

Studies on Management of Wet Bubble Disease of White Button Mushroom [*Agaricus bisporus* (Lange.) Sing.] in Kashmir

Shaheen Kauser Jan
(2007-191-D)

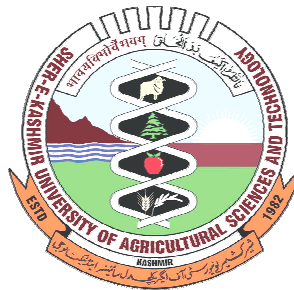


Division of Plant Pathology
Faculty of Postgraduate Studies
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir

2012

**Studies on Management of Wet Bubble Disease of White
Button Mushroom [*Agaricus bisporus* (Lange.) Sing.] in
Kashmir**

Shaheen Kauser Jan
(2007-191-D)



Thesis

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**The Faculty of Postgraduate Studies
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in partial fulfilment of requirement for the award of the degree of

**Doctor of Philosophy in Agriculture
(Plant Pathology)**

2012

Blessed are those who give without remembering

And blessed are those who take without forgetting

DEDICATED

*To my beloved Husband
And daughters
Marakeen and Maheen*

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Shalimar Campus, Srinagar

Certificate – I

This is to certify that the thesis entitled “**Studies on management of wet bubble disease of white button mushroom [*Agaricus bisporus* (Lange.) Sing.] in Kashmir**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)**, to the Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Mrs. Shaheen Kauser Jan (Regd. No. 2007-191-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Title of the Thesis : **“Studies on management of wet bubble disease of white button mushroom [*Agaricus bisporus* (Lange.) Sing.] in Kashmir”**

ABSTRACT

The status and management of wet bubble disease of white button mushroom (*Agaricus bisporus*) was studied at Mushroom Research and Training Centre of the SKUAST of Kashmir during spring and autumn seasons of the year 2008 and 2009. An extensive survey conducted revealed that wet bubble disease was prevalent in all mushroom growing districts in the range of 33.0 to 67.0 per cent. With the overall mean disease incidence of 9.09 per cent over years in three districts, the disease incidence was more (10.55-14.25%) in spring than in autumn season (3.61-8.05%). Similarly, the overall disease intensity was 17.82 per cent over the years in these districts with more (16.86 - 28.31%) in spring than in autumn season (9.81-15.46%). The per cent yield loss over the years due to the disease ranged from 13.53 -22.41 per cent with maximum loss reported in district Pulwama and minimum in district Srinagar. With an overall yield loss of 16.97 per cent over the years, the loss was highest (15.88-26.01%) in spring than in autumn season (8.63-14.92%). The fungal pathogen constantly associated with the disease was established as *Mycogone pernicioso*. The pathogen produced typical and characteristic symptoms of wet bubble disease. The initial source of inoculum was traced in contaminated casing before it reached the farm, or on the farm during storage and mixing. Garden soil and spent compost were found to harbour the wet bubble pathogen. Air currents, sciarid flies and water splashes were found to facilitate pathogen dispersal and spread from one production tray to another in mushroom farms. The *in vitro* efficacy of ten fungitoxicants against *A. bisporus* and *M. pernicioso* revealed that captan and prochloraz manganese with the mean inhibition of 95.71 and 99.52 per cent, respectively,

were highly inhibitory to the pathogen among non-systemics. Prochloraz manganese completely inhibited the mycelial growth of the pathogen at 50-500 $\mu\text{g ml}^{-1}$ with least inhibition (10.83%) of *A. bisporus* mycelium, whereas captan did so at only 500 $\mu\text{g ml}^{-1}$ concentration, with 15.41 per cent inhibition of *A. bisporus* mycelium. Among systemic fungicides carbendazim proved highly inhibitory to the pathogen (97.35%), , with the least inhibition (1.45%) of *A. bisporus* mycelium. Bitertanol and myclobutanil with the inhibition percentage of 93.05 and 93.70 per cent were the next best with least (7.08 to 9.78%) inhibition of *A. bisporus* mycelium. The *in vitro* evaluation of aqueous extract of eight different locally available plant species indicated that all the tested botanicals inhibited growth of the pathogenic fungus, but *Curcuma longa* with inhibition percentage of 65.19 per cent proved to be the best botanical with least(6.52%) inhibition of *A. bisporus* mycelium. *Metricaria* spp. with the inhibition percentage of 61.49 per cent against the pathogen was the next best botanical, but at the same time it also inhibited the growth of the host mycelium to 24.12 per cent. The test botanicals were highly effective at 3 per cent concentration. The ethanol extract of *C. longa*, *Metricaria* spp. and *Lavendulla officinalis* also behaved in a similar way exhibiting the inhibition percentages of 98.83, 89.80 and 66.72 per cent, respectively. However the ethanol extract of *Azadirachta indica* too exhibited appreciable levels of inhibition (45.67 %) against *M. perniciosia* with the least (9.89 %) inhibition of host mycelium. Among bacterial antagonists evaluated *in vitro*, all the test antagonists, *P. flourescens* , *B. subtilis* and *Azotobacter* sp., exhibited stimulatory effects of varying degrees on *A. bisporus* mycelium. *Pseudomonas flourescens*-103, *Bacillus subtilis*-116 and *Azotobacter* sp.-106 gave the highest mycelial growth inhibition of 100.0, 98.88 and 98.51 per cent of the pathogen fungus, respectively. The spraying of non-systemic or systemic fungicides or incorporation of powdered formulation of both bacterial antagonists or botanicals in casing mixture gave a good control of the pathogen fungus when applied separately *in vivo*. The integration of one or more of these components reduced the disease to a level significantly lower than check and appreciably enhanced the early pinhead formation, weight and number of healthy fruit bodies and the ultimate mushroom yield, as compared to check.

Key words : White button mushroom [*Agaricus bisporus* (Lange.) Sing.], wet bubble disease, *M. perniciosia*, integrated management

Signature of Student

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Signature of Major Advisor

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“Four things support the world: the learning of wise, the justice of the great, the prayers of the good, and the valour of the brave” Prophet Muhammad ^(PBUH)

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Place: Shalimar

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Chapter – 1

INTRODUCTION

Mushroom production represents one of the commercially important microbial technologies for large scale recycling of agro wastes and relieves pressure on arable land owing to its cultivation under controlled conditions. In the present scenario of economy, it has opened up new vistas of export earnings. Present world production is around 16 million tonnes. Among commercially cultivated mushrooms, *Agaricus bisporus* (Lange.) Imbach. popularly known as white button mushroom or European mushroom is extensively cultivated throughout the world. Its large scale production is centered in Europe (mainly western part), North America (USA, Canada), and Southeast Asia (China, Korea, Indonesia, Taiwan and India).

The production of white button mushroom accounts for 35 to 45 per cent of total world production. In India export oriented large units with production capacities between 2000-3000 tonnes per annum have been set up mainly in southern, western and northern regions. A large number of small production units without the temperature regulation equipment exist throughout India and function mostly during the autumn and winter months only.

In India the annual production of mushrooms is estimated to be around 1,20,000 metric tonnes with 85 per cent of this production being of button mushrooms (Anonymous, 2010). Commercial production of white button mushroom in India picked up in nineties and several high-tech export oriented farms were setup with foreign technology collaborations. But still a major share of mushroom production comes from small farms. Of late, much emphasis is being laid in production of mushrooms in the state of Jammu and Kashmir where 79,277 spawn bottles for laying about 150,000 trays/ poly bags were distributed in 2009-2010 and about 5051.61 quintals mushroom harvested under Rashtriya Krishi Vikas Yojna (RKVY) alone (Anonymous, 2011).

The main constraints in the speedy development of this crop in the valley to the cottage level industry are mushroom diseases. The crop is infested by a number of fungal, bacterial, and viral pathogens which result either in the partial or total failure of the crop or to the least deteriorates the quality of the produce. The cultivation of button mushrooms in the Jammu & Kashmir state is usually carried out in few rooms in residential houses but in a few isolated cases specially built mushroom houses without any environmental control system and without the provision of compost pasteurization have also been used for the cultivation, thus providing the conditions conducive for the growth and multiplication of pathogens and competitor moulds associated with mushroom culture. Although new farms with environmental control system and compost pasteurization facilities are coming up in the state, the number of such farms is very few (Munshi and Ghani, 2003). By the end of 2010, the mushroom production of Jammu & Kashmir state has reached 950 metric tonnes per annum of which valley contributed 250 metric tonnes. The mushroom industry in the valley is in the revival phase and more and more people and entrepreneurs are taking up this venture to earn their livelihood by adopting the year round cultivation.

The increase in the number of mushroom production units without the facilities of pasteurization coupled with the year round cultivation lead to the growth in the populations of a few fungal and bacterial pathogens, thus posing a serious threat in the profitable production of this crop. Major fungal diseases *viz.*, dry bubble, wet bubble and cobweb are responsible for inflicting varying degree of crop losses in mushroom farms (Forer *et al.*, 1974; Sharma and Vijay, 1996; Jandaik and Gularia, 1999; Bhat and Singh, 2002; Singh *et al.*, 2010). Among these wet bubble disease (*M. perniciosus*) causes extensive damage by bringing soft rot or decay of whole fruiting body. If not controlled well in time, the pathogen causes havoc damaging the entire crop. Serious outbreaks have been recorded in various farms in many mushroom growing countries including Serbia (Potocnik *et al.*, 2004). The disease is most prevalent in India in temperate areas although few reports are available from subtropics as well (Sharma and Kapoor, 2000; Bhatt and Singh, 2000). In Haryana it was the main problem and the

incidence up to 30 and 25 per cent in 2008-2009 and 2009-2010, respectively, in 14 mushroom farms of the state was reported (Singh and Sharma, 2010).

In 1998 the disease spread to almost all the mushroom units located in Solan district of Himachal Pradesh recording a high level of incidence (Sharma and Singh, 2003).

The disease is characterized by the development of whitish mouldy growth of mycelium on portions of fruit bodies. It spreads covering the entire cap eventually reducing the sporophore to a foul smelling white mass (Garcha, 1978). If the infection takes place before the differentiation of stipe and pileus the sclerodermoid masses are formed, whereas infection after differentiation results in the production of thickened stipe with deformation of gills (Smith, 1924). The disease causes pathological changes in fruit bodies of *A. bisporus* after a latency period of about one day. The reaction of undifferentiated primordia smaller than 6 mm at the time of inoculation were of a vastly hyperplastic, tumorous character. They produced large, irregular, confluent lumps of *A. bisporus* with no sign of differentiation or organogenesis (Umar *et al.*, 2000). These tumorous bodies often showed dripping amber liquid as teardrops. Wet bubble infected fruit bodies were often found to be infected by various types of bacteria around day 6 of infection and this bacterial infection was the actual cause of death of mushrooms which occurred around day 10 of infection (Fletcher *et al.*, 1995; Umar *et al.*, 2000).

Keeping in view the destructive nature of the disease on growing mushrooms crop, especially in temperate climatic conditions of the valley, the disease warrants control through effective management since any single method lacks the flexibility necessary to suppress dynamic pathogen population for a longer time, studies are taken up for the harmonious use of various botanicals, bio-control agents and chemicals for efficiently managing the disease with the following aims and objectives :

- 1) To ascertain status for the disease in important mushroom growing areas of Kashmir;
- 2) To assess influence of various factors on disease spread; and
- 3) To develop an integrated management strategy for the disease.

Chapter – 2

REVIEW OF LITERATURE

The cultivated white button mushroom (*Agaricus bisporus* L.) is an important cash crop which utilizes large quantities of farm residues, additives, and supplements for its cultivation and harbour a variety of microbial pathogens and competitor moulds. As in most farms seasonal cultivation of this mushroom is being practiced, where unpasteurized composted substrate is used and poor hygiene and insanitary practices are prevalent at farmer's level, invite a number of parasitic diseases which take considerable toll of the produce each year (Fletcher *et al.*, 1986; Vijay and Gupta, 1997; Bahl 2000;). The problem of this pathogenic fungus is a serious impediment in the planned development of mushroom cultivation and expansion programme in our state too. Of these the incidence of wet bubble disease (*Mycogone perniciosa*) of white button mushroom causes extensive damage by bringing soft rot or decay of whole fruiting bodies. The disease is most prevalent in India in temperate areas although reports of its occurrence exist in subtropics also.

An attempt has been made to review the available literature about wet bubble disease (*M. perniciosa*) of white button mushroom under the following headings:

- 2.1 Disease status and losses
- 2.2 Symptomology
- 2.3 Pathogen morphology
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2.1 Disease status and losses

Wet bubble disease of white button mushroom also called as La mole, white mould, bubble, *Mycogone* disease has been reported as one of the serious diseases from almost all major mushroom growing countries of the world. The disease was first described from Paris in 1888, and is stated to be responsible for the heaviest losses in mushroom beds in France, England and United States (Nielson, 1932). The disease has also been reported to assume serious proportions in other major mushroom growing countries of the world such as Hungary, Netherlands, China, Taiwan, South Africa, Brazil, Australia and Poland from time to time. In India this disease was reported for the first time in 1978 from some mushroom farms in Jammu and Kashmir (Kaul *et al.*, 1978), later this disease was reported from the states of Himachal Pradesh, Haryana and Maharashtra (Sharma, 1996; Sharma and Kumar, 2000; Bhatt and Singh, 2000; Sharma and Singh, 2003). Nielson (1932) stated that wet bubble disease caused heaviest losses among all diseases in mushroom beds in France, England and United States.

Mycogone pernicioso Magn. a mycoparasite responsible to cause this disease was isolated from 3.7 per cent samples collected from various mushroom farms. Forer *et al.* (1974), while estimating qualitative and quantitative losses caused by wet bubble and dry bubble in Pennsylvania (USA) reported that these two diseases caused \$ 2.2 million as qualitative and \$ 19.7 million as quantitative loss to mushroom. Nair (1977) conducted a survey of 24 mushroom farms in New South Wales during 1975-76 and observed that the most economically important disease in these farms include wet bubble disease.

The natural incidence of wet bubble disease of button mushrooms ranged from 1 to 100 per cent in northern India. Loss in yield in *A. bisporus* (S-11) due to this disease under artificial inoculation conditions has been reported to vary from 15.72 to 80.13 per cent. Bhatt and Singh (2000) have reported the yield loss up to 100 per cent as a result of artificial inoculation of *M. pernicioso*. All the strains of *Agaricus bisporus* U-3, S-11, S-791, S-910 and *A. bitorquis* NCB-6, NCB-13

were found susceptible to *M. perniciosus* under *in vivo* conditions (Sharma and Kumar, 2000).

2.2 Symptomology

The symptoms of wet bubble disease at different stages of development have been described by many workers (Smith, 1924; Fletcher and Ganney, 1968; Bech *et al.*, 1982; Geijan, 1977; Zaayen, 1982; Figueiredo and Mucci, 1985; Tu and Liao, 1989; Sharma and Kumar, 2000; Umar *et al.*, 2000). Smith (1924) recognized two main symptom types, infected sporophores and sclerodermoid masses. Garcha (1978) described the symptoms as white mouldy growth on the mushroom fructifications leading to their putrefaction, with emission of foul odour and exudation of golden brown liquid. Hsu and Han (1981) reported that the infected sporophores are recognized by two types of symptoms, one is a tumorous form arising as a result of infections of pin heads and the other is a malformation arising as a result of infection at a later stage of sporophore development. Both types of infections exude some liquid droplets on the surface of infected sporophores. These droplets later change into amber color. Tu and Liao (1989) observed that when young pinheads are infected they develop monstrous shapes which often do not resemble mushrooms. Sharma and Kumar (2000) described the symptoms as short, curly, pure white fluffy mouldy growth of the pathogen on malformed mushrooms which can be easily observed by the naked eyes. Cross section of deformed sporophores without cottony growth showed black circular area just beneath the upper layer. Fletcher and Ganny (1969) reported that infection in the form of black streaks sometimes also occurs at the base of stipe in apparently healthy sporophores. The hyphae of *M. perniciosus* have been found to coil around host hyphae with firm adhesions eventually penetrating the host cells (Umar *et al.*, 2000). The intra-hyphal growth is believed to have some important consequences for the host in particular when the invading parasite is already infected with viruses and mycoplasmas.

Umar *et al.* (2000) described dramatic cytological changes as a result of infection when young (up to 6 mm) pinheads were infected. Large, very irregular, nodular, tumorous fungal masses are formed and no differentiation or organogenesis of the cell masses takes place. Mycopathogen grew on the surface as fluffy mycelium but was absent deep in the lesions. Transmission EM revealed two kinds of cell wall reactions, Fletcher *et al.* (1995), focal swelling like cushion at the site of adhesion of *M. pernicioso* or focal lytic changes with swollen mitochondria. Mycogone-infected fruit bodies were often found to be infected by various types of bacteria and mycoviruses around day 6 of infection and this infection was the actual cause of death of mushroom which occurred around day 10 of infection (Albouy *et al.*, 1973; Fletcher *et al.*, 1995; Umar *et al.*, 2000). These viruses containing strains of *M. pernicioso* grew slower and induced typical, pathological features of developmental error in growing fruit bodies of mushrooms (Chaze and Sarazin, 1936; Fletcher *et al.*, 1995). The mycoviruses trigger the hyperplastic process which result in extreme increase of the hyphal reserve cells of the host (Umar *et al.*, 2000).

2.3 Pathogen morphology

Mycogone pernicioso Magn. (Perfect state; *Hypomyces pernicioso*) belongs to division Eumycota, sub-division Deuteromycotina, class Hypomycetes, order Moniliales and family Moniliaceae. Its teliomorph i.e. *Hypomyces* belongs to division Eumycota, sub-division Ascomycotina, class Pyrenomycetes, order Sphaeriales and family Hypomycetaceae. Mycelium is white compact, felt-like. The hyphae are branched, interwoven, septate, hyaline and 3-5 μ broad. It produces conidia and chlamydospores. Conidiophores are short, slender, branched, hyaline measuring 200 x 3-5 μ m and have subverticillate to verticillate branches, which bear thin walled, one celled conidia measuring 5-10 x 4-5 μ m. Large two-celled chlamydospores are also produced with upper cell warty, thick walled, globose, bright coloured measuring 15-30 x 10-20 μ m and the lower cell hyaline, smooth and measuring 5-10 x 4-5 μ m (Sharma and Singh, 2003).

2.4 Host range

Although *Mycogone pernicioso* is not species specific but the cultivated mushroom *A. bisporus* seems to be its main host worldwide. This mycopathogen has been reported to attack large number of wild fleshy fungi (Sharma, 1995). Figueiredo and Mucci (1985) revealed that *M. pernicioso* is capable of infecting other mushroom species like *A. compestris*. Among *pleurotus* species *P. eryngii* and *P. nebrodensis* are reported to be susceptible (Sisto *et al.*, 1997). All the strains (most commonly cultivated in India) of *A. bisporus* and *A. bitorquis* (K-32) were found to be susceptible to *M. pernicioso* infection under *in vivo* conditions. Sharma and Kumar (2000) reported that U-3, 791, S-310 and S-11 strains of *A. bisporus* and NCB-6 and NCB-13 strains of *A. bitorquis* are susceptible to *M. pernicioso*.

2.5 Pathogen survival and dispersal

The spread of *M. pernicioso* occurs primarily through casing soil but the introduction of the pathogen through other agencies, like spent compost and infection trash, is not ruled out. The infection can be air-or waterborne or carried mechanically by mites and flies (Garcha, 1978). Hsu and Hans (1981) reported water splash as an important factor for wet bubble spread on beds. Bech *et al.* (1982) reported that the spread through contact occurred readily during watering and especially harvesting. They also observed that contaminated containers can be a source of spread over greater distances.

Tu and Liao (1989) reported that the spores of *M. pernicioso* can also be spread by air currents. Kumar and Sharma (1998) reported that transmission percentage of *M. pernicioso* under *in vitro* conditions by sciarid and phorid flies was 100 percent on MEA medium and 4-12 per cent on compost. Chlamydospores have been reported to survive for a long time (up to 3 years) in casing soil and may serve as the primary source of inoculum. The aleuriospores

produced on the surface of monstrous structures are probably responsible for secondary infection.

2.6 Pathogen physiology

Like other organisms the growth and development of *M. pernicioso* is also influenced by various external factors. Hsu and Hans (1981) and Singh and Sharma (2000) reported that optimum temperature for mycelial growth, sporulation and conidial germination of *M. pernicioso* was 25°C. He also recorded pH 6.0 as optimum for conidial germination. Lambert (1930) showed that the mycoparasite is quite sensitive to prolonged exposure to high temperatures. The cardinal temperature for growth of the organism on Thaxter's agar are 8, 24 and 32°C. He also reported that in agar cultures *M. pernicioso* was killed by exposures to temperatures of 42°C (106°F) or higher for 6 hr or more. The thermal death point for *M. pernicioso* was established at 48°C (Zaayen and Rutigens, 1981). However, the aqueous suspension of *Mycogone* spores was found to withstand exposure to 42 and 36°C for 10 and 60 min respectively (Bech *et al.*, 1982). Hsu and Han (1981) and Singh and Sharma (2000) also recorded pH 6.0 as optimum for growth and conidial germination. On an average a pH range of 5-6 was reported optimum for overall growth (Sharma and Kumar, 2000).

The pH of the substrate is also known to significantly alter the growth and sporulation of *M. pernicioso*. As reported by Tu and Liao (1989) pathogen is tolerant to a wide pH range in acid side and able to grow at pH 4.4, however, the growth becomes weaker or rather restricted to pH 8.4.

The availability of the nutrients in the substrate is also important and determines the growth and sporulation of the mycoparasite/pathogen. According to Liao (1981), chlamydospore failed to germinate on various media *in vitro* even after heat (40-70°C) treatment or application of chemicals and solvent. However, germination occurred on potato dextrose agar (PDA) medium exposed to the gas produced by mushroom mycelia in compost for 36 hrs at 24°C. In another study

Bech and Kovacs (1981) found that aleuriospores are unable to germinate in water, Richard's solution, pressed mushroom juice or in PDA, but verticiloid spores showed a certain degree of germination in diluted mushroom juice and on PDA. The fact that PDA medium sustained maximum mycelial growth of *M. pernicioso* was also demonstrated by Singh and Sharma (2000).

The mycelia of *M. pernicioso* exposed to different temperatures for 30 min lost its viability beyond 40°C. Conidia failed to germinate after treatment at 44°C, whereas chlamyospores treated at 48°C failed to germinate, thus clearly indicating that thermal death points of mycelium, conidia and chlamyospores are between 40 to 42 °C, 42 to 44 °C and 46 to 49 °C, respectively (Sharma and Singh, 2002).

Holland and Cooke (1991) reported however, in malt extract agar medium, *M. pernicioso* formed abundant thin walled, hyaline phialo-conidia and thick walled pigmented verrucose conidia. During nutrient depletion, other propagules namely lateral smooth conidia, infected intercalary cells, chlamyospores and orthoconidia appeared. Mannose and asparagine have been reported as best sources respectively of carbon and nitrogen for the pathogen (Singh and Sharma, 2000). Compost extract agar medium was found best for sustaining mycelial growth whereas spore production was maximum on malt extract peptone dextrose agar medium (Sharma and Kumar, 2000).

2.7 Disease epidemiology

The primary source of infection on most farms is casing material containing dormant thick walled chlamyospores. Besides this infection can also occur through air infected trash and spent compost. Once the pathogen is established in the crop the secondary spread is by means of conidia which are carried by water splashes and run off water. Pickers may also spread the disease/pathogen through their hands. Conidia germinate within 4-6 hours in

water (Sharma *et al.*, 2001) and the latency period is about one day (Umar *et al.*, 2000). The pathogen remained viable up to 3 years in the post mushroom substrate under natural day conditions in the form of chlamydospores (Tu and Liao, 1989; Sharma and Singh, 2002).

Attempts to induce germination of chlamydospores under *in-vitro* conditions was not successful until 2000, when Sharma *et al.*(1999) reported 15 percent germination in 3 months old chlamydospores (Umar *et al.*, 2000) demonstrated that pieces of pileal tissue of *Agaricus* species incubated with spore suspension of *M. pernicioso* induced germination of the dormant, thick walled chlamydospores. Water borne spores may either splash or spread through runoff water from infected spores and Watering the cultivation surface/tray/bag with a high pressure water pump and/or excess watering could be a very important means of disease spread (Sharma and Singh, 2002). Fletcher and Ganny (1968) and Hsu and Han (1981) have reported the spread of mushroom pathogens through water splash and surface runoff water. When inoculum was contacted with casing material of the healthy bag, disease symptoms appeared on the fruit bodies after 12-13 days, whereas the stipe of these mushrooms showed infection after 10-11 days (Sharma and Singh, 2002). Crawling of mushroom flies (Sciarids and Phorids) and mites over pure culture of *M. pernicioso* results in its hundred per cent transmission to the agar slants (Kumar and Sharma, 1998). Transfer of these pests over casing material of mushroom bags showed very less transmission; a maximum of 30 per cent transmission was observed with Phorids. Bech *et al.* (1982) also reported that mushroom flies transmit wet bubble disease to distant places. Fletcher and Ganny (1968) concluded that fly borne spread of wet bubble disease did not appear to be very important. Phorid flies captured from fluffy surfaces (growth of *M. pernicioso*) showed presence of aleuriospores in their gastro-intestinal tract and on their extremities, thereby indicating the spread of disease (Umar *et al.*, 2001).

2.8 Disease management

2.8.1 Management through physical methods

Wuest and Moore (1972) suggested that aerated steam at 54.4°C for 15 minutes can eliminate *M. perniciosa* from casing soil. Munns (1975) suggested the use of plastic pots to cover mushroom showing wet bubble symptoms during the cropping season to prevent spread of disease. Tu and Liao (1989) while working to find out an integrated approach for the management of wet bubble disease revealed that the use of clean compost, pasteurization or sterilization of casing soil, good peak heating and fumigation of mushroom house and use of Benomyl or Mertect-40 per cent were effective in managing *M. perniciosa*. Zhang (1990) suggested three methods of prevention of wet bubble disease which include steam sterilization of mushroom beds, formaldehyde fumigation and fungicidal application.

2.8.2 Management through chemicals

A resounding success in the control of mushroom fungal diseases/pathogens has been achieved with the introduction of Benomyl containing fungicides. Fletcher (1971) found benomyl and thiabendazole as possible materials for the control of important mushroom diseases. The application of benzimidazole derivatives against all the fungal pathogenic diseases of *Agaricus bisporus* in cropping rooms has therefore been recommended (Wuest and Cole, 1970; Gandy, 1971). The pathogenic fungi *Verticillium malthousei*, *Mycogone perniciosa* and *Trichoderma viride* were sensitive *in vitro* to Benomyl and MBC (Methyl 1-2 benzimidazole carbamate) fungicides whereas several edible fungi, including *Agaricus bisporus* were tolerant (Kim, 1975).

Benomyl spray at 0.5-4 g m⁻² immediately after casing has been reported very effective for protecting the crop (Gandy, 1974; Stanek and Vojtechovska, 1972). Fletcher (1975) reported that adequate control of wet bubble was obtained by benomyl or thiophanate methyl at 10 g a.i. at casing, while thiobendazole was

less effective. Satisfactory control of wet bubble has also been obtained by spraying benomyl @ 0.5 g a.i m⁻² 3 days after casing (Kim, 1975). Geijn (1977) suggested the control of wet bubble disease by spraying the crop with carbendazim, benomyl or thiophanate methyl at 100-150 ppm immediately after casing. Basamid (Dazomet) and vapum (Methane sodium) applied @ 100 ppm to casing has been reported very effective (Kim *et al.*, 1978). Jandiak *et al.* (1978) reported that benomyl and MBC did not exert any adverse effect on the growth of mushroom mycelium even at high concentrations of 500 ppm. Similarly, Thapa and Raina (1989) tested carbendazim, thiophanate methyl *in vitro* and *in vivo* and found that 50 and 500 ppm concentrations had no effect on *A. bisporus* mycelium and also observed that the spawn run in treated beds was found to be faster more whitish and luxuriant as compared to control. Application of carbendazim, benomyl, chlorothalanil, TBZ, prochloraz manganese complex (Sportak 50 WP) into casing mixture have been reported very effective for the management of wet bubble by several workers (Hsu and Han, 1981; Zaayen and Adrichem, 1982; Fletcher, 1983; Zaayen *et al.*, 1983; Eicker, 1984; Jhune *et al.*, 1991; Sharma and Kumar, 2000; Singh and Sharma, 2001). It was reported that if casing is contaminated control can be achieved by treating it with 1 per cent formalin. Alternatively a spray of 0.8 per cent formalin on the casing surface, immediately after casing can be effective. However, this concentration can be injurious if used at a later stage in crop development. Sharma *et al.* (1999) have reported 62.5-100 per cent inhibition on *M. perniciosus* in culture when inoculum discs were drenched in 0.5-2 per cent formalin solution for 5 seconds. Exposure of *M. perniciosus* cultures to vapours of 1-4 per cent formalin for 6-24 hrs also resulted in 100 per cent inhibition of fungal growth on sub culturing. Maszkiewicz, (2001) reported that chlorothalanil (Gwarant 500 SC; at 1, 10, 50 and 100 mg/liter) significantly controlled the mushroom pathogens and did not reduce yield nor delayed occurrence of first mushroom flush. Bhatt and Singh, (2002) screened different fungicides under *in vitro* conditions at various concentrations and found that bavistin, captan, sporogon, ridomil MZ and formalin in addition to inhibiting

the growth of the pathogen did not inhibit the growth of *Agaricus bisporus*. They further found from *in vivo* studies that bavistin (0.075%) was found most effective in controlling the disease caused by *Verticillium fungicola* as well as giving significantly higher yields as compared to captan, ridomil MZ and check. Similarly Singh and Singh (2005) reported that chemical fungitoxicants, sporogon and bavistin (0.05%) were effective in controlling the wet bubble disease in mushroom beds as compared to check, without effecting the yield of mushrooms. Katyal *et al.*, 2008 tested the efficacy of carbendazim (bavistin 50% WP) against three competitor and parasitic moulds (*Populaspora byssina*, *Trichoderma* spp. and *Verticillium fungicola*) of button mushroom (*Agaricus bisporus*) by adding it in sick compost. Artificial inoculation of these mycopathogens @ 0.1% revealed yield losses on inoculations with *V. fungicola* (15-20%), *Trichoderma* spp. (16-17%) and *P. byssina* (8-9%). A comparison of yields in untreated and uninoculated or inoculated controls revealed that the use of carbendazim (25-50 ppm) improved the total yields (14-33%) and numbers of fruit bodies when mixed at both spawning and casing stages in strain P-1 and 15-40% in strain U-3 of button mushroom. *In vitro* experiments showed that sodium bicarbonate (SBC) was effective in inhibiting the growth of *Mycogone perniciosa*. The inhibition of radial growth, germination and germ tube elongation of chlamydospores was 100%, 73.9%, 79.1%, respectively, at 4% SBC. The *in vivo* efficacy of sodium bicarbonate and reduced dose of Prochloraz manganese either alone or in combination was applied to casing soil twice by drenching 2 hours before casing and 2 days after casing. The efficacy of combination of SBC with reduced dose (10% of label dose) of Prochloraz manganese was not significantly and consistently different from that of the label dose of Prochloraz manganese in inhibiting the disease and increasing the yield in three repeated experiments. Similarly, in the bags treated with the combination of sodium bicarbonate and reduced dose of Prochloraz manganese, the amount of sclerotoid masses were reduced to 309 g from 1048 g in the control. Sodium bicarbonate treatment neither impaired the quality of sporophores nor reduced the yield (Karabulut *et al.*, 2007).

Three different fungicides viz., Derosal, Sporogon and Indofil M-45 were evaluated each @ 25 ppm and 50 ppm as prophylactic treatments for control of mycopathogens of *Agaricus bisporus* by incorporating these in the compost at the time of spawning and in the casing mixture, at the time of casing. All the fungicides used were effective in improving the yield of *Agaricus bisporus* strains, P-1 and U-3. Derosal @ 50 ppm was the most effective, resulting in yields of 11.93 kg and 12.42 kgs per 100 kg on wheat straw based compost, prepared in 24 days, compared to yields of 9.9 kgs and 10.52 kgs per 100 kg compost in control for strains P-1 and U-3, respectively. Improved yields were also obtained with the wheat straw + paddy straw (1:2, w/w) compost for both the strains with the same prophylactic treatments used. A corresponding increase in the number of fruit bodies harvested and average weight of fruit body was also observed with the treatments (Kapoor *et al.*, 2008).

2.8.3 Biological management

Literature revealed that mycelial growth and sporophore formation of *Agaricus bisporus* can be stimulated by diverse range of bacteria simultaneously, these can be inhibitory to mushroom pathogens (Eger, 1963; Urayama, 1967; Park and Agnohotri, 1969; Hayes *et al.*, 1969; Stanek, 1972; Curoto and Favelli, 1972; Fermor and Wood, 1981; Sparling *et al.*, 1982; Arkan *et al.*, 1994; Ahlawat and Rai, 2000; Ahlawat *et al.*, 2002). Jhune *et al.* (1990) screened 12 isolates of bacteria and 91 isolates of actinomycetes from mushroom compost and casing mixture and observed different strains AJ-117, AJ-136 and AJ-139, as promising bioagents against *M. perniciosus*. Gandy (1979) has made an interesting observation and reported that *Acremonium strictum* produces a heat stable antibiotic possibly a cephalosporin, which is inhibitory to *M. perniciosus* but no attempts have been made to explore this approach as both the fungi are pathogenic to mushrooms. The control of *M. perniciosus* through bacterial antagonist, however, seems to show some promise and its efficacy has been reported by number of workers (Flierman, 1973; Michal and Judith, 1975). It has been

reported that the microbes present in casing exhibit antagonistic potential against pathogenic fungi of *A.bisporus* (Trogoff and Ricard, 1976; Gandy *et al.*, 1980). The bacterial isolates present in casing soil inhibit the mushroom pathogens by means of ferric ion competition instead of spatial (Elad and Baker, 1985). The usefulness of bacterial flora in control of pathogens affecting yield of *Agaricus bisporus* was also supported by (Kleopfer, 1992). Pseudomonads constitute a sizeable part of microbial populations of casing microflora and many of them produce siderophores which improve the growth of *A. bisporus* and also reduce mushroom parasites (Byer and Sikora, 1990).

Fermor *et al.* (1991) isolated 100 potential antagonistic Fluorescent pseudomonads from mushroom farms, screened them under *in vivo* against bacterial blotch of *A. bisporus* and reported 50 per cent reduction of the disease. Siderophore producing pseudomonads contributed in varying degree in the inhibition of *P. tolaasii* causing bacterial blotch of *A. bisporus*. The isolates of *P. fluorescens*-M. 15 and *P. putida* are effective against *Cladobotryum dendroides* (Henry *et al.*, 1991).

Singh *et al.* (2000) observed that the *Verticillium fungicola* var. *fungicola* causing dry bubble disease was reduced by bacterial isolates (CIc, CIIf, and CVa) and suggested that the micro flora existing in the casing could be used for management of different fungal diseases of *A. bisporus*. Ahlawat and Rai (2000) documented that the bacterial inoculants used under *in vitro* stimulates the growth of mushroom mycelium and under mushroom house conditions enhance early pinning, yield and reduce the false truffle disease incidence. Bora *et al.* (2000) demonstrated that the Fluorescent pseudomonad isolates were successful in inhibiting *Papulaspora byssina*, a competitive pathogen by 86.6-92.5 per cent, and colonized the compost faster than the pathogen. Various bacterial isolates tested under *in vitro* against *V. fungicola* inhibited the growth of pathogen by 40-60 per cent and on treating the *V. fungicola* infested casing soil, with bacterial isolates BIV and BII under mushroom house conditions suppressed the growth of

V. fungicola and enhanced the yield of white button mushroom (Bhatt and Singh 2000). While studying the affect of different casing isolates against *V. fungicola*, observed that most of the isolates suppressed the growth of the pathogen and increased the yield of *A. bisporus*. Fluorescent pseudomonads and actinomycetes were also found antagonistic to *M. pernicioso*, enhancing the yield of *A. bisporus* Mishra and Singh (2003).

2.8.4 Control through botanicals

The control of *M. pernicioso* through botanicals has shown some promise (Flierman, 1973; Micheal and Judith, 1975). The effect of incorporation of neem leaf extract in malt extract agar (MEA) medium on the mycelial growth of various competitors/pathogenic fungi of *Agaricus bisporus* and reported 30-60 per cent inhibition of *Rhizopus species*, *Gliocladium deliquescens*, *Sclerotium rolfsii* and *Trichoderma viride*, whereas *Cephalosporium species* and *Chaetomium globosum* were inhibited to the extent of 10-15 per cent, respectively (Anonymous, 1985). Sohi and Grewal (1987) reported that incorporation of some dried plant leaves in compost and casing inhibited the mycelial growth of *Trichoderma viride*, *Penicillium oxallicum* and *Gliocladium deliquescens*. Root extract of (10%) water hyacinth was highly inhibitory to the growth of *Coprinus kimuri* and increased the production of *Volvariella volvaceae* fruit bodies (Chakraborty and Purkayastha, 1987). Grewal (1988) observed that incorporation of dried leaves of Neem and Eucalyptus in mushroom compost reduced the population of competitor moulds including *Fusarium solani* and *Sepedonium species*. In second related experiment, Sarkar *et al.* (1988) treated the compost with extracts of casurina leaves and water hyacinth which reduced the incidence of weed fungi occurring in *Pleurotus* beds and also increased the yield of mushroom. Grewal and Grewal (1988) found that incorporation of dried leaves of some botanicals namely; *Azadirachta indica*, *Cannabis sativa*, *Eucalyptus hybrida* and *Ricinus communis* at 3kg/100kg of dry wheat straw in mushroom compost before composting displayed differential fungicidal properties against various weed moulds of mushroom. Sharma and

Jandiak (1994) evaluated 5 plant materials namely leaves of Neem, Eucalyptus, Tagetes, Water hyacinth and garlic cloves against 4 different competitor moulds such as *Trichoderma viride*, *Papulaspora byssina*, *Myceliophthora lutae* and *Chaetomium globosum* in *Agaricus bisporus*. All the plant materials inhibited the mycelial growth of these weed fungi by 0.8 to 29.6%. Similarly, Sharma (1998) documented that water hyacinth resulted in drastic reduction in the incidence of false truffle and significantly increased the yields of both *Agaricus bisporus* and *Agaricus bitorquis*. *In vitro* evaluation of different botanicals indicated that water hyacinth, neem cake and neem leaves resulted in significant inhibition of false truffle (*Diehliomyces microsporus*) in *Agaricus bisporus* and *Agaricus bitorquis* (Sharma and Jarial, 2000). Methanol extracts of 13 plant spp. that include *Erigeron bonariensis*, *Erigeron karvinskianus*, *Cleome viscosa*, *Lepidium apetalum*, *Mentha longifolia*, *Origanum vulgare*, *Valeriana wallichii* and *Vitex negundo* screened, *in vitro*, for their fungicidal activity against two mushroom fungal pathogens *Mycogone pernicioso* and *Verticillum fungicola* showed that the 12 of the plant extracts, were inhibitory against *M. pernicioso* (Arora *et al.*, 2003). Fungicidal activity of essential oil obtained from the exocarp of *Citrus sinensis* against 11 fungi, isolated from wheat straw based compost was used for the culture of *Agaricus bisporus* (Raina, 2004). The essential oil was effective against *Curvularia lunata* (*Cochliobolus lunatus*), *Fusarium oxysporum* and *Helminthosporium oryzae* (*Cochliobolus miyabeanus*) at 1000 ppm. Complete inhibition of *Alternaria alternata* and *Aspergillus flavus* was recorded for the essential oil at 2000 ppm. Complete inhibition of mycelial growth was obtained in *Aspergillus niger*, *Apergillus fumigates*, *Verticillum fungicola* and *Fusarium solani* with essential oil at 1500 ppm. He also reported that essential oil showed negligible toxicity against *Agaricus bisporus* mycelia, indicating the possibility of its use against pathogenic fungi in mushroom culture.

The water-methanol-and glycerol diluted plant extracts of 27 botanicals were evaluated *in vitro* against *Trichoderma viride* and *Agaricus bisporus*.

Among these water diluted extract (5%) of *Lantana camara* (WDELc) reduced the radial growth of *T. viride* by 28.57% along with 38.61% growth promotion of *A. bisporus* (Mishra and Singh, 2005). The antifungal activity of leaf extract of at least 6 angiospermic plants such as; *Aegle marmelos*, *Berberis aristata*, *Cannabis sativa*, *Cleome viscosa*, *Erigeron karvinskianus*, *Leonotis nepetaefolia* was used against the fungal pathogen of mushrooms (Singh and Singh, 2005). Among them *E. karvinskianus* was highly effective against the pathogen and the mushroom yield was enhanced from 11.69-13.25 kgs/q compost.

Glamocilige *et al.* (2006) analyzed the plants savory (*Satureja thymbra*) and sage (*Salvia pomifera*) using GC and GC-MS and reported that the main components of *S. thymbra* were gamma-terpinene (23.2%) and carvacrol (48.5%) and in *S. pomifera* were alpha-pinene (20.4%) and alpha-thujone (36.1%). The components were assayed for antifungal activity against *Mycogone pernicioso* causing wet bubble disease of white button mushroom and it was found that gamma-terpinene and carvacrol of *S. thymbra* showed complete inhibition of the pathogen. Essential oils of *Metricaria chamomilla*, *Mentha piperita*, *Lavendulla augustifolia*, *M. spicata*, *Ocimum basilicum*, *Thymus vulgaris*, *Origanum vulgare*, *Salvia officinalis*, *Citrus limon* and Limonene, alpha-pinene, beta-pinene, 1,8, cineole camphor, carvacrol, thymol and menthol were assayed for inhibitory activity against three major pathogens of the button mushroom (*Agaricus bisporus*) i.e. *Vetricillium fungicola*, *Trichoderma harzianum* and the bacterium *Pseudomonas tolaassi*. The highest and the broadest antifungal activity was shown by *Origanum vulgare* oil and Carvacrol among the components tested (Sokovic and Griensven, 2006).

Chapter – 3

MATERIALS AND METHODS

The present studies on the integrated management of wet bubble disease of *Agaricus bisporus* (Lange.) Imbach. were conducted during 2008 and 2009 at Mushroom Research and Training Centre, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar. The details of the materials used and methodology adopted in achieving the set objectives of study are described as under:

3.1 Disease status

Survey of the mushroom units located in three districts *viz.*, Srinagar, Budgam and Pulwama, of Kashmir Division was conducted in both spring and autumn crop seasons of 2008 and 2009, to ascertain the status of wet bubble disease (*Mycogone perniciosa*) of white button mushroom, *Agaricus bisporus* (Lange) Imbach. Nine representative locations/mushroom farms were randomly selected in each district. The frequency of prevalence of wet bubble disease was recorded on the basis of the number of mushroom units infected out of the total units surveyed, as per the method adopted by Singh and Sharma (2002).

For working out the disease incidence for each mushroom farm, the number of infected bags/trays out of the total number of bags/trays observed was recorded, and the per cent disease incidence was calculated by the formula given by (Fletcher *et al.*, 1983).

$$\text{Disease incidence} = \frac{\text{No. of infected trays/bags}}{\text{Total No. of trays/bags}} \times 100$$

For working out the disease intensity, the number of healthy and infected sporophores in each randomly selected tray/bag in a farm was counted and categorized on the following 0-5 scale.

Grade	No of infected sporophores (%)
0	0
1	1-5
2	6-10
3	11-25
4	26-50
5	More than 50

The percentage of the disease intensity was calculated using the formula:

$$\text{Disease intensity (\%)} = \frac{\Sigma \text{disease score}}{\text{Total No. of observations} \times \text{maximum grade value}} \times 100$$

From each infected mushroom farm surveyed, yield obtained was recorded and the percent yield loss due to disease was calculated using the following formula given by Zaayen and Adrichem (1982).

$$\text{Per cent yield loss} = \frac{\text{Expected yield} - \text{yield obtained}}{\text{Expected yield}} \times 100$$

Where,

$$\text{Expected yield} = \text{Total No. Fruit bodies formed} \times \text{Average weight of healthy fruit body}$$

$$\text{Yield obtained} = \text{No. of healthy fruit bodies} \times \text{Average weight of healthy fruit body}$$

During the survey, diseased material (sporophores along-with infected casing soil) was collected in paper bags/containers and kept in a refrigerator at (2-5 °C) for further studies.

Diseased samples were also preserved in formalin acetic acid alcohol (FAA) solution for microscopic examinations.

3.2 Isolation, identification and establishment of pure culture

3.2.1 Isolation of pathogen

Isolation of the associated micro-organism was made from the mushroom sporophores, showing typical symptoms of wet bubble disease following routine pathological techniques (Holliday, 1980). The diseased sporophores/sclerodermoid masses were first examined for the associated fungus by teasing the diseased portion with the help of a teasing needle and observed under microscope. For isolation of the causal fungus, 5 mm small flesh disc segments were taken from the infected sporophore, with the help of sterilized cork borer, surface sterilized with 0.1 per cent mercuric chloride for 30 seconds followed by rinsing thrice with sterilized distilled water, blotter dried and inoculated under aseptic conditions on Rose Bengal-amended potato dextrose agar (PDA) medium in sterilized petri-dishes, incubating the plates at 23 ± 2 °C.

3.2.2 Purification and maintenance of pathogen

The culture was purified by hyphal tip (Pathak, 1972) and single spore isolation (George, 1947) methods. The pure culture, thus obtained, was maintained by repeated sub-culturing at monthly intervals. The stock culture in PDA slants was stored at 4⁰C in a refrigerator. The isolations were made afresh in each cropping season to avoid possible loss of pathogenic behaviour of the isolated fungus by repeated sub-culturing.

2.2.3 Identification of pathogen

The isolated micro-organism was identified on the basis of its morphological and cultural characteristics comparing it with the standard descriptions of *Mycogone perniciosa* given by Hsu and Han (1981) and Singh and Sharma (2002). The identity of the pathogen was also got confirmed from National Bureau of Agriculturally Important Micro-organisms (NBAIMS) New Delhi, and the culture deposited under accession No. 7493.09.

3.3 Pathogenicity test

3.3.1 *In vitro* test

The isolated micro-organism and the *Agaricus bisporus* was grown in dual culture in petri-plates at 23 ± 2 °C and observed for the type of interaction. The slower growing *A. bisporus* was inoculated first and the colony was allowed to reach a diameter of 15 to 20 mm before the fast growing pathogen was inoculated on opposite sides of 90 mm petri-plates. The radial growth of the pathogen and host was measured at the time of colony contact. Upon contact between the host and the pathogen, the interaction between the two mycelial colonies was noted.

3.3.2 *In vivo* test

In order to prove the pathogenicity of the isolated micro-organism and also to record the symptoms and development of infection on the sporophores, two sets of experiments were carried out in plastic baskets of 5 kg compost capacity. The baskets were disinfected with 2 per cent formalin and rinsed thrice with sterilized distilled water. The baskets were filled with pasteurized compost, spawned and kept in spawn run room at 24 ± 2 °C. In the first set of experiment, chlamydospore suspension (2×10^6) chlamydospores ml^{-1} was inoculated in sterilized casing mixture at the time of casing.

In the second set of experiment, the isolated micro-organism was inoculated on healthy pinheads and fruit-bodies in the form of chlamydospore suspension (2×10^6 chlamydospore ml^{-1}) and the mycelial discs from the culture

plate, to observe the wet bubble symptom development. After inoculation the baskets were kept in an isolated room at a temperature $21\pm 1^{\circ}\text{C}$ with relative humidity of more than 85 per cent. An un-inoculated basket was also maintained under similar conditions to serve as check and kept apart to avoid contamination. Both the sets of pathogenicity tests were closely monitored for symptom development.

Re-isolations of the micro-organism from the artificially inoculated sporophores/sclerodermoid masses and diseased pinheads were made and the resultant cultures compared with the original inoculant to satisfy Koch's postulates.

3.4 Morphological characters of the isolated pathogen

The morphological characteristics of the causal organism on host and in artificial culture were studied in the laboratory. The important characters studied were the cultural characters such as mycelial colour and growth and the morphological characters such as shape, size, colour and septation of hyphae, conidiophores, conidia and chlamydo spores.

3.5 Assessment of factors responsible for disease development and spread

3.5.1 Contaminated compost

The pathogen was mixed with compost at different stages *viz.*, on stacking (0 day), and 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th turnings on 6th, 10th, 13th, 16th, 19th, 22nd and 29th day of composting. At each stage 10 ml of *M. perniciosus* spore suspension (1×10^7 spores/ml) prepared in sterilized distilled water from 7-day old culture, was admixed with 100 grams of composting mixture. The inoculated compost was filled in sterilized glass bottles, tight lidded, labeled and then kept in the centre of the pile. Five such bottles were maintained for each composting stage. The bottles were taken out from the compost heap on the filling day. For examining the presence of live pathogen in the compost, one gram of the compost was taken out from each bottle, serially diluted in sterilized distilled water and 1

ml aseptically poured on PDA in petri-plates. The plates were incubated at $23\pm 2^{\circ}\text{C}$ for 6 hours and the germinating conidia/chlamyospores counted.

3.5.2 Contaminated casing soil

3.5.2.1 Presence of pathogen in casing materials

Composite samples of different casing materials such as peat, garden soil, virgin soil, spent compost, farm yard manure (FYM) and sand were collected, sieved through a 200 mesh sieve and put in pre-sterilized plastic vials. One gram of the material was separately used for assessing the *M. perniciosus* propagules using serial dilution method (q.v. Section 3.5.1).

3.5.2.2 Spent compost as inoculum source

500 g of composite samples of spent compost were collected in triplicate from the production trays, showing the signs of wet bubble disease soon after termination of spring and autumn cropping season of 2008 and placed separately in plastic jars. The jars were kept outdoors under natural conditions and 5 g of compost samples drawn at monthly intervals for assessing the presence of pathogen *M. perniciosus* using serial dilution method (q.v. Section 3.5.1).

3.5.2.3 Pathogen survival in casing material

Sterilized casing material comprising of soil and peat in the ratio of 2:1 was admixed with spore suspension of test pathogen having spore load of 6×10^8 spore ml^{-1} and prepared from 7 days old culture in distilled sterilized water. The inoculated casing mixture was filled in perforated plastic jars and kept outdoors under natural conditions. From each jar samples were drawn at monthly intervals and examined for presence of the pathogen by dilution plate technique (q.v. Section 3.5.1)

3.5.3 Air currents

The infected fruit bodies/sclerodermoid masses having abundant conidia were placed 15 cm away from a small electric fan in the inoculation room. Petri-dishes containing PDA, amended with Rose Bengal were exposed for 5 sec at right angles to air flow at various distances from 20 to 100 cm away from infected sporophores, the plates were incubated at $24\pm 1^{\circ}\text{C}$ and the data on number of the pathogen colonies developed recorded after 48 hr. of incubation.

3.5.4 Spread through water splash

water was sprayed on to the mushroom trays containing *M. perniciosa* infected sporophores/sclerodermoid masses and the water splashes collected in pre-sterilized petri-plates at different distances such as 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 cm. The water collected was later examined for spore density by the method adopted by Hsu and Han (1981) and Singh and Sharma (2002) and expressed as number of spores per microscopic field.

3.5.5 Flies

To assess the influence of sciarid flies infestation on dispersal of wet bubble disease different population (10, 20, 50, 75 and 100) of flies collected from infested *Pleurotus* bags were allowed to acquire inoculum from wet bubble infected sporophores on diseased trays (kept in insect cages) for varied periods (30, 60, 90 and 120 minutes) and subsequently released on healthy trays also kept in insect cages. The percentage of infestation in each tray was calculated by the following formula :

$$\text{Percentage of infested sporophores} = \frac{\text{No. of infested sporophores}}{\text{Total number of sporophores per tray}} \times 100$$

3.6 Management studies

3.6.1 *In vitro* fungitoxics

Five non systemic and five systemic fungitoxics were assayed *in vitro* against *M. perniciosus* and *A. bisporus* mycelium using poisoned food technique Nene and Thapliyal (1979). Each non systemic fungitoxicant was evaluated at concentrations of 25, 50, 100, 200 and 500 $\mu\text{g ml}^{-1}$, whereas the systemic fungitoxics were evaluated at concentrations of 5, 10, 25, 50 and 100 $\mu\text{g ml}^{-1}$. 25 ml double strength sterilized PDA in 150 ml Erlenmeyer flask, was amended aseptically with 25 ml double strength test fungitoxicant concentration. The contents were thoroughly but gently shaken and aseptically poured in pre-sterilized petriplates. Mycelial discs of 7 and 14 day old culture, respectively, of *M. perniciosus* and *A. bisporus* were separately and aseptically placed at the centre of each petriplate and incubated at $23\pm 2^\circ\text{C}$. Each treatment was replicated thrice. *M. perniciosus* and *A. bisporus* allowed to grow separately on PDA amended with sterilized water in petriplates served as checks. Data on radial mycelial growth were recorded when the test pathogen nearly covered the petriplate in check. The mycelial growth inhibition as index of fungicidal efficacy was computed using the formula given by Vincent (1947).

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

Where PI = Percent inhibition

C = Growth of pathogen in control (mm)

T = Growth of pathogen in treatment (mm)

Systemic and non-systemic fungitoxicants used with their chemical names

Common name	Trade name	Chemical name
<u>a) Non systemic</u>		
1. Prochloraz manganese	Sportek WP	Manganese, dichlorotetrakis [N-propyl-N-[2-(2,4,6-trichlorophenoxy) ethyle]-1H-imidazole-1-carboximide]
2. Chlorothalanil	Kavach 75 WP	2, 4, 5, 6 tetrachloro isophthalonitrile
3. Mancozeb	Dithane M-45	Manganese ethylene bis dithiocarbamate plus zinc
4. Captan	Captaf 50 WP	N-(trichloromethylthio)-4-cyclo hexane-1, 2-dicarboximide
5. Propineb	Antracol 70 WP	3-(2-methylpirperidinno) propyl-3, 4-dichloro-benzoate
<u>b) Non systemic</u>		
1. Carbendazim	Bavistin 50 WP	[2 (methoxy-carbamoyl) benzimidazole]
2. Bitertanol	Baycor 25 WP	1-(1, 1-biphenyl)-1 H-I, 2, 4-triazole-1-1 ethanol
3. Myclobutanil	Systhane 10 WP	A-butyl- α (4-chlorophenyl)
4. Triademiphon	Bayleton 25 WP	1-(4-chlorophenoxy)-3,3-dimethyl-1 (1H-1, 2,4-triazol-1-yl)-2-butanone
5. Diniconazole	Sumi 25 WP	4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-pentene-3-1

3.6.2 In vivo evaluation of fungitoxicants

Fungicides which have shown promising results during *in vitro* evaluation and were least inhibitory to mushroom mycelium, were evaluated *in vivo* during the present studies. The systemic fungicides viz., carbendazim 50 WP, myclobutanil 10 WP and bitertanol 25 WP were evaluated at 0.025, 0.05 and 0.1% concentrations, whereas the non-systemic fungicides viz., prochloraz

manganese 50 WP, chlorothalonil 75 WP and captan 50 WP were evaluated at 0.05, 0.1 and 0.2% concentrations. Each fungicidal concentration was admixed with casing mixture at the rate of 100 ml/kg casing mixture before inoculation of the pathogen (*M. perniciosa*). Casing was done by spreading 2-3 cm thick layer of casing mixture on spawn-run compost in 10 kg polybags. Each bag received about 2 kg casing soil. Inoculation of the pathogen was done by spraying spore suspension (1×10^8 spores ml^{-1}) obtained from 10 day old culture, on casing soil at the rate of 20 ml/bag of 30 cm diameter. Each treatment represented by a single bag was replicated three times. The control was run without any fungicidal treatment with and without inoculation of pathogen. Per cent disease intensity and yield for a cropping period of one month and also other quality characters were recorded during first flush as per the procedure given earlier (q.v. Section 3.6.1).

3.7 Evaluation of bacterial antagonists

3.7.1 *In vitro* evaluation

The pure cultures *Pseudomonas fluorescens* isolates PS-103, PS-104 and PS-105, *Bacillus subtilis* isolates BS-101, BS-115 and BS-116 and *Azotobacter* sp. isolates Azt-106, Azt-108 and Azt-117 were obtained from the Division of Environmental Sciences, SKUAST-K Shalimar, the isolates were maintained and mass multiplied by subculturing on nutrient Agar (NA)/as well as on King's -B medium, incubating the culture at $25 \pm 2^\circ\text{C}$ for 48 hours. The antagonistic potentialities of these bacterial isolates were tested against the growth of *A. bisporus* and *M. perniciosa* using dual culture technique (Ahlawat and Rai, 1997). The PDA was prepared, autoclaved and poured in petri plates and the bacterial strains were separately streaked on PDA in the centre of the petri plate. After streaking, 5mm discs of both *M. perniciosa* (7-day old culture) and *Agaricus bisporus* (10-day old culture) were equidistantly placed on two sides of the bacterial streak and incubated at $23 \pm 2^\circ\text{C}$ for three days. The petri plates with pathogen *M. perniciosa* and host *A. bisporus* only served as controls. Each treatment was replicated thrice. The petri plates were incubated at $23 \pm 2^\circ\text{C}$.

Observations on colony diameter of both *M. pernicioso* and *A. bisporus* in treated plates was recorded and the per cent inhibition over control calculated according to the formula given by Vincent (1947).

$$\text{Percent mycelial growth inhibition} = \frac{(C-T)}{C} \times 100$$

Where C = Radial mycelial growth (mm) in check

T = Radial mycelial growth in the treatment (mm)

Based on the growth of the host and the pathogen, the bacterial isolates were grouped into various categories as proposed by Ahlawat and Rai (1997) with slight modifications (Munshi *et al.*, 2008) as I – Antagonistic, II. Neutralistic, III. Stimulatory, IV. More stimulatory.

3.7.2 *In vivo* evaluation

The test bio-control agents, which have shown maximum *in vitro* antagonism against *M. pernicioso* and no *in vitro*, inhibition against *A. bisporus*, were evaluated *in vivo* against wet bubble disease. The antagonists (1×10^8 cfu/g⁻¹ soil) were admixed separately with casing mixture at the rate of 0.5, 1 and 2 per cent before inoculation of the pathogen. The pathogen was inoculated and the antagonist admixed casing mixture spread over spawn-run compost, filled in 10 kg polythene bags. For each treatment three replications were run, each replication comprising a single bag. Treatments without inoculation of pathogen and/or bio-control agents served as controls. The per cent disease intensity and yield were calculated for one month cropping period, whereas other quality characters were recorded only during first flush (q.v. section 3.7.1).

3.8 Evaluation of botanicals

3.8.1 *In vitro* evaluation

The aqueous and ethanol extract of the following eight locally available botanicals were evaluated *in vitro* by poisoned food technique for their efficacy

against both *A. bisporus* and *M. pernicioso* at three different concentrations of 1, 2 and 3 per cent (Plate-1 and 2).

Botanical name	Common/local name	Part used for
<i>Metricaria</i> sp.	<i>Metricaria</i>	Whole plant (leaves and tender shoots)
<i>Curcuma longa</i>	Turmeric	Rhizome
<i>Mentha longifolia</i>	‘Ven’	Whole plant
<i>Artemesia annua</i>	‘Tethwan’	Foliage
<i>Juglans regia</i>	Walnut	Hull
<i>Levendulla officinalis</i>	Lavender	Whole plant
<i>Azadirachta indica</i>	Neem	Leaves
<i>Urtica dioica</i>	Nettle ‘soi’	Whole plant

3.8.2 Preparation of botanical extracts

3.8.2.1 Aqueous extract

The aqueous extracts of the botanicals were prepared by taking one kg of medium sized leaves & tender shoots of each botanical except for turmeric where rhizomes were used (Plate-3). The botanicals used were thoroughly washed with tap water and shade dried. Walnut hull and turmeric rhizomes were dried until brittle and crushed in electric grinder. The materials was diluted by adding one litre of distilled sterilized water and strained through four folds of muslin cloth. The filtrate was again filtered through Whatman’s filter No. 41 under aseptic conditions and passed through bacteria proof Sietz filter. The filtrate thus obtained was taken as 100 per cent aqueous extract. Double strength of the test concentrations of 1, 2 and 3% were obtained by adding appropriate amount of the extract in 50 ml sterilized distilled water and aseptically amended in 50 ml double strength molten PDA medium in 250 ml Erlenmeyer flask. The amended PDA



Metricaria spp



Curcuma longa



Artemisia annua



Lavendula officinalis



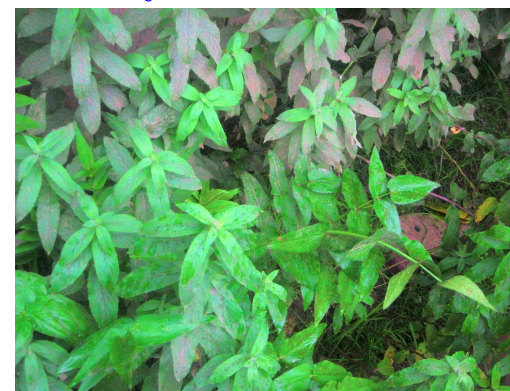
Urtica dioica



Azadirachta indica



Juglans regia



Mentha longifolia

Plate-1 : Locally available botanicals used in the study



Metricaria spp



Curcuma longa



Artemisia annua



Lavendula officinalis



Urtica dioica



Azadirachta indica



Juglans regia



Mentha longifolia

Plate-2 : Evaluation of dried botanicals



Powder formulation



Aqueous extract



Ethanol Extract

Plate-3 : Preparation of botanical extracts

was then aseptically poured in sterilized petriplates. A 5 mm diameter disc of *M. pernicioso* (7-day old culture) as well as *A. bisporus* (14 days old culture) were aseptically placed separately in the centre of the petriplates.

In both the cases treatments were replicated thrice. The comparative efficacy of the aqueous extract of the test botanicals was calculated as per cent mycelial growth inhibition of the test fungus in each treatment. The per cent inhibition was calculated by the formula :

$$\text{Percent mycelial growth inhibition} = \frac{(C-T)}{C} \times 100$$

Where C = Radial mycelial growth (mm) in check

T = Radial mycelial growth in the treatment (mm)

3.8.2.2 Ethanol extract

The ethanol extracts of the botanicals were obtained by the method adopted by Deshpande *et al.* (2004). The plants/plant parts were washed with tap water rinsed with sterile distilled water and then shade-dried. Walnut hull and turmeric rhizomes were also dried till brittle. The shade-dried plants/plant parts were ground with the help of electric grinder to obtain the fine powder of each botanical. The dried powder was then stored in plastic containers for further use.

The dried powder of the plant parts (20 g) was mixed with 200 ml solvent (70% ethanol) in 250 ml conical flask. Turmeric rhizomes were also crushed in electric grinder and then 20 g mixed with 200 ml solvent. All the flasks were tightly plugged with sterile cotton plugs, their mouth wrapped with aluminum foil and kept on a shaker for 36 hrs. After keeping the flasks undisturbed for 6 hours to allow suspended plant material to settle down, the extract was decanted, filtered and centrifuged at 500 rpm for 15 minutes. The supernatant was collected and the solvent allowed to evaporate at 40-50⁰C to make the final volume one-fourth of the original volume. The extract now taken as 100% standard extract was stored in

air tight containers/bottles for further use. The ethanol extract of each botanical was bioassayed for fungitoxic activity at 1, 2 and 3% concentration against both *A. bisporus* and *M. perniciosa* by employing poisoned food technique.(q.v. Section 3.7.3).

3.9 In vivo evaluation

The effective botanical extracts such as *Curcuma longa*, *Artemisia annua* and *Lavendulla officinalis* which were most inhibitory to *M. perniciosa* and least inhibitory to *A. bisporus* were evaluated *in vivo* against the wet bubble disease. The botanicals were dried under shade after collection till brittle and crushed into a fine powder in a grinder. The powder was passed through double layered muslin cloth and mixed with the casing mixture at the rate of 0.5, 1 and 2 per cent before inoculation of the disease. The botanical-admixed casing mixture was spread over the spawn-run compost (10 kg poly bags) before inoculation of the pathogen as in previous section (q.v. Section 3.7.2). For each treatment three replications were run, each replication represented by a single bag. The bags were kept in production room at a temperature of 22-24°C for four days after casing and 15-18°C for rest of the period till cropping was over. Treatments with no botanical admixture with and without pathogen inoculation served as controls. Per cent disease intensity and yield per bag were calculated for one month cropping period, whereas other quality characters were studied during first flush only.

3.10 Integrated management

Integrated management of wet bubble disease was attempted in two separate sets of experiments. In one set of integrated management most efficient systemic fungicide carbendazim 50 WP at 0.05% concentration, the most effective botanical *Curcuma longa* at 1% concentration and a potential bio-control agent *P. flourescens-103* at 1% concentration with minimum inhibition against *M. perniciosa* were evaluated in combination. In second set of experiment, the most effective non-systemic fungicide captan 50 WP was utilized in treatment

combinations. Treatment combinations in these two sets of experiments were as under:

Treatments in set-I	Treatments in set-II
Carbendazim 50 WP 0.05%	Captan 50 WP 0.1%
<i>Curcuma longa</i> 1%	<i>Curcuma longa</i> 1%
<i>P. flourescens</i> (PS-103) 1%	<i>P. flourescens</i> (PS-103) 1%
Carbendazim 0.05% x <i>C. longa</i> 1%	Captan 0.1% x <i>C. longa</i> 1%
Carbendazim 0.05% x PS-103 1%	Captan 0.1% x PS-103 1%
<i>C. longa</i> 1% x PS-103 1%	<i>C. longa</i> 1% x PS-103 1%
Carbendazim 0.05% x <i>C. longa</i> 1% x PS-103 1%	Captan 0.1% x <i>C. longa</i> 1% x PS-103 1%
Control-I (sterilized casing soil)	Control-I (sterilized casing soil)
Control-II (Infested casing soil)	Control-II (Infested. casing soil)
Control-III (un-infested . casing soil)	Control-III (Un-infested casing soil)

3.11 Screening of various button mushroom strains against wet bubble (*M. perniciosus*) disease

Seven strains viz., S-176, S-11, U-3, RRL-89, RRL-80, S-737 and NCS-102 of button mushroom (*A. bisporus*) obtained from Mushroom Research & Training Centre (SKUAST-K) and Indian type culture collection (IARI) Pusa, New Delhi through Dr. P.N. Choudhry, Ex. Principal Scientist were evaluated for their tolerance/susceptibility to wet bubble disease caused by *M. perniciosus* under epiphytotic conditions during spring season of 2009 and 2010. The spawn from these cultures was prepared on wheat grains as per the procedure given by Manjal (1973). Long method of composting (Mantel *et al.*, 1972) was adopted and the ingredients as per SKI-4 formula (wheat straw 300 kg; chicken manure 150 kg; rice bran, 50 kg; molasses 12 ½ kg; linseed meal 12.5 kg; urea 5 kg; potash 2

kg and gypsum 15 kg). The fully prepared compost was filled in polythene bags at the rate of 10 kg per bag. Layer spawning was done at the rate of 0.6%. After complete spawn run, 3-4 cm thick casing was uniformly laid on each tray. The casing consisted of a mixture of sick soil and peat in the ratio of 2:1. The soil was made sick by admixing spore suspension (1×10^{10} per ml water) of the pathogenic fungus at the rate of 10 ml/kg soil. For each treatment, three replications were maintained, each replication represented by a single bag of 10 kg compost. After casing, the bags were kept at 22-24°C in incubation room at 75-80% relative humidity (RH). On 6th day onwards, the temperature and the relative humidity were maintained at 10-20 °C and 75%, respectively.

The data on per cent disease intensity and the total mushroom weight and yield per quintal compost were recorded for 1½ months, whereas the other quality parameters were recorded only during 1st flush. The per cent decrease in fruit body weight due to the disease was calculated by recording the difference in weights obtained in the diseased bags and the healthy controls maintained for each strain through the use of pathogen-free casing material.

3.12 Statistical analysis

The data collected were subjected to statistical analysis wherever needed. The differences exhibited by the treatments in various experiments were tested for their significance as per the methods suggested by Gomez and Gomez (1984). The 'Minitab' computer software was used for data analysis.

Chapter – 4

EXPERIMENTAL FINDINGS

The studies on wet bubble disease of white button mushroom [*Agaricus bisporus* (Lange) Imbach] were carried out at Mushroom Research and Training Centre, Division of Plant Pathology SKUAST-K, Shalimar, Srinagar Kashmir during 2008 and 2009 with the objectives of ascertaining the status of the disease and developing an integrated management strategy for it.

4.1 Status of wet bubble Disease

Survey for ascertaining the status of wet bubble disease of white button mushroom was conducted in three districts-Srinagar, Budgam and Pulwama-of Kashmir Division in both spring and autumn crop seasons of the year 2008 and 2009. In each district, nine mushroom farms were randomly selected for assessing the disease incidence, disease intensity and yield losses.

4.1.1 Disease incidence

Data presented in Table-1 illustrated in Fig. 1 reveals that wet bubble disease on white button mushroom was prevalent during both the years (2008 and 2009) and its prevalence ranged from 33 to 67 per cent in mushroom farms in the three districts. The disease was most prevalent (67%) in district Budgam followed by that (56%) in district Pulwama, whereas district Srinagar exhibited the least disease prevalence (33%). On an overall basis, the disease incidence was more in spring (12.75%) than in autumn (5.22%) season. It was highest (11.07%) in district Pulwama and the lowest (7.27%) in district Budgam. The overall mean disease incidence over the years in all the three districts was 9.09 per cent. The disease incidence observed in spring seasons of the year 2008 and 2009 revealed maximum incidence of 14.10-14.25 per cent in district Srinagar and Pulwama, while as minimum incidence of 10.55 per cent was recorded in district Budgam. In spring 2008, wet bubble incidence was maximum (66.67%) in Bandzoo

Table-1 : Incidence of wet bubble disease of white button mushroom at different mushroom units of district Srinagar, Budgam and Pulwama during 2008 and 2009

Location/unit surveyed	Wet bubble incidence (%)						Overall Mean
	Spring			Autumn			
	2008	2009	Mean	2008	2009	Mean	
A) Srinagar							
Saidpora	60.0	35.0	47.5	11.0	12.0	11.5	29.5
Brain	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batmaloo	16.67	60.0	38.33	5.83	17.5	11.6	24.99
Zainakote	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nishat	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mehjoornagar	28.75	56.25	42.5	5.0	13.75	9.37	2.59
Botakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Harwan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amdakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	11.71	16.80	14.25	2.42	4.80	3.61	8.93
B) Budgam							
Pakherpora	19.0	20.0	19.5	3.0	9.0	6.0	12.75
Zaloowa	13.5	22.0	17.75	6.5	8.5	7.5	12.62
Parnewa	11.33	26.66	18.99	4.67	0.0	2.33	10.66
Shamsabad	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sonarkolipora	10.5	18.0	14.25	5.5	8.5	7.0	10.62
Arigam	14.0	15.0	14.5	4.5	7.0	5.75	10.12
Kanir	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Surasyar	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bandepora	8.30	11.65	9.97	5.30	9.5	7.4	8.68
Mean	8.51	12.59	10.55	3.27	4.72	3.99	7.27
C) Pulwama							
Parigam	13.5	19.0	16.25	43.5	6.0	24.75	20.5
Ladoo	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batapora	34.0	14.0	24.0	44.0	8.0	26.0	25.0
Midoora	0.0	12.85	12.85	0.0	0.0	6.42	9.63
Batpora	24.28	20.0	22.14	25.71	0.0	12.85	17.49
Bandzoo	66.67	36.67	51.67	5.0	0.0	2.5	27.08
Achhan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tral	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tandwal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	15.38	11.39	14.10	13.13	1.5	8.05	11.07
Grand Mean	11.87	13.59	12.75	6.27	3.67	5.22	9.09

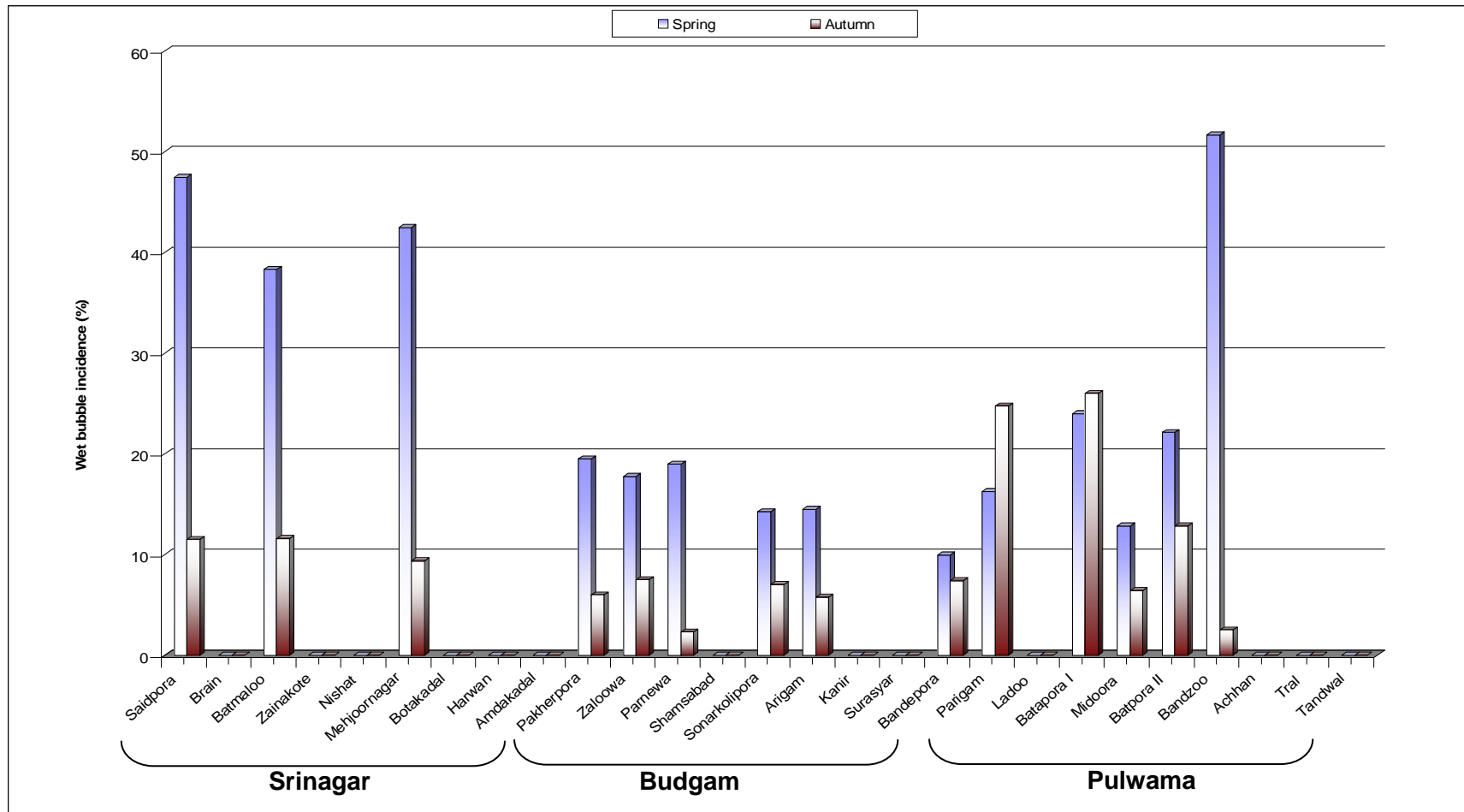


Fig.1 : Incidence of wet bubble disease of white button mushroom at different mushroom units of district Srinagar, Budgam and Pulwama during 2008 and 2009

Pulwama and Saidpora Srinagar (60%) followed by that in Batapora Pulwama (34%) and Mehjoornagar Srinagar (28.75%). Whereas, in 2009 spring the disease was more in Batmaloo (60%) and Mehjoornagar (56%) in Srinagar followed by Bandzoo (36.67%) in Pulwama district.

The disease incidence observed in Autumn seasons of the year 2008 and 2009 revealed maximum incidence of 8.05 per cent in district Pulwama followed by minimum 3.99 and 3.61 per cent recorded in district Budgam and Srinagar. In Autumn 2008, wet bubble was maximum (44%) in Batapora Pulwama followed by 43.5 and 25.71 per cent in Parigam and Batpora, respectively, of the same district, whereas in Autumn 2009 the disease incidence was maximum (17.5%) in Batmaloo Srinagar followed by that in Mehjoornagar Srinagar (13.75%) and Saidpora, Harwan (12.0%) . The minimum disease incidence of 6 to 8 per cent was recorded in Parigam and Batapora village of Pulwama district.

4.1.2 Disease intensity

The data presented in the Table-2 illustrated in Fig. 2 reveals that the intensity of wet bubble disease ranged from 0.0 to 83.0 per cent in 2008 and 2009 at different mushroom farms in the three districts. On an overall basis, the disease intensity was more (22.58%) in spring than in autumn (12.18%) season. It was the highest (21.86%) in district Budgam and the lowest (13.33%) in district Srinagar. The overall mean disease intensity in all the three districts was 17.82%.

The average disease intensity observed in spring seasons of the year 2008 and 2009 was maximum in district Budgam (28.31%) and Pulwama (22.59%) and minimum in district Srinagar (16.86%). In 2008 spring, wet bubble intensity was maximum (56.67-66.67 %) in Parigam and Bandzoo areas of Pulwama district followed by that in Batpora Pulwama, Saidpora Srinagar and Zaloowa and Sonarkalipora villages of district Budgam (46.67-50.00%) whereas in 2009 spring the disease intensity was maximum (80.00-83.33 %) in Mehjoornagar Srinagar and Parnewa Budgam followed by that in Saidpora and Batmaloo Srinagar and

Table-2 : Intensity of wet bubble disease of white button mushroom at different mushroom units of district Srinagar, Budgam and Pulwama during 2008 and 2009

Location/unit surveyed	Per cent disease intensity						Overall Mean
	Spring			Autumn			
	2008	2009	Mean	2008	2009	Mean	
A) Srinagar							
Saidpora	46.67	60.00	53.33	33.33	26.66	29.99	41.66
Brain	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batmaloo	23.53	63.33	43.33	26.67	26.67	26.67	35.00
Zainakote	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nishat	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mehjoornagar	30.0	80.00	55.0	13.33	50.00	31.66	43.33
Botakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Harwan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amdakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	11.13	22.59	16.86	8.14	11.48	9.81	13.33
B) Budgam							
Pakherpora	43.33	50.00	46.66	26.67	20.0	23.33	34.99
Zaloowa	46.67	66.33	56.5	33.33	23.33	28.33	39.91
Parnewa	40.0	83.33	61.66	23.33	0.0	11.66	36.66
Shamsabad	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sonarkolipora	50.0	60.0	55.0	33.33	36.67	35.0	45.0
Arigam	23.33	46.66	34.99	20.0	26.67	23.33	29.16
Kanir	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Surasyar	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bandepora	36.67	23.33	30.00	18.33	16.67	17.50	23.75
Mean	26.67	29.96	28.31	17.22	13.70	15.46	21.86
C) Pulwama							
Parigam	56.67	46.67	51.67	50.0	20.0	55.0	43.33
Ladoo	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batapora	43.33	53.33	48.33	30.0	33.33	31.67	39.99
Midoora	0.0	33.33	16.66	0.0	0.0	0.0	8.33
Batpora	46.67	43.33	45.0	33.33	0.0	16.67	30.83
Bandzoo	66.67	16.67	41.67	36.67	0.0	18.33	30.0
Achhan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tral	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tandwal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	23.70	21.48	22.59	16.67	5.92	11.29	16.94
Grand Mean	20.49	24.67	22.58	14.01	10.36	12.18	17.82

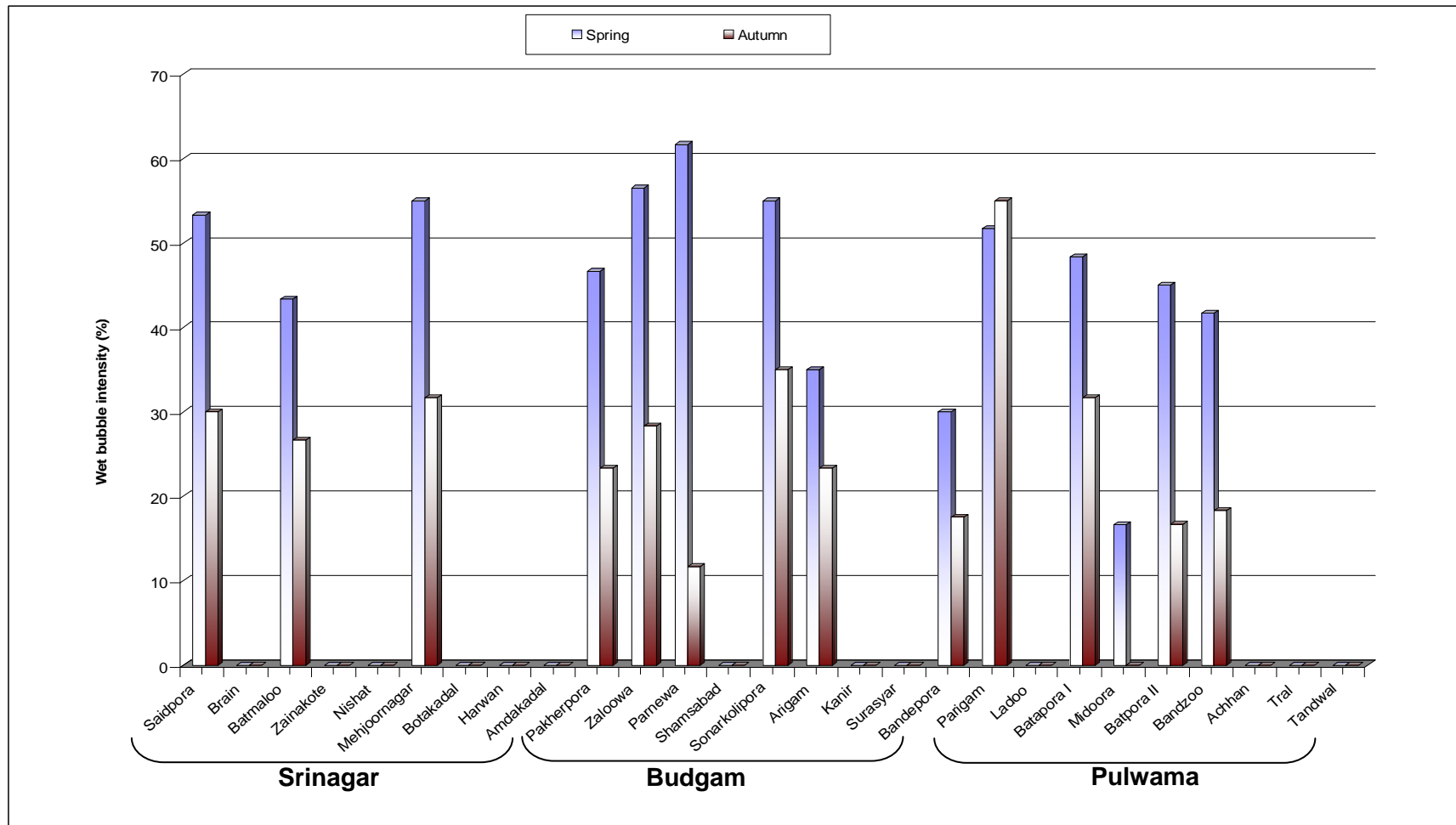


Fig. 2 : Intensity of wet bubble disease of white button mushroom at different mushroom units of district Srinagar, Budgam and Pulwama during 2008 and 2009

Zaloowa Budgam (60.00-60.33%). The disease intensity observed in autumn seasons of the year 2008 and 2009 revealed maximum intensity of 11.29 to 15.46 per cent in district Pulwama and district Budgam and minimum intensity of 9.81% in district Srinagar. In autumn 2008, wet bubble intensity was maximum (50%) in Parigam Pulwama followed by that in Saidpora Srinagar, Zaloowa and Sonarkalipora Budgam and Batapora Pulwama (33.33%). In autumn 2009, the disease intensity was maximum (50%) in Mejoornagar Srinagar followed by that in (36.67%) Sonarkalipora of district Budgam. The minimum disease intensity (20.00 %) was recorded in Parigam village of Pulwama district.

4.1.3 Yield Loss

Data presented in the Table-3 illustrated in Fig. 3 reveals that the extent of yield loss due to wet bubble disease ranged from 11.33 to 65.00 per cent in different mushroom growing units in the three districts of the valley. On an overall basis, the yield loss was the highest (21.08%) in spring than in autumn season (11.58%). It was the highest (22.41%) in district Pulwama followed by that (14.99%) in district Budgam, and the lowest (13.53%) in district Srinagar. The overall mean yield loss over the years in all the three districts was 16.97 per cent.

The yield loss observed in spring seasons of the year 2008 and 2009 revealed maximum yield loss of 21.35 to 26.01 per cent in district Budgam and Pulwama and minimum yield loss of 15.88 per cent in district Srinagar. In 2008 spring, the yield loss due to wet bubble disease was maximum (65%) in Parigam Pulwama followed by that (50.00-53.33%) in Batpora Pulwama and Saidpora Srinagar, whereas in spring 2009 the yield loss was maximum (60.67%) in Saidpora Srinagar followed by that (52.00-54.67%) in Mehjoornagar Srinagar and Parigam Pulwama. The per cent yield loss observed in Autumn seasons of the year 2008 and 2009 revealed maximum yield loss of 11.19 to 14.92 in districts Srinagar and Pulwama and a minimum of 8.63 per cent in district Budgam. In autumn 2008, the per cent yield loss due to wet bubble was maximum (55.00%) in Saidpora Srinagar followed by that (43.67 %) in Parigam Pulwama and minimum

Table 3 : Yield loss of white button mushroom inflicted by wet bubble disease at various mushroom units of district Srinagar, Budgam and Pulwama during 2008-2009

Location/unit surveyed	Yield loss (%)						Overall Mean
	Spring			Autumn			
	2008	2009	Mean	2008	2009	Mean	
A) Srinagar							
Saidpora	53.33	60.67	57.00	55.00	49.00	52.00	54.5
Brain	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batmaloo	27.54	42.67	35.10	24.33	17.50	20.91	28.00
Zainakote	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nishat	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mehjoornagar	49.75	52.00	50.87	28.67	27.0	27.83	39.35
Botakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Harwan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amdakadal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	14.51	17.26	15.88	12.0	10.39	11.19	13.53
B) Budgam							
Pakherpora	23.00	34.67	28.83	11.33	9.00	10.16	19.49
Zalooa	30.67	45.00	37.83	16.67	20.33	18.50	28.16
Parnewa	40.00	27.67	33.83	18.67	0.0	18.67	26.25
Shamsabad	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sonarkolipora	15.16	34.0	24.58	18.5	12.00	15.25	19.91
Arigam	28.67	36.67	32.67	8.0	14.67	11.33	22.00
Kanir	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Surasyar	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bandepora	40.0	28.83	34.41	24.58	18.67	21.62	28.01
Mean	19.72	22.98	21.35	9.97	7.29	8.63	14.99
C) Pulwama							
Parigam	65.00	54.67	59.83	43.67	24.00	33.83	46.83
Ladoo	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Batapora	50.29	49.67	49.98	37.00	40.67	38.83	44.40
Midoora	0.0	35.00	35.00	0.0	0.0	0.0	35.00
Batpora	51.67	48.33	50.0	25.67	0.0	25.67	37.83
Bandzoo	38.67	40.0	39.33	36.00	0.0	36.0	37.66
Achhan	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tral	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tandwal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	22.84	25.29	26.01	15.81	7.18	14.92	22.41
Grand Mean	19.02	21.84	21.08	12.59	8.28	11.58	16.97

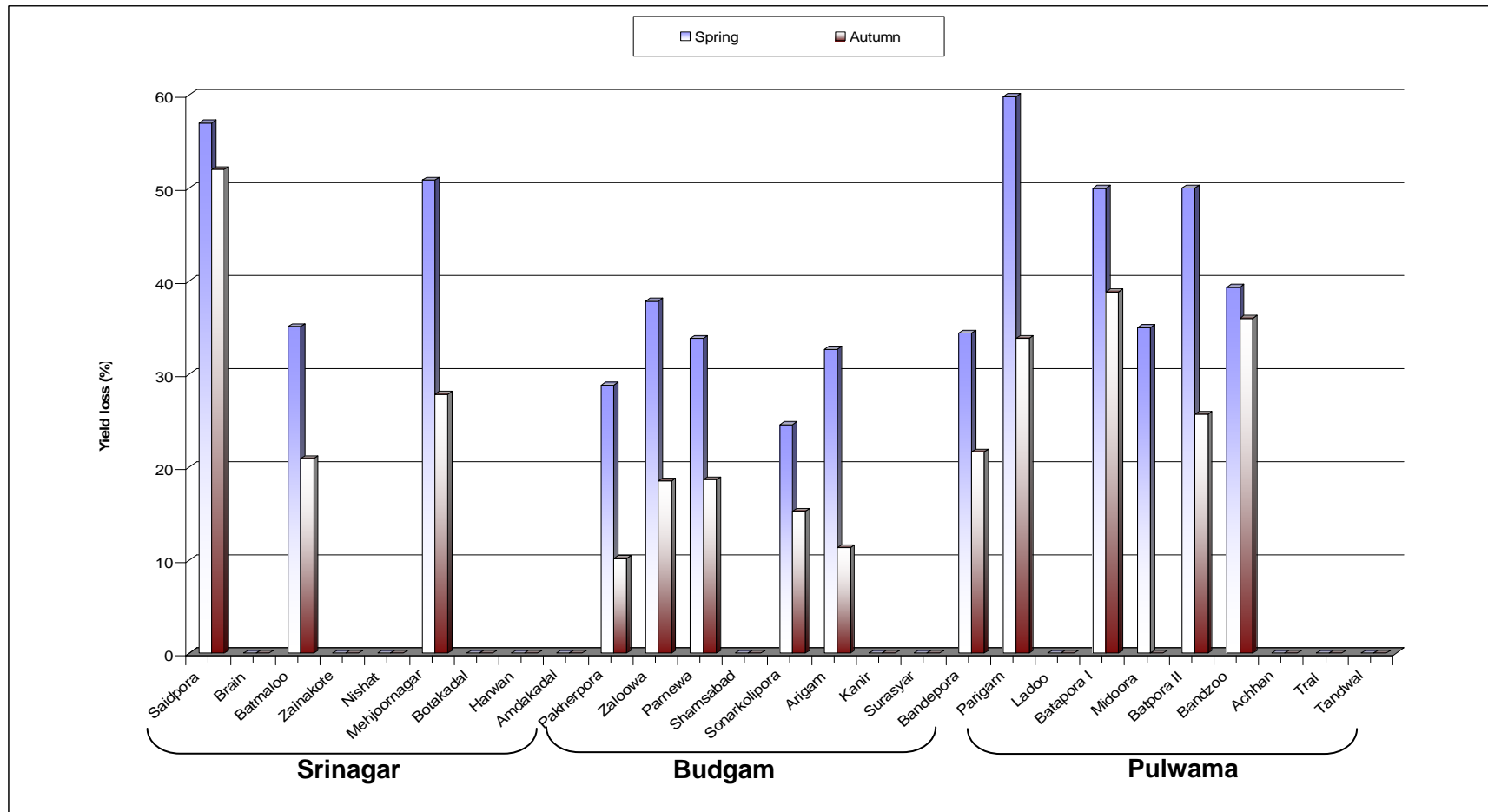


Fig. 3 : Yield loss of white button mushroom inflicted by wet bubble disease at various mushroom units of district Srinagar, Budgam and Pulwama during 2008-2009

(11.33 %) in Pakherpora Budgam, whereas in Autumn 2009 the per cent yield loss was maximum (49.00 %) again in Saidpora Srinagar followed by that (40.67 %) in Batapora Pulwama. The minimum (9.00%) per cent yield loss was recorded in Pakherpora location of district Pulwama.

4.2 Symptomology

The symptoms of wet bubble disease included development of whitish mouldy growth of the mycelium on the casing surface and on portions of fruit bodies, which eventually spreads covering the entire cap and causing distortion of the affected mushrooms (Plate-4). These distorted masses or sclerodermoid masses are upto 8-10 cm across. When young pin heads were infected, they developed amorphic shapes, not at all resembling the typical mushrooms. Similar types of symptoms were produced on inoculation of pinheads and mature fruit bodies with mycelial bits of spore suspension sprays or inoculation with agar culture discs of the pathogen. However, the appearance of symptoms and the subsequent disease development was much quicker when fruit bodies of the mushroom (*A. bisporus*) were inoculated with mycelial bits. Characteristic symptoms in this case appeared within 1 to 2 days of inoculation. The reactions of undifferentiated primordia were vastly hyperplastic and of tumorous nature. Finally, large irregular lumps of mushroom tissues were formed with no sign of differentiation or organogenesis. Dripping out of foul smelling amber liquid as teardrops on the tumorous bodies was consistently a typical sign of the wet bubble disease.

4.2.1 Pathogenicity

Observations recorded on the response of white button mushroom to the isolated fungus (Plate-5) inoculated in sterilized casing mixture at the time of casing indicated its pathogenic behaviour, which ultimately resulted in the development of typical symptoms (Plate-6). The symptoms started appearing as white fluffy patches of mycelium on the surface of casing from the 7th day after



On-developing-pin heads



Un-differentiated tissue



Sclerodermoid mass



Amber exudation on fluffy mycelium



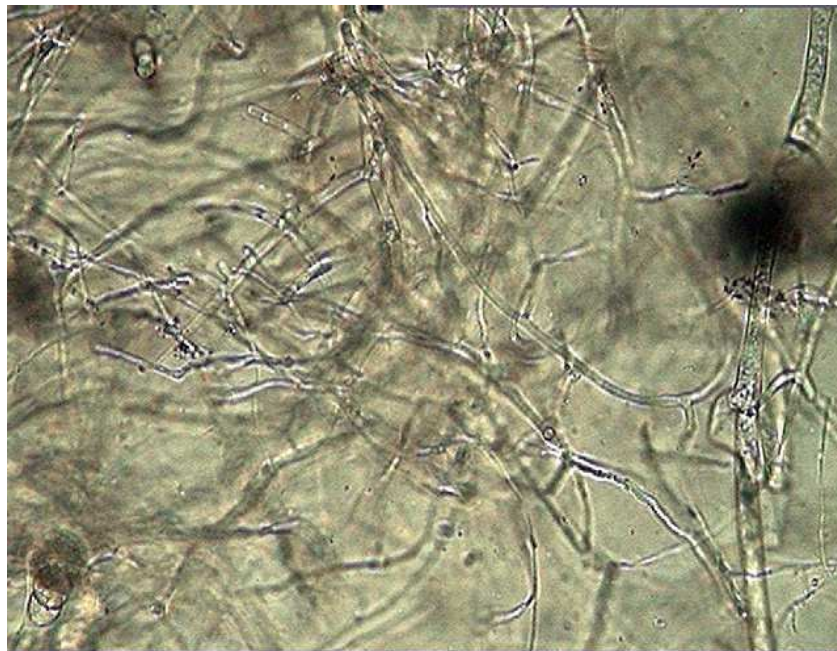
Cross section of sclerodermoid mass v/s healthy fruit body



Plate-4 : Symptoms of wet bubble disease of white button mushroom



Dual culture of *Mycogone pernicioso* and *Agaricus bisporus*



Lysis of *Agaricus bisporus* mycelium

Plate-5 : *In vitro* pathogenicity test



Inoculation at pin head stage



Inoculation at casing



Symptom development after inoculation

Plate-6 : *In vivo* Pathogenicity test

inoculation, whereas when inoculation was done on young pinheads, symptoms started appearing within 1 to 2 days after inoculation. Mushrooms attacked before differentiation looked as a blob. Inoculation of mature stages of mushroom showed an initial browning. A large portion of the gill tissue got colonized when the mature mushrooms got infected, well after the differentiation of stalk, cap and gills. When the infected mushrooms were incubated at 18 to 20°C at a high relative humidity of 95 per cent maintained, enormous number of spores appeared on the mycelial mat of the pathogen. The large mushrooms when inoculated also showed swollen stipes or deformed caps with tissue discolouration and also fuzzy growth on mushroom cap. The blobby surface of mushroom became wet, and slightly tinted matericeal fluid was discharged. Re-isolations from these infected sporophore masses yielded typical culture of the fungus and satisfied the Koch's postulates.

4.2.3 Morphology of the fungus

The cultural and morphological characters of the pathogen were studied both in culture and on host.

4.2.3.1 Morphology of fungus in culture

The isolated fungus cultured on potato dextrose agar medium (Plate-7) produced copious flocculent mycelium with even or uneven edges. The amount of aerial mycelium varied from dense to sparse, while the colour of colonies turned from white to pale brown and finally dark brown after 12-14 days of incubation at $24\pm 1^\circ\text{C}$ (Plate-8). The maximal radial growth (90 mm) was recorded on compost agar medium after 15 days, followed by potato dextrose agar (75.5 mm) and malt extract agar (72.0 mm).

The pathogen in culture produced septate and hyaline mycelium. The hyphae were branched and interwoven, 3-5 μm broad. Conidiophores were short, slender, branched, hyaline sub-verticillate to verticillate with apical conidium measuring 150 to 180 x 3 to 5 μm . Conidia were oval, single, 2-celled, thin



Mycelial growth in test tube



Mycelial growth on PDA

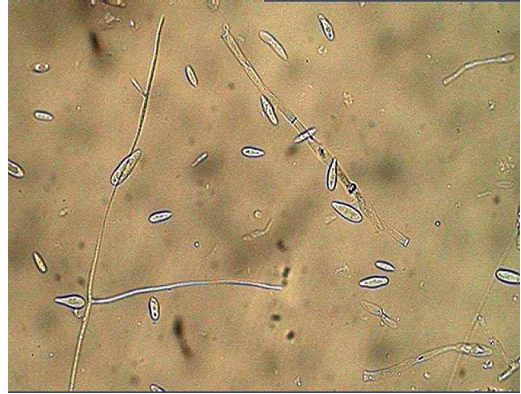


Mycelial growth on MEA

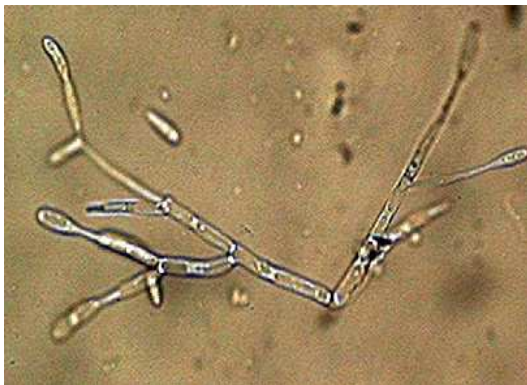
Plate-7 : *In vitro* growth of *Mycogone perniciosa*



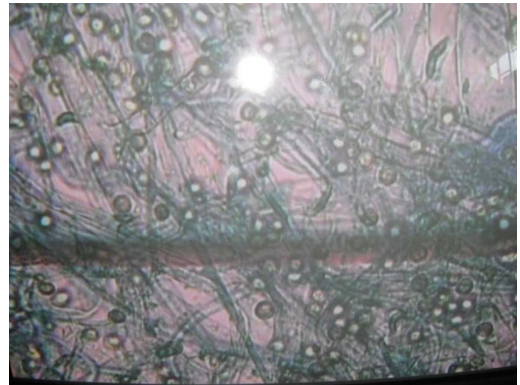
Mycelium



Single celled Conidia



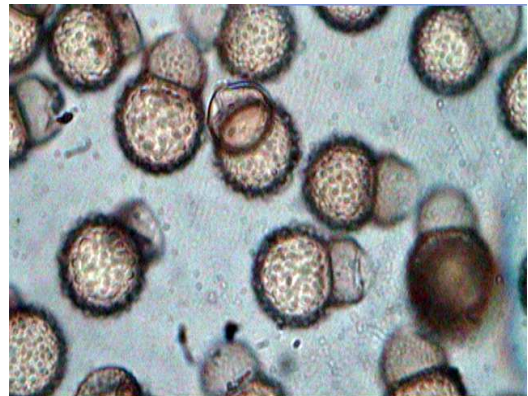
Sub-verticillate to verticillate conidiophores



Chlamydo spores on host



Terminal chlamydo spore



Bi-cellular chlamydo spore

Plate-8 : Morphological characters of *Mycogone perniciosa*

walled, 12-14 x 4-5 μm and relatively short lived. Chlamyospores or aleurospores were bi-celled with upper cell thick walled, warty, globose, bright coloured, measuring 12-15 x 24-26 μm and lower cell hyaline, smooth and measuring 12 x 14 μm . The chlamyospores are produced terminally.

4.2.3.2 Morphology of fungus on host

The mycoparasite is both inter and intra-cellular, the conidiophores are erect, long and verticillately branched. Under humid conditions, aerial growth of mycelium continued over the surface of infected fruit bodies. However, there was no variation in the colour, size, shape and septation of conidiophores, conidia and mycelium either on the host or in culture medium.

4.3 Factors affecting disease development and spread

4.3.1 Contaminated compost

The recovery and re-isolation of the pathogenic fungus from the infested compost samples kept in the central core of compost heap at stacking and during various turnings were made through dilution plate technique. The results (Table-4) reveal that the pathogen admixed with compost either at stacking (0 day of composting) or at 1st (6th day of composting), 2nd (10th day of composting), 3rd (13th day of composting), 4th (16th day of composting) or 5th turnings (19th day of composting) could not be re-isolated. However, the pathogen was successfully re-isolated when admixed with the compost at 6th, 7th or 8th turnings i.e. on 22nd, 25th and 29th day of composting. The pile temperature recorded during composting indicated that the temperature was minimum at stacking (0-30⁰ C), which increased to 65-70⁰C from 1st to 3rd turnings and gradually decreased thereafter reaching minimum (25-30⁰C) at 8th turning, when the pile was ready for filling.

Table 4 : Isolation of *M. perniciosus* incorporated in compost at different stages of composting

Pathogen incorporation stage	Temperature of pile (°C)	Isolation status
Stacking (0 day)	0-30	–
1 st turning (6 th day)	65-70	–
2 nd turning (10 th day)	65-70	–
3 rd turning (13 th day)	65-70	–
4 th turning (16 th day)	60-65	–
5 th turning (19 th day)	50-55	–
6 th turning (22 nd day)	40-45	+
7 th turning (25 th day)	30-35	+
8 th turning (29 th day)	25-30	+

+ = Successful isolation

– = Pathogen could not be isolated

4.3.2 Contaminated casing soil

4.3.2.1 Pathogen presence in casing mixture (soil and peat)

Samples were drawn from the pathogen-infested casing soil kept outdoors under shade at monthly intervals starting from May 2008 to March 2009, and examined for the presence of viable pathogen propagules through dilution plate technique. It is evident from the Table-5 that the pathogen was present in viable form in all the test samples in varied populations. The population was maximum (6×10^4 cfu g^{-1} soil) in the first month of sampling i.e. in May 2008 which decreased gradually as the season advanced reaching minimum (3×10^1 cfu g^{-1} soil) in February 2009. Thereafter, the population again increased slightly (1.0×10^2 cfu g^{-1} soil) in March and April, 2009.

4.3.2.2 Spent-compost as a source of inoculum

The spent compost collected from healthy and diseased mushroom production trays at the end of spring crop of 2008 was kept outdoors and examined for the presence and population dynamics of *M. perniciosus* at the end of each month by dilution plate technique. It is clear from Table-6 that the pathogen was continuously present in samples from spent compost of diseased trays compared to samples from spent compost of healthy trays where no pathogen was detected. However, the pathogen population was maximum (3×10^7 cfu g^{-1} compost) immediately after placing outdoors in May 2008, the population gradually decreased every month reaching appreciably low (6×10^3 g^{-1} compost) in October 2008 and the lowest (3×10^2 g^{-1}) in March-April 2009.

4.3.2.3 Population of *M. perniciosus* in different casing materials

Composite samples of different casing materials utilized for preparation of casing mixture, collected from growers houses, were examined for the presence and population density of *M. Perniciosus*. The result (Table-7) reveals that only the garden soil and the spent compost carried the wet bubble pathogen in populations varying each year. The garden soil harboured maximum pathogen population of 2

Table 5 : Population of *Mycogone pernicios*a propagules in samples drawn at monthly intervals from casing mixture (soil and peat) placed outdoors under natural conditions during May 2008 to April 2009

Month in which sample drawn	Pathogen population (cfug ⁻¹ casing mixture)
May 2008	6 x 10 ⁴
June 2008	4 x 10 ³
July 2008	8 x 10 ²
August 2008	3 x 10 ²
September 2008	6 x 10 ²
October 2008	2.5 x 10 ²
November 2008	2 x 10 ²
December 2008	2 x 10 ²
January 2009	1 x 10 ²
February 2009	3 x 10 ¹
March 2009	1 x 10 ²
April 2009	1 x 10 ²

Table 6 : Population of *M. perniciosus* propagules in samples drawn at monthly intervals from mushroom spent-compost collected from apparently diseased and healthy production trays of spring crop 2008 and placed outdoors

Sampling month	Pathogen population (cfug ⁻¹ compost)
May 2008	3 x 10 ⁷
June 2008	3 x 10 ⁷
July 2008	6 x 10 ⁵
August 2008	4 x 10 ⁵
September 2008	3.5 x 10 ⁵
October 2008	6 x 10 ³
November 2008	5 x 10 ³
December 2008	3 x 10 ³
January 2009	8 x 10 ²
February 2009	4 x 10 ²
March 2009	3 x 10 ²
April 2009	3 x 10 ²

No pathogen propagule was detected from spent compost of healthy production trays placed outdoors

Table-7 : Population of *Mycogone pernicioso* in different casing materials during 2008 and 2009

Casing material	<i>M. pernicioso</i> population (cfug ⁻¹ casing mixture)	
	Spring 2008	Autumn 2009
Peat soil	–	–
Garden soil	2 x 10 ⁴	5.5 x 10 ²
Virgin soil	–	–
Spent compost	4.5 x 10 ⁴	3.5 x 10 ²
Farmyard manure	–	–
Sand	–	–

– = Not isolated

$\times 10^4$ and 5.5×10^2 cfu g^{-1} soil, compared to 4.5×10^2 and 3.5×10^2 cfu g^{-1} during 2008 and 2009, respectively. The peat soil, virgin soil, farm yard manure and sand did not yield any *M. perniciosus* propagules during both the years.

4.3.3 Air currents

The dispersal of *M. perniciosus* spores from the site of production with the help of varied air currents was assessed by counting the number of pathogen colonies developed on PDA plates exposed to the air currents at different distances. The results (Table-8) revealed an average maximum dispersal (7 colonies/plate) occurring at high air current velocity (7 m/sec) followed by that (3 colonies/plate) at medium air current velocity (5 m/sec), with minimum dispersal (0.87 colonies/plate) occurring at low velocity (2 m/sec). The high air velocity (7 m/sec) could drift *M. perniciosus* spores to a distance of upto 80 cms; however, the spores were dispersed upto a maximum distance of 40-60 cm only with air currents of low to medium velocity.

4.3.4 Sciarid fly infestation

Different populations of sciarid flies were allowed to acquire inoculum from wet bubble infected sporophores for varied periods and subsequently released on healthy mushroom trays to assess their influence on the spread of the disease. The results (Table-9) revealed an average maximum sporocarp infection (39.80%) occurring with the maximum acquisition period of 120 minutes followed by that (31.33%) with 90 minutes acquisition period. Least average sporocarp infection (12.33%) was recorded with acquisition period of 30 minutes. The population of sciarid flies also influenced the disease spread symptoms significantly. On an average, the maximum test population of 100 flies/tray yielded maximum sporocarp infection (35.83%) followed by a population of 75 flies/tray (31.25%); the minimum fly population of 10/tray even resulted in 15.25% sporocarp infection. A significant interaction also existed between fly population and acquisition period in spreading the wet bubble disease. The

Table-8 : Number of *Mycogone pernicioso* colonies developed on PDA plates exposed to air currents of different velocities at different distances

Air current velocity	Distance from inoculum source					Mean
	20 cm	40 cm	60 cm	80 cm	100 cm	
High (7 meters/sec.)	15.67	11.33	7.00	1.00	0.00	7.00^a
Medium (5 meters/sec.)	9.33	3.67	2.00	0.00	0.00	3.00^b
Low (2 meters/sec.)	2.67	1.33	0.33	0.00	0.00	0.87^c
Mean	9.22^A	5.44^B	3.11^C	0.33^D	0.00^E	
		SE±				CD(p=0.05)
Distance		0.53				1.08
Speed		0.41				0.83
Distance x Speed		0.91				1.87

*Figures are the number of *M. pernicioso* colonies

Table-9 : Per cent *Agaricus bisporus* sporocorps infected with *Mycogone pernicioso* after release of different populations of sciarid flies fed on diseases sporocorps

Acquisition time (minutes)	Sciarid fly population					Mean
	10	20	50	75	100	
30 min	5.33*	8.33	11.00	18.00	19.00	12.33
60 min	12.00	15.67	16.67	23.00	29.67	19.40
90 min	16.67	25.00	35.00	37.33	42.67	31.33
120 min	27.00	31.33	42.00	46.67	52.00	39.80
	15.25	20.08	26.16	31.25	35.83	
		S.E±		CD(p=0.05)		
Fly population (FP)		1.09		2.20		
Acquisition time (AT)		0.97		1.97		
Pop. x Time (FP x AT)		2.18		4.40		

*Figures are the per cent infected sporocorps

maximum fly population of 100/tray with the maximum acquisition time of 120 minutes resulted in the maximum sporocarp infection of 52% followed by a fly population of 75/tray with the same acquisition period (46.67%). The least fly population of 10-20/tray with minimum acquisition period of 30 minutes resulted in the least sporocarp infection (5.33-8.33%).

4.3.5 Water splashes

The influence of water spray on the dispersal of *M. pernicioso* spores was observed by collecting water splashes at different distances from the infected mushroom sporophore and counting the spores per ml. The result (Table-10) reveal that the population was maximum (176 cfu/ml of splash water) nearest the source (0 cm) and minimum (2 cfu/ml) at a distance of 80 cm from the infected mushroom sporophore. None of the pathogen spores was observed in water splashes collected beyond 80 cm distance from the infected mushroom sporophore.

4.4 Management of wet bubble disease

4.4.1 Use of fungicides

4.4.1.1 *In vitro* evaluation of non-systemics

Five non-systemic fungicides *viz.*, prochloraz manganese, chlorothalonil, mancozeb, captan and propineb were evaluated at 25, 50, 100, 200 and 500 $\mu\text{g ml}^{-1}$ concentrations by poisoned food technique for their inhibitory effects on pathogen *Mycogone pernicioso* and the host *Agaricus bisporus* mycelium (Plate-9).

a) Effect on mycelial growth of pathogen

Prochloraz manganese proved most effective and provided a growth inhibition of 99.52% followed by captan exhibiting inhibition percentage of 95.71 per cent. Mancozeb was the next best fungicide showing an inhibition of 86.56 per cent, whereas propineb was the least inhibitory (41.20%) fungicide against the test

Table-10 : Effect of water sprays on dispersal of *M. perniciosa* spores from infected sporophores of white button mushroom

Distance from infected mushroom sporophores (cms)	0	10	20	30	40	50	60	70	80	90	100	
No. of aleurospores/chlamydo spores/ml water splash	176.00	149.33	94.67	74.33	42.67	29.00	12.00	8.00	2.00	0.0	0.0	
			S.E±				CD(p=0.05)					
Distance				2.16				4.47				

Table-11 : *In vitro* evaluation of different non-systemic fungitoxicants against *Mycogone pernicioso*, the causal agent of wet bubble disease of white button mushroom (*Agaricus bisporus*)

Fungicide	Conc. ($\mu\text{g ml}^{-1}$)	Growth inhibition over control (%)					Mean
		25	50	100	200	500	
Prochloraz manganese 50 WP		97.46 (80.63)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	99.52 (88.12)^a
Chlorothalonil 75 WP		59.58 (50.18)	68.64 (55.75)	76.75 (60.89)	80.75 (63.67)	95.51 (77.07)	76.24 (61.51)^d
Mancozeb 75 WP		78.07 (62.07)	82.31 (65.12)	85.85 (67.90)	88.68 (70.33)	97.91 (81.68)	86.56 (69.42)^c
Captan 50 WP		86.79 (68.03)	94.10 (75.48)	97.91 (81.43)	99.76 (88.08)	100.00 (90.00)	95.71 (80.60)^b
Propineb 75 WP		19.84 (25.82)	27.15 (31.08)	32.10 (34.02)	51.19 (45.38)	75.71 (60.23)	41.20 (39.30)^e
Mean		72.35 (56.08)^E	73.59 (62.82)^D	77.81 (66.25)^C	83.51 70.92)^B	91.98 (77.40)^A	
		S.E\pm		CD(p=0.05)			
Fungicide		(0.50)		(0.83)			
Concentration		(0.50)		(0.83)			
Fungicide x Concentration		(1.13)		(1.89)			

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical

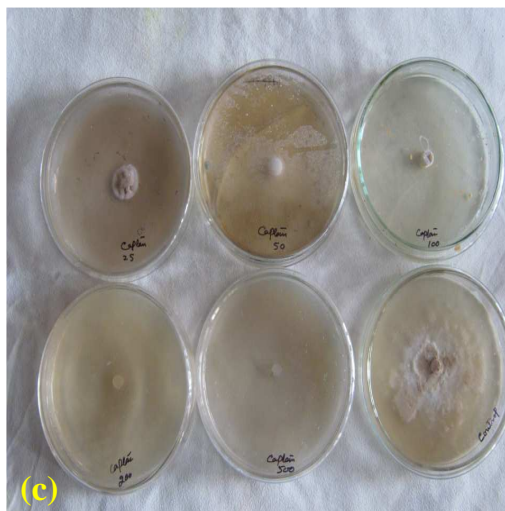
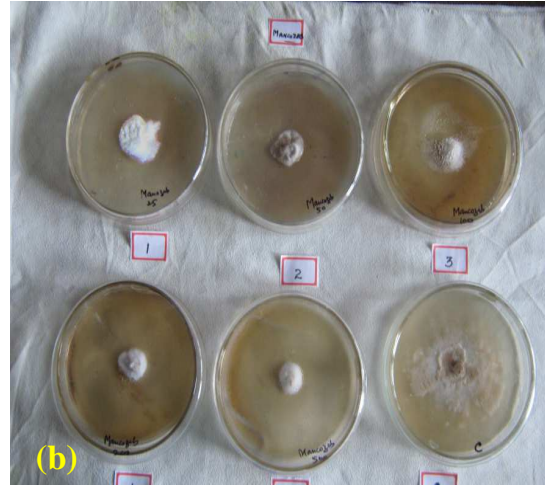
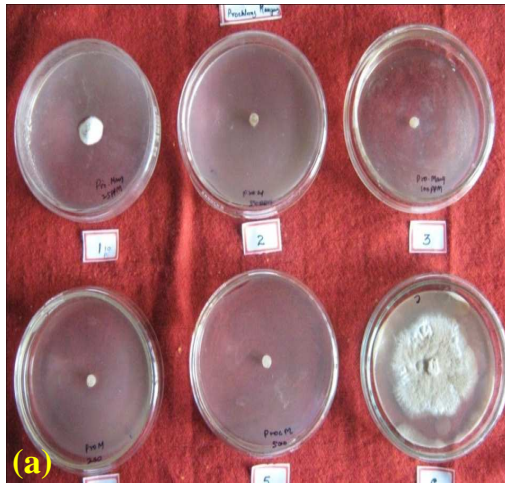


Plate-9 : *In vitro* evaluation of non-systemic fungicides
a, b, c = Against pathogen *M. perniciosa*
d = Against host *A. bisporus*

pathogen (Table-11; Fig. 4). A significant interaction between fungicides and their concentrations also existed. The results further revealed that an increase in fungicide concentration resulted in a corresponding increase in per cent growth inhibition of test pathogen, such that a maximum overall inhibition of 91.98% was obtained at the highest concentration of 500 $\mu\text{g ml}^{-1}$, whereas 25 $\mu\text{g ml}^{-1}$ fungicide concentration exhibited overall growth inhibition of 72.35%.

Prochloraz manganese at a concentration of 50 $\mu\text{g ml}^{-1}$ and more and captan at a concentration of 500 $\mu\text{g ml}^{-1}$ completely restricted the growth of the test pathogen. Captan at 200 $\mu\text{g ml}^{-1}$ concentration was next best fungicide exhibiting a growth inhibition of 99.76 per cent followed by again prochloraz manganese at 25 $\mu\text{g ml}^{-1}$, captan at 100 $\mu\text{g ml}^{-1}$ and mancozeb at 500 $\mu\text{g ml}^{-1}$. Propineb was the least effective fungicide in checking the growth of the test pathogen.

b) Effect on mycelial growth of host

The results (Table-12; Fig. 4) revealed that the test fungicides varied significantly in their inhibitory effects on the host mycelium. In overall comparison, prochloraz manganese recorded minimum (10.83%) growth inhibition of *A. bisporus* mycelium followed by chlorothalonil and captan (13.22-15.41%), whereas propineb exhibited maximum growth inhibition (38.01%) of the test fungus. On an average, the per cent growth inhibition increased with increase in fungicide concentration such that minimum (1.87%) inhibition was obtained at 25 $\mu\text{g ml}^{-1}$ and maximum (54.36%) at 500 $\mu\text{g ml}^{-1}$. A significant interaction between fungicides and their concentrations also existed. Prochloraz manganese, chlorothalonil, mancozeb and captan at upto 50 $\mu\text{g ml}^{-1}$ and mancozeb at 25 $\mu\text{g ml}^{-1}$ did not show any growth inhibition of host mycelium. Prochloraz manganese and captan at 100 $\mu\text{g ml}^{-1}$ and mancozeb at 50 $\mu\text{g ml}^{-1}$ were the next least inhibitory (3.15-3.64%) fungicide concentrations followed by chlorothalonil 100 $\mu\text{g ml}^{-1}$ (7.29%) and propineb 25 $\mu\text{g ml}^{-1}$ (9.37%). Mancozeb and propineb at 500

Table-12 : *In vitro* evaluations of different non-systemic fungicides against *Agaricus bisporus* mycelium

Fungicide	Conc. ($\mu\text{g ml}^{-1}$)	Growth inhibition over control (%)					Mean
		25	50	100	200	500	
Prochloraz manganese 50 WP	0.0 (2.86)	0.0 (2.86)	3.64 (10.94)	12.49 (20.67)	38.02 (38.06)	10.83 (15.07)^a	
Chlorothalonil 75 WP	0.0 (2.86)	0.0 (2.86)	7.29 (15.63)	15.10 (21.53)	43.74 (41.40)	13.22 (16.85)^b	
Mancozeb 75 WP	0.0 (2.86)	3.62 (10.55)	14.06 (21.99)	39.06 (38.68)	68.22 (54.07)	24.99 (25.63)^c	
Captan 50 WP	0.0 (2.86)	0.0 (2.86)	3.15 (10.21)	14.57 (22.35)	59.37 (50.40)	15.41 (17.73)^b	
Propineb 75 WP	9.37 (17.71)	22.39 (28.22)	43.22 (41.10)	52.60 (46.48)	62.49 (52.23)	38.01 (37.14)^d	
Mean	1.87 (5.83)^A	5.20 (9.47)^D	14.27 (19.97)^C	26.76 (29.94)^B	54.36 (47.23)^A		
		S.E\pm		CD(p=0.05)			
Fungicide	(0.61)		(1.23)				
Concentration	(0.61)		(1.23)				
Fungicide x concentration	(1.22)		(2.46)				

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical .

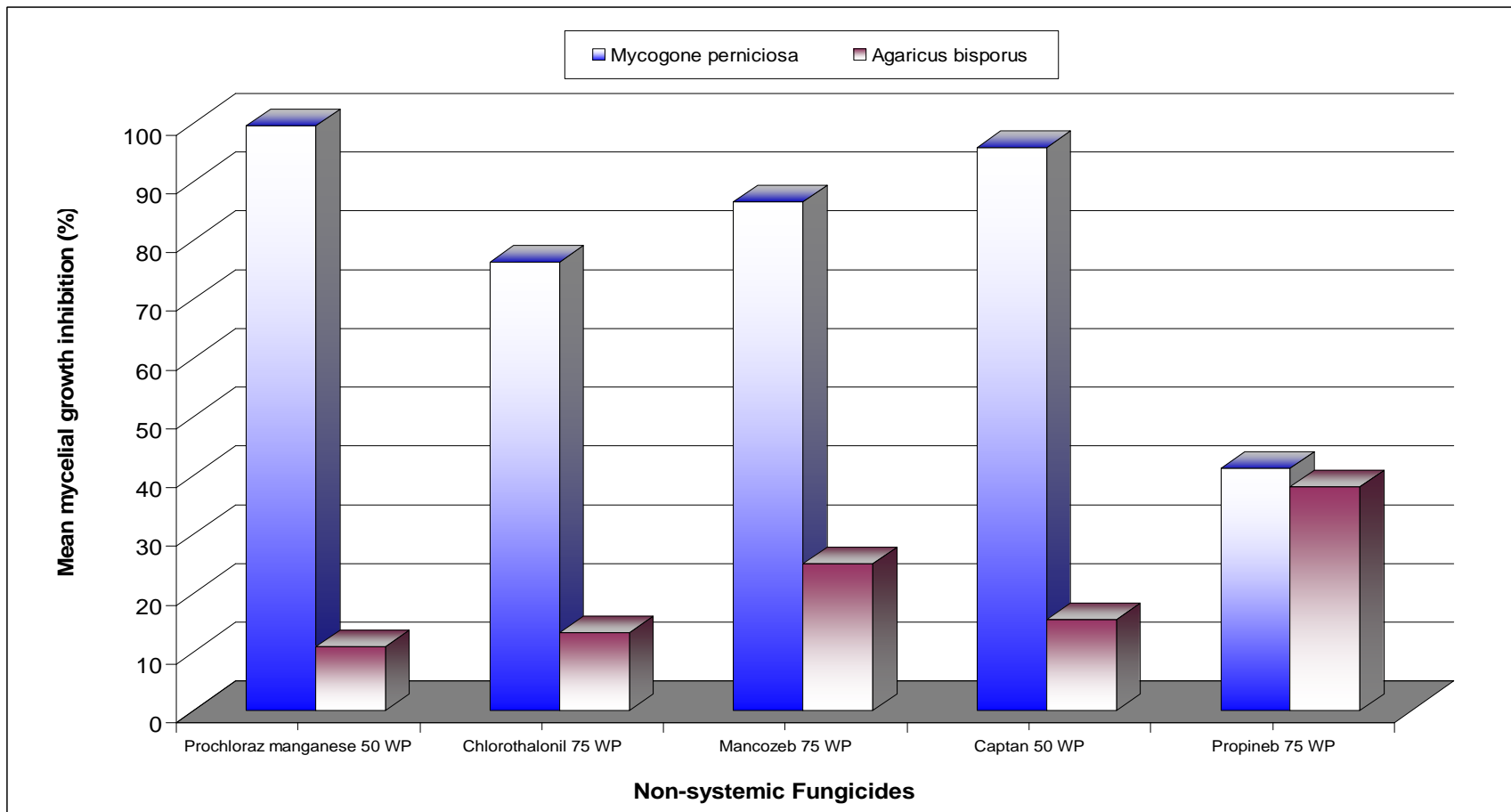


Fig. 4 : *In vitro* evaluation of different non-systemic fungicides against *Mycogone perniciosa* and *Agaricus bisporus* mycelium

$\mu\text{g ml}^{-1}$ exhibited maximum inhibition (62.49-68.22%) of the test fungal mycelium.

4.4.1.2 *In vivo* evaluation of non-systemics

a) Effect on disease development

The data (Table-13) revealed that all the fungicidal treatments reduced the per cent disease intensity as compared to pathogen-infested and untreated check-I. Compared to a wet bubble intensity of 16.67 per cent obtained in pathogen infested and untreated check-I, the disease was reduced to 0.0-0.37 per cent by application of captan 50 WP at 0.1-0.2% or prochloraz manganese 50 WP at 0.2%, exhibiting the disease control of 97.78-100%, which was statistically at par with uninfested and untreated check-II. Captan 50 WP at 0.05%, prochloraz manganese 50 WP at 0.1% and chlorothalonil 75 WP at 0.2% were the next best treatments exhibiting wet bubble intensity of 1.11-1.85 per cent with a disease control of 88.90-93.34 per cent. Prochloraz manganese 50 WP and chlorothalonil 75 WP at 0.05 and 0.1% were the least effective fungicidal treatments exhibiting wet bubble intensity of 4.07-5.92 with a disease control of 64.48-75.58 per cent.

b) Effect on yield and yield components

It is evident from the (Table-14) that the application of captan 50 WP at 0.2% concentration exhibited minimum (90.16) number of fruit-bodies per kg mushroom followed by prochloraz manganese 50 WP and chlorothalonil 75 WP at 0.2% and captan 50 WP at 0.1% yielding 91.50-92.50 fruit-bodies per kg mushroom, lesser than 94.16 and 92.83 fruit-bodies per kg mushroom obtained in infested-untreated and un-infested, untreated checks, respectively.

The average fruit-body weight also varied significantly with different fungicidal treatments. Prochloraz manganese 50 WP and captan 50 WP each at 0.2% concentration exhibited maximum (10.86-10.89 g) average weight of single fruit-body similar to that obtained in uninfested-untreated check (10.93 g). Captan 50 WP (0.10%) and chlorothalonil 75 WP (0.20%) were the next best treatments

Table-13 : Effect of non-systemic fungitoxicants incorporated in casing on per cent intensity of wet bubble disease (*Mycogone perniciosa*) of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatments		Spring 2009	Spring 2010	Mean	Disease control (%)
P. manganese 50 WP	0.05%	5.18 (13.09)	6.66 (14.82)	5.92 (13.96) ^d	64.48
	0.1%	1.48 (6.66)	1.48 (6.66)	1.48 (6.66) ^b	91.12
	0.2%	0.0 (2.86)	0.74 (4.76)	0.37 (3.81) ^a	97.78
Chlorothalonil 75 WP	0.05%	4.44 (12.16)	7.41 (15.75)	5.92 (13.96) ^d	64.48
	0.1%	3.70 (10.96)	4.44 (12.16)	4.07 (11.56) ^c	75.58
	0.2%	2.22 (8.56)	1.48 (6.66)	1.85 (7.61) ^b	88.90
Captan 50 WP	0.05%	0.74 (4.76)	1.48 (6.66)	1.11 (5.71) ^b	93.34
	0.1%	0.00 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	100
	0.2%	0.0 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	100
Check I (infested - untreated)		16.29 (23.73)	17.04 (23.24)	16.67 (24.03) ^a	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	-
S.E±		(1.05)	(1.26)	(0.94)	
CD(p=0.05)		(2.14)	(2.57)	(1.89)	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table- 14 : Effect of non-systemic fungicides incorporated in *M. perniciosa* infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Fungicide		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield (kgs/q compost)
P. Manganese 50 WP	0.05%	95.83 ^f	10.38 ^e	8.89 ^d
	0.1%	94.00 ^{de}	10.60 ^d	10.59 ^c
	0.2%	91.50 ^b	10.86 ^a	14.25 ^a
Chlorothalanil 75 WP	0.05%	96.00 ^f	10.30 ^e	8.51 ^d
	0.1%	94.50 ^e	10.59 ^d	10.90 ^c
	0.2%	92.66 ^{bc}	10.77 ^b	13.48 ^b
Captan 50 WP	0.05%	94.33 ^e	10.62 ^{cd}	13.47 ^b
	0.1%	92.50 ^b	10.69 ^{bc}	14.23 ^a
	0.2%	90.16 ^a	10.89 ^a	14.83 ^a
Control I (infested - untreated)		94.16 ^e	10.68 ^c	6.18 ^e
Control II (uninfested - untreated)		92.83 ^{cd}	10.93 ^a	12.84 ^b
SE±		(0.62)	(0.04)	(0.50)
CD (p=0.05)		(1.25)	(0.08)	(1.01)

Means of three replications; means followed by similar letter(s) are statistically identical.

showing average fruit-body weight of 10.69-10.77 g compared to 10.68 g obtained in infested-untreated check.

The button yield per quintal compost also improved significantly with the application of non-systemic fungitoxicants. Captan 50 WP (0.10-0.20%) and prochloraz manganese 50 WP (0.20%) exhibited maximum button yield of 14.23-14.83 kg / quintal compost as compared to that of 6.18 and 12.84 kg / quintal compost obtained in infested-untreated and uninfested-untreated checks, respectively. Prochloraz manganese 50 WP (0.10%) or captan 50 WP (0.05%) were the next best treatments with the average yield of (12.59-13.48) kg / quintal compost. Prochloraz manganese 50 WP and chlorothalonil 75 WP each at 0.05% concentration were the least effective fungicides.

c) **Effect of quality parameters of sporophores**

The non-systemic fungicidal applications on infested casing also significantly affected the quality parameters of sporophores such as pileus weight, pileus dia, stipe weight and stipe dia (Table-15)

- **Pileus weight :-** The pileus weight of 5.32 g as obtained in infested-untreated check was found to significantly improve to 6.01-6.02 g in the treatments receiving captan 50 WP (0.20%) or prochloraz manganese 50 WP (0.20%). Chlorothalonil 75 WP (0.10%) or captan 50 WP (0.10%) or Prochloraz manganese 50 WP (0.10%) were the next best treatments providing the pileus weight of 5.70-5.79 g.
- **Pileus dia :-** The maximum pileus dia (3.54 cm) among different fungicidal treatments was exhibited by captan 50 WP (0.20%) which was statistically at par with uninfested-untreated check (3.56 cm). Prochloraz manganese 50 WP (0.20%), chlorothalonil 75 WP (0.20%) and captan 50 WP (0.05-0.10%) were the next best treatments providing pileus dia of 3.50-3.51 cm.

Table: 15 Effect of non-systemic fungicides incorporated in *M. perniciosa* infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010 (pooled over years)

Fungicide		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
P. manganese 50 WP	0.05%	5.62 ^c	3.29 ^e	4.57 ^e	1.26 ^a
	0.1%	5.79 ^b	3.37 ^d	4.62 ^d	1.29 ^a
	0.2%	6.02 ^{ab}	3.50 ^b	4.70 ^c	1.31 ^a
Chlorothalanil 75 WP	0.05%	5.26 ^e	3.37 ^d	4.57 ^e	1.23 ^a
	0.1%	5.70 ^{cd}	3.44 ^c	4.64 ^d	1.25 ^a
	0.2%	5.58 ^{bc}	3.51 ^b	4.77 ^b	1.25 ^a
Captan 50 WP	0.05%	5.63 ^c	3.45 ^{bc}	4.61 ^{de}	1.19 ^a
	0.1%	5.71 ^b	3.50 ^b	4.68 ^c	1.21 ^a
	0.2%	6.01 ^a	3.54 ^a	4.80 ^a	1.24 ^a
Control I (infested - untreated)		5.32 ^{bc}	3.29 ^e	4.29 ^f	1.23 ^a
Control II (uninfested - untreated)		6.26 ^a	3.56 ^a	4.26 ^e	1.25 ^a
SE±		(0.16)	(0.02)	(0.02)	(0.03)
CD (p=0.05)		(0.31)	(0.05)	(0.05)	(0.07)

Means of three replications; means followed by similar letter(s) are statistically identical

- **Stipe weight :-** The stipe weight was maximum (4.80 g) in treatment receiving captan 50 WP (0.20%) as compared to that (4.29 g) in infested-untreated check. Captan 50 WP (0.10%) or chlorothalonil 75 WP (0.20%) or prochloraz manganese 50 WP (0.20%) were the next best treatments providing stipe weight of 4.68-4.77g. The average weight of stipe in uninfested-untreated check was 3.56 cm.
- **Stipe dia:-** The maximum stipe dia of 1.31 cm was exhibited by the treatments receiving captan 50 WP (0.20%) and prochloraz manganese 50 WP (0.20%), as compared to that of 1.23 cm observed in infested-untreated check.

4.4.1.3 *In vitro* evaluation of systemics

Five systemic fungicides *viz.*, carbendazim, myclobutanil, bitertanol, triademephon and diniconazole were evaluated at 5, 10, 25, 50 and 100 $\mu\text{g ml}^{-1}$ concentrations by poisoned food technique for their inhibitory effects on pathogen *M. perniciosa* and host *A. bisporus* mycelium (Plate-10).

a) **Effects on pathogen**

On an average, carbendazim proved most effective and provided growth inhibition of 97.35 per cent followed by myclobutanil and bitertanol exhibiting inhibition percentage of 93.05-93.70, respectively. It is also clear from Table-16 and Fig. 5 that with increase in concentration, the per cent inhibition over control also increased significantly, such that a maximum growth inhibition of 99.02 per cent was obtained at 100 $\mu\text{g ml}^{-1}$ concentration and a minimum of 81.39 per cent at 5 $\mu\text{g ml}^{-1}$ concentration. A significant interaction between fungicides and their concentrations also existed. Myclobutanil at 50-100 $\mu\text{g ml}^{-1}$ and carbendazim and bitertanol at 100 $\mu\text{g ml}^{-1}$ concentration exhibited maximum (100%) growth inhibition of the test pathogen. Carbendazim and bitertanol 50 $\mu\text{g ml}^{-1}$ were the next best fungicide concentrations exhibiting the growth inhibition of 99.16-99.53 per cent followed by again carbendazim 25 $\mu\text{g ml}^{-1}$ giving the inhibition

Table-16 : *In vitro* evaluation of different systemic fungicide against *Mycogone pernicioso*, the causal agent of wet bubble disease of white button mushroom (*Agaricus bisporus*)

Fungicide	Growth inhibition over control (%)					Mean
	5	10	25	50	100	
Carbendazim 50 WP	93.70 (75.09)	95.80 (77.53)	98.13 (80.63)	99.16 (84.76)	100.00 (90.00)	97.35 (81.60)a
Myclobutanil 10 WP	84.61 (66.42)	85.31 (67.39)	98.60 (82.04)	100.00 (90.00)	100.00 (90.00)	93.70 (79.17)b
Bitertanol 25 WP	83.94 (60.90)	87.41 (68.87)	94.40 (75.84)	99.53 (86.17)	100.00 (90.00)	93.05 (65.17)e
Triademiphon	68.76 (55.54)	75.05 (59.56)	81.81 (64.39)	87.41 (68.87)	95.80 (77.53)	81.76 (65.17)e
Diniconazole	75.98 (60.22)	82.27 (64.89)	89.74 (70.76)	97.43 (99.50)	99.30 (85.37)	88.94 (72.18)d
Mean	81.39 (63.63)^E	85.16 (67.64)^D	92.53 (74.77)^C	96.70 (81.86)^B	99.02 (86.58)^A	
		S.E±		CD(p=0.05)		
Fungicide	(0.69)		(1.15)			
Concentration	(0.69)		(1.15)			
Fungicide x concentration	(1.55)		(2.59)			

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

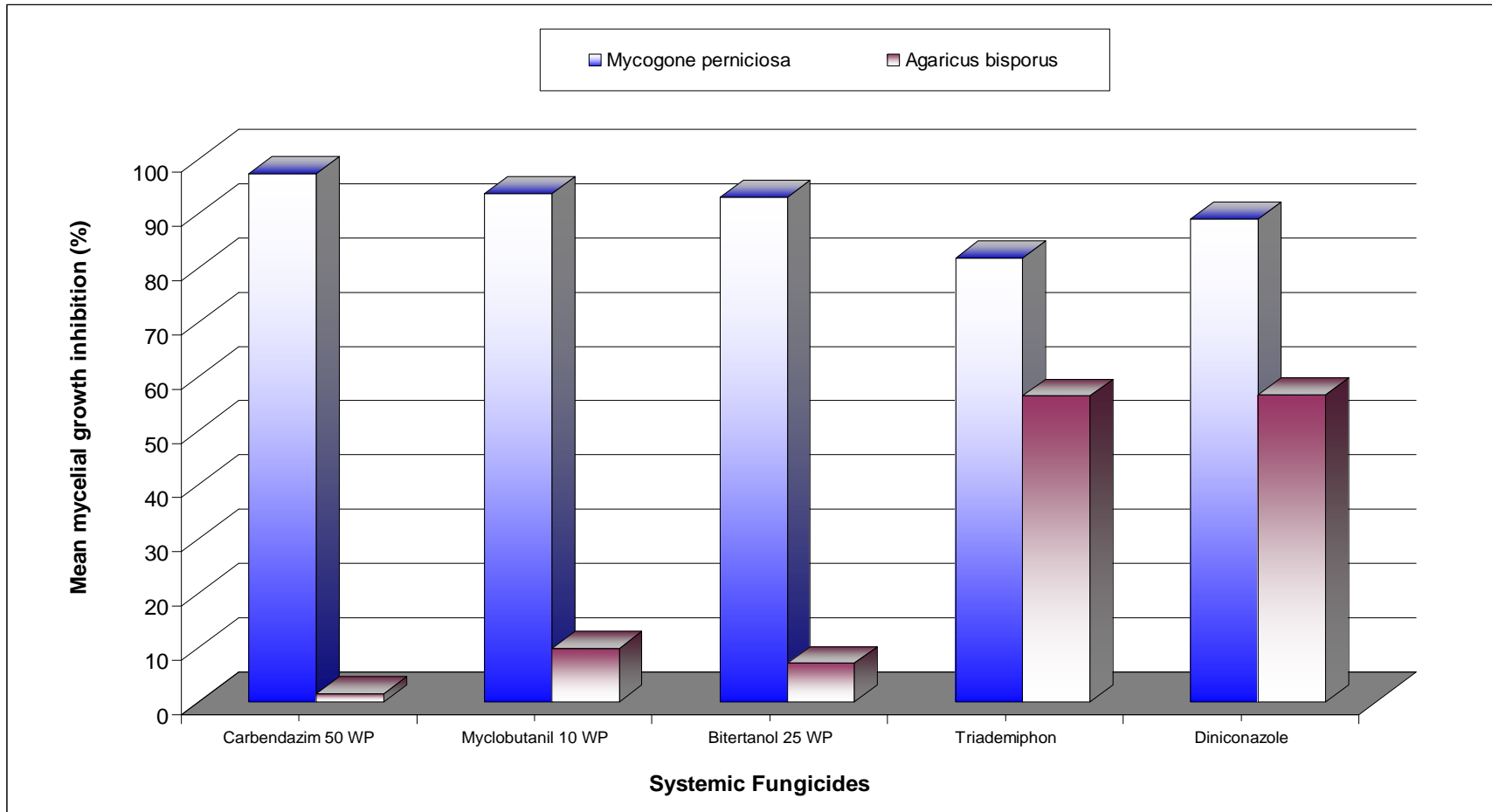


Fig. 5 : *In vitro* evaluation of different systemic fungicides against *Mycogone perniciosa* and *Agaricus bisporus* mycelium

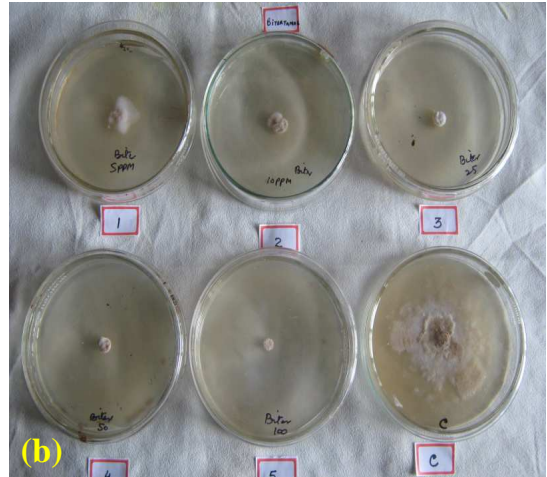


Plate-10 : *In vitro* evaluation of systemic fungicides
a, b, c = Against pathogen *M. perniciosa*
d = Against host *A. bisporus*

percentage of 98.13 per cent. The other fungicides in order of their increasing inhibitory effect were triademephon at $10 \mu\text{g ml}^{-1}$ (75.05%) < diniconazole at $5 \mu\text{g ml}^{-1}$ (75.98%) < triademephon at $25 \mu\text{g ml}^{-1}$ (81.81%) < diniconazole $10 \mu\text{g ml}^{-1}$ and bitertanol $5 \mu\text{g ml}^{-1}$ (82.27-83.94) < myclobutanil at $5 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$ (84.61-85.31%) < triademephon at $50 \mu\text{g ml}^{-1}$ and diniconazole $25 \mu\text{g ml}^{-1}$ (87.41-89.74%) < carbendazim at $5 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$ (93.70-95.80%) and bitertanol $25 \mu\text{g ml}^{-1}$ (94.40%) < carbendazim $\mu\text{g ml}^{-1}$ (98.13%) and bitertanol $50 \mu\text{g ml}^{-1}$ (99.53%).

b) Effects on host mycelium

All the test fungicides varied significantly for their inhibitory effects against host mycelium (Table-17; Fig. 5). In overall comparison, carbendazim recorded minimum (1.45%) growth inhibition of *A. bisporus* followed by myclobutanil (7.08%). Bitertanol was the next least inhibitory (9.78%) to the host fungus, whereas triademephon and diniconazole exhibited maximum inhibition (56.34-56.55%). The mycelial inhibition was found to show a continuous increase with increase in fungicide concentration. A significant interaction between fungicides and their concentrations also existed. Carbendazim at upto $25 \mu\text{g ml}^{-1}$ and bitertanol at $5 \mu\text{g ml}^{-1}$ did not show any inhibition of the host fungus. The same fungicides respectively at 50-100 and 10-25 $\mu\text{g ml}^{-1}$ and bitertanol at 5-10 $\mu\text{g ml}^{-1}$ were next least inhibitory (2.60-4.68%) to the test fungus. The other fungicides in order of their increasing inhibitory effects on *A. bisporus* were bitertanol $25 \mu\text{g ml}^{-1}$ < bitertanol and myclobutanil $50 \mu\text{g ml}^{-1}$ < myclobutanil $100 \mu\text{g ml}^{-1}$ < bitertanol $100 \mu\text{g ml}^{-1}$, triademephon $5 \mu\text{g ml}^{-1}$ and diniconazole $10 \mu\text{g ml}^{-1}$ < triademephon and diniconazole $25 \mu\text{g ml}^{-1}$ < diniconazole $50 \mu\text{g ml}^{-1}$ < triademephon $50 \mu\text{g ml}^{-1}$ < triademephon $100 \mu\text{g ml}^{-1}$ < diniconazole $100 \mu\text{g ml}^{-1}$

Table-17 : *In vitro* evaluation of systemic fungicide against *Agaricus bisporus* mycelium

Fungicide	Conc. ($\mu\text{g ml}^{-1}$)	Growth inhibition over control (%)					Mean
	5	10	25	50	100		
Carbendazim 50 WP	0.0 (2.86)	0.0 (2.86)	0.0 (2.86)	2.60 (9.27)	4.68 (12.38)	1.45 (6.91)^a	
Myclobutanil 10 WP	2.60 (9.27)	3.64 (10.73)	7.29 (15.65)	12.49 (20.69)	22.91 (28.59)	9.78 (16.98)^c	
Bitertanol 25 WP	0.0 (2.86)	2.60 (9.27)	4.68 (12.38)	11.45 (19.74)	16.67 (24.00)	7.08 (13.63)^b	
Triademiphon	22.91 (28.55)	36.97 (37.40)	55.72 (48.30)	78.12 (62.08)	88.02 (69.75)	56.34 (49.21)^d	
Diniconazole	22.91 (28.55)	41.14 (39.89)	54.16 (47.39)	72.39 (58.30)	92.18 (73.81)	56.55 (49.58)^d	
Mean	9.68 (14.41)^E	16.87 (20.03)^D	24.37 (25.31)^C	35.41 (34.01)^B	44.89 (41.70)^A		
		S.E\pm		CD(p=0.05)			
Fungicide	(0.80)		(1.62)				
Concentration	(0.80)		(1.62)				
Fungicide x concentration	(1.61)		(3.24)				

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

4.4.1.4 *In vivo* evaluation of systemics

a) Effect on disease development

The data on the effect of systemic fungicides incorporated in casing on wet bubble disease of white button mushroom (Table-18) revealed that all the fungicidal treatments reduced the per cent disease intensity as compared to pathogen infested-untreated check-I. Compared to a wet bubble intensity of 16.67 per cent obtained in pathogen uninfested-untreated check-I, the disease was reduced to 1.11-1.48 per cent by applications of carbendazim 50 WP or bitertanol 25 WP at 0.1%, exhibiting the disease control of 91.12-93.34 per cent. Carbendazim 50 WP or bitertanol 25 WP @ 0.05% or myclobutanil 10 WP @ 1% were the next best treatments exhibiting wet bubble intensity of 2.59-3.70 per cent with a disease control of 77.80-84.46 per cent. Myclobutanil 10 WP @ 0.025 - 0.05% and carbendazim 50 WP and bitertanol 25 WP @ 0.025% were the least effective fungicidal treatment exhibiting wet bubble intensity of 5.55-7.41 per cent with a disease control of 55.54-62.62 per cent. However, a wet bubble-free crop without any disease intensity was obtained in pathogen uninfested-untreated check-II.

b) Effect on yield and yield components

The fungicidal application on pathogen-infested casing was found to show significant effects on yield and yield components such as number and weight of fruit-bodies. It is evident from Table-19 that minimum (88.83-91.67) number of fruit-bodies per kg mushroom was recorded in treatments which received carbendazim 50 WP or bitertanol 25 WP as compared to infested-treated check (92.16). Myclobutanil 10 WP (0.05%) and carbendazim 50 WP (0.05%), were next best fungicides providing as many fruit-bodies per kg mushroom (93.17-93.50) as obtained in uninfested-untreated check. Carbendazim 50 WP and bitertanol 25 WP each at 0.025% did not significantly influence this yield

Table- 18 Effect of systemic fungitoxicants incorporated in casing on per cent intensity of wet bubble disease (*Mycogone perniciosa*) of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatments		Spring 2009	Spring 2010	Mean	Diseases control (%)
Carbendazim 50 WP	0.025%	6.67 (14.96)	5.92 (14.02)	6.92 (14.49) ^{de}	62.26
	0.05%	5.18 (13.09)	2.22 (8.56)	3.70 (10.82) ^c	77.80
	0.1%	1.48 (6.66)	0.74 (4.76)	1.11 (5.71) ^b	93.34
Myclobutanil 10 WP	0.025%	7.41 (15.75)	7.41 (15.75)	7.41 (15.75) ^a	55.54
	0.05%	5.92 (14.02)	5.18 (13.09)	5.55 (13.56) ^d	66.70
	0.1%	2.96 (9.76)	2.22 (7.86)	2.59 (8.81) ^c	84.46
Bitertanol 25 WP	0.025%	6.67 (14.96)	5.92 (14.02)	6.23 (14.49) ^{de}	62.62
	0.05%	4.44 (11.89)	2.96 (9.06)	3.70 (10.48) ^c	77.80
	0.1%	1.48 (6.66)	1.48 (5.96)	1.48 (6.31) ^b	91.12
Check I (infested - untreated)		16.29 (23.73)	17.04 (24.34)	16.67 (24.03) ^f	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	-
S.E±		(1.17)	(1.51)	(1.08)	
CD(p=0.05)		(2.40)	(3.08)	(2.16)	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table-19 : Effect of systemic fungicides incorporated in *M. perniciosa* infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Fungicide		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
	0.025%	96.33 ^{cd}	10.33 ^c	9.18 ^c
Carbendazim 50 WP	0.05%	93.50 ^{bc}	10.57 ^{bb}	10.25 ^b
	0.1%	88.83 ^a	11.09 ^a	12.29 ^a
	0.025%	95.67 ^c	10.57 ^b	6.43 ^e
Myclobutanil 10 WP	0.05%	93.17 ^b	10.78 ^{ab}	8.00 ^d
	0.1%	90.00 ^a	11.12 ^a	10.49 ^b
	0.025%	97.50 ^d	10.23 ^c	8.25 ^{cd}
Bitertanol 25 WP	0.05%	95.50 ^c	10.46 ^{bc}	10.23 ^b
	0.1%	91.67 ^{ab}	10.90 ^a	12.97 ^a
	Control I (infested -untreated)	96.33 ^{cd}	10.23 ^c	6.18 ^e
Control II (uninfested -untreated)		92.16 ^b	10.83 ^a	12.84 ^a
SE±		(1.46)	(0.20)	(0.47)
CD (p=0.05)		(2.93)	(0.40)	(0.95)

Means of three replications; means followed by similar letter(s) are statistically identical.

component compared to infested-untreated check (96.33-97.50 fruit-bodies per kg mushroom).

The application of systemic fungicides in *M. pernicios*a-infested casing significantly affected the average weight of fruit-bodies. The average weight of fruit-body of 10.23 g as obtained in infested-untreated check was found to improve significantly to 10.46-11.12 g, with the application of myclobutanil 10 WP (0.05-0.1%), carbendazim 50 WP (0.1%) or bitertanol 25 WP (0.1%) similar to that (10.83 g) obtained in infested-untreated control. The application of carbendazim 50 WP (0.025-0.05%), myclobutanil 10 WP (0.025-0.05%) or bitertanol 25 WP (0.05%) were the next best fungicide treatment providing fruit body weight of 10.46-10.69 g.

The button yields (kgs per quintal compost) also improved significantly with the application of fungicides; carbendazim 50 WP and bitertanol 25 WP each at 0.1% concentration exhibited maximum yield 12.29-12.97 kg/ctl compost, as good as that obtained in uninfested-untreated check (12.84 kg / ctl. compost). Bitertanol 25 WP, carbendazim 50 WP each at 0.05% and myclobutanil 10 WP at 0.1% concentration were the next best fungicides showing the average yield of 10.23-10.49 kg q⁻¹ compost compared at 6.18 kg q⁻¹ compost obtained in infested-untreated check. Of all the evaluated fungicidal concentrations, myclobutanil 10 WP application at 0.025% did not significantly improve the button yield compared to infested-untreated check.

c) **Effect on quality parameters of sporophores**

The fungicidal application on infested casing significantly affected some of the sporophore quality parameters such as pileus dia and stipe weight, whereas the pileus weight and stipe dia did not experience any significant change (Table-20).

- **Pileus weight:**-The fungicidal application in pathogen-infested casing did not significantly change the pileus weight of the *A. bisporus* sporophores.

Table-20 : Effect of systemic fungicides incorporated in *M. pernicios*-infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010 (pooled over years)

Fungicide		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
Carbendazim 50 WP	0.025%	5.69	3.36 ^c	4.66 ^c	1.27
	0.05%	5.83	3.29 ^c	4.69 ^b	1.30
	0.1%	6.06	3.63 ^b	4.80 ^a	1.32
Myclobutanil 10 WP	0.025%	5.32	3.38 ^c	4.60 ^c	1.22
	0.05%	5.74	3.47 ^b	4.61 ^{bc}	1.25
	0.1%	5.97	3.53 ^b	4.72 ^{ab}	1.26
Bitertanol 25 WP	0.025%	5.70	3.46 ^{bc}	4.63 ^b	1.20
	0.05%	5.91	3.47 ^b	4.69 ^b	1.23
	0.1%	6.10	3.58 ^b	4.83 ^a	1.26
Control I (infested - untreated))		5.69	3.36 ^c	4.23 ^d	1.24
Control II (uninfested- untreated)		6.20	3.82 ^a	4.59 ^c	1.26
SE±		(0.48)	(0.09)	(0.05)	(0.00)
CD (p=0.05)		(NS)	(0.18)	(0.11)	(NS)

Means of three replications; means followed by similar letter(s) are statistically identical.

- **Pileus dia:**-The pileus dia of 3.36 cm as obtained in infested-untreated check was found to significantly improve to 3.58-3.63 cm in treatments receiving bitertanol 25 WP (0.025-0.1%) or myclobutanil 10 WP (0.05-0.1%) or carbendazim 50 WP (0.1%). Carbendazim 50 WP (0.025 - 0.05%) or myclobutanil 10 WP (0.025%) applications were the next best treatments providing the pileus dia of 3.36-3.39 cm, similar to that obtained in infested-untreated check. However, none of the fungicidal applications provided the pileus dia of 3.82 cm obtained in uninfested-untreated check.
- **Stipe weight:**-The stipe weight significantly improved to 4.72-4.83g in treatments which received carbendazim 50 WP or myclobutanil 10 WP or bitertanol 25 WP each at 0.1% as compared to that of 4.23 g obtained in infested-untreated check and 4.59 obtained in uninfested-untreated check. Carbendazim 50 WP (0.025-0.05%), myclobutanil 10 WP (0.05%) and bitertanol 25 WP (0.025-0.05%) were the next best treatments providing the average stipe weight of 4.61-4.00g.
- **Stipe dia:**-The fungicidal application did not significantly change the stipe diameter of *A. bisporus* sporophores

4.4.2 Use of bacterial antagonists

4.4.2.1 *In vitro* evaluation

Perusal of the data (Table-21) reveals that none of the test isolates of the three bacterial genera exhibited any antagonism to the growth of *A. bisporus* mycelium. While *P. fluorescens* isolate PS-103 and *Azotobacter sp.* Isolate, Azt-106 were more stimulatory, *Azotobacter sp.* isolates Azt-108 and Azt-117 showed stimulatory effect on *in vitro* growth *A. bisporus* (Plate-11). The *P. fluorescens* isolates PS-104, PS-105 and *B. subtilis* isolate BS-101 exhibited neutralistic effects on *A. bisporus* growth. On the contrary, all the test isolates, except *B. subtilis* isolate BS-101 and *Azotobacter sp.* isolate Azt-117, exhibited antagonism

Table-21 : *In vitro* mycelial growth of *Agaricus bisporus* and *Mycogone perniciosa* in presence of bacterial antagonists

Bacterial isolate	Radial mycelial growth (mm) of <i>A. bisporus</i>	Per cent growth stimulation	Radial mycelial growth (mm) of <i>M. perniciosa</i>	Per cent growth inhibition	Interaction	
					<i>M. perniciosa</i>	<i>A. bisporus</i>
<i>P. flourescens</i> -103	53.15	(39.79)	0.50	(98.88)	A	MS
<i>P. flourescens</i> -104	34.50	(07.07)	2.50	(94.44)	A	N
<i>P. flourescens</i> -105	36.17	(11.49)	5.33	(88.14)	A	N
<i>B. subtilis</i> -101	34.0	(05.69)	11.16	(75.18)	N	N
<i>B. subtilis</i> -115	38.13	(26.55)	4.0	(91.11)	A	S
<i>B. subtilis</i> -116	41.13	(22.09)	0.0	(100)	A	S
<i>Azotobacter</i> -106	50.47	(36.50)	0.67	(98.51)	A	MS
<i>Azotobacter</i> -108	38.18	(26.45)	5.5	(87.77)	A	S
<i>Azotobacter</i> -117	40.50	(28.08)	11.5	(74.44)	N	S
Control	32.0	-	45.0	-		

S = Stimulatory;

MS = More stimulatory;

N = Neutralistic;

A = Antagonistic – with clear inhibition zone

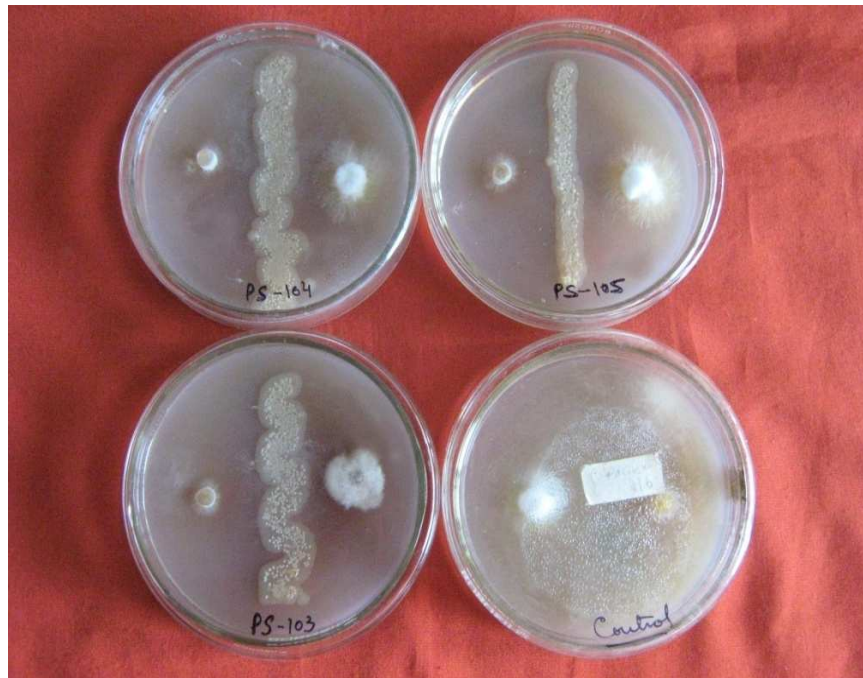


Plate-11 : *In vitro* evaluation of bacterial antagonists

of varying degrees against *M. pernicioso*. The isolate BS-101 and Azt-117 showed only neutralistic effects on the growth of the pathogen *M. pernicioso*.

4.4.2.2 In vivo evaluation

a) Effect on disease development

The data (Table-22) reveals that all the bacterial antagonists reduced the per cent disease intensity as compared to pathogen infested-untreated check-I. Compared to a wet bubble intensity of 16.23 per cent obtained in pathogen infested-untreated check-I, the disease was reduced to 1.85 per cent, with the disease control of 88.60 per cent in treatment receiving *P. flourescens* at 2.0%, followed by disease intensity of 4.81-5.18, with the disease control of 68.08-70.36 per cent in the treatments receiving *P. flourescens* at 1% or *B. subtilis* at 2% concentrations. *P. flourescens* at 5% and *Azotobacter* sp. at 2% concentrations were the next best treatments exhibiting wet bubble intensity of 7.04-7.41 per cent, with the disease control of 54.34-56.62 per cent. Out of all the bacterial antagonist treatments, *Azotobacter* sp. at 0.5% concentration was least effective treatment showing disease intensity of 13.33 per cent, with the disease control of 37.76 per cent.

b) Effect on yield and yield components

The incorporation of bacterial antagonists in pathogen-infested casing was found to show significant effects on yield and yield components such as number and weight of fruit bodies. It is evident from the (Table-23) that minimum number (82.83 - 83.00) of fruit bodies per kg mushroom was recorded in treatments which received *B. subtilis*-116 or *P. flourescens*-103 each at 2% concentration as compared to that (92.83) in uninfested-untreated check. *B. subtilis*-116 and *P. flourescens*-103 each at 1% concentration and *Azotobacter* sp.-106 at 2% concentration were the next best treatments providing 84.33 - 84.50 fruit bodies per kg mushroom, compared to 94.16 obtained in untreated check.

Table-22 : Effect of bacterial antagonists incorporated in *Mycogone pernicioso*- infested casing on per cent intensity of wet bubble disease of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatment		Spring 2009	Spring 2010	Mean	Diseases control (%)
<i>P. flourescens</i> -103	0.5%	7.41 (15.57)	6.67 (14.96)	7.04 (15.36) ^d	56.62
	1.0%	5.18 (13.09)	4.44 (12.16)	4.81 (12.62) ^c	70.36
	2.0%	2.22 (8.56)	1.48 (5.96)	1.85 (7.26) ^b	88.60
<i>B. Subtilis</i> - 116	0.5%	9.63 (18.05)	10.37 (18.76)	10.0 (18.40) ^e	38.38
	1.0%	6.67 (14.82)	8.15 (16.54)	7.41 (15.68) ^d	54.34
	2.0%	5.18 (13.09)	5.18 (13.09)	5.18 (13.09) ^c	68.08
<i>Azotobacter</i> -106	0.5%	15.56 (23.23)	11.11 (19.47)	13.33 (21.35) ^f	37.76
	1.0%	10.37 (18.76)	9.63 (18.05)	10.0 (18.40) ^e	38.38
	2.0%	8.15 (16.54)	6.67 (14.96)	7.41 (15.75) ^d	54.34
Check I (infested - untreated)		16.23 (23.75)	16.23 (23.75)	16.23 (23.75) ^g	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	-
S.E±		(1.16)	(0.90)	(0.73)	
CD (p=0.05)		(2.37)	(1.83)	(1.47)	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table: 23 Effect of bacterial antagonists incorporated in *M. pernicioso*-infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Bact. antagonists		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
<i>P. flourescens</i> -103	0.5%	88.16 ^c	11.38 ^c	8.78d ^e
	1.0 %	84.50 ^d	12.04 ^a	10.99 ^b
	2.0%	83.00 ^e	12.14 ^a	12.42 ^a
<i>B. subtilis</i> -116	0.5%	88.66 ^c	11.33 ^c	7.83 ^f
	1.0%	84.33 ^{de}	11.93 ^{ab}	9.58 ^{cd}
	2.0%	82.83 ^f	12.01 ^a	10.09 ^{bc}
<i>Azotobacter</i> -106	0.5%	90.50 ^b	11.05 ^d	5.14 ^g
	1.0%	88.50 ^c	11.33 ^c	6.70 ^g
	2.0%	84.66 ^d	11.74 ^b	7.94 ^{ef}
Control I (infested-untreated)		94.16 ^a	10.68 ^e	6.18 ^g
Control II (uninfested-untreated)		92.83 ^a	10.93 ^d	12.84 ^a
SE±		0.69	0.10	0.45
CD (p=0.05)		1.38	0.21	0.90

Means of three replications; means followed by similar letter(s) are statistically identical.

The application of bacterial antagonist treatments in pathogen-infested casing significantly affected the average weight of fruit-bodies. The average weight of fruit-body (10.68 g) as obtained in untreated check was found to improve significantly (12.01 - 12.14g) in treatments receiving, *P.fluorescens*-103 at 1 and 2% concentration or *B. subtilis*-116 at 2% concentrations. *Azotobacter*-106 at 2% concentration and *B. subtilis*-116 at 1% concentration were the next best treatments providing the fruit-body weight of 11.74 - 11.93 g. The average weight of fruit-bodies in next treatments ranged from 11.05 - 11.38 g compared to 10.93 g in uninfested-untreated check.

The button yields also improved significantly with the application of bacterial antagonist treatments. *P. fluorescens*-103 at 2% concentration exhibited maximum yield (12.42 kg/quintal) as good as that obtained in uninfested-untreated check (12.84 kg/quintal compost). The same antagonist @ 1.0% concentration or *B. subtilis*-116 at 2.0% concentration were the next best treatments with the average yield of 10.09 - 10.99kg/quintal compost. Applications of *Azotobacter* sp at 0.5-1.0% concentration was the least effective antagonist providing button yield of 5.14-6.70 kg / q compost similar to that obtained in infested-untreated check (6.18 kg/q compost).

c) **Effect on quality parameters of sporophores**

The incorporation of bacterial antagonists in pathogen-infested casing significantly affected the sporophore quality parameters such as pileus weight, pileus diameter, stipe weight and stipe diameter (Table-24).

- **Pileus Weight:**-The maximum pileus weight (6.11 g) among the different bacterial antagonist treatments was exhibited by *P. fluorescens*-103 at 2% concentration followed by pileus weight by that (5.38-5.51 g) in the treatments receiving *P. fluorescens* at 1.0%, *Azotobacter* sp.-106 and *B. subtilis*-116 at 2.0% concentration, compared to the pileus weight (5.32 g) obtained in untreated check.

Table-24: Effect of bacterial antagonists incorporated in *M. pernicios*-infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010 (pooled over years)

Bacterial antagonist		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
<i>P. flourescens</i> - 103	0.5%	5.34 ^b	3.29 ^d	4.66 ^{cd}	1.25 ^c
	1 %	5.38 ^b	3.35 ^c	4.69 ^{bc}	1.28 ^{bc}
	2%	6.11 ^a	3.48 ^{ab}	4.80 ^a	1.31 ^a
<i>B. subtilis</i> -116	0.5%	5.29 ^b	3.24 ^{de}	4.60 ^e	1.27 ^c
	1%	5.35 ^b	3.31 ^d	4.61 ^d	1.31 ^b
	2%	5.51 ^b	3.45 ^b	4.72 ^b	1.32 ^a
<i>Azotobacter</i> - 106	0.5%	5.29 ^b	3.21 ^e	4.63 ^d	1.24 ^d
	1%	5.35 ^b	3.26 ^d	4.69 ^{bc}	1.27 ^c
	2%	5.47 ^b	3.33 ^{cd}	4.83 ^a	1.31 ^a
Control (untreated)		5.32 ^b	3.42 ^{bc}	4.29 ^f	1.23 ^d
Control II (uninfested- untreated)		6.26 ^a	3.56 ^a	4.26 ^f	1.25 ^{cd}
SE±		0.16	0.05	0.02	0.01
CD (p=0.05)		0.32	0.10	0.05	0.03

Means of three replications; means followed by similar letter(s) are statistically identical.

- **Pileus dia:-**The maximum pileus dia (3.45 - 3.48 cm) among the treatments was exhibited by *B.subtilis*-106 and *P. flourescens*-103 both at 2.0% concentration statistically similar to that obtained in uninfested - untreated check (3.56 cm). The next best pileus dia of 3.29 - 3.35 cm was obtained in the treatment receiving *P. flourescens*-103 at 0.5 - 1.0% concentrations.
- **Stipe weight:-**The stipe weight of 4. 29 g was obtained in untreated check was found to significantly improve to 4.80 - 4.83 g in treatments receiving *P. fluorescens*-103 or *Azotobacter* sp-106 each at 2.0% concentration. *P. fluorescens*-103 and *Azotobacter* sp-106 each at 1.0% concentration were in the next best treatments showing stipe wt of 4.69-4.72g. The average weight of stipe in uninfested-untreated check was (4.26 g).
- **Stipe dia:-**The maximum dia of stipe 1.35 cm was exhibited in treatment receiving *B. subtilis*-116 at 2.0% concentration as compared to that (1.23 cm) obtained in untreated check. *P. fluorescens*-103 and *Azotobacter*-106 each at 2.0% concentration were the next best treatments providing the stipe dia of 1.31 cm compared to stipe dia of 1.25 cm obtained in uninfested-untreated check.

4.4.3 Use of botanicals

4.4.3.1 *In vitro* evaluation against pathogen

Eight botanicals viz., *Metricaria* sp., *Curcuma longa*, *Mentha longifolia*, *Artemesia annua*, *Juglans regia*, *Lavendulla officinalis*, *Azadirachta indica* and *Urtica dioca*, were evaluated *in vitro* for their possible inhibitory action against the pathogen *M. perniciosus* and host *Agaricus bisporus* mycelium (Plate-12).

a) Aqueous extract

The aqueous extract of the botanicals evaluated for their inhibitory effects on mycelial growth of *M. perniciosus* at 1, 2 and 3 per cent concentration through poisoned food technique (Table-25; Fig. 6) revealed significant inhibitory effects

Table-25 : Evaluation of aqueous extract of botanicals against *Mycogone pernicioso* causing wet bubble disease of *Agaricus bisporus*

Botanical	Conc. (%)	Per cent inhibition over control			Mean
		1	2	3	
<i>Metricaria</i> spp.		51.15 (45.66)	59.48 (50.47)	74.99 (60.00)	61.49 (52.04)^b
<i>Cureuma longa</i>		54.16 (47.38)	64.58 (53.48)	76.84 (61.24)	65.19 (54.03)^a
<i>Mentha longifolia</i>		13.88 (21.81)	59.48 (50.46)	70.13 (56.87)	47.83 (43.04)^d
<i>Artemesia annua</i>		47.91 (43.80)	59.02 (50.19)	69.90 (56.73)	58.94 (50.24)^c
<i>Juglans regia</i>		27.08 (1.34)	54.16 (47.38)	63.88 (53.05)	48.37 (43.92)^d
<i>Lavendulla officinalis</i>		46.29 (42.87)	63.88 (53.05)	69.67 (56.59)	59.94 (50.83)^c
<i>Azadirachta indica</i>		28.23 (32.08)	36.11 (36.93)	72.68 (58.48)	45.67 (42.49)^d
<i>Urtica dioica</i>		5.55 (13.55)	11.11 (19.44)	28.23 (2.08)	14.96 (21.69)^e
Mean		34.28 (34.81)^a	50.97 (45.17)^b	65.79 (54.38)^c	
		S.E±		CD(p=0.05)	
Botanical		0.53		0.89	
Concentration		0.33		0.55	
Botanical x concentration		0.93		1.56	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

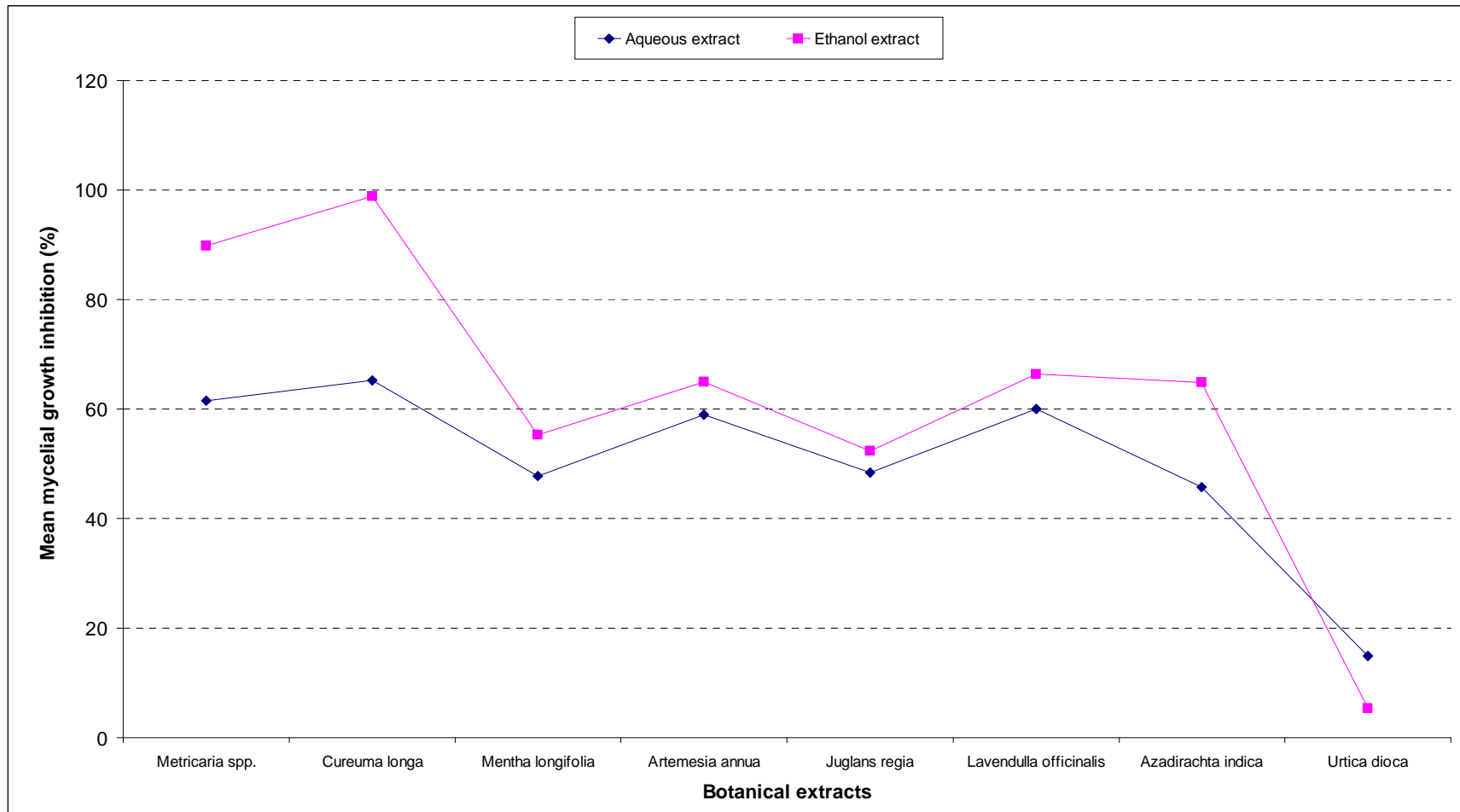
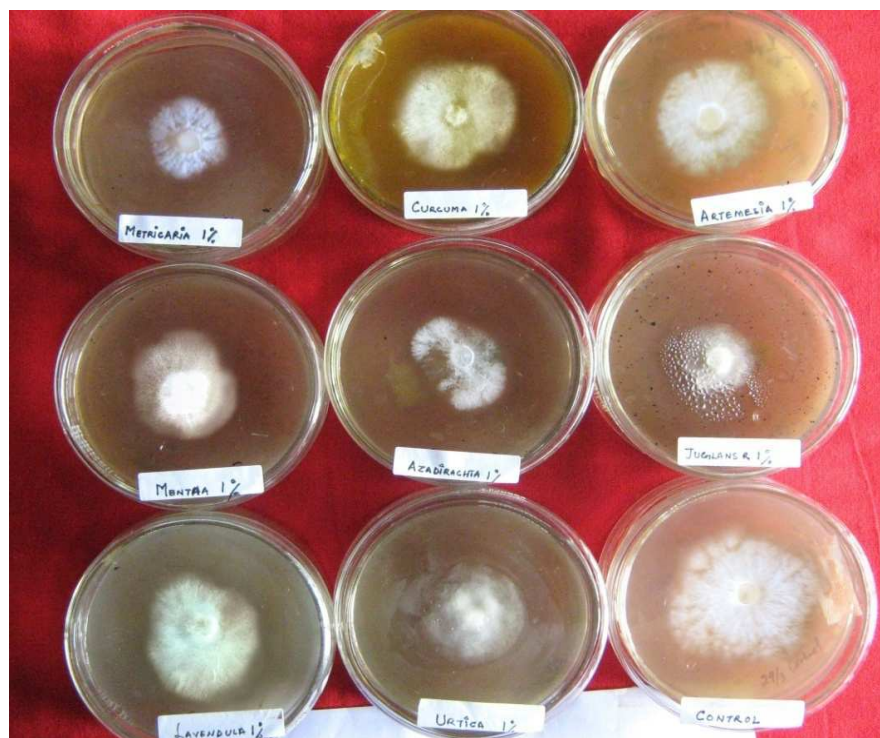


Fig. 6 : *In vitro* evaluation of aqueous and ethanol extract of botanicals against *Mycogone perniciosa*



Against pathogen *Mycogone perniciosa*



Against host *A. bisporus*

Plate-12 : *In vitro* evaluation of Botanicals extracts

of the test botanicals. On an average, the aqueous extracts of *C. longa*, *Metricaria* sp., *L. officinalis* exhibited maximum inhibition (59.94-65.19%) of *M. pernicioso* mycelium. Aqueous extract of *A. annua* was next maximum inhibitory (58.94%), whereas *A. indica* and *M. longifolia* showed the average inhibition of 45.67-47.83%. The test fungus *U. dioca* was least inhibitory (14.96%) against *M. pernicioso* out of all the tested botanicals. The mycelial growth inhibition significantly increased with increase in the extract concentration such that a minimum average inhibition (34.28%) was recorded at the lowest test concentration of 1% and the highest average inhibition (65.79%) was recorded at the highest test concentration of 3 per cent. A significant interaction between botanicals and their aqueous extract concentrations also existed. *C. longa* and *Metricaria* sp. at 3% concentration provided maximum growth inhibition (74.99-76.84%) of *M. pernicioso*. *A. indica* at 3% concentration was the next best botanical providing a growth inhibition of 72.68% followed by *M. longifolia*, *A. annua* and *L. officinalis* at the same concentration with growth inhibition of 69.67-70.13%. *U. dioca* at 1% concentration provided the least growth inhibition of 5.555 only.

b) Ethanol extract

The ethanol extract of the botanicals evaluated for their possible inhibitory effects against *M. pernicioso* at three different concentrations of 1, 2 and 3 per cent through poisoned food technique revealed that all the botanicals at each level of concentration have shown significantly varied inhibitory effects (Table-26; Fig. 6). In overall comparison, *C. longa* and *Metricaria* sp. exhibited maximum mycelial growth inhibition of 98.83 and 89.80 per cent followed by *L. officinalis* and *A. indica* with the inhibition percentage of 66.32 and 64.81 per cent, respectively. *Juglans regia* showed the inhibition percentage of 55.24, whereas *U. dioca* was least inhibitory (5.39%) against the test pathogen. On an average, the lower concentrations exhibited minimum inhibition (49.01%) and the higher concentrations the maximum inhibition (73.57%). A significant interaction

Table-26 : *In vitro* evaluation of aqueous extract of botanicals against host *Agaricus bisporus* mycelium

Botanical	Conc. (%)	Per cent inhibition over control			Mean
		1	2	3	
<i>Metricaria</i> spp.		8.33 (16.47)	19.26 (26.00)	44.78 (42.00)	24.12 (28.16)^c
<i>Cureuma longa</i>		04.16 (11.38)	06.24 (14.40)	09.16 (17.56)	06.52 (14.44)^a
<i>Mentha longifolia</i>		20.30 (26.74)	29.16 (32.68)	41.14 (39.89)	30.20 (33.10)^d
<i>Artemesia annua</i>		04.68 (12.04)	06.76 (14.98)	10.93 (19.18)	07.45 (15.40)^a
<i>Juglans regia</i>		21.35 (27.49)	33.33 (35.25)	43.74 (41.40)	32.80 (34.71)^d
<i>Lavendulla officinalis</i>		03.95 (11.09)	14.58 (22.35)	17.18 (24.76)	11.90 (19.40)^b
<i>Azadirachta indica</i>		04.29 (11.86)	07.80 (16.13)	14.06 (22.00)	8.71 (16.66)^a
<i>Urtica dioica</i>		31.76 (34.28)	42.18 (40.49)	48.95 (44.39)	40.96 (39.72)^e
Mean		12.35 (18.92)^a	19.91 (25.28)^b	28.74 (31.40)^c	
		S.E±		CD(p=0.05)	
Botanical		(1.12)		(2.26)	
Concentration		(0.68)		(1.38)	
Botanical x concentration		(1.94)		(3.91)	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

between the botanicals and their ethanol extracts concentrations also existed. *C. longa* exhibited maximum inhibition of 100% at 3% concentration followed by the same botanical at 2% concentration providing 98.60 per cent growth inhibition. *C. longa* even at 1% concentration providing a growth inhibition of 97.91% proved better than the next best botanical *Metricaria* sp. evaluated at 3% concentration and providing only 93.74% growth inhibition of *M. perniciososa*. *Urtica dioica* at 1% concentration was the least inhibitory botanical against the pathogen providing only 1.84% growth inhibition.

4.4.3.2 *In vitro* evaluation against host mycelium

a) Aqueous extract

The aqueous extract of botanicals evaluated for their inhibitory effect on mycelial growth of *A. bisporus* at 1, 2 and 3 per cent concentration through poisoned food technique (Table-27; Fig. 7) revealed significant inhibitory effects of the test botanicals. On an average, the aqueous extract of *C. longa*, *A. annua* and *A. indica* exhibited minimum inhibition (6.52-8.71%) of *A. bisporus* mycelium. Aqueous extract of *L. officinalis* was the next least inhibitory (11.09%), whereas *U. dioica* extract showed the maximum average inhibition (40.96%) of *A. bisporus* mycelium. The inhibition significantly increased with increase in the extract concentration such that a minimum average inhibition (12.35%) was recorded at the lowest test concentration of 1 per cent and the highest average inhibition (28.74%) was recorded at the highest test concentration of 3 per cent. A significant interaction between botanicals and their aqueous extract concentrations also existed. *A. annua* at concentration of upto 2 per cent and *L. officinalis* & *A. indica* at 1 per cent concentration resulted in the least mycelial inhibition (3.95-6.76%) of *A. bisporus* followed by *Metricaria* at 1%, *A. indica* at 2% and *C. longa* & *A. annua* at 3% each showing mycelial growth inhibition of 7.80-10.93 per cent. *Urtica dioica* exhibited maximum inhibition of *Agaricus bisporus* mycelium at all the test concentrations.

Table-27 : *In vitro* evaluation of ethanol extract of botanicals against *Mycogone pernicioso* causing wet bubble disease of *Agaricus bisporus*

Botanical	Conc. (%)	Per cent inhibition over control			Mean
		1	2	3	
<i>Metricaria</i> spp.		85.41 (67.54)	90.27 (71.83)	93.74 (75.53)	89.80 (71.63)^b
<i>Cureuma longa</i>		97.91 (81.77)	98.60 (83.36)	100.00 (90.00)	98.83 (85.04)^a
<i>Mentha longifolia</i>		44.44 (41.80)	53.24 (46.85)	68.05 (55.59)	55.24 (48.08)^e
<i>Artemesia annua</i>		49.53 (44.73)	63.42 (52.78)	78.94 (62.68)	64.96 (53.39)^d
<i>Juglans regia</i>		35.18 (36.37)	51.84 (46.05)	70.09 (56.84)	52.37 (46.42)^f
<i>Lavendulla officinalis</i>		51.38 (45.79)	71.17 (57.52)	76.42 (60.94)	66.32 (54.75)^c
<i>Azadirachta indica</i>		26.39 (30.89)	76.38 (60.94)	91.66 (73.25)	64.81 (55.02)^c
<i>Urtica dioica</i>		1.84 (7.70)	4.62 (12.39)	9.72 (18.13)	5.39 (12.74)^g
Mean		49.01 (44.57)^C	62.72 (53.37)^B	73.57 (61.62)^A	
		S.E±	CD(p=0.05)		
Botanical		(0.53)	(0.89)		
Concentration		(0.33)	(0.55)		
Botanical x concentration		(0.93)	(1.56)		

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

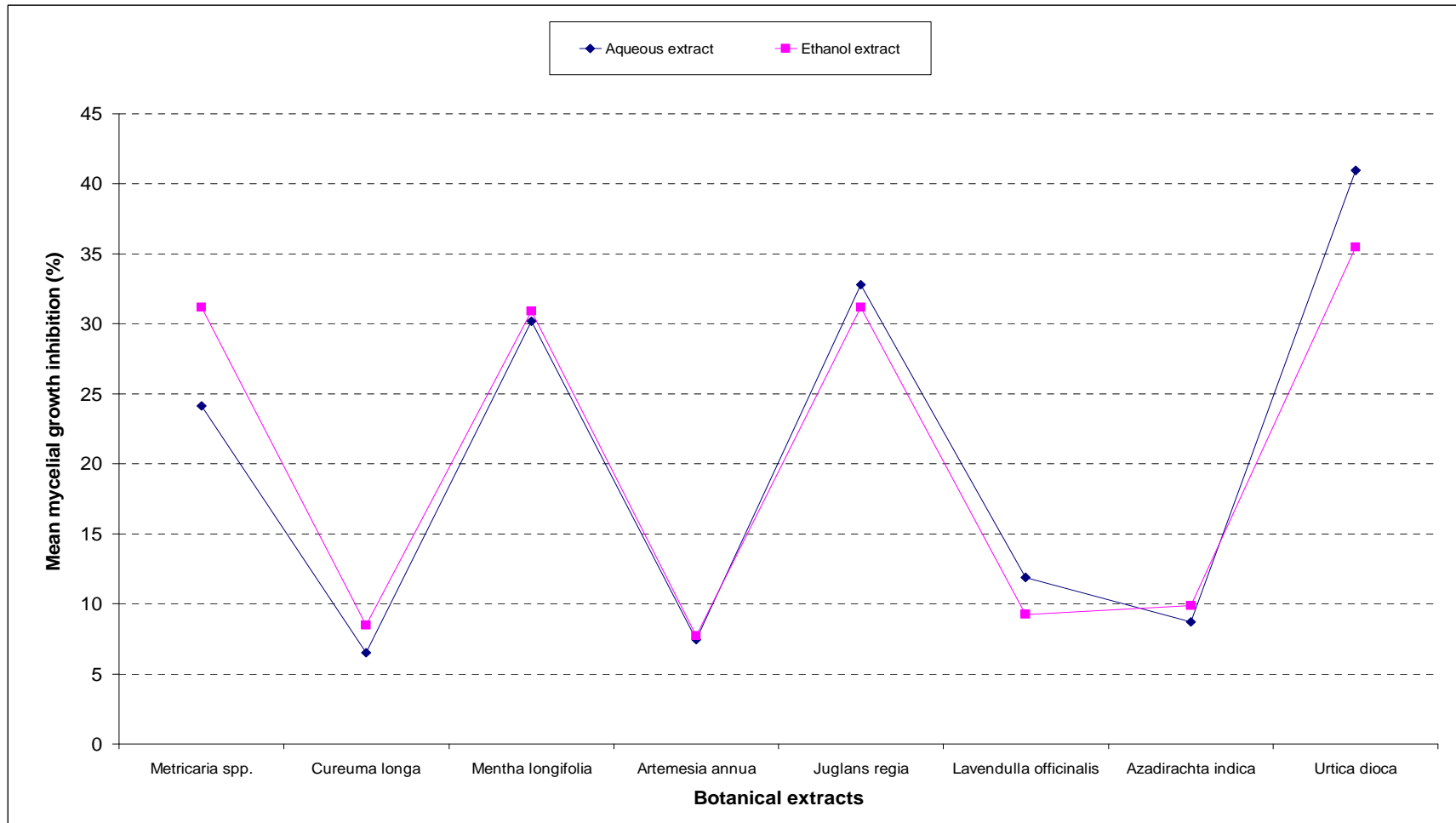


Fig. 7 : *In vitro* evaluation of aqueous and ethanol extract of botanicals against *Agaricus bisporus* mycelium

b) Ethanol extract

The ethanol extract of the botanicals evaluated for their possible inhibitory effect against *A. bisporus* at three different concentrations of 1, 2 and 3 per cent through poisoned food technique revealed that all the botanicals at each level of concentration have shown significantly varied inhibitory effect (Table-28; Fig. 7). In an overall comparison, *C. longa*, *A. annua* and *L. officinalis* exhibited minimum mycelial growth inhibition (7.70-9.22%) of *A. bisporus* followed by *A. indica* (9.89%). *Urtica dioica* exhibited minimum inhibition of (35.48%). On an average, the lower concentrations again exhibited minimum inhibition of 12.42% and the higher concentrations maximum inhibition of 28.18%. A significant interaction between the botanicals and their ethanol extract concentrations also existed. *A. annua*, *L. officinalis* and *A. indica* each at 1% concentration exhibited minimum growth inhibition of 2.70-3.33 per cent followed by *C. longa* at upto 2 per cent concentration and *A. annua* at 2 per cent concentration exhibiting growth inhibition of 5.20-7.18 per cent. *Metricaria* sp., *M. longifolia* and *U. dioica* each at 3 per cent concentration exhibited maximum growth inhibition of 43.74-44.26 per cent.

4.4.3.3 In vivo evaluation

a) Effect on disease development

The data (Table-29) revealed that all the botanical treatments reduced the per cent disease intensity as compared to untreated check-I. Compared to a wet bubble intensity of 20.0 per cent obtained in untreated check-I, the disease was reduced to 1.85-3.70 per cent by the incorporation of *C. longa* or *A. annua* at 3% concentration in casing material exhibiting the disease control of 81.50-90.75 per cent. *C. longa* at 2.0% and *L. officinalis* at 3.0% concentrations were the next best treatments showing disease intensity of 5.55-6.29 per cent, with the disease control of 68.55-72.25 per cent. *C. longa* and *A. annua* at 1.0% concentration and *L. officinalis* at 2.0% concentration were the least effective botanical

Table-28 : *In vitro* evaluation of ethanol extract of various botanicals against host *Agaricus bisporus* mycelium

Botanical	Conc. (%)	Per cent inhibition over control			Mean
		1	2	3	
<i>Metricaria</i> spp.		21.14 (27.69)	28.12 (32.01)	44.26 (41.70)	31.17 (33.80)^c
<i>Cureuma longa</i>		05.20 (12.97)	07.18 (15.54)	13.01 (21.09)	8.46 (16.53)^a
<i>Mentha longifolia</i>		16.14 (23.68)	32.80 (34.92)	43.74 (41.40)	30.89 (33.33)^c
<i>Artemesia annua</i>		03.33 (10.29)	06.76 (14.98)	13.01 (21.09)	07.70 (15.45)^a
<i>Juglans regia</i>		20.72 (27.39)	35.31 (36.41)	37.49 (37.75)	31.17 (33.85)^c
<i>Lavendulla officinalis</i>		02.70 (09.09)	10.41 (18.74)	14.57 (22.36)	09.22 (16.73)^{ab}
<i>Azadirachta indica</i>		03.12 (09.95)	10.93 (19.27)	15.62 (23.26)	09.89 (17.49)^b
<i>Urtica dioica</i>		27.08 (31.34)	35.63 (36.75)	43.74 (41.40)	35.48 (36.50)^d
Mean		12.42 (19.05)^C	22.13 (27.19)^B	28.18 (31.25)^A	
		S.E±		CD(p=0.05)	
Botanical		(0.96)		(1.93)	
Concentration		(0.58)		(1.18)	
Botanical x concentration		(1.66)		(3.35)	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table-29: Effect of botanicals incorporated in casing on per cent intensity of wet bubble disease (*Mycogone pernicioso*) of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatment		Spring 2009	Spring 2010	Mean	Diseases control (%)
<i>C. longa</i>	0.5%	11.11 (19.40)	9.63 (18.05)	10.37 (18.72) ^f	48.15
	1.0 %	7.41 (15.75)	5.18 (13.09)	6.29 (14.42) ^{de}	68.55
	2.0%	2.22 (8.56)	1.48 (6.66)	1.85 (7.61) ^b	90.75
<i>A. annua</i>	0.5%	10.37 (18.76)	10.37 (18.69)	10.37 (18.69) ^f	48.15
	1.0%	6.67 (14.96)	7.41 (15.75)	7.04 (15.36) ^e	54.80
	2.0%	4.44 (12.16)	2.96 (9.06)	3.70 (10.61) ^c	81.50
<i>L. officinalis</i>	0.5%	14.81 (22.62)	14.07 (22.01)	14.44 (22.31) ^g	27.80
	1.0%	9.63 (18.05)	8.89 (17.34)	9.26 (17.69) ^f	53.70
	2.0%	5.92 (14.02)	5.18 (12.82)	5.55 (13.42) ^d	72.25
Check-I		22.96 (23.73)	17.04 (23.34)	20.00 (24.03) ^g	-
Check-II		0.0 (2.86)	0.0 (2.86)	0.00 (2.86) ^a	-
S.E±		0.77	1.23	0.96	
CD(p=0.05)		1.57	2.50	11.91	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

treatments exhibiting wet bubble intensity of 9.27-10.37 per cent with a disease control of 48.15-53.70 per cent. However, a wet bubble free crop without any disease intensity was obtained from pathogen uninfested-untreated check-II.

b) Effect on yield and yield components

It is evident from the (Table-30) that the application of *L. officinalis*, *A. annua* or *Curcuma longa* each at 2.0% concentration exhibited minimum number (86.00-86.66) of fruit-bodies per kg mushroom followed by *L.officinalis* and *C. longa* each at 2.0% concentration yielding 88.00 and 89.00 number of fruit-bodies per kg mushroom, lesser than 94.16 and 92.83 fruit-body per kg mushroom obtained in un-treated and uninfested-untreated checks, respectively.

The average fruit-body weight also varied significantly with the application of different botanical treatments. *A.annua* and *L. officinalis* each at 3.0% concentration exhibited maximum (11.16 -11.20g) average weight of single fruit-body followed by *C.longa* and *L. officinalis* (10.87-10.90), each at 2.0% concentration.

The button yield also improved significantly with the application of botanicals. *C. longa* and *A. annua* each at 2.0% concentration exhibited maximum button yield of 10.84 - 11.06 kg/quintal compost as compared to that of 6.18 and 12.84 kg/quintal compost obtained in untreated and un-infested-untreated checks, respectively. *C. longa* and *A. annua* each at 1.0% and *L. officinalis* at 2.0% concentration were the next best treatments with the average yield of 9.09 - 9.75 kg/quintal compost. *L. officinalis* at 0.5% was the least effective botanical yielding only 7.33 kg mushroom/quintal compost.

c) Effect on quality parameters of sporophores

The application of botanicals in pathogen-infested casing material also significantly affected the quality parameters of sporophores such as pileus weight, pileus diameter, stipe weight and stipe diameter (Table-31).

Table-30 : Effect of botanical biocides incorporated in *M. perniciosa* infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Botanical		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
<i>C. longa</i>	0.5%	91.33 ^d	10.63 ^{de}	8.38 ^e
	1.0 %	89.00 ^c	10.70 ^d	9.09 ^d
	2.0%	86.66 ^{ab}	10.90 ^c	10.84 ^b
<i>A. annua</i>	0.5%	91.83 ^d	10.47 ^f	8.67 ^e
	1.0%	88.50 ^c	10.88 ^c	9.75 ^{cd}
	2.0%	86.16 ^a	11.16 ^a	11.06 ^b
<i>L. officinalis</i>	0.5%	92.00 ^{de}	10.52 ^{ef}	7.33 ^f
	1.0%	88.00 ^{bc}	10.87 ^c	8.42 ^e
	2.0%	86.00 ^a	11.20 ^a	9.21 ^{de}
Control I (Infested-untreated)		94.16 ^f	10.68 ^d	6.18 ^g
Control II (uninfested -untreated)		92.83 ^{ef}	10.93 ^c	12.84 ^a
SE±		0.68	0.06	0.45
CD (p=0.05)		1.37	0.13	0.90

Means of three replications; means followed by similar letter(s) are statistically identical.

Table-31 : Effect of botanical biocides incorporated in *M. pernicioso*-infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010

Botanical		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
<i>C. longa</i>	0.5%	5.81 ^b	3.34 ^f	4.71 ^d	1.25 ^d
	1.0 %	5.95 ^{ab}	3.44 ^e	4.81 ^{bc}	1.28 ^c
	2.0%	6.17 ^a	3.53 ^{bc}	4.95 ^a	1.31 ^b
<i>A. annua</i>	0.5%	5.31 ^d	3.42 ^e	4.64 ^e	1.27 ^c
	1.0%	5.75 ^b	3.50 ^{cd}	4.76 ^{cd}	1.31 ^b
	2.0%	6.14 ^a	3.62 ^a	4.90 ^a	1.35 ^a
<i>L. officinalis</i>	0.5%	5.60 ^{c^d}	3.43 ^e	4.83 ^b	1.24 ^d
	1.0%	5.70 ^{b^c}	3.47 ^{de}	4.59 ^b	1.27 ^c
	2.0%	6.07 ^a	3.57 ^{ab}	4.73 ^d	1.31 ^b
Control I (infested-untreated)		5.32 ^d	3.42 ^e	4.29 ^f	1.23 ^d
Control II (uninfested-untreated)		6.26 ^a	3.56 ^b	4.26 ^f	1.25 ^d
SE±		0.16	0.02	0.02	0.01
CD (p=0.05)		0.32	0.05	0.05	0.02

Means of three replications; means followed by similar letter(s) are statistically identical.

- **Pileus Weight:-**The pileus weight 5.32 g as obtained in untreated check was found to significantly improve to 6.14-6.17 g in the treatments receiving *A. annua* or *C. longa* at 2.0% concentration. *C. longa* at 0.5% and 1.0%, and *A. annua* or *L. officinalis* both at 1.0% concentrations were the next best botanicals providing the pileus weight of 5.70 - 5.95 g.
- **Pileus dia:-**The maximum pileus dia 3.62cm among different botanical treatments was exhibited by *A. annua* at 2.0% concentration followed by treatment receiving *A. annua* at 1.0% or *C. longa* or *L. officinalis* each at 2.0% concentration with the pileus dia of 3.50 -3.57 cm as compared to 3.42 cm pileus dia obtained in untreated check.
- **Stipe weight:-**The stipe weight of 4.29g as obtained in infested, untreated check was found to significantly improve to 4.83 - 4.95g in the treatment receiving *L. officinalis*, *A. annua* or *C. longa* each at 2.0% concentration. The same botanical treatments at 1.0% concentration exhibited the stipe weight of 4.73 - 4.81 g as compared to average weight of 4.26g obtained in uninfested-untreated check.
- **Stipe dia:-**The maximum stipe dia of 1.35cm was exhibited by the treatment receiving *A.annua* at 2.0% concentration followed by stipe dia of 1.31cm obtained in treatments receiving *A.annua* at 1.0% or *C. longa* and *L. officinalis* each at 2.0% concentration, as compared to 1.23 cm untreated check.

4.4.4 Integrated Management

A botanical (*C. longa*, 1%) and a bacterial antagonist (*P. flourescens*-103, 1%) were admixed with pathogen infested casing soil mixture individually and also in combinations with each other, together with either a non-systemic (captan) or systemic (carbendazim) fungicidal spray for the management of wet bubble disease of button mushroom (*A. bisporus*) (Plate-13). Treatments without admixtures of any management agents was maintained as check.

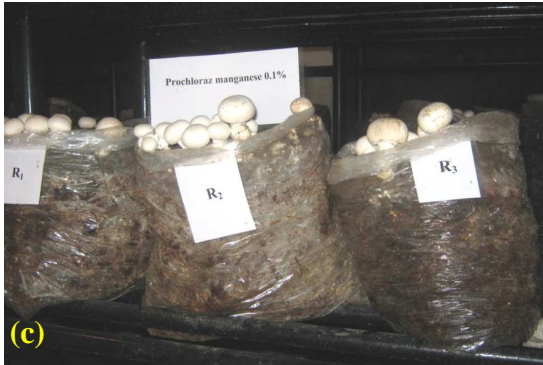


Plate-13 : *In vivo* bio-assay of fungitoxicants botanicals and bacterial antagonists

- a, b) = Layout plan of experiment
- c, d) = Production bags applied with *P. managanese* and carbendazim
- e, f, g) = Integrated management components
- h) = Un-infested casing soil

4.4.4.1 Integration with non-systemic fungicide

a) Effect on disease development

It is evident from the data (Table-32) that all the three management agents applied individually or in different combinations reduced the disease intensity as compared to the control. Minimum disease intensity (8.29%) was recorded in treatment which received all the three management agents, whereas maximum disease intensity (73.42%) was observed in control which did not receive any management application. However, treatments which received captan 50 WP (0.1%) + *C. longa* (1.0%) or captan 50 WP (0.1%) + *P. flourescens* (1.0%) recording 9.45% and 9.57% disease intensity, respectively were identical with treatments which received all the three agents in combination. Captan 50 WP 0.1% individually or *C. longa* (1.0%) + *P. flourescens* (1.0%) or *P. flourescens* (1.0%) and *C. longa* (1.0%) recording 11.38, 13.96, 16.56 and 17.16 percent disease intensity were statistically inferior to other treatments.

b) Effect of yield and yield components

The results (Table-33) revealed that the incorporation of *C. longa* (1%) and *P. flourescens* -103 (1%) in the casing mixture resulted in the minimum number of fruit bodies per kg mushroom. Incorporation of *P. flourescens*-103 alone and in combination with captan were the next best treatments yielding 92.17 - 92.33 fruit bodies/kg mushroom similar to infested check (92.67 fruit bodies/kg mushroom)

The highest weight of fruit body (12.21g) was exhibited by the treatment where *C. longa* and *P. flourescens*-103 were incorporated with captan spray in the casing soil followed by the treatment receiving *P. flourescens*-103 alone and in the combination with *C. longa* and (sterilized casing soil) check-I (10.84-10.97), compared to that of obtained in infested Check-II and uninfested Check-III (10.31 and 10.68). The treatment receiving *C. longa* 1% with captan (0.1%) spray proved the next best treatment with fruit body weight of 10.78 g.

Table-32 : Effect of integration of some management components with captan sprays on per cent disease intensity of wet bubble disease during spring 2009-2010

Treatment	Disease intensity (%)		Mean
	2009	2010	
Captan 50 WP 0.1%	11.06 (19.38)*	11.71 (19.95)	11.38 (19.66)bc**
<i>Curcuma longa</i> 1.0%	17.82 (24.95)	16.51 (23.93)	17.16 (24.44)e
<i>Pseudomonas fluorescens</i> -103 1.0%	17.83 (24.94)	15.30 (22.98)	16.56 (23.96)de
Captan 0.1% + <i>C. longa</i> 1.0%	9.31 (17.72)	9.84 (18.25)	9.57 (17.98)ab
Captan 0.1% + <i>P. fluorescens</i> -103 1.0%	9.63 (18.06)	9.27 (17.72)	9.45 (17.89)a
<i>C. longa</i> 1.0% + <i>P. fluorescens</i> -103 1.0%	15.43 (23.11)	12.49 (20.68)	13.96 (21.89)cd
Captan 0.1% + <i>C. longa</i> 1.0% + <i>P. fluorescens</i> -103 1.0%	9.11 (17.54)	7.47 (15.83)	8.29 (16.68)a
Control (untreated infested casing soil)	73.30 (59.90)	73.54 (59.34)	73.42 (59.17)f
Mean	20.43 (25.58)	19.51 (24.83)	
	SE±	SEM±	CD(p=0.05)
Treatments	1.40	0.99	2.37
Year	0.85	0.60	NS
Treatments x year	1.98	1.40	NS

*Figures in parenthesis are angular transformed values; **means followed by similar letter(s) are statistically identical ; *Curcuma longa* and *Pseudomonas fluorescens*-103 were incorporated in casing mixture as dry powder at the time of casing; mean per cent disease intensity recorded in pathogen un-infested sterilized casing soil and un-infested- unsterilized casing soil were recorded as 0.00 and 2.0 respectively.

The data (Table-33) further revealed that none of the treatments provided as much button yield as obtained in check-I (sterilized casing soil) and check-III (uninfested casing soil). However, the incorporation of *C. longa* and *P. flourescens* -103 in casing soil along with a spray of captan exhibited highest (14.08kg / q compost) button yield. Spray of captan 50WP (0.1%) along with incorporation of *C. longa* or *P. flourescens* -103 in casing were the next best treatments with regard to button yield (11.45-12.21 kgs/ q compost).

c) Effect on sporophore quality parameters

The integrated management components significantly affected the sporophore quality parameters such as pileus weight, pileus dia, stipe weight and stipe dia.

- **Pileus weight:** The data (Table-34) revealed maximum pileus weight (5.90-5.95 g) exhibited by the treatments receiving both *C. longa* and *P. flourescens* -103 1% with or without captan 0.1% spray. Applications of captan (0.1%) or *C.longa* (1.0%) singly or in combination were next as good as check I (sterilized casing soil) with respect to pileus weight (5.74-5.79g).
- **Pileus dia:** The dia of pileus did not vary much in different treatments and their combinations, and thus proved to be non-significant.
- **Stipe weight:** The maximum stipe weight 4.85-4.92 g was exhibited by the treatments receiving *P. flourescens* -103 or *C.longa* alone and in the combinations. The next best stipe weight (4.70-4.73 g) was provided by the treatment receiving captan (0.1%) in combination with either *C.longa* or *P. flourescens* -103.
- **Stipe dia:** The maximum stipe dia (1.43 cm) was exhibited in treatment receiving *P. flourescens*-103 (1%) compared to that (1.27 cm) obtained in infested check II. The next best stipe dia of (1.36

Table-33: Effect of integration of some management components with captan 50 WP spray on the yield parameters of white button mushroom (*Agaricus bisporus*) during 2009 and 2010 (data pooled over years)

Treatment	No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
Captan 50 WP 0.1%	95.00 ^d	10.49 ^d	11.97 ^d
<i>C. longa</i> 1.0%	94.17 ^d	10.62 ^d	9.95 ^e
<i>P. flourescens-103</i> 1.0%	92.17 ^b	10.97 ^b	10.21 ^e
Captan 50 WP 0.1% x <i>C. longa</i> 1.0%	94.00 ^{cd}	10.70 ^c	11.45 ^d
Captan 50 WP 0.1% x PS- 103 1.0%	92.33 ^b	10.90 ^b	12.21 ^d
<i>C. longa</i> 1% x PS-103 1.0%	90.67 ^a	10.95 ^b	10.30 ^e
Captan50 WP 0.1% x <i>C. longa</i> 1.0% x PS-103 1.0%	94.17 ^d	12.21 ^a	14.08 ^c
Control I (Sterilized casing soil)	93.83 ^c	10.61 ^d	17.97 ^a
Control II (infested. casing. soil)	92.67 ^{bc}	10.84 ^{bc}	4.66 ^f
Control III (uninfested casing. soil)	93.50 ^c	10.68 ^{cd}	15.30 ^b
SE±	(0.74)	(0.10)	(0.38)
CD (p=0.05)	(1.49)	0.20)	(0.81)

Means of three replications; means followed by similar letter(s) are statistically identical.

Table-34 : Effect of integration of some management components with captan 50 WP spray on quality parameters of white button mushrooms (*Agaricus bisporus*) during 2009 and 2010 (data pooled over years)

Treatment	Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
Captan 50 WP 0.1%	5.74 ^c	3.30	4.61 ^c	1.24 ^h
<i>C. longa</i> 1.0%	5.70 ^{cd}	3.34	4.83 ^{ab}	1.29 ^f
<i>P. fluorescens</i> -103 1.0%	5.51 ^e	3.35	4.92 ^a	1.43 ^a
Captan 0.1% x <i>C. longa</i> 1.0%	5.74 ^c	3.40	4.73 ^b	1.27 ^g
Captan 0.1% x PS- 103 1.0%	5.58 ^{de}	3.34	4.70 ^b	1.34 ^d
<i>C. longa</i> 1% x PS-103 1.0%	5.90 ^{ab}	3.36	4.85 ^a	1.36 ^c
Captan 0.1% x <i>C. longa</i> 1.0% x PS-103 1.0%	5.95 ^a	3.34	4.78 ^b	1.32 ^e
Control I (sterilized casing soil)	5.79 ^{bc}	3.39	4.68 ^b	1.27 ^g
Control II (infested. casing. soil)	5.57 ^e	3.30	4.43 ^d	1.38 ^b
Control III (uninfested casing. soil)	5.57 ^e	3.34	4.47 ^d	1.37 ^b
SE±	(0.06)	(0.06)	(0.04)	(0.00)
CD(p=0.05)	(0.12)	(NS)	(0.09)	(0.01)

Means of three replications; means followed by similar letter(s) are statistically identical.

cm) was obtained in treatments receiving both *C. longa* x *P. flourescens*-103.

4.4.4.2 Integration with systemic fungicide

a) Effect on disease development

It is evident from the data (Table-35) that all the three management components applied individually or in different combinations significantly reduced the disease intensity as compared to the control. The minimum disease intensity (8.77%) was recorded in treatment which received all the three management agents, whereas maximum disease intensity (73.42%) was observed in control which did not receive any management application. However, treatments which received carbendazim 50% WP 0.05% + *C. longa* (1%) or carbendazim 50% WP (0.05%) + *P. flourescens* or carbendazim 50% WP (0.05%) alone recorded 9.55%, 10.39% and 10.96% disease intensity, respectively, were identical with treatment which received all the three agents in combinations. *C. longa* 1% + *P. flourescens* together or *P. flourescens* (1%) and *C. longa* (1%) individually recording 13.96%, 16.56% and 17.16% disease intensity were statistically inferior to other treatments.

b) Effect of yield and yield components

The results (Table-36) revealed that the incorporation of *P. flourescens*-103 (1%) in the casing mixture resulted in the minimum (84.33) number of fruit bodies per kg mushroom, following by the combination treatment receiving *C. longa* (1%) with *P. flourescens*-103 (1%) providing 86.83 number of fruit bodies per kg mushroom. Incorporation of *C. longa* (1%) alone or in combination with carbendazim and *P. flourescens* in the casing mixture recording 89.33- 90.44 fruit bodies per kg mushroom was the next best treatment compared to that of 96.33 fruit bodies per kg mushroom obtained in infested check II.

The highest weight of fruit body (12.01 g) was exhibited by the treatment receiving *P. flourescens*-103 (1%) following by treatments receiving *C. longa*

Table-35 : Effect of integration management components with carbendazim sprays on the per cent disease intensity of wet bubble disease during spring 2009-2010

Treatment	Per cent disease control		Mean
	2009	2010	
Carbendazim 50 WP 0.05%	11.78 (20.06)*	10.14 (18.53)	10.96 (19.29)a**
<i>Curcuma longa</i> 1.0%	17.82 (24.95)	16.51 (23.93)	17.16 (24.44)c
<i>Pseudomonas flourescens</i> -103 1.0%	17.83 (24.94)	15.30 (22.98)	16.56 (23.96)bc
Carbendazim 50 WP 0.05% + <i>C. longa</i> 1.0%	9.57 (17.99)	9.53 (17.93)	9.55 (17.96)a
Carbendazim 50 WP 0.05% + <i>P. flourescens</i> -103 1.0%	9.08 (17.53)	11.70 (19.98)	10.39 (18.75)a
<i>C. longa</i> 1.0% + <i>P. flourescens</i> -103 1.0%	15.43 (23.11)	12.49 (20.68)	13.96 (21.89)b
Carbendazim 50 WP 0.05% + <i>C. longa</i> 1.0% + <i>P. flourescens</i> -103 1.0%	8.78 (17.23)	8.76 (17.21)	8.77 (17.22)a
Control (pathogen infested casing soil)	73.30 (59.00)	73.54 (59.34)	73.42 (59.17)d
Mean	20.45 (25.60)	19.74 (25.07)	
	SE±	SEM±	CD(p=0.05)
Treatments	1.35	0.96	2.28
Year	0.68	0.47	NS
Treatments x year	1.92	1.40	NS
Uninfested-sterilized casing soil	0.00 (2.86)	0.00 (2.86)	0.00 (2.86)
Uninfested-unsterilized casing soil	2.50 (9.09)	150 (7.03)	2.00 (8.06)

* Figures in parenthesis are angular transformed values ;**means followed by similar letter(s) are statistically identical ; *Curcuma longa* and *Pseudomonas flourescens*-103 were incorporated in casing mixture as dry powder at the time of casing; mean per cent disease intensity recorded in pathogen un-infested sterilized casing soil and un-infested- unsterilized casing soil were recorded as 0.00 and 2.0 respectively.

(1%) alone or in combination with either *P. flourescens* (1%), or *P. flourescens*-103 and carbendazim 11.00-11.23 g compare to that 10.36 g obtained in infested check II.

The data (Table-36) further revealed that none of the treatments provided as much button yield as obtained in check-I (sterilized casing soil) or check III (un-infested casing soil). However, the incorporation of *C. longa* (1%) and *P. flourescens*-103 in casing soil with the spray of carbendazim (0.05%) exhibited highest (14.27 kg/q compost) yield, followed by the incorporation of *P. flourescens*-103 only and with the spray of carbendazim (12.83 kgs/ q compost) compared to the yield (4.53 kg/q compost) obtained in infested check II.

c) Effect on sporophore quality parameters

- **Pileus weight:** The data (Table-37) revealed that maximum pileus weight (5.96g) among the different treatment combinations was exhibited by the treatment receiving only *P. flourescens*-103 (1%) followed by that (5.87 g) obtained in the treatment receiving *P. flourescens*-103 along with carbendazim 50 WP (0.05%) spray, compared to pileus weight (5.46 g) obtained in infested casing soil check I.
- **Pileus dia :** The diameter of pileus did not vary significantly among different treatments and their combinations in the present studies,.
- **Stipe weight :** The maximum stipe weight of (4.86-4.91 g) was recorded in the treatment receiving *P. flourescens*-103 (1%) alone and in combination with *C. longa* (1%) followed by stipe weight of (4.79-4.80 g) obtained in the treatments receiving *C. longa* (1%) alone and in combination with carbendazim 50WP (0.05%) and *P. flourescens*-103 (1%) compared to a stipe weight (4.46 g) obtained in infested check II.

Table-36: Effect of integration of some management components with carbendazim 50 WP spray on the yield parameters of white button mushrooms (*Agaricus bisporus*) during 2009 and 2010 (data pooled over years)

Treatments	No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
Carbendazim 50 WP 0.05%	97.67 ^{fg}	10.34 ^e	11.04 ^e
<i>C. longa</i> 1.0%	89.33 ^c	11.00 ^{bc}	10.25 ^e
<i>P. flourescens</i> -103 1%	84.33 ^a	12.01 ^a	10.29 ^e
Carbendazim 0.05% x <i>C. longa</i> 1.0%	93.50 ^e	10.67 ^d	10.80 ^e
Carbendazim 0.05% x PS-103 1.0%	91.00 ^d	11.08 ^b	12.83 ^d
<i>C. longa</i> 1% x PS-103 1.0%	86.83 ^b	11.23 ^b	10.70 ^e
Carbendazim 0.05% x <i>C. longa</i> 1.0% x PS-103 1.0%	90.44 ^{cd}	11.11 ^b	14.27 ^c
Control I (Sterilized casing soil)	94.67 ^c	10.75 ^{cd}	17.97 ^b
Control II (infested casing soil)	96.33 ^f	10.36 ^e	4.53 ^f
Control III (uninfested casing soil)	99.00 ^g	10.51 ^{de}	15.47 ^a
SE±	(0.65)	(0.14)	(0.47)
CD(p=0.05)	(1.37)	(0.29)	(0.99)

Means of three replications; means followed by similar letter(s) are statistically identical.

Table-37: Effect of integration of some management components with carbendazim 50 WP spray on the quality parameters of white button mushrooms (*Agaricus bisporus*) during 2009 and 2010 (data pooled over years)

Treatments	Wt. of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
Carbendazim 50 WP 0.05%	5.76 ^c	3.31 ^d	4.66 ^d	1.26 ^f
<i>C. longa</i> 1.0%	5.38 ^h	3.38	4.80 ^{bc}	1.29 ^e
<i>P. fluorescens</i> -103 1.0%	5.96 ^a	3.37	4.91 ^a	1.43 ^a
Carbendazim 0.05% x <i>C. longa</i> 1.0%	5.57 ^f	3.28	4.75 ^c	1.28 ^e
Carbendazim 0.05% x PS- 103 1.0%	5.87 ^b	3.36	4.67 ^d	1.35 ^c
<i>C. longa</i> 1.0% x PS-103 1.0%	5.67 ^{de}	3.38	4.86 ^{ab}	1.37 ^{bc}
Carbendazim 0.05% x <i>C. longa</i> 1.0% x PS-103 1.0%	5.70 ^{cd}	3.35	4.79 ^c	1.33 ^d
Control I (Sterilized casing soil)	5.83 ^{be}	3.39	4.76 ^c	1.27
Control II (infested. casing. soil)	5.46 ^g	3.42	4.46 ^e	1.35
Control III (uninfested casing. soil)	5.63 ^{ef}	3.48	4.70 ^{cd}	1.39 ^b
SE+	(0.03)	(0.04)	(0.05)	(0.01)
CD(p=0.05)	(0.06)	(NS)	(0.10)	(0.02)

Means of three replications; means followed by similar letter(s) are statistically identical.

- **Stipe dia :** The maximum stipe dia (1.45 cm) was exhibited in treatments receiving *P. flourescens*-103 (1%) alone followed by stipe dia of 1.35-1.37 obtained in treatments receiving *P. flourescens*-103 in combination with either carbendazim 50WP(0.05%) or *C. longa* as compared to (1.27 cm) obtained in infested casing check II.

4.5 Screening of *A. bisporus* strains

All the seven strains of button mushroom screened for their tolerance/susceptibility to wet bubble disease (*M. perniciosa*) during spring season of 2009 & 2010 were found equally and highly susceptible to the pathogen (Table-38; Plate-14). The per cent disease intensity varied non-significantly and ranged from 58.81 to 67.14% among different strains, strain U-3 recording comparatively lesser disease intensity and the strain RRL-80 the maximum intensity. The yield of mushroom per quintal compost was highly influenced by the disease in all the test strains. The yield was maximum (4.92 kg q⁻¹ compost) in strain S-176 compared to all other test strains (2.86-3.32 kg q⁻¹ compost). The per cent decrease in yield over control ranged from 68.31 to 79.23% with minimum in strains S-176 and maximum in strain S-737. The test strains did not vary significantly in other quality characters (Table-39) viz., number of fruit bodies kg⁻¹ mushroom, weight of single fruit body and weight and diameter of pileus and of stipe.

Table-38: Evaluation of different strains of *Agaricus bisporus* with regard to wet bubble (*Mycogone perniciososa*) intensity and button yield during 2009 and 2010 (pooled over years)

<i>A. bisporus</i> strain	Wet bubble intensity (%)	Button yield (kg q ⁻¹ compost)	Yield loss (%)
S-176	61.95 (51.94)	4.92 ^a	68.31
S-11	61.65 (51.76)	3.07 ^b	76.18
U-3	58.81 (50.14)	3.15 ^b	78.97
RRL-89	65.11 (53.87)	3.32 ^b	74.55
RRL-80	67.14 (56.01)	2.92 ^b	76.60
S-737	64.50 (53.45)	2.86 ^b	79.23
NCS-102	63.09 (52.78)	3.28 ^b	77.67
<hr/>			
CD (0.05)	NS	(0.71)	

*Figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table-39: Quality parameters of different strains of button mushroom (*Agaricus bisporus*) recorded in 2009 and 2010 (pooled over years)

<i>A. bisporus</i> Strain	Fruit bodies (No. /kg mushroom)	Weight of single fruit body (g)	Weight of pileus (g)	Diameter of pileus (cm)	Weight of stipe (g)	Diameter of stipe (cm)
S-176	77.0	20.33	10.66	6.06	6.30	1.30
S-11	70.33	19.00	11.0	6.50	6.06	1.16
U-3	70.33	18.66	10.0	6.10	6.16	1.20
RRL-89	69.0	19.0	10.0	6.13	6.13	1.16
RRL-80	74.0	21.0	9.66	6.16	5.80	1.23
S-737	65.67	18.67	10.66	5.76	5.36	1.36
NCS-102	70.00	20.67	11.33	6.0	6.06	1.26
SE ±	2.95	1.30	0.95	0.04	0.23	0.13
CD (p=0.05)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

Means of three replications

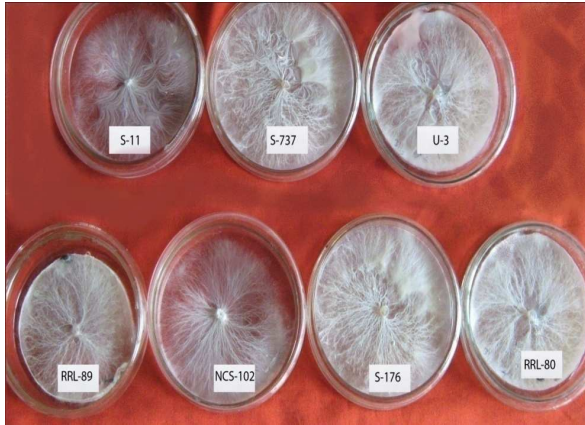


Plate-14 : Screening of *A. bisporus* strains against *M. perniciosa*

Chapter – 5

DISCUSSION

Wet bubble disease is a severe impediment in the profitable cultivation of white button mushroom (*Agaricus bisporus* L.). It is incited by an important and cosmopolitan fungal pathogen, *Mycogone perniciosa* Magn. which is responsible for frequent crop failures in Kashmir valley. The disease has been reported to assume serious proportions in mushroom growing countries of the world like the United Kingdom (Nielson, 1932; Russel, 1984), Netherlands (Zaayen, 1983), USA (Nielson, 1932; Gray and Morgan, 1981), France (Nielson, 1932), China (Zhi *et al.*, 1995), Taiwan (Tu and Liao, 1982), South Africa (Eicker, 1987), Brazil (Figueiredo and Mucci, 1985), Hungary (Szalay *et al.*, 1982), Australia (Nair and Baker, 1978) and Poland (Maszkiewicz, 1992). It was reported for the first time in the Jammu and Kashmir state in 1978 from Pinglin area of Pulwama (Kaul *et al.*, 1978). It is believed that the frequent incidence of mould disease including that of wet bubble disease in Kashmir valley and the resultant crop failure of maximum frequency, owing to lack of knowledge to effectively and efficiently manage the disease led to abandoning of its cultivation in early eighties. The mycoparasite parasitizes the fruit bodies/sporophores and/or growing mycelium and causes variable yield losses depending upon the stage of infection, amount of inoculum and the prevailing ecological factors inside the production rooms. This disease was later reported from the states of Himachal Pradesh, Haryana, and Maharashtra (Sharma, 1994; Sharma and Kumar, 2002; Bhatt and Singh, 2000) as well.

Establishing the status of the disease and the resultant yield losses due to it are the pre-requisites for deciding at the adoption of disease management practices and form the basic components of decision making in integrated disease management. The objective was achieved by undertaking surveys of three predominant mushroom growing districts *viz.*, Srinagar, Budgam, and Pulwama,

of Kashmir valley during the present investigation. The disease was found prevalent in varying proportions in all the districts and locations across seasons indicating thereby the presence of the mycoparasite inoculum in abundance in the valley throughout the year, emanating most probably from the infections of wild mushrooms growing in the forests and also from the intensive cultivation of *A. bisporus* in production rooms which provide to the pathogenic fungus the readily available host round the year. The disposal and utilization of spent compost could also account for the primary spread of infection in different production houses across districts in different villages / locations. The spent compost from the production houses infected with wet bubble pathogen, provide inoculum for the infection of future mushroom crops. There are reports of pathogen remaining viable upto 3 years in the post mushroom substrate under natural conditions in the form of chlamydospores (Tu and Liao, 1989; Sharma and Singh, 2002). The levels of sanitation adopted and the method of composting followed by different growers in different villages, as observed during the present surveys, also seem to have contributed to variations in wet bubble incidence and intensities in those locations. That the incidence and intensity varied over different locations surveyed was also observed by several other workers in India and abroad (Nielson, 1932; Forer *et al.*, 1974; Nair and Baker, 1978; Zaayen, 1983; Zhi *et al.*, 1995; Sharma and Kumar, 2000; Singh and Sharma, 2002).

It was also observed during the present surveys that the wet bubble disease incidence was more in spring season compared to that in autumn season. The onset of pathogen-favourable environmental conditions relating to temperature and high relative humidity outside mushroom production houses seem to promote *M. perniciosus* growth and proliferation in contaminated spent compost, casing materials and wild *A. bisporus* (Wood, 1958; Sinden, 1971). The initial source of the pathogen on most farms is often traced in contaminated casing (Fletcher and Gaze, 1989). Generally symptoms in the first-flush indicate contamination of the casing ingredients either before it reaches on the farm or on the farm itself during

storage or mixing. The absence of wild mushrooms in open fields and forests in summers, reducing in chances of availability of pathogen inoculum in autumn coupled with the prevalence of temperatures reducing the pathogen populations in spent compost and casing materials lying outside production rooms seem to have resulted in reduced availability of pathogen inoculum in mushroom production rooms in autumn crop (Gandy, 1972; Fletcher and Atkinson, 1977; Fletcher and Ganny, 1969; Cross and Jacobs, 1969; Hsu and Han, 1982; Tu and Liao, 1989; Umar *et al.*, 2000; Sharma and Singh, 2002) also reported the means of spread and variations in wet bubble incidence in different growing seasons.

Like other diseases, the wet bubble of button mushrooms is also identified by the manifestations of certain characteristic symptoms. Two main types of symptoms were observed during the present studies—infected sporophores and brown tumorous undifferentiated sclerodermoid masses, which resulted due to infection at different stages in the development of sporophores. Sporophores inoculated with pathogen culture bits developed characteristic symptoms within 24 hours, whereas the infestation with spore and mycelial suspension symptoms developed in symptoms in 9 to 12 days after inoculation depending upon the time of infestation of casing soil. Shortest incubation period of 4-5 days was recorded when the inoculum was sprayed at pinhead formation stage and the longest of 12 days when sprayed one day after casing. The findings indicate maximum susceptibility and vulnerability of pinheads and buttons. The symptoms produced in inoculated material were similar to those recorded under natural conditions during survey. The undifferentiated primordia which were less than 5-6 mm in diameter, responded to the inoculation with hyperplasia and tumour induction. Large, irregular confluent *A. bisporus* lumps developed with no signs of differentiation or organogenesis. These tumorous bodies were often studded with coloured tear drops especially in conditions of very high humidity. Sometimes partial differentiation of the primordia resulted in partly formed caps showing protuberances on their surfaces. At later stages (10-12th day of infections, these

tumerous masses were found infected the various types of bacteria. Similar description of the disease was also given by several other researchers (Smith, 1924; Bech and Kovacs, 1977; Geijn, 1982; Zaayen, 1982; Figueiredo and Mucci, 1985; Korovljev, 1986; Ruan, 1990; Garcha, 1978; Tu and Liao, 1989; Fletcher and Ganny, 1968; Sharma and Kumar, 2000; Umar *et al.*, 2000).

Isolation of the pathogen was made from infected sporophores and pathogenicity proved in accordance with the Koch's postulates both *in vivo* and *in vitro*. The pathogen was found to attack all the growth stages of this mushroom but the immature stages were found more susceptible than the mature ones, and characteristic symptoms developed on all the inoculated sporophores. The morphological characters of the associated pathogen were studied and compared its the standard authentic description from the literature (Smith, 1924; Atkins and La Touche, 1948; Hsu and Han, 1981; Kaul *et al.*, 1978), establishing its identity as *Mycogone perniciosa* authority etc. and re-confirming through from ITCC, New Delhi, India under Accession No 7493.09

The *in vitro* interaction between *A. bisporus* and *M. perniciosa* mycelia indicated the collapse of *A. bisporus* hyphae at the point of its contact with *M. perniciosa* hyphae without production of any zone of inhibition, indicating thereby operation of mycoparasitism between the two fungi. Hyphal collapse at the point of contact between the two fungi was also reported earlier (Gary and Morgan, 1981; Sharma and Vijay, 1996). Khanna *et al.* (2003) also reported that no clearing zone of inhibition occurred between *A. bisporus* and *Verticillium fungicola* mycelium.

The effective management of wet bubble disease in mushroom production houses requires preferably the use of components other than chemicals (fungicides) such as botanicals and bio-control agents. Some fungicides can also be used for economical management of the disease. However, all these applications and amendments aim at checking or inhibiting the growth and proliferation of *M. perniciosa* with no or least such effects on *A. bisporus*. These

components were, therefore, evaluated both *in vitro* and *in vivo* against both these fungi to select the most suitable ones for containing the disease. During the present investigations, prochloraz manganese followed by captan showed maximum *in vitro* inhibition against *M. pernicioso* and least against *A. bisporus* amongst non-systemic fungicides; whereas among systemic carbendazim, bitertanol and myclobutanil were *in vitro* most inhibitory to *M. pernicioso* and least inhibitory to *A. bisporus in vitro*. These fungicides, therefore, show promise for controlling wet bubble disease of white button mushroom. Similarly, inhibitory effects of these fungicides have also been reported by several other workers (Jandiak *et al.*, 1978; Thapa and Raina, 1989; Dhar and Kapoor, 1990; Bhat and Singh, 2002; Bhardwaj, 1983; Sharma and Jarial, 2000; Butt and Singh, 2002).

Of the botanicals, the aqueous extract of *C. longa*, *Metricaria* sp. and *L. officinalis* were least inhibitory to *A. bisporus* mycelium and most inhibitory to *M. pernicioso* mycelium. The ethanol extracts of *C. longa* and *Metricaria* as well behaved in a similar way. However, the ethanol extract of *A. indica* too exhibited appreciable levels of inhibition against *M. pernicioso* and least inhibition against *A. bisporus*. The findings indicate usefulness of the *C. longa*, *Metricaria* sp. and *A. indica* as sprays or as amendments in compost or casing soil to keep the wet bubble disease at low ebb. Similar results with botanicals have also been reported by other workers Sharma and Jandiak (1994), Grewal and Grewal (1988), Sharma and Kumar (2005), Raina *et al.* (2003) reported differential fungicidal property of leaf extract of *A. indica*, *Chrysanthemum indicum* and *Tagetes erecta* against weed moulds of mushroom.

Another component of wet bubble management such as bacterial antagonists, evaluated under the present investigation, revealed that all the test antagonists – *Pseudomonas* spp., *B. subtilis* and *Azotobacter* spp. – exhibited no adverse effects *in vitro* on *A. bisporus* with simultaneous inhibitory effects of varying degrees on *M. pernicioso* except for *B. subtilis* isolate BS-101 and

Azotobacter isolate Azt-117 which were neutral towards the pathogen. Attempts have been made by several workers to control *M. pernicioso* and other pathogens of the *A. bisporus* under *in vitro* conditions and almost all of them have reported similar findings (Henry *et al.*, 1991; Bora and Ozaktan, 1996; Bora *et al.*, 2000; Bhat *et al.*, 2000; Mishra and Singh, 2003; Ahlawat *et al.*, 2002).

The effectiveness of any disease management component can better be assessed by their *in vivo* evaluation against these diseases. The *in vivo* evaluation of the fungitoxicants revealed the efficacy of carbendazim and bitertanol (systemic) or captan and prochloraz manganese (non-systemic) incorporated in pathogen infested casing material in controlling wet bubble disease with concomitant increase in mushroom button yield. The incorporation of bacterial antagonists such as *P. fluorescens*, *B. subtilis* or *Azotobacter* sp. at different concentrations in pathogen-infested casing also yielded appreciable disease control with corresponding yield gains. Similarly the incorporation of *C. longa* or *A. annua* in pathogen-infested casing soil also exhibited wet bubble control with corresponding yield gains. The incorporation of effective fungicides, botanicals or bacterial antagonists in casing soil for the control of wet bubble disease of the button mushrooms have also been demonstrated by other researchers (Stanek and Vojtechorska, 1972; Gandy, 1974; Geijn, 1977; Kim *et al.*, 1978). The effectiveness of prochloraz manganese and captan against *M. pernicioso* mould of edible fungi has also been earlier documented (Hsu and Han, 1981; Zaayen and Adrichem, 1982; Fletcher, 1983; Zaayen *et al.*, 1983; Eicker, 1984; Jhune *et al.*, 1991; Sharma and Kumar, 2000). Similarly, the usefulness of *P. fluorescens* and *B. subtilis* in the control of moulds/diseases observed in the present investigations are in conformity with those of Fleriman (1973), Michal and Judith (1975), Gandy (1979), Schippers *et al.* (1987) and Campell (1989). The antagonistic behaviour of fluorescent pseudomonads against mushroom pathogens with increase in mushroom yields has been reported by many other workers (Byer and Sikora, 1990; Fermor, 1991; Bora *et al.*, 2000; Ahlawat *et al.*, 2002; Mishra *et al.*, 2003). Singh *et al.* (2000) also claimed good success of siderophore-producing isolate (C116) of fluorescent pseudomonads against *M. pernicioso*. The incorporation of

C. longa/L. officinalis/A. annua for successfully managing the wet bubble disease as observed during the present study is in unison with those of Sharma *et al.* (1998), Sharma and Jarial (2000), Mishra and Singh (2003), Sharma and Kumar (2005).

Identifying a white button strain having fair level of resistance/tolerance to *M. perniciosa* infections with considerably good yield and yield components shall help in devising an economical and eco-friendly management of wet bubble disease. To accomplish this objective, seven *A. bisporus* strains were evaluated for their tolerance to *M. perniciosa* infections. However, none of these strains was statistically more tolerant to the pathogen. The comparative advantages in only the qualitative and quantitative characters of these strains can, therefore, be considered for identifying the preferable strains. The wet bubble disease of the button mushroom can not therefore be managed by mere cultivation of a particular *A. bisporus* strain (You *et al.* 1978), however, reported that a strain No. 703 having cream coloured fruiting body has been reported less sensitive to *M. perniciosa* compared to other strains. This strain needs to be evaluated under our conditions for its adaptability and profitability.

Sharma and Kumar (2000), also recorded more loss in S11 strain where as in present case yield loss ranged from 74.55 to 79.23 in all tested strains. These errors might be due to the difference in inoculum loads used and growing conditions prevailing during experiment.

The integration of two or more management components, thus identified, is likely to provide a cumulative or additive effect in controlling the wet bubble disease. The integration is sure to help reduce the use of fungitoxicants. The integration of non-chemical components such as bio-agents and botanicals coupled with adoption of compost and casing pasteurization, sanitation and avoidance of prevalence of pre-disposing factors shall help raise a profitable and organic crop.

Chapter – 6

SUMMARY AND CONCLUSION

The investigations on “Integrated Management of Wet Bubble Disease of White Button Mushroom [*Agaricus bisporus* (Lange) Imach]” was carried out at Mushroom Research and Training Centre, Division of Plant Pathology, during 2008 and 2009. The results obtained during the course of this study are summarised as under:

Survey of the mushroom farms in Srinagar, Budgam and Pulwama districts of Kashmir valley during 2008 and 2009, indicated maximum wet bubble prevalence of 67.0 per cent in district Budgam followed by 56.0 per cent in district Pulwama, whereas district Srinagar exhibited in least disease prevalence of 33.0 per cent. The overall mean disease incidence ranged from 7.29 to 11.07 per cent in three districts with maximum incidence in district Pulwama and minimum in district Budgam with the overall mean disease incidence of 9.09 per cent over years in three districts, the incidence was more (10.55-14.25%) in spring than (3.61-8.05%) in Autumn season. Similarly, the overall mean disease intensity ranged from 13.33 to 21.86 per cent in the three districts with maximum intensity in districts Budgam and minimum in district Srinagar. With an overall mean disease intensity of 17.82 per cent over years in these districts, the disease intensity was more 16.86 to 28.31 per cent in spring than in autumn (9.81-15.46 %) season. The per cent yield loss due to the wet bubble disease ranged from 13.53 to 22.41 per cent with maximum loss reported in district Pulwama and minimum in district Srinagar. With an overall yield loss of 16.97 per cent over years, the loss was highest 15.88 to 26.01 per cent in spring than in autumn (8.63-14.92 %) season.

The pathogen was found to attack all the developmental stages of the button mushroom *A. bisporus*. The disease is mainly characterised by the presence of white mouldy growth on the sporophores, leading to their putrefaction

with the production of golden brown exudates. The mushrooms became malformed with swollen stipes and with reduced or deformed caps. From time of infection to the appearance of distortion symptoms is 10 to 14 days. When these distorted masses were cut across, the affected tissue is generally dark in colour and wet in appearance. When differentiated, sporophores are attacked, the stalk is colonised, with a brown streak, reaching the cap and the gills.

Isolation of the pathogen was made from diseased sporophores and the pathogenicity proved in accordance with the Koch's postulates both *in vivo* as well as *in vitro*. The pathogen was found to attack all the growth stages of this mushroom but immature stages were found to be more suitable than the mature ones and characteristic symptoms developed on all the inoculated sporophores. Mycelium of the pathogen was initially compact, felt like, septate, hyaline but later turning amber brown with age, produced conidia and chlamydospores, conidiophores sub-verticillate to verticillately branched, well septated bearing thin walled 1-2 celled conidia which were relatively short lived. The chlamydospores are terminal, two celled, with a very thick walled terminal cell. The average time between inoculation and symptom expression varied between 2-12 days depending upon the time of infestation. Further the interaction between *A. bisporus* and *M. perniciosus* mycelia studied in duel cultures revealed the hyphal collapse of *Agaricus bisporus* at the point of contact with *M. perniciosus* without any zone of inhibition, indicating thereby mycoparasitism between the two fungi.

On the basis of cultural and morphological characters of the isolates fungus as compared with the authentic description together with its pathogenicity on *A. bisporus*, the fungus was identified as *Mycogone perniciosus* Magn. and its identity confirmed through Indian type collection centre, New Delhi under Accession No. 7493.09.

The pathogenic fungus of wet bubble disease was not re-isolated from the pathogen inoculated compost samples at the time of filling when kept within central region of the heap at the time of stacking, 1st, 2nd, 3rd, 4th and 5th turning however it got easily isolated when the samples were kept during 6th, 7th and 8th turning. The peak heating temperature eliminated the pathogen from the sick compost samples.

Pathogen infested casing soil serves the main source of infection of wet bubble disease. The pathogen survived and remained viable for all months worked out in infested casing soil when kept outdoors. The population of pathogen remained maximum in the first month, the experiment was laid out and later declined gradually. Diseased spent compost also serves source of infection of wet bubble disease. Samples drawn each month from healthy and diseased spent compost recorded presence of pathogen during the month observed. No pathogen was isolated from healthy spent compost. The pathogen population in diseased spent compost was maximum ($3 \times 10^7 \text{ g}^{-1}$ compost) immediately after placing outdoor then declined gradually.

Only the garden soil and spent compost was found to harbour the wet bubble pathogen. The peat soil, virgin soil, farm yard manure and sand did not yield any propagule of *M. perniciosus*.

Dispersal of *M. perniciosus* spores was found to be carried out by air currents, sciarid flies and water splash. The dispersal by air current is highly influenced by air velocity (7 colonies/plate) was carried out by higher air current velocity (7 m/sec) and minimum (0.87 colonies/plate) at low velocity (2 m/sec).

Sciariid fly also carried the spores to different distances, dispersal by sciarid flies are influenced by inoculum acquiring period and number of flies visiting diseased sporophores. Maximum sporocorps got infected with maximum

acquisition period of 120 minutes and higher population of flies visiting diseased sporocorps.

The dispersal was also found be carried by water splashes during water spray on trays each day. The maximum colony forming units were observed during spray nearest the infected sporocorps and it declined with the increase in distance of splash.

The *in vitro* efficacy of ten fungitoxicants against *A. bisporus* and *M. perniciosa* revealed that prochloraz manganese was highly fungitoxic among non-systemics completely inhibiting the mycelial growth of the pathogen at 50-500 $\mu\text{g ml}^{-1}$ with least inhibition of *A. bisporus* mycelium. The other fungitoxicants inhibiting the mycelial growth of pathogen in order of their efficacy were captan, mancozeb, chlorothalanil and propineb.

Among systemic fungicides carbendazim proved highly fungitoxic, inhibiting the mycelial growth of the pathogen at different test concentrations. The other systemic fungicides inhibiting the growth of the pathogen in order of their efficacy were myclobutanil, bitertanol, diniconazole and triademiphon. Carbendazim, myclobutanil and bitertanol at concentration 100 $\mu\text{g ml}^{-1}$ completely inhibited the pathogen mycelium, whileas myclobutanil did so at 50 $\mu\text{g ml}^{-1}$. Carbendazim also recorded no growth inhibition of *A. bisporus* mycelium at 5-25 $\mu\text{g ml}^{-1}$. The other fungitoxicants in order of their least inhibitory effects against host fungus are bitertanol, myclobutanil, triademiphon and diniconazole.

The *in vitro* evaluation of aqueous extract of eight different locally available plant species indicated that *Curcuma longa*, *Metricaria* sp. and *L. officinalis* were least inhibitory to *A. bisporus* mycelium and most inhibitory to *M. perniciosa* mycelium. The test botanicals were highly effective at 3 per cent concentrations. The other aqueous extract of botanicals inhibiting the mycelial

growth of pathogen in order of their efficacy were *Artemesia annua*, *Azadirachta indica*, *Juglans regia*, *Mentha longifolia* and *Urtica dioca*. The ethanol extract of *Curcuma longa*, *Metricaria* sp. and *Lavendulla officinalis* as well behaved in a similar way. However the ethanol extract of *A. indica* too exhibited appreciable levels of inhibition against *M. pernicioso* and least inhibition against *A. bisporus*. Ethanol extract of botanicals proved to be most effective than aqueous extract of botanicals so much so that *Curcuma longa* at 3 per cent concentration completely inhibited the pathogen fungus, with least inhibition of host fungus. The other ethanol extracts of botanicals in order of their efficacy against the pathogen fungus are *Mentha longifolia*, *Juglans regia* and *Urtica dioca*.

Among spore cultures of bacterial antagonists evaluated *in vitro*, revealed that all the test antagonists *Pseudomonas fluorescens* ., *Bacillus subtilis* and *Azotobacter* sp. exhibited stimulatory effects of varying degree on *Agaricus bisporus* mycelium with antagonistic effects on *M. pernicioso* mycelium, *Bacillus subtilis*-116, *Pseudomonas fluorescens*-103 and *Azotobacter*-106 gave the highest mycelial growth inhibition of 100.0, 98.88 and 98.51 per cent of pathogen fungus, respectively. The *in vivo* evaluation of the fungitoxicants revealed the efficacy of carbendazim and bitertanol (systemic) or captan and prochloraz manganese (non-systemic) incorporated in pathogen infested casing material in controlling wet bubble disease with concomitant increase in mushroom button yield. The incorporation of bacterial antagonists such as *P. fluorescens*, *B. subtilis* or *Azotobacter* sp. at different concentrations in pathogen-infested casing also yielded appreciable disease control with corresponding yield gains. Similarly the incorporation of *C. longa* or *A. annua* in pathogen-infested casing soil also exhibited wet bubble control with corresponding yield gains.

CONCLUSION

In light of the present investigations, it can be deduced that:

- White button mushroom cultivation in Kashmir valley is primarily in the hands of resource-poor growers. The prevalence of unhygienic conditions in and around the mushroom farms and lack of pasteurization facility result in recurrence of wet bubble and other mould diseases causing heavy economic losses to these growers. The disease can be managed at farmer's field levels by the use of safer fungicides, botanicals and bio agents on or in the casing soils and adhering to farm sanitation.
- Sprays on casing with non-systemic fungicides prochloraz manganese 50 WP or captan 50 WP @ 0.2 per cent and systemic fungicides carbendazim 50 WP or bitertanol @ 0.1 per cent, respectively are most effective in controlling wet bubble disease without causing toxicity to the mushroom mycelium. Incorporation of dry formulations of bacterial antagonists in casing mixture at the time of casing @ 2 to 3 per cent can be much helpful for the management of the disease.
- Incorporation of botanicals such as *C. longa*, *A. annua*, or *L.officinalis* as dry powder @ 1 to 3 per cent with casing mixture at the time of casing has indicated broad spectrum fungicidal activity against *M. perniciosa* without affecting the quality and yield of fruiting bodies. These formulations have much scope for commercialization for production of an organic mushroom crop, in view of lesser choice available for selective fungicides and the need to avoid pesticide residues in this short duration high value crop.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. B.M. Sharma, Professor, Department of Plant Pathology, CSKHPKV, Palampur during Viva-Voce examination held on 10th of September, 2012 have been incorporated in the manuscript entitled “**Studies on management of wet bubble disease of white button mushroom [*Agaricus bisporus* (Lange.) Sing.] in Kashmir**” submitted by Mrs. Shaheen Kauser Jan (Regd. No. 2007-191-D).

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