a] (2.8)	260.0[b] (16.1)

Figures in parenthesis are square root transformed values

In each column figures followed by same letter do not differ significantly according to DMRT

A similar trend was observed in the case of pinhead emergence also. An early pinhead emergence (10 days) was noticed in chemical sterilization along with $CaCO_3$ followed by chemical sterilization, boiling and boiling + $CaCO_3$. In treatment chemical sterilization + NaOH milky mushroom took 14 days for pinhead emergence. The beds with chemical sterilization + $CaCO_3$ and chemical sterilization method produced significantly more number of sporophore (8.5 and 7.5) compared to other treatments. The least number (4.0) of sporophore was produced in Boiling+ $CaCO_3$.

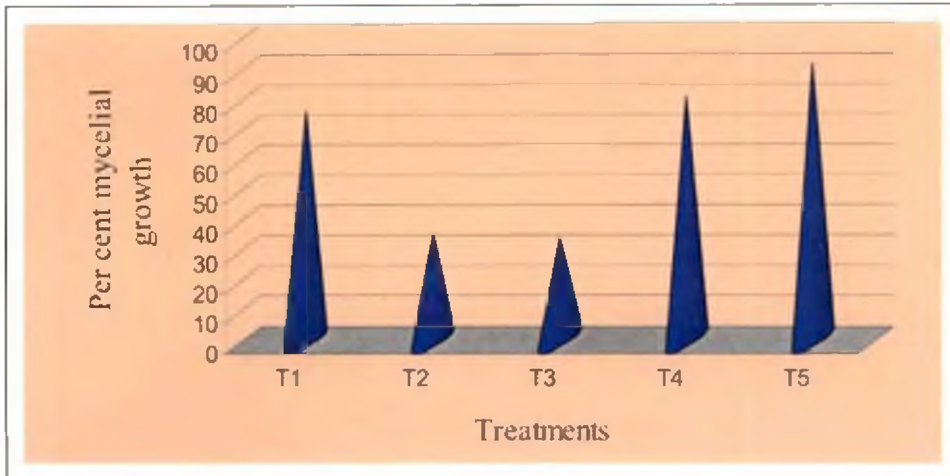


Fig. 5a Effect of different methods of sterilization of substrate on per cent mycelial growth

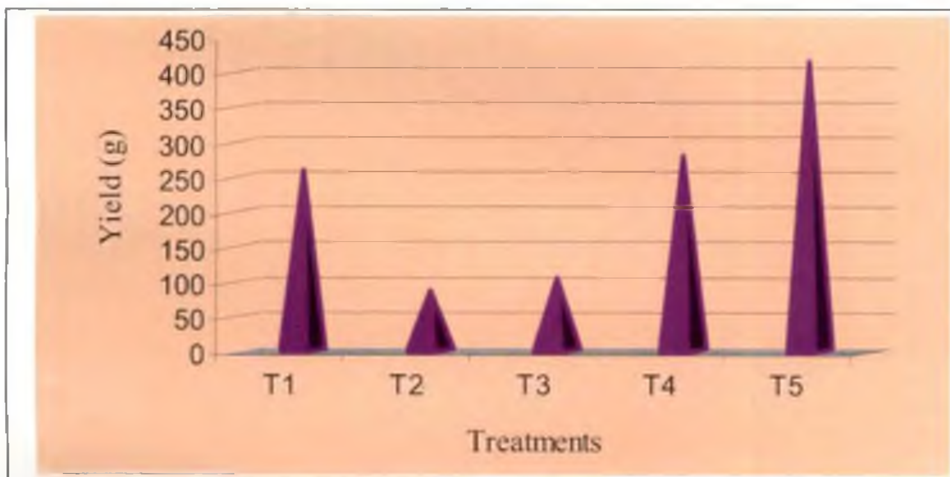


Fig. 5a Effect of different methods of sterilization of substrate on yield

T_1-Chemical sterilization
T_2-Boiling + $CaCO_3$
T_3-Chemical sterilization+ NaOH
T_4-Boiling
T_5-Chemical sterilization + $CaCO_3$

The data on yield of mushroom showed a significant difference among the treatments. The treatment chemical sterilization + $CaCO_3$ recorded a significantly higher yield (415 g) compared to all others. This was followed by boiling (280g) and chemical sterilization (260g), which were on par. Minimum yield was recorded in boiling + $CaCO_3$ (88.0 g), which was on par with chemical sterilization + NaOH (Fig. 5b).

In general, all the beds were free from weed fungi and the incidence of bacterial blotch on sporophore was comparatively less in all treatments.

Based on the farm evaluation two sterilization methods, chemical sterilization + $CaCO_3$ and boiling were selected for the disease management studies.

4.15 *In vitro* effect of phytoextracts on pathogens and milky mushroom

Effect of water extract of garlic cloves, turmeric rhizomes and leaves of neem, ocimum and marigold were tested against fungal and bacterial pathogens by poison food method. The results of the study are presented in Table 20 and 21.

4.15.1 *In vitro* effect of phytoextracts against bacterial pathogens

The inhibitory effect of five phytoextracts at different concentrations, against the bacterial pathogens was studied. The efficiency of phytoextracts was measured by the reduction in number of colony forming units (cfu) compared to control (Table 20).

Among the different plant extracts tried, turmeric extract was found significantly superior in giving 100 per cent inhibition at all concentrations against all the four bacterial isolates. This was followed by higher concentrations of neem leaf and garlic extract. At five per cent concentration neem leaf extract

Table 20: *In vitro* effect of phytoextracts against bacterial pathogens

Sl.No	Isolates	Per cent reduction in cfu over control															Control
		Garlic extract			Ocimum extract			Turmeric extract			Neem extract			Marigold extract			
		5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	% inhibition
1	B_1	16.6^{i} (4.1)	100^{a} (10.0)	100^{a} (10.0)	25.7^{g} (5.1)	47.7^{e} (6.9)	80.7^{c} (9.0)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	97.0^{b} (9.9)	100^{a} (10.0)	100^{a} (10.0)	19.5^{h} (4.5)	35.1^{f} (5.9)	58.1^{d} (7.7)	0^{a} (0.7)
2	B_2	75.6^{c} (8.7)	100^{a} (10.0)	100^{a} (10.0)	27.5^{g} (5.3)	60.0^{e} (7.8)	75.0^{c} (8.7)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	91.7^{b} (9.6)	100^{a} (10.0)	100^{a} (10.0)	11.1^{h} (3.4)	42:3^{f} (6.5)	63.7^{d} (8.0)	0^{a} (0.7)
3	B_3	68.7^{d} (8.3)	100^{a} (10.0)	100^{a} (10.0)	27.8^{h} (5.3)	54.7^{f} (7.4)	75.9^{c} (8.7)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	81.3^{b} (9.0)	100^{a} (10.0)	100^{a} (10.0)	98.2^{a} (9.9)	100^{a} (10.0)	100^{a} (10.0)	0^{a} (0.7)
4	B_4	68.1^{c} (8.3)	100^{a} (10.0)	100^{a} (10.0)	46.4^{f} (6.8)	54.4^{e} (7.4)	92.0^{b} (9.6)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	98.2^{a} (9.9)	100^{a} (10.0)	100^{a} (10.0)	10.7^{g} (3.4)	58.2^{d} (7.7)	57.5^{d} (7.6)	0^{a} (0.7)

Figures in parenthesis are square root transformed values

In each column figures followed by same letter do not differ significantly according to DMRT

was more inhibitory compared to garlic extract. At five per cent concentration garlic was giving more than 60 per cent inhibition of isolates B_2, B_3 and B_4. Leaf extracts of marigold was having least inhibitory properties against all the bacterial isolates. Ocimum leaf extract at 15 per cent concentration was giving 75 per cent inhibition for isolates B_2 and B_3 and more than 80 per cent inhibition for B_1 and B_4.

4.15.2 *In vitro* effect of phytoextracts on pathogenic/ weed fungi and milky mushroom

The efficacy of different phytoextracts at various concentrations on the growth of fungal pathogens and weed fungi are given in the Table 21. From the data it is evident that there was significant difference among the treatments in inhibiting the growth of pathogenic / weed fungi.

Among the five plant extracts tested garlic and ocimum were found to be most inhibitory at five per cent concentration against all pathogenic/ weed fungi except *Curvularia* sp. At five per cent concentration neem leaf extract was significantly superior to all other treatments in giving maximum inhibition (84.1 per cent) to the growth of *Curvularia* sp. All other treatments differ significantly in inhibitory effect against *Curvularia* sp. Least inhibition (27.4 per cent) was offered by turmeric extract. Garlic and neem leaf extract gave 100 per cent inhibition to *Curvularia* sp. at 10 per cent concentration.

Among the different plant extracts only garlic, ocimum and marigold at higher concentrations exhibited inhibitory property against *Trichoderma* sp. Garlic extract at five per cent concentration recorded 100 per cent inhibition followed by ocimum (81.5 per cent). At higher concentrations slight inhibitory effect was noticed for marigold extract. The inhibitory effect was absent for neem leaf extract even at the highest concentrations tried. All the phytoextracts at lowest concentration gave 100 per cent inhibition against *D. dendroides*.

Table 21: *In vitro* effect of phytoextracts against pathogenic /weed fungi

Pathogenic/ weed fungi	Per cent inhibition over control*															Control
	Garlic extract			Ocimum leaf extract			Neem leaf extract			Turmeric extract			Marigold leaf extract			
	5 %	10%	15%	5 %	10 %	15 %	5 %	10 %	15 %	5 %	10%	15 %	5 %	10 %	15 %	% inhibition
Curvularia sp.	72.9^{f} (8.5)	100^{a} (10)	100^{a} (10)	78.1^{e} (8.9)	83.1^{cd} (9.1)	87.1^{b} (9.4)	84.1^{c} (9.2)	100^{a} (10)	100^{a} (10)	27.4^{j} (5.3)	41.4^{i} (6.5)	59.6^{g} (7.8)	44.8^{h} (6.7)	61.5^{g} (7.9)	81.9^{d} (9.1)	0^{a} (0.7
Trichoderma sp.	100^{a} (10.0)	100^{a} (10)	100^{a} (10)	81.5^{c} (9.1)	82.2^{bc} (9.1)	83.7^{b} (9.2)	0^{g} (0.7)	0^{g} (0.7)	0^{g} (0.7)	0^{g} (0.7)	0.4^{g} (0.9)	3.4^{f} (2.0)	0^{g} (0.7)	20.0^{e} (4.5)	35.9^{d} (6.0)	0^{a} (0.7
D.dendroides	100^{a} (10.0)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10.0)	100^{a} (10	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	0^{a} (0.7
C. indicum	100^{a} (10.0)	100^{a} (10)	100^{a} (10	100^{a} (10)	100^{a} (10)	100^{a} (10)	20.3^{f} (4.6)	50.7^{e} (7.2)	76.2^{d} (8.8)	84.8^{c} (9.3)	87.0^{b} (9.4)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10.0)	0^{a} (0.7
Coprinus sp.	100^{a} (10.0)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10.0)	71.9^{c} (8.5)	79.6^{b} (8.9)	100^{a} (10.0)	0^{a} (0.7
Penicillium sp.	100^{a} (10.0)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	85.1 ^{b} (9.3)	100^{a} (10)	100^{a} (10)	66.3^{f} (8.2)	78.4^{e} (8.9)	82.9^{c} (9.1)	80.0^{d} (9.0)	86.3^{b} (9.3)	100^{a} (10.0)	0^{a} (0.7
Aspergillus sp.	81.8^{d} (9.0)	85.8^{c} (9.2)	87.4^{b} (9.3)	100^{a} (10)	100^{a} (10)	100^{a} (10)	0^{i} (0.7)	20.4^{h} (4.6)	34.0^{g} (5.9)	61.5^{f} (7.9)	67.0^{e} (8.2)	81.5^{d} (9.1)	84.8^{c} (9.2)	100^{a} (10.0)	100^{a} (10.0)	0^{a} (0.7
R. solani	100^{a} (10.0)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	100^{a} (10)	66.29^{f} (8.1)	72.6^{e} (8.5)	78.8^{d} (8.9)	85.6^{b} (9.3)	100^{a} (10)	100^{a} (10.0)	79.3^{d} (8.9)	82.6^{c} (9.1)	100^{a} (10.0)	0^{a} (0.7
Sclerotium sp.	100^{a} (10.0)	100^{a} (10.0)	100^{a} (10)	63.70^{e} (8)	75.9^{d} (8.7)	81.9^{c} (9.1)	17.0^{h} (4.2)	42.2^{f} (6.5)	62.6^{e} (7.9)	86.3^{b} (9.3)	87.4^{b} (9.4)	100^{a} (10.0)	15.9^{h} (4.1)	32.2^{g} (5.7)	40.4^{f} (6.4)	0^{a} (0.7
C. indica	0^{b} (0.7)	0^{b} (0.7)	17.7^{a} (4.2)	0^{b} (0.7)	0^{b} (0.7)	3.6^{a} (2.0)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7)	0^{a} (0.7

* Mean of three replications
Figures in parenthesis are square root transformed values
In each column figures followed by same letter do not differ significantly according to DMRT

Against *C. indicum* garlic extract, ocimum leaf extract and marigold leaf extract gave 100 per cent inhibition at five per cent concentration, which was followed by turmeric extract (84.8 per cent). At 15 per cent level turmeric was also giving 100 per cent inhibition. Neem leaf extract at five per cent concentration was the least effective treatment to *C. indicum* (20.3 per cent).

All treatments except marigold extract showed 100 per cent inhibition in growth of *Coprinus* sp. at all concentrations tried. Marigold leaf extract could provide the same effect at 15 per cent concentration. Almost same trend was observed against *Penicillium* sp. Extracts of garlic and ocimum recorded 100 per cent inhibition against the organism. Turmeric was the least effective among the different treatments.

Ocimum extract was the best treatment against *Aspergillus* sp. which showed 100 per cent inhibition at all concentrations tried and neem leaf extract was the least effective treatment. Against *R. solani* garlic and ocimum were the most effective with 100 per cent inhibition at all concentrations tested. Higher concentrations of turmeric and marigold (15 per cent) also recorded 100 per cent inhibition. Neem leaf extract was least effective and could not provide 80 per cent inhibition even at the highest concentrations tried.

Apart from other organisms *Sclerotium* sp. could tolerate leaf extracts. All the concentrations of garlic extract and turmeric extract at 15 per cent level could inhibit the growth of *Sclerotium* sp. completely. Marigold leaf extract was least effective at all concentrations tried. More than 80 per cent inhibition was given by turmeric extract even at five per cent concentrations.

Among the five plant extracts garlic and ocimum extract at the highest concentrations showed slight inhibition of 17.7 and 3.6 per cent respectively to the growth of milky mushroom. All other treatments have no inhibitory effect against *C. indica*.

Garlic extract gave 100 per cent inhibition to all pathogenic/ weed fungi at five per cent level except against *Curvularia* sp. and *Aspergillus* sp. Ocimum extract gave 100 per cent inhibition towards all fungi except *Curvularia* sp, *Trichoderma* sp. and *Sclerotium* sp. at five per cent level. These two treatments found to be best in inhibiting the growth of fungal pathogens followed by turmeric extract.

Based on the *in vitro* study the following phytoextarcts were selected for field evaluation, garlic and ocimum (5 per cent), turmeric (10 per cent), neem and marigold leaf extract (15 per cent).

4.16 *In vivo* evaluation of selected phytoextracts

Effect of the selected phytoextracts on mycelial growth and yield were studied and the results are given in Table 22.

Among the different treatments T_1 (Chemical sterilization along with $CaCO_3$ +Garlic extract) and T_2 (Chemical sterilization along with $CaCO_3$ +Ocimim extract) recorded significantly higher mycelial growth (79.2 and 79.3), compared to others, which were on par with T_3 (Chemical sterilization along with $CaCO_3$ +Turmeric extract). Least mycelial growth was recorded by T_{11} (Boiling+ Marigold leaf extract), which was lower than T_{12} (Boiling+ distilled water).

Among the treatments an early pin head emergence was noticed in T_2 and T_3, 16 days after casing. The time taken for mushroom production in T_8 (Boiling + Ocimum extract) was found to be the maximum (26 days). In all other treatments pinhead emergence was noticed between 18- 24 days after casing.

Application of T_1 (Chemical sterilization along with $CaCO_3$ +Garlic extract) and T_2 (Chemical sterilization along with $CaCO_3$ +Ocimim extract)

Table 22: *In vivo* effect of selected phytoextracts on growth and yield of *C. indica*

Treatments	Mycelial growth (%)	Pinhead emergence (days after casing)	Number of Sporophore	Yield (g)/ 500 g substrate
T1	79.2[a] (8.9)	19.3[abc] (4.5)	13.3[a] (3.7)	325.0[a] (18.0)
T2	79.3[a] (8.9)	16.3[c] (4.1)	14.7[a] (3.9)	266.8[b] (16.)
T3	75.4[ab] (8.7)	16.7[c] (4.1)	8.7[b] (3.0)	226.2[b] (15.1)
T4	66.5[cd] (8.2)	18.3[bc] (4.3)	6.3[bc] (2.6)	148.1[cd] (12.2)
T5	62.0[de] (7.9)	18.3[bc] (4.3)	6.7[bc] (2.6)	133.7[cd] (11.6)
T6	70.4[bc] (8.4)	24.7[ab] (5.0)	5.0[c] (2.3)	122.2[cd] (11.1)
T7	46.0[f] (6.8)	19.3[abc] (4.5)	4.3[c] (2.2)	104.3[def] (10.2)
T8	45.9[f] (6.8)	26.0[a] (5.1)	4.0[c] (2.1)	90.3[ef] (9.5)
T9	56.8[e] (7.5)	18.7[bc] (4.3)	8.3[b] (2.9)	166.8[c] (12.9)
T10	46.2[f] (6.8)	19.3[abc] (4.4)	5.7[bc] (2.5)	119.6[def] (10.9)
T11	36.7[g] (8.9)	24.3[ab] (4.4)	4.3[c] (3.7)	91.7[ef] (18.0)
T12	47.4[f] (8.9)	20.7[abc] (4.1)	4.0[c] (3.9)	76.5[f] (16.3)

T1- Chemical sterilization along withCaCO3+Garlic extract (5%)
T2- Chemical sterilization along withCaCO3+Ocimum extract (5%)
T3- Chemical sterilization along withCaCO3+Turmeric extract (10%)
T4- Chemical sterilization along withCaCO3+Neem leaf extract (15%)
T5- Chemical sterilization along withCaCO3+Marigold extract (15%)
T6-Chemical sterilization along withCaCO3+ Distilled water
T7-Boiling + Garlic extract (5%)
T8 -Boiling + Ocimum extract (5%)
T9-Boiling + Turmeric extract (10%)
T10-Boiling + Neem leaf extract (15%)
T11-Boiling + Marigold extract (15%)
T12-Boiling + Distilled water

Figures in parenthesis are square root transformed values
In each column figures followed by same letter do not differ significantly according to DMRT

produced significantly higher number of sporophore 13.3 and 14.7 respectively; this was followed by turmeric extract. The least sporophore number (4) was observed in T_{12} and T_8.

The data on the yield revealed that there was significant variation among the treatments. T_1 (Chemical sterilization along with $CaCO_3$ + Garlic extract) recorded an average yield of 325.0 g which was significantly superior to all other treatments. This was closely followed by T_2 (Chemical sterilization along with $CaCO_3$ +Ocimum extract) and T_3 (Chemical sterilization along with $CaCO_3$ +Turmeric extract). The least yield of 76.5 g recoded by T_{12} (Boiling+ distilled water).

4.16.1 Effect of phytoextracts on weed fungi incidence

All treated beds were free from natural incidence of weed fungi.

4.16.2 Effect of phytoextracts on bacterial disease incidence

Effect of phytoextracts in controlling natural incidence of bacterial disease in farm was studied after second and third emergence of pinheads. The disease incidence and severity was recorded during first, second and third harvest and the data obtained are presented in Table 23.

During the first harvest (with out spraying) maximum disease incidence was noticed in T_9 (100 per cent), which was on par with T_1 (Chemical sterilization along with $CaCO_3$+Garlic extract) and T_{10} (Boiling +Neem leaf extract). Lowest incidence and severity was noticed in T_2, T_4, T_6 and T_8. The maximum disease severity was noticed in T_1 and T_9 (63.8 and 63.3 per cent), which was on par with T_3 (59.9).

Table 23: Effect of phytoextracts on incidence and severity of bacterial diseases

Sl. No	Treatments	Disease incidence			Diseases severity		
		1 Harvest	2 Harvest	3 Harvest	1 Harvest	2 Harvest	3 Harvest
1	T_1	75 (8.7)^{ab}	51.6(7.2)^{c}	8.3 (2.9)^{g}	63.8(8.0)^{a}	33.9(5.9)^{abc}	8.3(2.9)^{b}
2	T_2	0(0.7)^{e}	69.0(8.3)^{b}	50.0 (7.1)^{d}	0(0.7)^{d}	53.8(7.24)^{ab}	50.0(7.1)^{ab}
3	T_3	58.3(7.7)^{b}	11.0(3.4)^{e}	63.6(8.0)^{c}	59.9(7.7)^{ab}	11.1(2.40)^{bc}	55.5(7.5)^{ab}
4	T_4	0(0.7)^{e}	33.3(5.8)^{d}	0(0.7)^{f}	0.0(0.7)^{d}	29.6(3.62)^{abc}	0(0.7)^{b}
5	T_5	33.3 (5.8)^{c}	66.6 (8.2)^{b}	16.6(4.1)^{f}	33.3(5.8)^{c}	66.6(8.19)^{ab}	16.6(4.1)^{ab}
6	T_6	0(0.7)^{e}	88.8 (9.4)^{a}	0(0.7)^{h}	0(0.7)^{d}	77.7(8.62)^{ab}	0(0.7)^{b}
7	T_7	33.3(5.8)^{c}	0(0.7)^{f}	16.6(4.1)^{f}	27.6(5.3)^{cd}	0(0.7)^{c}	16.6(4.1)^{ab}
8	T_8	0(0.7)^{e}	50.0(7.1)^{c}	16.6(4.1)^{f}	0(0.7)^{d}	50.0(7.1)^{abc}	16.6(4.1)^{ab}
9	T_9	100 (10.0)^{a}	47.2 (6.9)^{c}	83.3(9.2)^{b}	63.3(7.9)^{a}	55.5(7.5)^{ab}	50(7.1)^{ab}
10	T_{10}	83.3(9.2)^{ab}	83.3(9.2)^{a}	33.3(5.8)^{e}	50.0(7.1)^{b}	66.6(8.1)^{ab}	33.3(5.8)^{ab}
11	T_{11}	11.0(3.4)^{d}	66.6(8.2)^{b}	33.3(5.8)^{e}	33.3(5.8)^{c}	83.8(9.2)^{a}	100(10.0)^{a}
12	T_{12}	33.3(5.8)^{c}	66.6(8.2)^{b}	100(10.0)^{a}	33.3(5.8)^{c}	55.5(7.5)^{abc}	66.6(8.2)^{ab}

T_1- Chemical sterilization along with$CaCO_3$+Garlic extract (5%)
T_2- Chemical sterilization along with$CaCO_3$+Ocimum extract (5%)
T_3- Chemical sterilization along with$CaCO_3$+Turmeric extract(10%)
T_4- Chemical sterilization along with$CaCO_3$+Neem leaf extract(15%)
T_5- Chemical sterilization along with$CaCO_3$+Marigold extract (15%)
T_6-Chemical sterilization along with$CaCO_3$+ Distilled water
T_7- Boiling+Garlic extract (5%)
T_8- Boiling+Ocimum extract (5%)
T_9- Boiling+Turmeric extract(10%)
T_{10}-Boiling+Neem leaf extract(15%)
T_{11}- Boiling+Marigold extract (15%)
T_{12}-Boiling+ Distilled water

Figures in parenthesis are square root transformed values

In each column figures followed by same letter do not differ significantly according to DMRT

During the second harvest no disease incidence was noticed in T_7 (Boiling + Garlic extract), which was closely followed by T_3 (Chemical sterilization along with $CaCO_3$+Turmeric extract). Garlic extract spraying could reduce the disease incidence to zero per cent from 33 per cent obtained during the first harvest (Fig. 6a). About 25 per cent reduction in bacterial disease incidence was noticed T_1. Spraying of turmeric extract (T_9) also reduced the disease incidence from 100 per cent to 47.2 per cent and 58.3 per cent to 11 per cent in T_3 (Chemical sterilization along with $CaCO_3$+Turmeric extract). Maximum disease incidence was noticed in T_6 and T_{10}, which were followed, by T_2, T_5, T_{11} and T_{12}. Maximum disease severity of 83.8 per cent was recorded in T_{11}, which was on par with all other treatments except T_3 and T_7. An increase in disease incidence and severity was noticed on beds sprayed with ocimum, neem and marigold extract and in control.

In third harvest T_1 (Chemical sterilization along with $CaCO_3$+Garlic extract) was found to be best with 8.3 per cent disease incidence and severity, this was followed by T_7 (Boiling+ Garlic extract). Lowest incidence and severity was also noticed in treatments T_4, T_5 and T_8, which is not due to treatment effect but because of the less number of sporophore production during the third harvest (Fig. 6b). Maximum disease incidence was noticed in T_{12} and T_9 followed by T_3 and T_2. Disease severity was maximized in T_{11} (Marigold leaf extract).

4.17 Biochemical changes in bacteria infected mushroom

Changes in content of crude protein, fibre, sugar and phenol in healthy and diseased mushrooms were studied (Table 24).

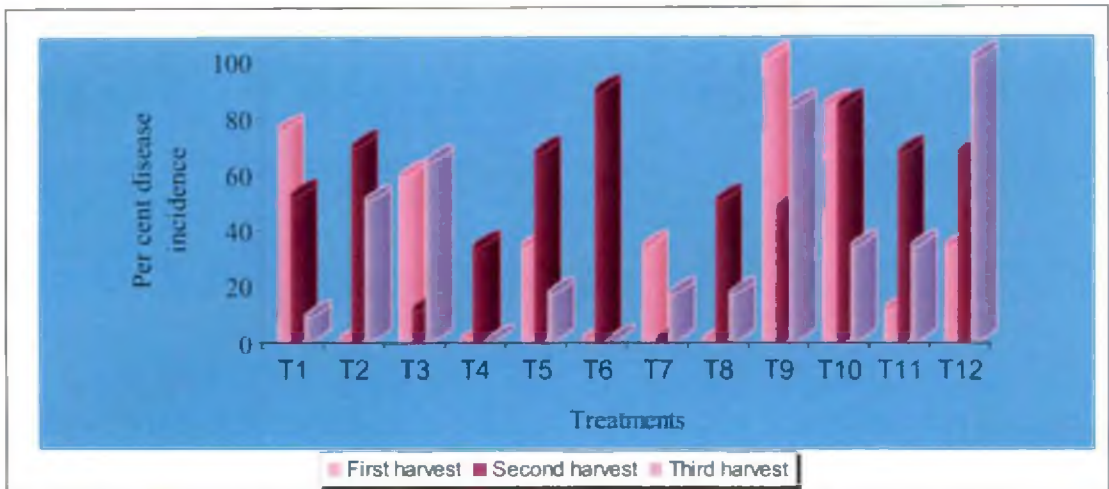


Fig. 6a. Effect of phytoextracts on bacterial disease incidence

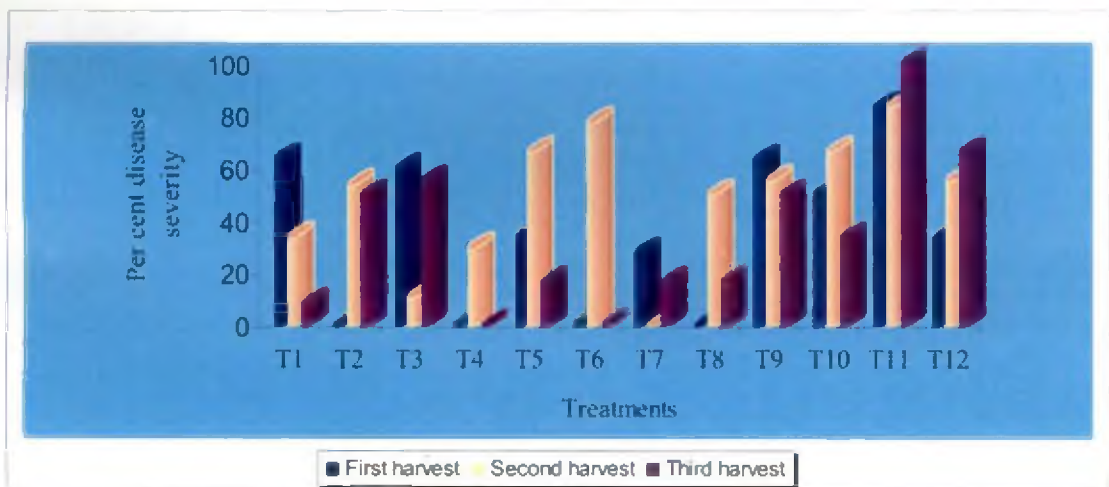


Fig. 6a. Effect of phytoextracts on bacterial disease severity

T_1- Chemical sterilization along with$CaCO_3$+Garlic extract (5%)
T_2- Chemical sterilization along with$CaCO_3$+Ocimum extract (5%)
T_3- Chemical sterilization along with$CaCO_3$+Turmeric extract (10%)
T_4- Chemical sterilization along with$CaCO_3$+Neem leaf extract (15%)
T_5- Chemical sterilization along with$CaCO_3$+Marigold extract (15%)
T_6- Chemical sterilization along with$CaCO_3$+ Distilled water
T_7- Boiling+ Garlic extract (5%)
T_8- Boiling +Ocimum extract (5%)
T_9- Boiling+ Turmeric extract (10%)
T_{10}-Boiling+Neem extract (15%)
T_{11}-Boiling+ Marigold extract (15%)
T_{12}-Boiling+ Distilled water

Table 24: Biochemical constituents in bacteria infected mushroom

Sl.No	Biochemical constituents	Healthy	Diseased
1	Crude protien (Per cent)	23.5	28.1
2	Crude fibre (Per cent)	19.3	15.5
3	Sugar (µg/g)	8.5	8.7
4	Phenol (µg /g)	8.8	7.3

From the table it was clear that there was an increase in crude protein content in diseased mushroom compared to healthy. In healthy mushroom 23.5 per cent crude protein content was recorded against 28.1 per cent in diseased. Crude fibre content was reduced in diseased (15.5 per cent) compared to healthy (19.3 per cent). Similar trend was observed in phenol. Phenol content in healthy mushroom was high (8.8 µg /g of mushroom tissue) compared to 7.3 µg /g of diseased mushroom. There was only slight change in sugar content in healthy and diseased mushroom, it was 8.7 µg/g in diseased against 8.5 µg/g in healthy mushroom.

Discussion

5. DISCUSSION

Mushrooms are prone to a number of biotic and abiotic stresses. Milky mushroom, *Calocybe indica* (P&C) is comparatively new to the world of mushroom growers. As commercial cultivation of the milky mushroom is growing at rapid rate, pest and disease problems during cropping are also emerging at a faster rate. As scanty literature are available on the diseases of milky mushroom, a study was taken up on the various diseases of this mushroom, their causal agents, weed fungi associated with cultivation and to evolve ecofriendly management of these problems.

As part of the study, a survey was conducted in milky mushroom farm, of College of Horticulture, Vellanikkara and also in private farms at Eravimangalam, Kodungalloor and Kattilapoovum in Thrissur district on the occurrence of diseases of milky mushroom. Both biotic and abiotic problems were identified during the survey. Biotic problems include fungal and bacterial diseases, among this bacterial blotch is the most problematic one, noticed in all locations. Several workers reported bacterial blotch disease in other cultivated mushrooms (Guleria, 1976; Biswas *et al.*, 1983; Besette *et al.,* 1985; Femour, 1986 and Atkey *et al.*, 1991). Incidence of bacterial blotch can be serious if the humidity is more than 90 per cent and if there is free water available on sporophore. According to Sharma (1995) temperature above 20^0C and relative humidity of more than 85 per cent lead to water condensation over the pileus in white button mushroom. Jandaik and Jandaik (2003) reported that high relative humidity and continuous persistence of water film on the surface of pilei enhanced bacterial multiplication.

Incidence of cobweb disease was noticed during June and July in a farm at Kattilapoovum, where the conditions are most conducive for the development of the disease. The cultivation was carried out in damp shed without proper hygiene and low temperature was prevailed inside the farm. Incidence of cobweb disease in milky mushroom was reported by Pandey *et al.* (2003) and the disease was

generally found to occur under high humid conditions. Sharma (1995) reported that the cobweb disease was more severe on white button mushroom cultivated in damp and unhygienic conditions with high relative humidity.

Greenish fungal infection on pinheads of milky mushroom was noticed at Kodungalloor farm, where a dry condition exists inside the farm. This may be because the mushroom farm was erected on the terrace of a building where natural dry conditions can occur if not watered sufficiently. Bayer *et al.* (n.d) reported *Penicillium* infection on basidiocarp of white button mushroom.

Weed fungi incidence on bed during the spawn run stage was also noticed in all the milky mushroom farms. Many workers reported weed fungi incidence during spawn running stage of milky mushroom (Pathak *et al.,* 1998; Pandey and Tewari, 2002; Pandey *et al.*, 2003; Krishnamoorthy, 2004; Gogoi *et al.*, 2006 and Heera, 2006). The intensity and extent of damage depends on the conditions like temperature, ventilation inside the spawn running room, moisture content of the substrate, along with hygienic conditions in and around the farm. If these conditions are congenial for the growth of weed fungi their population increases in an alarming rate. Pandey and Tewari (2002) reported that high contamination of substrate even after pasteurization, air in the chopping room and spawn running room, contaminated spawn were the reasons for growth of weed moulds in milky mushroom beds. According to Thapa *et al.* (1979) olive green mould *Chaetomium globosum* never appeared in the compost, which had been pasteurized properly with adequate ventilation. Sharma (1995) and Sharma and Vijay (1996a) reported that improper pasteurization of the substrate, frequent use of formalin and unhygienic conditions of cultivation aggravate the incidence of *Trichoderma* sp. in *Agaricus* sp. In *Agaricus* sp. high substrate moisture and free ammonia present in substrate favour the infection by *Coprinus* sp. (Sharma, 1995; Sharma and Vijay, 1995 and Pani, 2000).

The Agrowe mushroom farm at Eravimangalam has been regularly surveyed once in a month for one year. The most important and consistent problem in the farm was the incidence of bacterial blotch on sporophore. The incidence of the disease was severe due to relay cropping system practiced in this farm, where sporophores of different age group are available. Further, suitable conditions provided for cultivation also help in continuity of the infection chain. The high relative humidity of more than 90 per cent, high temperature around 32-34^{0}C and free water drops available on the sporophore by the condensation of water vapour favours the incidence of bacterial blotch. These conditions can retain all the favourable factors necessary for the multiplication of the bacterial pathogen. Once the pathogen is introduced to the farm it survives between crops, in debris, on tools and on various structures inside the farm. According to Jandaik and Jandaik (2003) high relative humidity and continuous persistence of water film on the surface of pilei enhances bacterial multiplication in white button mushroom.

The abiotic problems at the Agrowe farm were commonly observed during the fag end of the crop in August or September when farm is almost in a neglected condition. The common abiotic problems noticed are scaling, splitting and malformation of mushroom, which are associated with high temperature and moisture stress. The unfavourable dry condition can cause a light browning of the surface of the young sporophore and as the sporophore expands these tissues breaks and results in the scaling and splitting of sporophore. According to Sharma (1995) the main reason for scales being formed on white button mushroom was the poor climate control, especially too much drying inside the farm. Sharma and Vijay (1996b) also reported scaling of cap, as an abiotic disorder in most of the seasonal button mushroom farms mainly because of low humidity.

Isolation from the diseased sporophores collected from different farms yielded bacterial and fungal organisms. Sporophores showing bacterial blotch symptoms collected from different farms showed association of six different

bacterial isolates. Isolate B_1 B_3 and B_4 from Agrowe mushroom farm and B_2 and B_5 from Kodungalloor. The isolate B_6 was obtained from Vellanikkara. The inoculum source of the bacterial isolates can be from casing soil or from air in and around the farm. At Agrowe farm and farm at Kodungalloor, neopeat was used as the casing material, whereas the farm at Vellanikkara used potting mixture with 1:1:1 ratio of soil, sand and dried cow dung. This can be the reason for the variation in bacterial isolates from different locations. According to Sharma (1995) and Jandaik and Jandaik (2003) casing material and air born dust are the primary means of introducing the bacterial pathogen in to a mushroom farm.

Pathogenicity of all the bacterial isolates was proved by inoculating bacterial suspension on healthy sporophore.

Studies on symptamatology of the bacterial blotch showed that the symptoms produced by all the isolates are identical; the extent depends on the severity of the disease. Initial symptoms of bacterial blotch appeared as light brown discolouration on sporophore, it appeared both on pileus and stipe. Later underlying tissues become damaged and typical water soaked lesions are produced. Finally these lesions were deepened and widened and whole sporophore become rotten. Pathak *et al.* (1998) reported yellowing of the stipe and killing of young sporophores in milky mushroom due to bacterial infection. According to Guleria (1976) initial symptoms of the blotch disease appeared as pale yellow lesion on mushroom tissue. Biswas *et al.* (1983) reported that the first symptoms of bacterial rot disease in oyster mushroom are water soaked areas and yellow brown discolouration of the young sporophores. Similar symptoms were also reported by many workers in other species of cultivated mushrooms (Jandaik *et al.*, 1993; Sharma, 1995; Jandaik and Jandaik, 2003 and Desuramaux *et al.*, 2005).

Two fungal pathogens were also isolated from diseased mushroom samples collected from Kattilapoovum and Kodungalloor. From Kattilapoovum cobweb disease was noticed and the fungus associated with the disease was

isolated in PDA medium. Similarly the samples from Kodungalloor, upon isolation yielded *Penicillium* sp. on PDA.

Pathogenicity of the fungal isolates was proved by inoculating mycelial disc into casing soil of healthy milky mushroom beds. The mycelial growth of the fungus was observed on casing surface after 9-10 days of inoculation. Symptoms of the disease first appeared as small circular, white patches of mycelial growth on the surface of casing soil. Later it spreads very fast and attacks the basal portion of the stipe producing white fluffy fungal growth. Finally the infected mushroom develops pink colour at the point of infection, the tissues became softened and rotted. The incidence of cobweb disease with similar symptoms on milky mushroom was reported by Pandey *et al.* (2003) and Buvaneswari (2004). On *Agaricus bisporus* Seth, 1977; Dar, 1985 and Sharma, 1995, observed similar symptoms of cobweb disease.

In the case of Penicillium infection on emerging pinheads also, the pathogenicity was proved by the same method. The growth of the fungus was observed on casing surface after 6-7 days of inoculation. The symptomatological studies revealed that the infection initiated as a brown discolouration and greenish fungal growth on small emerging pinheads. At later stages it prevents further development as well as production of fresh pinheads. Bayer *et al.* (n.d) reported that secondary invaders like *Penicillium* sp covered the basidiocarp of white button mushroom and resulted brown discoloration.

Weed fungi like, *Trichoderma* sp., *Aspergillus* sp., *Chaetomium indicum*, *Curvularia* sp., *Sclerotium* sp., *Coprinus* sp. and *Rhizoctonia solani* were isolated from contaminated mushroom beds. Pathak *et al.* (1998) reported incidence of *Trichoderma* spp., *Aspergillus* spp., *Rhizopus* spp. and *Coprinus* spp. in milky mushroom beds. Similar reports were made by Pandey and Tewari (2002) with respect to *Trichoderma harzianum* and *C. globosum*. Pandey *et al.* (2003) and Heera (2006) in the case of *Trichoderma* sp. Krishnamoorthy (2004) and Gogoi

et al. (2006) also reported that *Coprinus* sp. infection restricts the spawn running of milky mushroom.

Inoculating mycelial disc into test tubes containing paddy straw bits and milky mushroom spawn proved the colonization of the weed fungi on paddy straw. All weed fungi inhibited the mycelial growth of the milky mushroom to varied extent. Similar reports were also made by Pandey and Tewari (1990) in oyster mushroom.

The nature of damage by the weed fungi was almost the same, where patchy growth of the mushroom mycelium was observed in infected beds. Some of them grew in association with spawn grains, whereas some had faster growth compared to mushroom mycelium. Ultimately they produced their survival structures and make the bed unsuitable for growth of the mushroom mycelium. Initial growth of the weed fungi was identical with white mycelial growth similar to milky mushroom growth except *Curvularia* sp., which produced black coloured mycelium on spawn grain. Due to the similarity in growth detection of weed fungi in early stages of spawn running was difficult. The growth of *Aspergillus* sp. and *Trichoderma* sp. could be detected after the production green coloured spores. The fungus *R. solani* produced orange coloured soft irregular structures one week after spawn run, whereas *Sclerotium* sp. produced dark brown coloured sclerotia. The weed fungus *Coprinus* sp. produced black coloured fruiting bodies inside the bed and on autolysis it produced black slimy ink mass. The isolates of *T. harzianum* from the beds of *C. indica*, *P. sajor-caju* and *A.bisporus* have good activity of all the extra cellular lignocellulolytic enzymes (Sharma *et al.*, 2005b). Many workers have reported the nature of damage by weed fungi on other cultivated mushrooms. Weed fungi deplete the necessary assimilatory substances from the substrate, required for the growth of mushroom mycelium (Sharma and Jandaik, 1980). Das and Purkayastha (1994) reported higher protease activity by *Coprinus cinereus* than *Volvariella volvaceae.* Sharma and Vijay (1995) reported that the slimy ink mass produced after autolysis of *Coprinus* sp. interfered with spawn run of *A.*

bisporus. High cellulolytic activity and faster growth helps *Trichoderma* sp. to colonize the substrate easily Pandey and Tewari (1990 and 2002). Competition for space and nutrients, antibiosis and release of hydrolytic enzymes were the mechanisms by which *Trichoderma* sp. parasitizes *A. bisporus* (Rinker and Castle, 2007).

An attempt was made to identify the pathogenic organism involved in the diseases of milky mushroom. Further, tentative identification of weed fungi associated with milky mushroom also carried out. Among the six bacterial isolates B_1, B_5 and B_6 showed same result for all the tests, hence the three isolates were considered as single isolate B_1. Pathogenic bacterial isolates B_1, B_2 and B_4 produced cream coloured, punctiform colonies and that of B_3 was yellow. In King's B medium only isolate B_2 produced diffusible pigments. Except isolate B_4 all others were gram negative rods. The isolate B_4 produced endospores.

The bacterial isolates B_3, B_4 and B_5 recorded maximum growth at 30^0C where as the isolates B1, B_2 and B_6 recorded maximum growth at 35 ^{0} C. At 25 ^{0}C all the isolates recorded poor growth. All the bacterial isolates recorded maximum growth at pH 5 and minimum growth at pH 8.

All the isolates showed positive reaction for catalase, oxidase, citrate utilization, arginine dihydrolase reaction, while they were negative for levan production. Isolates B_1, B_2 and B_3 were aerobic in nature and were positive for lecithinase test and they did not hydrolyzed starch. The isolate B_3 was giving negative reaction for denitrification and positive for lipase test. Isolate B_4 showed growth both in aerobic and anaerobic condition and was negative for lecithinase test and hydrolysed starch.

Utilization of sugar/ carbohydrate by different isolates was also studied. Isolates B_1 and B_2 showed uniformity in utilization of all the 12 sugars tested except maltose and lactose and both failed to utilize sucrose. The isolate B3

utilized all the 12 carbon sources tested, whereas the isolate B_4 utilized only maltose and manitol. Most of these results are in agreement with those reported by many workers, while characterizing the *Pseudomonas* sp. and *Bacillus* sp. (Paine, 1919; Harrigan and Mc cane, 1966; Bradshaw, 1973; Krieg and Holt, 1984; Bradhury, 1986 and Schaad, 1992). Several workers reported the association of different *Pseudomonas* sp. like *P. tolaasii, P. alcaligens, P. fluorescens, P. aeruginosa, P. agarici, P. fluorescence* biotype III and *P. putida* with bacterial blotch diseases of cultivated mushrooms (Guleria, 1976; Biswas *et al.* 1983; Poppe *et al,* 1985; Jandaik *et al.*, 1993 and Rinker and Castle, 2007). The genus *Bacillus* was reported as a cellulolytic bacteria from mushroom compost by Shirkot *et al.* (1994). Pathak *et al.* (1998) reported reduction of mycelial growth by *Bacillus* sp. in *Lentinula* sp. Hence based on these characters coupled with pathogenicity the isolates B_1, B_2 and B_3 were tentatively identified as *Pseudomonas* sp. and isolate B_4 as *Bacillus* sp.

Cultural and morphological characters were studied in detail for the identification of pathogenic and weed fungi. Cobweb pathogen produced branched, hyaline hyphae and colour of the media changes to pink or red. Conidiphores erect and verticilliately branched. Conidia were elongate, single, 3-4 septate and measure 15.8-23.7 μm x 7.9-10.3 μm. These characters were in conformity with those described for *Dactylium dendroides* by Pandey *et al.* (2003) Buvaneswari (2004) and Sharma *et al.* (2006). Thus the pathogen causing cobweb disease was identified as *Dactylium dendroides.*

Pathogenic fungus *Penicillium* sp. isolated from pinheads produced wrinkled greyish green colonies on PDA. Conidiophores terminating in a verticel of metulae bearing conidia in chains and the conidia measures 1.88-2.6 μm. Pandey and Tewari (1989 and 1990), Vijay and Sohi (1989) and Pani (2000) reported *Penicillium* sp. with similar characters from mushroom beds.

Seven weed fungi interfering with mycelial colonization of mushroom fungus was isolated from mushroom beds. In PDA medium *Aspergillus* sp. produced yellowish green colonies. Hyphae hyaline, conidial heads radiating on long conidiophore, vesicles globose to subglobose with 39.5-47.4 μm diameter and conidia globose, measure 3.95μm diameter. Black coloured fluffy colonies were produced by *Curvularia* sp. Hyphae branched with 3.95-5.26 μm width. Conidiophores arising singly or in groups, conidia 3-4 septate, dark brown and the third cell from the base always curved, darkest and larger than others and measures 19.75- 27.6 μm long and 11.85-19.75 μm wide. Colonies of *Trichoderma* sp. was smooth, initially white and gradually turned to bright green. Mycelium septate, hyaline and smooth walled. Phialides arise in group, philaospores short obovoid, pale green and measure 3 x 2.2 μm. The characters were in conformity with that described by Gilman (1957) and Subramanian (1971). Thus the three weed fungi were identified as *Aspergillus* sp. Micheli, *Curvularia* sp. Boedijn and *Trichoderma* sp. (Persoon) Harz.

The *Coprinus* sp. produced sporophores of cylindrical or conical pileus with wooly scales 2.5-3 cm diameter. Gills are free and 3-4 gills/mm. Stipe centrally placed, 3.5-8cm long. Black coloured spores and spore print. The characters observed were compared with the reports of Singer (1975), Geetha (1982) and Purkayastha and Chandra (1985) and fungus was identified as *Coprinus* sp. Smooth, white, rapid growing colonies were produced by *Sclerotium* sp. Hyphae hyaline with small white sclerotia. Later colour of the sclerotia was changed to dark brown. These characters were confirmed with that of *Sclerotium* sp. reported by earlier workers (Gilman, 1957; Subramanian, 1971 and Ou, 1972). The organism was thus identified as *Sclerotium* sp. Tode ex Fries. The weed fungus *Rhizoctonia* sp. showed rapid growth in PDA medium. Colony light orange in colour, hyphae hyaline, septate with a width of 3.95 μm. Later orange coloured soft irregular shaped bodies of varying size were produced in medium. Greyish white colonies with thin, raised hairy mycelium was produced by *Chaetomium* sp. Perithecia opaque, globose, with apical tuft of dark bristle or

setae. Ascospores dark brown ovoid and measuring 11.85 x 10.5 µm. Based on these characters these two fungui were identified as *Rhizoctonia* sp. and *Chaetomium* sp. Later identity of these two weed fungi was confirmed as *Rhizoctonia solani* and *Chaetomium indicum* from "National centre of fungal taxonomy" New Delhi (NCFT-1310.07 and NCFT - 1311.07).

Effect of pH and temperature on the growth of pathogenic / weed fungi and on milky mushroom was studied. All the pathogenic/ weed fungi and milky mushroom prefers the same temperature (30^0C) for their faster growth except *Penicillium* sp. which prefers a higher temperature of 35 ^{0}C. The *Penicillium* sp. attack on sporophore at Kodungalloor was noticed only during dry condition inside the farm. According to Krishnamoorthy and Muthusamy (1997) the preferred temperature for the growth of milky mushroom lies in between 30-35^0C.

Similarly except *Coprinus* sp. all other pathogenic/ weed fungi prefer a neutral pH for faster growth. Geetha (1982) reported that *Coprinus* sp. can grow in a wide range of pH from acidic to alkaline and best at pH 5. The weed fungus *Sclerotium* sp. can grow in a wide range of pH. Most preferred pH of milky mushroom, *Penicillium* sp. *and Curvularia* sp. lies in between the range of 7-8. But these two are least problematic ones. This property of milky mushroom can be exploited to reduce the weed fungal attack on bed. Deepthi (2003) reported that addition of lime to soaking water enhanced the colonization of mushroom mycelium. The preferred pH for the growth of milky mushroom was 8 (Krishnamoorthy, 1995 and Buvaneswari, 2004).

In interaction studies between bacterial pathogen and *C. indica,* the *Pseudomonas* sp. (Eravimangalam) showed maximum inhibition (74.4 per cent) and was closely followed by *Bacillus* sp. (73.3 per cent). Other two isolates recorded 67.7 per cent inhibition. In dual culture the growth of *C. indica* was arrested from fifth day onwards due to inhibitory effect by bacterial pathogen.

According to Buvanewari (2004) and Heera (2006) monoculture of *C. indica* recorded nine cm growth after 14 days of incubation.

In interaction studies between pathogenic / weed fungi and milky mushroom, *Sclerotium* sp. showed maximum inhibition by over growth in three days followed by *R. solani, D. dendroides* and *Trichoderma* sp. These organisms could not produce any zone of inhibition in culture, only over growth. Inhibition by over growth of mushroom mycelium was reported by Sharma and Jandaik, 1980; Doshi and Singh, 1983; Pandey and Tewari, 1990; Dar, 1997 and Pandey *et al.*, 2003. Other weed fungi like *Aspergillus* sp., *Curvularia* sp., *Chaetomium indicum, Penicillium* sp. and *Coprinus* sp. recorded 100 per cent inhibition by over growth after 7-8 days of incubation. On PDA medium all fungi showed faster growth rate than the milky mushroom. The growth of *C. indica* mycelium ceased only after the medium has been completely covered up by weed fungi. These findings are in agreement with the earlier reports (Vijay and Sohi, 1989; Pandey and Tewari, 1990 and Pandey *et al.*, 2003).

Culture filtrates of different weed fungi showed adverse effect on the mycelial growth of *C.indica*, thus indicating the production of certain metabolites inhibitory to the mycelial growth of milky mushroom. Among the seven weed fungi tested maximum inhibition was recorded by *C. indicum* followed by other weed fungi like *Curvularia* sp., *Aspergillus* sp. and *Trichoderma* sp. The fungi *R.solani* and *Sclerotium* sp. recorded low inhibition and least by *Coprinus* sp. From the result it is clear that inhibition by the slow growing weed fungi like *C. indicum*, *Curvularia* sp. and *Aspergillus* sp. was due to the metabolites produced by these organisms during their growth. Seshagiri and Eswaran (2002) reported the effect of culture filtrates of the contaminants on the growth of *C. indica* mycelium and the effect was proportional to the concentration of cultural filtrate. Vijay and Sohi (1989) reported that inhibition by culture filtrates of weed fungi was due to the shift in pH of the medium below three and there by inhibiting the growth of mushroom mycelium. The inhibitory effect of culture filtrate of

various weed fungi like *T. harzianum, T. viride, Aspergillus niger, C. globosum* was reported by many workers against the growth of *A. bisporus* and *Pleurotus* sp. (Sharma and Jandaik, 1980; Vijay and Sohi, 1989; Pandey and Tewari, 1990 and Raina, 2004a).

Antagonistic effect of weed fungi on linear growth of milky mushroom was studied and from the result it was clear that inhibitory effect depends on the substrate or medium of growth. Compared to other weed fungi, growth of *Trichoderma* sp. was more inhibitory on straw. This may be because of its high cellulolytic activity as reported by Pandey *et al.* (2003). The fungus *Sclerotium* sp. recorded similar growth rate in medium as well as on substrate. All other weed fungi recorded less than 50 per cent inhibition in paddy straw. The fast growing weed fungi inhibited the growth of milky mushroom due to the depletion of the substrate and competition for space and nutrients. Similar line of work was also conducted by Vijay and Sohi, (1989); Pandey *et al.* (2003).

Weed fungi incidence in mushroom beds were recorded in all months from August 2006 to July 2007. From the study it was revealed that weed moulds were always associated with mushroom beds in a continuously cultivating farm. Similar observations were reported by Siddique *et al.* (2004b) in oyster mushroom beds. Variation in population of weed fungi was observed in relation to season. The most frequent weed fungus observed during the period of study was *R. solani*. Other weed fungi observed were *C. indicum, Sclerotium* sp., *Aspergillus* sp. *Coprinus* sp. and *Curvularia* sp. Weed fungi incidence was maximum in the month of June and minimum during April. Weather parameters prevailing in June was most congenial for the incidence, multiplication and spread of weed fungi. The higher incidence of weed fungi in the month of June may be due to the high relative humidity and optimum temperature around 30^0C recorded inside the farm. The preference in temperature was also evidenced by lab study. Incidence of weed fungi was found maximum with in temperature range of 28-31^0C and relative humidity of 88-99 per cent inside the farm. These conditions are congenial for the

germination and perpetuation of the weed fungi. Moorthy and Mohan (1996) found maximum contamination in oyster mushroom beds during monsoon season. Siddique *et al.* (2004b) reported that frequency of contaminants in *P. sajor-caju* was maximum in the month of June with an average temperature of 29.2-32.7^0 C and 81-87 per cent relative humidity. Pandey and Tewari (2002) reported that constant availability of decaying straw and small bits of rotting mushrooms help in the faster growth and sporulation of weed moulds. In addition to these weather factors, number of beds inside the farm and the unhygienic conditions in and around the farm also contribute to the incidence of weed fungi. Some of these factors might have contributed to the lesser weed fungi incidence during September and October. Moorthy and Mohan (1996) reported that when the maximum capacity (96.5 per cent) of farm was utilized for cultivating oyster mushroom high per cent contamination was recorded in a year irrespective of the season.

Investigations on the effect of weed fungi on the mycelial growth and yield of *C. indica* revealed that all the weed fungi had an inhibitory effect on the mycelial growth and yield. Among the seven weed fungi tested *Sclerotium* sp. and *C. indicum* recorded maximum inhibition of mycelium growth and highest reduction in yield. Other weed fungi like *R. solani, Aspergillus* sp. and *Trichoderma* sp. recorded less than 50 per cent mycelial growth and considerable reduction in yield. Least inhibition was recorded by *Coprinus* sp. All weed fungi grew faster than the mushroom mycelium covering up the entire substrate in shortest time, which results in competition for space and nutrients and this might have contributed to reduction in yield. Similar observations were also recorded by earlier workers (Rajarathnam *et al.,* 1992; Pani and Patra 1997). Liberation of some diffusible toxin or inhibitory substances by weed fungi also found to inhibit the growth of milky mushroom as evidenced by laboratory study on the effect of cultural filtrates of weed fungi on mycelial growth of *C. indica.* Sharma and Jandaik, 1980 and Goyal *et al.*, 1994a also reported similar results in other edible mushroom species. The findings on reduction in mycelial growth and yield by

weed fungi are also in agreement with earlier reports. Pandey and Tewari (1988) reported 73 per cent reduction in mycelial growth by *Trichoderma* sp. and *C. globosum* during oyster mushroom cultivation and by *Sclerotium rolfsii* 74.4 per cent yield loss was recorded in paddy straw mushroom (Pani, 2000).

Among the different sterilization techniques maximum mycelial growth was observed in chemical sterilization along with $CaCO_3$. *In vitro* study also revealed that milky mushroom preferred pH around 8 for faster growth on PDA medium. In all treatments maximum growth rate was observed during the first five days after spawning. But Sharma *et al.* (2006) reported that pasteurization was the best method of substrate sterilization and *C. indica* failed to grow on chemically treated straw and on untreated straw.

In farm trial also chemical sterilization of substrate along with 0.2 per cent $CaCO_3$ gave maximum mycelial growth number and yield of sporophore. The effectiveness of the treatment may be due to the alkaline pH of soaking water. The mycelial growth of milky mushroom was faster in alkaline pH and it favours the faster colonization of the mushroom mycelium over the substrate. It also may be due to the increased solubilization of lignin and cellulose content of the substrate at alkaline pH. Anthony *et al.* (1986) reported that lignocellulose degradation with lignin solubilization was promoted by alkaline pH. Macedo *et al.* (1999) also reported that addition of calcium carbonate enhanced the lignin peroxidase activity. Increase in pH of soaking water to alkaline range results in faster mycelial growth in oyster mushroom Balakrishnan (1994) and Deepthi (2003). Boiling of the pre wetted substrate produced higher yield compared to chemical sterilization alone but lower than chemical sterilization along with $CaCO_3$. Reason for the failure of boiling may be due to improper sterilization and also excess moisture present in the substrate after cooking. A similar observation was also reported by Heera (2006). But Krishnamoorthy (1995) reported that boiling of pre wetted paddy straw as the best method of sterilization for milky mushroom cultivation.

Application of chemical fungicides to control the mushroom diseases was restricted for fear of residue problems. Prophylactic measures are the viable solution for management of mushroom diseases and hence trails have been taken up using plant extracts to contain the diseases of mushroom. Many workers have reported use of plant extracts for control of fungal and bacterial diseases (Raina and Jandaik, 1994; Rao *et al.*, 1994; Sharma and Jandaik, 1994b; Pani and Patra, 1997; Singh, 1999; Raina *et al.*, 2003; Bhanwar and Thakur, 2004 and Siddique *et al* 2004a).

Among the five extracts tested against bacterial isolates under *in vitro* condition turmeric extract was found to be the best followed by higher concentrations of garlic and neem leaf extract giving 100 per cent inhibition. From the result it was also clear that turmeric, garlic and neem leaf extract have inhibitory effect against *Bacillus* sp. The effect of garlic extract and neem cake against the growth of *Pseudomonas* sp. was reported by Raina and Jandaik (1994).

Different plant extracts have varied effect on the radial growth of pathogenic and weed fungi. Among the different plant extracts tested garlic was the best followed by ocimum in inhibiting the growth of all the pathogenic / weed fungi even at five per cent concentrations. But Sharma and Jandaik (1994b) reported that the garlic extracts gave only 2.9 to 3.7 per cent inhibition against *C. globosum* and *T. viride*. Similarly ocimum at all concentrations gave 100 per cent inhibition against all fungi except *Curvularia* sp., *Trichoderma* sp. and *Sclerotium* sp. Against *Trichoderma* sp. and *Sclerotium* sp. only garlic extract was able to give 100 per cent inhibition at five per cent concentration.

Neem leaf extract at lowest concentration also gave 100 per cent inhibition against *D. dendroides*. and *Coprinus* sp. Higher concentrations of neem leaf extract was effective against *Curvularia* sp., *Penicillium* sp., *C. indicum* and *R. solani.* But it was not effective against *Trichoderma* sp. Effectiveness of neem leaf extract against *T. viride* and *C. globosum* was reported by Sharma and

Jandaik (1994b). Higher concentrations of neem leaf extract were effective against weed fungi (Pani and Patra, 1997; Raina *et al.*, 2003; Bhanwar and Thakur, 2004 and Siddique *et al.*, 2004a).

Turmeric extract gave 100 per cent inhibition against *D. dendroides*. and *Coprinus* sp even at five per cent concentrations, it was also effective against *C. indicum*, *Penicillium* sp, *R. solani* and *Sclerotium* sp. But it was not at all effective against *Trichoderma* sp. Raina *et al.* (2003) also reported the same result against *Trichoderma* sp.

Marigold leaf extract gave 100 per cent inhibition against *D. dendroides*. and *C. indicum* at all concentrations tried. Earlier workers also reported the effectiveness of marigold leaves on the growth of weed fungi (Pani and Patra, 1997 and Raina *et al.*, 2003). Sharma and Jandaik (1994b) reported inhibitory effect of marigold extract on the growth of *C. globosum* and neem leaf, turmeric and marigold extracts were not effective against *Trichoderma* sp. Raina *et al.*(2003) reported that extracts of neem leaf, turmeric rhizome and marigold gave only 0.37 to 3.6 per cent inhibition against *Trichoderma* sp. According to Siddique *et al.* (2004a) extract of neem leaf was less effective against *T. harzianum*.

All the plant extracts tested were safe to mushroom mycelium, only garlic and ocimum extracts recorded slight inhibition against milky mushroom mycelium at the highest concentration tested. According to Sharma and Jandaik (1994b) addition of dried neem leaves into the substrate enhanced the growth of *A. bisporus*. Raina *et al.* (2003) reported that water extracts of the marigold and neem leaves enhanced the mycelial growth of *A. bisporus*.

On farm evaluation of phytoextracts was conducted to study the effect of treatments on growth, yield and disease incidence. Among the five extracts tested garlic extract treated substrate gave higher mycelial growth and yield followed by

ocimum and turmeric extracts. Incidence of weed fungi was absent in all the mushroom beds sprayed with effective concentrations of different plant extracts. Similar results were reported in oyster mushroom, white button and paddy straw mushroom. The reports showed an increase in mycelial growth, yield and reduction in contaminants as result of the application of phytoextracts on the substrate (Pani and Patra, 1997; Kumar, 2001; Raina *et al.*, 2003 and Bhanwar and Thakur, 2004).

The effectiveness of the phytoextracts in reducing the natural incidence and severity of bacterial disease on sporophore in the farm was also studied. The result showed that reduction in disease incidence could be obtained by prophylactic spraying of garlic and turmeric extracts. A perusal of literature showed that no such reports are available on this aspect.

Biochemical changes in the bacteria infected and healthy sporophore were compared. There was difference in phenol, crude protein, carbohydrate and crude fibre in diseased and healthy sporophore. Phenol content in healthy sporophore was high compared to diseased. The accumulation of phenols may act as an active resistant factor in defense mechanism (Sharma *et al.*, 1983). Buvaneswari (2004) reported increase in phenol content in *Dactylium dendroides* infected milky mushroom. An increase in crude protein content was observed on bacteria infected mushroom compared to healthy one. Buvaneswari (2004) reported an increase in protein content in cobweb affected milky mushroom compared to healthy one. She also reported that the increase in protein content in diseased mushroom might be due to the production of newer protein molecules in diseased mushroom. The crude fibre content of the diseased sporophore was less compared to healthy. Heera (2006) reported crude fibre content in healthy milky mushroom ranged between 16.02 to 24.1 per cent. There was only slight change in carbohydrate content in healthy and diseased mushrooms.

During this study on diseases of milky mushroom and their management, two fungal pathogen and four bacterial isolates were identified causing considerable damage to *C. indica* sporophore. Other severe fungal problems noticed were the weed fungi, which inhibited the growth of mushroom mycelium during the spawn run stage, thereby reducing the yield of the crop considerably. As it is an indoor crop the weather parameters have a good influence on the crop as well as on the incidence of the diseases. Quantification of weather parameters like temperature, humidity, ventilation/ air circulation, light intensity etc will give a clear picture on the effect of these factors on mushroom yield and disease incidence. Other deciding factors are quality of the substrate, pasteurization of the substrate, moisture content of the substrate at the time of bed preparation and general cleanliness of the farm. If all the factors are optimum for mushroom growing, the incidence of diseases will be minimum with maximum yield. It is also desirable to provide short break of two to three days with thorough cleaning and disinfection of the farm after each crop. This will provide a break in infection chain and minimize the build up of pathogen inoculum in a mushroom farm. Spraying of botanicals like garlic / turmeric extract at right time after the removal of all the sources of inoculum will help in the management of both fungal and bacterial diseases.

Summary

6. SUMMARY

The present study on "Diseases of milky mushroom (*Calocybe indica* P&C) and their management" was carried out to understand various aspects of diseases such as etiology, symptomatology and seasonal occurrence and to evolve ecofriendly management practices for the diseases.

A survey was conducted at four milky mushroom farms at different locations viz., Eravimangalam, Kodungalloor, Kattilapoovum and at Vellanikkara, in Thrissur district. Based on the survey the Agrowe farm at Eravimangalam was selected for monthly visit. The selected farm was visited once in a month from 2006 April to 2007 March. Bacterial blotch, malformation, scaling and splitting of sporophores were the major problems identified during the survey. Incidence of cobweb disease was a serious problem in farm at Kattilapoovum and infection of small pinheads with a fungal pathogen was noticed at Kodungalloor. In all farms incidence of weed fungi was observed during the spawn running stage. From the diseased samples collected, two fungal and six bacterial pathogens from sporophore and seven types of weed fungi interfering with mycelial colonization of mushroom fungus were isolated.

Studies on symptomatology of different bacterial isolates showed that no variation in symptoms was produced by the six isolates. The infection was noticed both on pileus and stipe as light brown discolouration later developed into water soaked lesions. Finally the whole mushrooms get rotten. The symptoms of cobweb disease appeared as circular white patches of fluffy mycelial growth on casing surface. Gradually it attacked the sporophore, resulted in browning and rotting of sporophore. The infection of *Penicillium* sp. on sporophore noticed from Kodungalloor showed brown discolouration of young pinheads. Nature of damage caused by various weed fungi was studied and the initial symptoms produced by

various weed fungi were same and later each fungus produced their survival structures on substrate and restricted the growth of milky mushroom mycelium.

Cultural, physiological and biochemical characters of bacterial isolates were studied. Based on the study the bacterial isolates were tentatively identified into two groups *viz., Pseudomonas* sp. *and Bacillus* sp. The cultural characters of pathogenic/ weed fungi such as rate of growth, colour and formation of fruiting bodies in PDA medium and morphological characters like size of hyphae, size and shape of conidia and conidiophores were studied in detail. Based on these the pathogen causing cobweb disease was identified as *Dactylium dendroides* and other fungus which infects sporophore was identified as *Penicillium* sp. The weed fungi isolated from contaminated mushroom beds belong to seven different genera namely, *Trichoderma* sp., *Rhizoctonia* sp., *Chaetomium* sp., *Aspergillus* sp., *Sclerotium* sp., *Curvularia* sp. and *Coprinus* sp. Effect of temperature and pH on the growth of fungal pathogenic/ weed fungi and milky mushroom were studied. Most of the fungi and milky mushroom prefers a neutral to alkaline range. Most of fungal pathogens/ weed fungi recorded a faster growth at 30^0C.

In vitro interaction between the bacterial isolates and *C. indica* was studied. The *Pseudomonas* sp. (B_3) recorded maximum inhibition followed by *Bacillus* sp. (B_4). Interaction between the pathogenic / weed fungi and *C. indica* was examined by dual culture technique. Among them *Sclerotium* sp. recorded 100 per cent inhibition with in three days after incubation followed by *R. solani, Trichoderma* sp. and *D. dendroides.* They recorded 100 per cent inhibition on four to five days of incubation. All other fungi recorded slow growth and inhibited mushroom mycelium after seven to eight days of incubation.

When the effect of culture filtrate of weed fungi on *C. indica* was studied, it was found that culture filtrate of weed fungi can inhibit the growth of mushroom mycelium. Among the seven weed fungi *C. indicum* recorded maximum inhibition indicating its ability to produce metabolites inhibitory to *C. indica*. Least inhibition was shown by *Coprinus* sp.

Antagonistic effect of weed fungi on linear mycelial growth of milky mushroom was studied by inoculating mycelial discs of weed fungi into test tubes filled with sterilized paddy straw bits and spawned with milky mushroom spawn. All the seven weed fungi inhibited mycelial growth with maximum inhibition by *Trichoderma* sp. and *Sclerotium* sp. followed by *R. solani* and least inhibition by *Curvularia* sp.

To find out the influence of season on the incidence of weed fungi in milky mushroom, ten numbers of mushroom beds were laid out in mushroom production unit, Department of Plant Pathology at fortnightly interval for a period of one year. This study revealed that weed fungi incidence was maximum in the month of June when the weather parameters like, temperature and relative humidity are most favourable and it was minimum (20 per cent) in April. During the period of study *R. solani* was identified as major weed fungus, which occurred through out the year.

The effect of weed fungi on the mycelial growth and yield of milky mushroom was studied by inoculating the mycelial discs of weed fungi into spawned beds. Among the seven weed fungi *Sclerotium* sp. recorded maximum reduction in mycelial growth and 100 per cent reduction in yield followed by *C. indicum*. Least inhibition was observed in mushroom beds inoculated with *Coprinus* sp.

Effect of different methods of substrate sterilization on mycelial growth of C. *indica* was studied both under *in vitro* and *in vivo* conditions. The study revealed that chemical sterilization of paddy straw with 0.2 per cent $CaCO_3$ was the best treatment. Under *in vivo* study the same treatment produced maximum yield followed by boiling.

The efficiency of five phytoextracts *viz.,* Garlic, ocimum, turmeric, neem and marigold against bacterial and pathogenic / weed fungi were studied by using poison food technique. For each extract 5, 10 and 15 per cent concentrations were tried. This study showed that turmeric extract was the best treatment which gave 100 per cent inhibition against all the bacterial isolates followed by higher concentrations of garlic and neem leaf extract. Against the fungal pathogens/ weed fungi garlic and ocimum extract was found to be the best followed by turmeric extract. Based on the *in vitro* study phytoextarcts were selected for field evaluation. The selected concentrations of extracts are garlic and ocimum (5 per cent), turmeric (10 per cent), neem and marigold leaf extract (15 per cent).

In vivo evaluation of selected phytoextracts on mycelial growth, pinhead emergence, sporophore yield and weed fungi incidence were studied by spraying phytoextract on sterilized substrate. In this study garlic extract sprayed on substrate sterilized by chemicals (carbendazim (75 ppm), formaldehyde (500ppm) and 0.2 per cent $CaCO_3$) recorded maximum mycelial growth and yield, followed by spraying with ocimum extract. All the treated beds were free from natural incidence of weed fungi. The effect of phytoextract in controlling natural incidence of bacterial pathogens in the farm was studied after the second and third pinhead emergence. The selected concentrations of phytoextracts were given as a spray on sporophores. Spraying of garlic, turmeric extract resulted in reduction in incidence of bacterial blotch on sporophores

Biochemical changes in healthy and bacteria infected sporophore were analysed. There was an increase in crude protein in infected mushroom than healthy. Phenol and crude fibre content was reduced in diseased mushroom. There was only a slight difference in carbohydrate content between healthy and diseased

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* Original not seen

Appendices

APPENDIX I

MEDIA COMPOSITION

(Ingredients per litre)

I. NUTRIENT AGAR MEDIUM

Peptone	: 5.0g
Beef extract	: 1.0g
NaCl	: 5.0g
Agar	: 20.0g
pH	: 6.5 to 7.5

II . POTATO DEXTROSE AGAR

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar	: 20.0

III. KING'S B MEDIUM

Peptone	: 20.0 g
Glycerol	:10.0 ml
K_2HPO_4	:10.0 g
$MgSO_4.7H_2O$	: 1.5 g
Agar	: 20.0 g
pH	: 7.2 – 7.4

IV. THORNLEY'S SEMI SOLID MEDIUM

Peptone	: 1.0g
K_2HPO_4	: 0.3 g
NaCl	: 5.0g
Agar	: 3 g

V.PEPTONE BEEF EXTRACT MEDIUM

Peptone	: 10 g
Beef extract	: 5 g
Sucrose	: 50 g
Agar Agar	: 20 g
Distilled water	: 1000ml

VI. SIERRA'S MEDIUM

Peptone	: 10.0 g
NaCl	: 5.0 g
$CaCl_2.7H_2O$	: 0.1 g
Agar Agar	: 20 g
Distilled water	: 1000ml
pH	: 7.0

VII. NITRATE REDUCTION MEDIUM

KNO_3 (Nitrate free)	: 1.0 g
Peptone	: 10.0 g

Beef extract	: 5.0g
Agar	: 15 g
pH	: 7

VIII. HAYWARD'S SEMISOLID MEDIUM

$NH_4H_2PO_4$ -	: 1.0g
KCl	: 0.2g
$MgSO_4.7H_2O$	:0.2g
Bactopeptone	: 1.0g
Bromothymol blue	: 0.08g
Agar	: 3.0g
pH	: 7-7.1

IX. PADDY STRAW AGAR MEDIUM

Powdered paddy straw	: 20g
Agar	: 20g

X. MALT EXTRACT AGAR MEDIUM

Maltextract	: 30g
Mycological peptone	: 5.0g
Agar	: 15g

XI. SIMMON'S CITRATE AGAR

Ammonium dihydrogen phosphate	: 1.0g
Dipotassium phosphate	: 1.0g

NaCl	: 5.0 g
Sodium citrate	: 2.0g
$MgSO_4$	: 0.2g
Agar	: 15.0 g
Bromothymol blue	: 0.08g

XII. STARCH MEDIUM

Peptone	: 10.g
Beef extract	: 5.0g
Starch solution	: 2.0g
Agar	: 15.0g
pH	: 7.0

X111. KING'S A MEDIUM

Peptone	:20g
Glycerol	:10ml
K_2SO_4	:10g
MgCl2	:1.4g
Agar	:15g
pH	:7.2

APPENDIX II

STAINS USED IN MICROBIOLOGICAL STUDIES

I. CRYSTAL VIOLET

One volume saturated alcohol solution of crystal violet in four volumes of 1 per cent aqueous ammonium oxalate.

II. GRAM'S IODINE

Iodine crystals	-	1.0 g
Potassium iodide	-	2.0 g
Distilled water	-	300 ml

III. SAFRANIN

Safranin O	-	0.25 g
Ethanol (95%)	-	10.0 ml
Distilled water	-	100 ml

Dissolve safranin in ethanol and then in water and filter.

IV. AMIDO BLACK

Methonol	-	50 ml
Acetic acid	-	10 ml
Amidoblack	-	1.5g
Distilled water	-	40 ml

DISEASES OF MILKY MUSHROOM (*Calocybe indica* P & C) AND THEIR MANAGEMENT

By

SAMEERA POTHUKATTIL

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Plant Pathology

COLLEGE OF HORTICULTURE
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2007

ABSTRACT

A study on "Diseases of milky mushroom (*Calocybe indica* P. &C.) and their management" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara, during the year 2006-2007.A survey was conducted on the occurrence of major diseases in four milky mushroom farms in Thrissur district The study revealed that two bacterial pathogens *viz., Pseudomonas* sp. and *Bacillus* sp. causing bacterial blotch and *Dactylium dendroides* and *Penicillium* sp. are the two fungi causing diseases on sporophore. Seven weed fungi were found to interfere with spawn running of milky mushroom namely *Trichoderma* sp., *Coprinus* sp., *Chaetomium indicum, Rhizoctonia solani, Curvularia* sp., *Sclerotium* sp., and *Aspergillus* sp.

In vitro interaction studies between the milky mushroom and both fungal/ bacterial pathogens on medium and substrate showed that they can inhibit the mycelial growth. Culture filtrate of the weed fungi has inhibitory activity against *C. indica*. Seasonal effect on incidence of weed fungi was studied. It showed that maximum incidence in June, was correlated with high humidity and the major weed fungus observed was *R. solani*. Different substrate sterilization methods have an influence on mycelial growth and yield. Both *in vitro* and *in vivo* studies showed that chemical sterilization along with $CaCO_3$ was the best treatment for the sterilization of paddy straw. Effect of weed fungi on mycelial growth and yield was tested; the study revealed that *Sclerotium* sp and *C. indicum* were showing more inhibitory effect by reducing the mycelial growth and yield. *In vitro* effect of phytoextracts was tested by poison food method. Phytoextracts of garlic, ocimum, turmeric, neem and marigold at 5, 10 and 15 per cent concentrations were tried. Among the five phytoextracts garlic and ocimum extracts were the best treatments against fungal pathogens / weed fungi.

Turmeric and garlic extracts were most inhibitory to bacterial pathogens. On farm evaluation of the extracts reduced the weed fungi incidence. Prophylatic spraying of phytoextracts on sporophores decreased the bacterial disease incidence. There was a slight change in biochemical constituents between healthy and diseased sporophores

General cleaning, use of good quality substrate, maintenance of optimum conditions *etc.* will help to get substantial yield and reduction in disease incidence. It is desirable to provide a short break of two to three days after each crop and prophylactic spraying with phytoextracts after the destruction of inoculum will help in the management of both fungal and bacterial diseases.

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