STUDIES ON DRY BUBBLE DISEASE OF WHITE BUTTON MUSHROOM *Agaricus bisporus* (Lange.) Sing.

BY

Sandeep Kumar G.M.

(Regn.No.05/216)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,

RAHURI-413 722. DIST-AHMEDNAGAR.

MAHARASHTRA STATE (INDIA)

In partial fulfilment of the requirements for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY,

COLLEGE OF AGRICULTURE,

Pune - 411 005.

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Approved by the advisory committee

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2007

This thesis work is affectionately dedicated to

My beloved parents,

Shri Manje Gowda K.

and

Sharadha, B.R.

Sandeep…

CANDIDATE'S DECLARATION

I here by declare that this thesis or part there of has not been submitted by me or any other person to any other university or institute for a degree or diploma.

Place: Pune

Date : 19 May 2007

(Sandeep Kumar G. M.)

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON DRY BUBBLE DISEASE OF WHITE BUTTON MUSHROOM *Agaricus bisporus* (Lange.)Sing."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** embodies the results of a piece of bonafide research carried out by **Sandeep Kumar G. M.** under my guidance and supervision and that no part of thesis has been submitted for any other Degree or Diploma.

The assistance and help received during the course of investigation and source of literature referred to have been duly acknowledged.

Place: Pune

Date: 19/05/2007

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Research Guide and Chairman

Dr. R. N. Sabale
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CERTIFICATE

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Place: Pune

Date: 19/5/2007

(R. N. Sabale)

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PUNE
19 / 05 /2007

(SANDEEP KUMAR G.M.)

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ABSTRACT

"STUDIES ON DRY BUBBLE DISEASE OF WHITE BUTTON MUSHROOM *Agaricus bisporus* (Lange.) Sing."

BY

Mr. SANDEEP KUMAR G.M.

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

Research guide : Dr. T.K. Narute

Department : Plant Pathology

The present investigation was intended to study the incitant of dry bubble disease in respect of survey and pathogenicity, its morphological, cultural, physiological characters and management aspects.

Survey revealed the incidence of the disease upto 2.5 to 13 per cent. Maximum incidence was recorded in Nutan Mushrooms, Pune on U-3 strain.

The fungus proved to be pathogenic on *Agaricus bisporus* and expressed the characteristic brown spot, stipe blast, harelip and dry bubble symptoms on mushrooms. Morphologically the

pathogen was found to be identical to *Verticillium fungicola* (Preuss) Hassebr.

The Richard's agar media was more preferential for growth and sporulation of the fungus. Carbon compounds *viz.*, sucrose, glucose, fructose, mannitol and starch were superior in support of mycelial growth to rest of the compounds. Among the nitrogenous compounds D-alanine, urea, ammonium phosphate and L-asparagine supported best growth of the fungus. Excellent sporulation was induced by D-methionine, urea and D-alanine.

The optimum temperature required for the fungal growth was 25^0C. No growth occurred at 5 and 40^0C. The optimum pH for maximum growth of the fungus was found to be 5 to 6.

Spore germination was favoured in different substrates tried. More preferential being in mushroom tissue extract (85.50 %) followed by 1% sucrose solution (83.00 %) and potato dextrose broth (80.50 %).

In dual culture of *A. bisporus* and *V. fungicola*, growth of *A. bisporus* was inhibited by 56.25 % as compared to 4.58 % inhibition of *V. fungicola.*

In *in-vitro* fungicidal assay, cent per cent mycelial inhibition was observed in all treatments at their higher concentrations. At lower concentrations, mancozeb + prochloraz and mancozeb + carbendazim were found to be highly inhibitory to the fungus.

In *in-vivo* evaluation of fungicides, the overall performance of fungicides in controlling dry bubble disease was best in mancozeb + prochloraz treatment with 82.22% disease reduction and highest yield of 1616.67g. Carbendazim and prochloraz in combinations with mancozeb gave significantly higher yields and disease reduction as compared to their combinations with chlorothalonil. Chlorothalonil spray alone failed to control disease with only 44.30% reduction in disease incidence.

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INTRODUCTION

1. INTRODUCTION

Button mushroom, *Agaricus bisporus* (Lange.) Sing. is popularly known as White button mushroom or European mushroom or Temperate mushroom. Among all the cultivated edible mushrooms, button mushroom is universally accepted by wide range of people and cultivated throughout world due to its high nutritive value, typical exotic flavour and attractive shape. The cultivation practice of button mushroom is an old agricultural practice which originated in France around 1650, where melon growers observed spontaneous appearance of buttons on used compost of melon crops. Then after indoor cultivation of *Agaricus bisporus* in caves was practiced in France and Holland around 1810. In 1894, the first structure specifically designed to grow mushrooms was built in Chester County, Pennsylvania, which is usually referred to as the mushroom capital of the world (Anonymous, 2002). In India, commercial mushroom growing was first initiated at Solan in 1961 and later the cultivation technology spread to Maharashtra and other parts of the country (Vijay and Gupta, 1997).

Mushrooms provide a rich addition to the diet in the form of protein, carbohydrate, valuable salts and vitamins. As food, the nutritive value of mushroom lies between meat and vegetables. Amino acid score of mushroom ranks at par with the nutritive value with those calculated for meat and milk. It contains tryphtophan and lysine amino acids and vitamins; folic acid and B12, which are normally absent in majority of the vegetables, hence it has been rightly referred to as "Vegetable meat" (Nita Bahl, 2002). It possesses low fat within the range of only 1.9-2.0% crude fat on dry weight basis and helps in reducing the cholesterol if consumed regularly. It also contains a very less amount of starch. Hence it is recommended for cardiac and diabetic patients. Besides this mushroom possess low caloric value, high protein, high fibre contents and high K:Na ratio which are ideally suited for the patients suffering from hypertension, pneumonia and underfeeding. Button mushrooms contain high levels of selenium, tyrosinase, ergosterol, aromatase inhibitors and immuno-modulating and anti-tumour polysaccharides, which are known to have anti-carcinogenic properties (Robert *et al.*, 2003).

Annual yields of dry protein per unit area utilized for *Agaricus bisporus* farming is 65,000 Kg/ha which is far better than beef (78 Kg/ha) and fish (675 farming Kg/ha) farming (Cooke, 1977). In developing and over populated countries like India the problem of malnutrition is quite obvious. Mushroom cultivation therefore needs to be popularized to fight protein malnutrition. The productivity of mushroom is higher than any other crop. Apart from food, nutritional and medicinal values, mushroom growing is an efficient means of waste disposals like agricultural and industrial waste.

Agaricus bisporus cultivation is also a mean of diversification for making agriculture profitable through efficient use of agriculture by-products which are main ingredients of *Agaricus bisporus* compost. The mushroom farming with about 6.2 Million metric tonnes production is an intensely managed form of food production practiced in over hundred countries of the world. Of this, the commercially cultivated button mushroom, *Agaricus bisporus* (Lange.) Sing. constitutes the major portion (31.8%) of total global production of mushrooms. (Chang, 1999). In India annual button mushroom production during 2002-2003 was 40,000 tones of which Maharashtra contributed 7000 tonnes (17.5 %) to total production (Anonymous, 2006).

The button mushroom cultivation is very sophisticated, sensitive and practiced under controlled atmospheric conditions. The successful cultivation depends upon the manipulation of ecological factors such as temperature, relative humidity, pH and hygiene for disease management. Fluctuations and disturbances in ecological factors invite many biotic and abiotic diseases. It is invaded by fungal, bacterial and viral diseases. Among the fungal diseases dry bubble disease caused by *Verticillium fungicola*(Preuss) Hassebr. is the most devastating disease reported from almost all mushroom growing areas. It is a very damaging disease and has been a pertinent problem for the mushroom industry since it was first reported in 1892 by Costantin and Dufour (Dragt *et al.*, 1996). The yield loss due to this disease was recorded as much as 69.75% by Bhatt and Singh in 2002.

Dry bubble disease on white button mushroom is known to cause the qualitative damage by drastically reducing the sugars and ascorbic acid content. Discolouration of fruiting bodies by the incitant of the disease reduces the market value of the produce there by causing economic losses to the growers (Thapa, 1985). The successful control of the disease is unlikely to be achieved without a sound knowledge of the etiology of the causal organism. The disease still take a toll of the mushroom crops and the measure used to control is a part of the cost of production to be reckoned with.

Considering the serious nature and limited information available on the disease, it was decided to undertake the detail investigations with following objectives:

1. Survey and collection of dry bubble diseased samples.
2. Isolation, identification, and proving the pathogenicity of the fungus.
3. Morphological, cultural and physiological studies.
4. *In- vitro* and *in- vivo* management studies.

The materials used, methods followed, results obtained and discussed are presented in succeeding chapters.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The disease dry bubble infecting button mushroom was reported for the first time in 1892 by Costantin and Dufour. Malthouse (1901) found species of *Verticillium* to be associated with the disease causing brown spots on white button mushroom in Edinburgh. Later on the disease had been reported from various countries *viz.,* Great Britain (Ware, 1933), U.S.A. (Lambert, 1932), South Africa (Bottomley, 1939), Romania (Hulea and Mateescu, 1959), Australia (Conroy, 1962), Bulgaria (Choleva, 1962), Switzerland (Fekete and Kuhn, 1965), Holland (Zaayen, 1976), Denmark (Bech and Kovacs, 1978), China (Chu, 1982), Taiwan (Tu *et al.,* 1989), Venezuela (Cedeno and Carrero, 1997), Spain (Gea *et al.,* 2003) and Serbia (Potocnik, 2004).

Seth *et al.* (1973) reported dry bubble disease for the first time in India. He observed incidence of brown spot in cropping trays at Chail and Taradevi in Himachal Pradesh.

Infection by *Verticillium* spores at early stage causes serious deformations of the sporocarps, which will appear as large, formless, puffball like masses. The cap becomes indistinguishable from the stem. Growers commonly refer to this type of symptom as dry bubble (Anonymous, 2002). The disease is variously named as fungal spot, brown spot, *Verticillium* disease and La mole (Khanna *et al.*, 2003). It was Ware who described and named the casual organism of dry bubble disease as *Verticillium malthousei* Ware (Ware, 1933). Atkins (1975) has described *Verticillium psalliotae* as identical to *Verticillium malthousei* except that the spores are sickle shaped and a distinctive red pigment is produced in culture on malt extract agar medium.

Gams (1971) reported *Verticillium malthousei* Ware. to be a synonym of *Verticillium fungicola* (Preuss) Hassebr. and hence forth *Verticillium malthousei* is generally known as *Verticillium fungicola.*

Gams and Zaayen (1982) divided the genus *Verticillium* into four sections and a residual group. The new section Albo- erecta is characterized by white or yellowish colonies and erect conidiophores and contains mainly fungicolous species. The common causal agent of dry bubble in *Agaricus bisporus* is *Verticillium fungicola var. fungicola,* described and defined by its maximum growth temperature requirement below 27°C. A similar fungus with a maximum growth temperature requirement 33°C, causing brown spots in *A. bitorquis* is described as *Verticillium fungicola var. aleophilum.* However, isolates from wild agarics with a strongly reduced growth at 24°C and a maximum growth below 27°C, a yellowish mycelium and inconspicuous sclerotia, are described as *Verticillium fungicola var. flavidum.*

2.1. Survey, Occurrence and Losses

The disease dry bubble infecting button mushroom was reported for the first time in 1892 by Costantin and Dufour. Seth *et al.* (1973) reported dry bubble disease for the first time in India. During the course of survey they observed incidence of brown spot in cropping trays at Chail and Taradevi. The incidence at Taradevi was considerable, out of 1000 trays, 550 trays were affected. The disease was mainly confined to mushroom farms during monsoon season where the cropping rooms were badly ventilated, trays were over watered, sanitation measures were insufficient and temperatures in the cropping rooms were not maintained.

Thapa (1985) recorded the incidence of dry bubble from 25-50 % at Solan and Kasauli and up to 15% at Shimla and Chail in a survey conducted regularly during 1981-1983.

Gea *et al.* (1995) carried out assessment of dry bubble incidence in Spain. A total of 11812 diseased mushrooms samples were studied from 17 crops grown on 5 farms. Of these, 67.7% had the typical symptoms of dry bubble. Further, he observed that *Verticillium fungicola* was present in all the farms and the diseased mushrooms infected by this pathogen was up to 74.3% and was observed throughout the duration of the crops.

Sharma and Vijay (1996) studied the prevalence and interaction of competitor and parasitic moulds infecting *Agaricus bisporus* and observed that *Verticillium* was the predominant mould which caused 12.88 to 53.65 % loss in mushroom yield on artificial inoculation in compost.

Kerni and Gupta (1997) made comparative studies of mycoparasites commonly occurring during cropping of white button mushroom under traditional method of mushroom cultivation in Jammu and Kashmir. They noticed incidence of mycoparasites was up to 37.23% and the species observed were *Papulospora byssina, Chaetomium, Myceliophthora lutea, Sepedonium sp., Trichoderma, Dactylium dendroides, Diehliomyces microporus, Scopulariopsis fimicola* and *Verticillium fungicola.*

Vijay *et al.* (2000) recorded 51% reduction in button mushroom yield when mesophilic fungi including *Verticillium fungicola* were inoculated in the compost at spawning and after spawning at an interval of 5, 10 and 15 days.

Bhatt and Singh (2002) conducted survey in North India during 2002 and reported 2.17 to 25.00 % incidence of *Verticillium fungicola* in button mushroom crop. Further they also recorded 69.75 % loss in button yield when 1.0 % inoculum load was mixed in casing soil, while it was 50.98 % when inoculum was mixed in compost with same inoculum load used during spawning.

2.2. Isolation, Identification, Pathogenicity and Symptomatology

Ware (1933) was the first to establish pathogenicity of *Verticillium malthousei* causing dry bubble disease of mushroom under controlled conditions both to the mushrooms grown in beds and the cut mushrooms.

Cross and Jacobs (1969) observed that conidia mixed in the casing soil gave maximum infection and reported that *Verticillium malthousei* spores germinate and grow in close association with mushroom mycelial strands in the casing material.

Holmes (1971) reported that mushrooms inoculated with *Verticillium malthousei* spores in casing materials and 7, 14, 21 and 28 days later varied in amount of disease incidence. Inoculum introduced earlier than the 21^{st} day resulted in the highest disease incidence and lowest mushroom yield.

Gandy (1972) studied the effect of spore concentrations and time of inoculation on development of dry bubble disease and observed that serial dilutions of conidial suspensions in distilled water sprayed over the surface of mushroom beds either immediately after casing or three, six or nine days later developed disease incidence with high spore concentrations, irrespective of the time of inoculation.

Seth *et al.* (1973) reported two types of symptoms of dry bubble disease. In case of first type of symptoms, he observed white spots initially on the casing soil which had a tendency to turn greyish-yellow. Later, light brown superficial spots appeared on the caps. These spots increased in area and finally coalesced to become bigger brown blotches. Affected areas became sunken and the centre of each lesion appeared lighter in colour where a grey - bloom developed later as a result of heavy sporulation of the causal organism. Caps got shrunk to several millimeters in blotched area thereby turning them leathery, dry and causing cracking to a certain extent. In another type of symptoms, the stalk of the infected mushroom showed an abnormal swelling and downward peeling of the stem tissue resulting in distortions and misshapenness in mushrooms.

Gams and Zaayen (1982) from Netherlands reported *V. fungicola var. fungicola* causing considerable damage to crops of *Agaricus bisporous* whereas crop of *Agaricus bitorquis* was damaged severely by *Verticillium fungicola var. aleophilum* and an isolate of *Verticillium psalliotae.*

North and Wuest (1993) reported that *Verticillium* disease of *Agaricus bisporus,* produces several symptoms in its host, including dry bubble, necrotic lesions and stipe blow out. Primordia, developing sporophores and maturing sporophores of four cultivars of *Agaricus bisporus* were inoculated with 5µL droplets of spore suspensions of

Verticillium fungicola containing 2 x 10^5, 2 x 10^6 and 10 x 10^6 spores mL^{-1}. The type of symptoms produced depended on the age of the sporophore at the time of inoculation, inoculum density and post inoculation incubation time and dry bubble developed after inoculation of primordial. He further observed necrotic lesions in all treatments. Stipe blow out developed only in sporophores inoculated with the highest dose of spores.

Dragt *et al.* (1996) studied the infection of *Agaricus bisporus* by the mycoparasite *Verticillium fungicola var. fungicola* using light microscopy and TEM. In light microscopy they observed *Verticillium fungicola* hyphae growing on and in the hyphae of *Agaricus bisporus* and appressoria of *Verticlillium.* However in TEM studies *Verticillium fungicola* hyphae.Visible symptoms of the disease were present within 3 days after inoculation. Brown lesions developed and surfaces of the basidiomes were covered with mycelium of *Verticillium fungicola.* After 7 days they observed basidiomes grey brown and severely infected.

Cedeno and Carrero (1997) isolated and identified *Verticillium fungicola* as the cause of deformations and cracks on sporophores of the common mushroom (*Agaricus bisporus* cv. Pennsylvnia-130) produced in Venezuela.

2.3. Morphological studies

Ware (1933) reported in detail the morphological description of *Verticiliium malthousei* characterized by creeping, septate, hyaline, branched hyphae, 1-3 µm in diameter; conidiophores lateral or terminal, erect, septate and 10-200 x 1.5-2 µm but generally verticillately branched and upto 910 x 1.5-5 µm. The secondary branches usually septate at the base, rarely with an additional septum in the middle, arising in whorls and 20-40 x 2-3 µm tapering to 1.0 µm at the tip; conidia oblong, cylindrical occasionally irregularly fusoid with obtuse ends, continuous and 3-16 x 1.5-5 µm in size, abstricted singly at the tip of the branch but remained clustered with mucilage in globular masses, 4-14 µm in diameter which swell and dissociate in water.

Treschow (1941) described *Verticillium psalliotae* as fungus producing pure white mycelium composed of septate, branched, repent hyphae, 1-2 µm in diameter; conidiophores erect, septate, usually with verticillate branches, 1-2 µm in diameter; verticel in the main axis number 1-10 and consists of 1-4, generally 2 or 3 phialides, 18-30 x 1-1.5 µm, tapering slightly towards the apex and septate at the base, conidia 6-10.5 x 2-3.5 µm are abstricted singly from each of the phialides, a distinctive feature of the former being their transversal attachment to the phialides by the longitudinal axis, with apices turned towards the hyphae.

Seth *et al.* (1973) described the morphology of the fungus causing dry bubble disease, in which the fungus produced unicellular hyaline spores borne in terminal clusters on slender branched and verticillate sporophores. Further they described lateral or terminal upright conidiophores having size of 15.0-180.0 x 1.5-2.0 µ and generally verticillately branched. Conidia oblong and cylindrical, occasionally irregularly fusoid, ends obtuse, hyaline, unicellular, measuring 3.5-15.6 x 1.6-5.3 µ.

Cedeno and Carrero (1997) isolated and identified *Verticillium fungicola* as the cause of deformations and cracks on sporophores of the common mushroom *Agaricus bisporus* cv.Pennsylvnia-130 produced in Venezuela. On potato-dextrose-agar, the fungus produced white colonies, which were pale-yellow on the underside, in Petri dishes. The conidiophores were hyaline, erect and showed groups of divergent phialides with a verticillate form. The phialides were hyaline, cylindrical, with lightly inflated base and acute tip. The conidia were hyaline, unicellular, elypsoid to cylindrical and measured 5.6 (4.0-7.0) X 1.9(1.5-2.0) mm.

2.4. Cultural studies

Thapa (1985) found that among various solid and liquid media tried, best growth of *Verticillium fungicola* was supported by Oat meal agar and Richard's solution respectively.

Rinker (1993) developed a chemically defined, selective agar medium containing raffinose, an antibiotic, mineral salts, dyes and fungicides for isolating *Verticillium fungicola*.On this medium, colonies of *V. fungicola* appeared flat and velvety, initially off white in colour when small and white when larger.

Cedeno and Carrero (1997) observed white colonies of the fungus on potato-dextrose-agar, which were pale-yellow on the underside, in petri dishes. The conidiophores were hyaline, erect and showed groups of divergent phialides with a verticillate form.

2.5. Physiological studies

Treschow(1941) reported that the optimum temperature for growth of *Verticillium malthousei* and *Verticillium psalliotae* was found to be 23 and 22^{0}C, respectively under *in-vitro* conditions. Further he observed that the pH of 6.7 and 5.6 were found to be optimum for the growth of *Verticillium malthousei* and *Verticillium psalliotae* respectively.

Fekete (1967) stated that for growth of various isolates of *Verticillium malthousei* the optimum temperature was 18-24^{0}C and maximum 33-36^{0}C.

Wuest and Forer (1975) carried out different experiments on the influence of temperature, time and volatiles on spore germination of *Verticillium malthousei* and observed maximum 90-98 per cent spore germination in an isolate from mushroom in 24 hours at 12^0 C, in 12 hours at 18^{0}C and in 19 hours at 24 and 34^{0}C. However, Volatiles, horse manure and soil substrates colonized by *Agaricus bisporus* had no significant effect on germination after 12 hours at 24^{0}C.

Thapa (1985) recorded maximum growth of *Verticillium fungicola* in Oat meal agar medium having pH 5.5 and incubated at a temperature of 20-25^{0}C. Among different carbon and nitrogen sources tried, best growth was noticed in medium supported by galactose and L + Valine respectively. Further, he studied the effect of different natural extracts and nutrient solutions on spore germination of *Verticillium fungicola* and recorded maximum spore germination in extracts of mushroom fruit bodies followed by sucrose solution (5000 ppm), Farm yard manure and spent compost. In dual culture of

Agaricus bisporus and *Verticillium fungicola*, he found that growth of *Agaricus bisporus* was inhibited more up to 40% as compared to the *Verticillium fungicola* 4.98 per cent.

Bech *et al.* (1989) studied the effect of temperature on growth and spore formation of *Verticillium fungicola.* He observed mycelial growth of *V. fungicola* on potato dextrose agar at 14 to 30^0C and found optimum temperature of 22 and 26^0C for growth of *V. fungicola* and mushrooms, respectively. Further he found 16 to 26^0C as best temperature for spore production but failed to grow at 28°C. However, *V. fungicola* spores could survive at higher temperature in dry and aqueous suspension and in aqueous suspension these spores could withstand 44^0C for ten minutes and 38^0 C for an hour.

Coetzee *et al.* (1991) observed best growth of *Verticillium fungicola* in media containing glucose, mannitol, sucrose, galactose and mannose as sole carbon sources. In case of nitrogenous sources good growth was occurred on all the nitrogen sources except sodium nitrite. The optimum temperature for fungal growth and sporulation was noticed between 22 and 24^0C. However at 32^0C conidia were germinated but mycelial growth inhibited. Further they observed significant sporulation in light than in darkness.

Vijay and Gupta (1992) in found that mycelial growth of *Agaricus bisporus* was reduced by 80.80% when grown in dual culture with *Verticillium fungicola.*

Rinker (1993) developed a chemically defined selective agar medium containing different ingredients like raffinose, an antibiotic, mineral salts, dyes and fungicides for isolating *Verticillium fungicola.*

Colonies of fungus were visible with in 7 days when incubated at 24^0C.

Jandaik and Guleria (2002) found highest growth of *Verticillium fungicola* at 20^0C and 6.00 pH, whereas, maximum sporulation at 25^0C and 6.00 pH. In case of the combined effect of temperature and relative humidity, they observed growth of pathogen at 25^0C and 95 per cent relative humidity while sporulation was favoured at same temperature.

2.6. Disease Management Studies

2.6.1. *In-vitro* evaluation of fungicides

Samuels and Johnston (1980) isolated *Verticillium fungicola and Verticillium psalliotae* from diseased samples from commercially grown mushrooms in New Zealand where benomyl was used as major chemical method of control. In *in vitro* tests with benomyl the isolates had low ED50 values of 0.8-2.1 mu g/ml as determined by radial growth on agar but high ED95 values (>500mu g/ml). Further they found that the ED values of Delsene MX (84% mancozeb and 6.2% carbendazim) were higher (ED50 value, 5-14mu g/ml and ED95 value <50mu g/ml).

Gea *et al.* (1997) exposed twenty isolates of *Verticillium fungicola* to a wide range of concentrations of chemicals *viz.,* benomyl, chlorothalonil, formaldehyde, ipridione, prochloraz-Mn-complex and prochloraz + carbendazim to study their *in-vitro* sensitivity and observed that all these isolates were more sensitive to prochloraz-Mn- complex (EC50 values <5 mg / litre) than to the other fungicides and only 7 isolates were moderately sensitive (EC50 values between 5 and 50 mg / litre) to prochloraz + carbendazim. However, majority of isolates were very resistant to the other fungicides *viz.,* benomyl, chlorothalonil and ipridione.

Bhatt and Singh (2002) found complete *in-vitro* inhibition of *V. fungicola* at 168 h after inoculation using Bavistin (10 μg /ml), Sporogon (10μg /ml), ridomil MZ (200μg/ml) and bavistin + formalin (5+ 5μg /ml). Even at the lower doses of bavistin (1-7 μg /ml) and sporogon (1-7μg /ml) both the chemicals were at par in inhibiting the growth of the pathogen to the extent of 89.59 per cent.

Tokousbalides *et al.* (2006) studied selective fungitoxicity of prochloraz, famoxadone, tebuconazole and trifloxystrobin between a pathogenic strain *Verticillium fungicola* (Preuss) Hassebr. and *Agaricus bisporus* (L) Sing in *in-vitro* and mushroom growing rooms and observed that none of the above fungicides was found to be as selective as prochloraz which could inhibit the growth of *Verticillium fungicola in-vitro* at very low concentrations (ED50 of 0.002ig/ml) but was fungitoxic to *Agaricus bisporus* at higher levels (ED50 of 5.08ig/ml). However, tebuconazole was found least selective, followed by trifloxystrobin and famoxadone.

2.6.2. *In-vivo* evaluation of fungicides

Zaayen (1979) obtained good control of mushroom dry bubble disease with two applications of Daconil 2787 @ 3 g / 1.0 litre water / m^2 without any adverse effect on yields. The highest yields were obtained with Daconil 2787 @ 3g /1.0 litre water /m^2 applied directly after casing and again two weeks later.

Gandy and Spencer (1981) compared twelve different fungicides with chlorothalonil, thiabendazole and benomyl for efficacy against *Verticillium fungicola* infecting mushroom crop and observed triforine, dimethirimol and tridemorph as phytotoxic. However, prochloraz and a carbendazim maneb mixture ("Delsene M") gave significantly higher yields of mushrooms than untreated control. Further they noticed healthy and greater yields from prochloraz and "Delsene M" treatment than chlorothalonil.

Russell (1984) recorded 83% control of *Verticillium fungicola* with prochloraz application@ 1.5g a.i. /m2 at 1, 3 and 5 wk after casing as compared with only 1% control obtained with chlorothalonil.

Eicker, (1987) from South Africa reported satisfactory control of dry bubble disease of cultivated mushrooms with a prochloraz-manganese w.p. complex application @ 1.5g a.i. /m^2 9 days after casing and a similar application at the end of first break. Further he observed that the fungicide was non-toxic to mushrooms and gave acceptably low crop residues when applied at recommended dosage.

Bonnen and Hopkins (1997) examined *in-vivo* response of *Verticillium fungicola* to four different fungicides *viz.,* benomyl, thiabendazole, chlorothalonil and diethofencarb. Of the isolates, 88% were cross tolerant between benomyl and thiabendazole. The level of tolerance to chlorothalonil was relatively high.

Coutinho *et al.* (2000) carried out field trial to evaluate the efficacy of benomyl, thiabendazole, chlorothalonil and prochloraz to control dry bubble disease infecting edible mushrooms and observed that chlorothalonil was inadequate for disease control and identified two benomyl tolerant *Verticillium fungicola* isolates.

Grogan *et al.* (2000) tested the *in-vivo* efficacy of prochloraz-manganese against two main isolates of mushroom crop from British farms and observed good control of both isolates by prochloraz-manganese as compared with untreated control. Single spray of bavistin @ 0.1 % after casing could effectively control the dry bubble disease (Anonymous, 2002).

Sharma and Jandaik (2003) obtained good control of dry bubble with an application of mancozeb @ $3 g/m^2$ at casing and two applications of prochloraz manganese @ $1 g/m^2$ after 15 days and 30 days of casing.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The present investigations were undertaken at the Plant Pathology Section and All India Coordinated Mushroom Improvement Project, College of Agriculture, Pune. The material and the methods followed during the course of research work are presented in this chapter.

3.1 Material

3.1.1 Source of isolate

The samples of diseased fruiting bodies of button mushroom infected with dry bubble were collected for isolation from the private mushrooms farms located in and around Pune district.

3.1.2 Source of spawn

Genetically pure spawn of U-3 strain of *Agaricus bisporus* was procured from the Mushroom Mycologist, All India Coordinated Mushroom Improvement Project, Pune centre and was used for *in-vivo* evaluation of different fungicides.

3.1.3 Fungicides

The following fungicides were used for *in-vitro* and *in-vivo* evaluation studies.

Fungicide	Chemical name	Formulation
Mancozeb	Manganous ethylene bisdithiocarbamate	75 % W.P.
Chlorothalonil	2, 4, 5, 6-tetrachloro - isophthalonitrile	75 % W.P.
Carbendazim	Methyl-2-benzimidazole carbamate	50 % W.P.
Prochloraz	1-N-propyl-N-carbamoyl imidazole	50 % W.P

3.1.4 Laboratory instruments and equipments

Various laboratory instruments required during the study were autoclave, B.O.D., incubator, hot air oven, compound microscope, refrigerator, pH meter, water bath, shaker, top pan balance, hand sprayers, etc. Among the other pretty items used include moist chamber, automizer, inoculating needle, cork borer, scissors, scalpel, spirit lamp, various glassware, filter papers and chemicals required for preparation of media.

3.2 Methods

3.2.1 Survey

Survey of different private mushroom units was carried out during Aug-Sept 2006. Incidence of dry bubble was recorded by counting the total number of bags and infected bags in a unit and incidence was expressed in percentage. Individual diseased fruits were collected in polythene bags and kept in a refrigerator for isolation of fungus and further studies.

3.2.2 Isolation, identification and Pathogenicity of the fungus

Isolation of the fungus was carried out by using spotted pilei by adopting standard tissue isolation method. Diseased mushroom tissue cut into 2mm bits were dipped in 0.1% $HgCl_2$ solution and subsequently washed in 3-4 plates containing distilled water. Diseased tissue bits were plated on potato dextrose agar and incubated at 25^0C for 7 days. The well isolated fungal growth free from contamination was transferred to agar slants by hyphal tip method. By frequent sub-culturing, pathogen was purified and agar slants showing pure fungal growth were maintained for further studies and culture was identified and got confirmed.

The pathogenicity was proved under *in-vivo* conditions. Compost was prepared by short method of composting (Wheat straw-1000Kg + Poultry manure 500Kg + Urea-16Kg + Wheat bran-120Kg + Gypsum-30 Kg). The compost was then analyzed for different quality parameters.

Quality of compost used

Parameters	**Contents**
N-initial	1.8%
N-at spawning	2.0%
pH	7.0
Moisture	70%
Bulk density	0.55g/ml
Colour	Dark brown
C: N ratio	27:1

Polyethylene bags of 150 gauge (18″ x 24″) were filled with 10 Kg compost/bag and spawning of U-3 strain of white button mushroom @ 0.7% of weight of compost was done. After colonization by the mushroom mycelium in compost at temperature of 25^0C, the bags were cased with sterilized casing mixture consisting of coir pith. The coir pith was water leached for 8hrs and then sterilized with steam. An uniform casing layer of 4 cm thickness was placed over spawn run compost. Temperature of the cropping room was maintained at 23 ± 2^0C for about a week to allow mushroom mycelium to spread in to casing layer. During cropping, air temperatures were maintained between $16\text{-}18^0$C and the casing layer was watered daily. Relative humidity of 85-90% was maintained in the cropping room.

Inoculum of mycelial and spore suspension of the isolated pathogen was sprayed on casing mixture with the help of an automizer during casing. In addition to this other bags were inoculated at pinheads stage. Un-inoculated bags were sprayed with equal volume of sterile water. Inoculated bags were observed regularly for the development of pinheads and appearance of the disease. The diseased mushrooms appeared in the inoculated bags, were collected for comparison of symptoms and reisolation. The culture raised from the infected material was compared with the original culture used for inoculation.

3.2.3 Morphological studies

The morphological characters of the pathogen were studied both on the host (*Agaricus bisporus*) as well as on potato dextrose agar medium. The thin sections of the diseased spots on fruiting bodies were cut and examined under the microscope for various infection structures. The morphological characters of the pathogen were also studied by growing it on PDA medium and observed under the microscope for colour, shape and septation of mycelium and sporulating structures. Size of fungal structures was recorded using Ocular and Stage micrometer.

3.2.4 Cultural Studies

The isolate of *Verticillium fungicola* was grown on different media in order to find best media and to study its growth characters and ability to sporulate on different media. The following different agar media were used for the study.

(1) Corn meal agar (5) Malt extract agar

(2) Compost agar (6) Mushroom tissue agar

(3) Czapek-Dox agar (7) Potato-dextrose agar

(4) Glucose-asparagine agar (8) Richard's agar

The above media were prepared as per their standard compositions and were sterilized in autoclave at 1.54Kg/cm^2. Triplicate plates for each medium were poured. Inoculated plates were then incubated at 25^{0}C for 7 days. Observations regarding growth characters and sporulation were recorded.

3.2.5 Physiological Studies

3.2.5.1 Utilization of carbon compounds

The ability of the fungus under study in utilizing various carbon compounds was judged by adding different carbon compounds separately on the molecular weight basis equivalent to molecular weight of sucrose in Richard's medium (without sucrose) as basal medium. The different carbon sources used under study were: (1) Citric acid, (2) D-Fructose, (3) D-Glucose, (4) D-xylose, (5) Mannitol, (6) Sorbitol, (7) Starch, and (8) Sucrose.

The stock solution of Richard's medium without sucrose was prepared and distributed in 100 ml lots in 250 ml Erlenmeyer flasks to which different carbon compounds were added in triplicate flasks for each compound. The media were autoclaved. The flasks were then incubated at 25^{0}C. The Richard's medium without sucrose served as a control. Observations on dry mycelial weight were recorded 25 days after inoculation and the data were statistically analyzed.

3.2.5.2 Utilization of nitrogenous compounds

Nitrogen requirement of the fungus was studied in Richard's medium as a basal medium without potassium nitrate to which different nitrogenous compounds were added separately on the molecular weight basis equivalent to molecular weight of potassium nitrate. The nitrogenous compounds added were: (1) Ammonium phosphate (2) D-Methionine (3) D-Alanine (4) Potassium nitrate (5) Sodium nitrate (6) Sodium nitrite (7) L-asparagine and (8) Urea.

The basal Richard's medium without potassium nitrate was prepared and distributed in 100 ml lots in 250 ml Erlenmeyer flasks. To each flask, required quantities of different nitrogenous compounds were added separately equivalent to the amount of nitrogen contained in potassium nitrate in basal medium. Richard's medium without potassium nitrate served as control. A set of triplicate flasks was maintained for each nitrogen source and incubated at 25^0C. Observations on dry mycelial weight were recorded 25 days after inoculation.

3.2.5.3 Effect of temperature

The effect of temperature on the growth and sporulation was studied on potato dextrose agar medium. Triplicate plates were poured for each temperature and inoculated with uniform bit of fungus of 7 days old culture and incubated at different temperature (5, 15, 20, 25, 30, 35 and 40^0C) for 7 days. Observations on the colony diameter and sporulation were recorded 7 days after the inoculation.

3.2.5.4 Effect of H-ion concentration

Stock solution of Richards's medium without agar was prepared and distributed in 250 ml Erlenmeyer flasks. The pH levels of medium *viz.*, 2, 3, 4, 5, 6, 7 and 8 were adjusted by adding approximate quantities of 0.1N HCl and 0.1N NaOH solution. The media were autoclaved at 1.54 kg/cm^2. Triplicate flasks were maintained for each pH. The final pH after sterilization was recorded with the aid of pH meter. The flasks were then inoculated with a uniform bit of fungus and maintained at room temperature. The mycelial mats were harvested after incubation of 25 days. The harvested mycelial mats were dried in hot air oven at 60^0 C for 48 hours to obtain constant weight.

3.2.5.5 Recording of mycelial growth and sporulation

In solid media, growth was measured by taking average of linear growth of the colony across two diameters at right angles. In liquid media studies, mycelial mats were dried in hot air oven at 60^{0}C for 48 hours to obtain constant weight and dried mycelial mats were weighed quickly in an electric balance. Calcium chloride was kept earlier inside to avoid absorption of moisture during weighment. Average of three replications in a treatment was worked out and used as a quantitative measure for comparing the growth of fungus in different treatments.

On the basis of visual observations as well as on the number of conidia present under low magnification, the degree of sporulation was classified into five categories as

+ + + +	=	Excellent sporulation.
+ + +	=	Good sporulation.
+ +	=	Moderate sporulation.
+	=	Scanty sporulation.
-	=	No sporulation.

3.2.5.6 Spore germination

Spore germination studies were undertaken to observe the effect of various substrates and time required for spore germination with the aid of series of hanging drop preparations of 15 days old culture grown on potato dextrose agar. Different substrates *viz.*, distilled water, sterilized water, tap water, sucrose solution (1 %), potato dextrose broth, Richard's solution, mushroom tissue extract and compost extract were used. Vantieghem cells with hanging drop slides were then placed in sterilized petri plates lined with thin layer of sterilized moist cotton to provide humid atmosphere for spore germination. These petri plates were incubated at 25^0C. The spore germination percentage was recorded at an interval of 2 hours up to 8 hours. Spores with length of the germ tube exceeding half the smaller diameter were considered as germinated.

3.2.5.7 Interaction studies

In order to study the inhibitory effect of *Verticillium fungicola* on *Agaricus bisporus* mycelium, both the pathogen and the host were grown together in petri dishes by keeping similar discs at a distance of 25 mm in petri plates containing Richard's agar. Observations on the growth of *Agaricus bisporus* were taken after 96 hour. Per cent inhibition of the growth of *Agaricus bisporus* mycelium due to presence of *Verticillium fungicola* was calculated by following the formula given by Vincent (1947). Four replications were maintained for each treatment.

3.2.6 Disease management studies

3.2.6.1 *In-vitro* effect of fungicides on growth and sporulation

The *in-vitro* efficacy of fungicides against dry bubble fungus was evaluated by adopting poison food technique (Nene and Thapliyal, 1993).

Following fungicides from different groups and their combinations at three different concentrations were used.

Fungicides	Concentrations (%)
1) Mancozeb	0.01, 0.15, 0.2
2) Chlorothalonil	0.01, 0.15, 0.2
3) Carbendazim	0.050, 0.075, 0.1
4) Prochloraz	0.050, 0.075, 0.1
5) Mancozeb+Carbendazim	
6) Mancozeb + Prochloraz	
7) Chlorothalonil + Carbendazim	
8) Chlorothalonil + Prochloraz	

To the presterilized PDA medium, fungicides of required quantity were aseptically added so as to get the required final concentrations. The medium was then poured into petriplates of size 9cm. For inoculation, pure culture of the pathogen obtained from the periphery of actively growing stock culture with sterile cork borer was transferred aseptically in the centre of petri dishes containing the medium with required concentration of the fungicides. Triplicates petridishes of each treatment were maintained. The treatment without fungicide served as check. All test plates were incubated at 25^0 C for 7 days.

The linear growth of the colony was measured in millimeters 7 days after inoculation in two directions at right angle to each other and the average of two measurements was taken as the diameter of the fungal colony. Antifungal activity was determined as per cent inhibition of radial growth with respect to PDA without fungicide. The inhibition of the fungus on the culture media was calculated with the formula given by Vincent (1947).

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

Where,

I = Percent inhibition.

C = Rate of growth in control in mm.

T = Rate of growth in treatments in mm.

3.2.6.2 *In-vivo* evaluation of fungicides.

Chemical control trial was conducted at All India Coordinated Mushroom Improvement Project, Pune centre.

Polyethylene bags of 150 gauge (18″ x 24″) were filled with 10 Kg compost/bag and spawning of U-3 strain of white button mushroom @ 0.7% of weight of compost was done. After colonization by the mushroom mycelium in compost at temperature of 25^0C, the bags were cased with sterilized casing mixture consisting of coir pith. Inoculum of pathogen was multiplied on wheat grains and 4 gm inoculum/kg of casing mixture was used for inoculation.

The *in-vivo* evaluation trial was laid in Completely Randomized Design (Plate 1) with the following treatment details:

Treatments : 10

Replication : 3

T1 : Mancozeb (0.2%)

T2 : Chlorothalonil (0.2%)

Plate 1. Experimental set up for *in-vivo* evaluation of fungicides.

T3 : Carbendazim (0.1%)

T4 : Prochloraz (0.1%)

T5 : Mancozeb + Carbendazim

T6 : Mancozeb + Prochloraz

T7 : Chlorothalonil + Carbendazim

T8 : Chlorothalonil + Prochloraz

T9 : Control I (Inoculated Un-sprayed)

T10 : Control II (Un-inoculated Un-sprayed)

Three sprays of fungicides were applied on the casing surface at a rate of 2 lit/m^2. First spray of fungicidal treatment was given immediately after the application of casing layer and second and third were followed at 15 and 30 days respectively. Two checks were maintained, one with inoculum but without fungicide and another without inoculum and fungicide. Three replications were maintained in each treatment and data on disease incidence, yield, number of fruit bodies and weight were recorded. Buttons were harvested at 3 to 4cm size. Each treatment was replicated three times. Per cent disease control from each treatment was worked out on the basis of the disease incidence in control I (Inoculated Un-sprayed) and yield loss was obtained by comparison with control II (Un-inoculated Un-sprayed).

3.2.7 Statistical Analysis

The data recorded were subjected to statistical analysis wherever considered essential. Before analyzing data, percentages derived from count data were converted in to arcsine values. The differences exhibited by the treatments in various experiments were tested for their significance at 5% level as per the methods suggested by Panse and Sukhatme (2000).

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The experiments were conducted at Plant Pathology section, College of Agriculture, Pune and in All India Coordinated Mushroom Improvement Project, Pune centre. The results obtained during the course of investigation are presented in this chapter.

4.1 Survey

The survey was conducted in the month of Aug-Sept 2006 to observe the disease incidence of dry bubble in different farms. Different private farms used varied strains of mushrooms. It was observed that the percent disease was ranged from 2.5 to 13%. Maximum incidence of 13% was noticed at Nutan Mushrooms on U-3 strain. The details of survey is presented in Table 1.

Table 1. Incidence of dry bubble disease at different mushroom farms

Sr. No	Name of firm	Strain grown	No. of bags observed	No. of bags infected	Per cent disease
1	AICMIP, Pune	U-3 S-11	200 200	15 5	7.5 2.5
2	Nutan Mushrooms, Pune	U-3 P-310	500 1000	65 83	13 8.3
3	Weikfield Agro Product, Pune	Salvan-30 Salvan-512	1000 1000	25 60	2.5 6.0

4.2 Isolation, Pathogenicity and Symptomatology

4.2.1 Isolation

Diseased samples collected during survey from different firms were used to isolate the fungus. On frequent transfer under aseptic conditions the pathogen was isolated and purified on potato dextrose agar.

4.2.2 Pathogenicity

The pathogenic nature of fungus was studied under *in-vivo* condition by inoculating mycelial and spore suspension of the pathogen on U-3 strain of button mushroom grown in polythene bags. The mycelial and spore suspension of the pathogen was inoculated at two stages *viz.*, at casing and during pinheads initiation. The observations recorded are presented in Table 2 and Plate 2.

Table 2. Per cent infection of fruiting bodies with artificial inoculation

Sr. No	Inoculation stage	No. of bags inoculated	No. of mushroom fruits harvested		Per cent disease incidence
			Healthy	Diseased	
1	At casing	6	371	319	46.23
2	Pinhead stage	6	544	256	32.00
3	Control	6	1020	0	0

Plate 2. Pathogenicity of the fungus.

It is evident form the data presented in Table 2 that, the pathogen could cause infection when inoculated either in the casing soil or on young pinheads. However, per cent infection was high (46.23%) in casing inoculation where as it was 32% in pinheads inoculation.

The inoculated pathogen was re-isolated from diseased samples and on comparison with the original isolate it was found to be identical, thus pathogenicity was proved.

4.2.3 Symptomatology

The symptoms observed on infected samples during survey and exhibited during artificial inoculations were identical. It was observed that the symptoms initiate as localized light brown coloured superficial depressed spots on fully developed sporophores. These spots increased in area and coalesced to form irregular brown blotches. Later on affected areas become sunken and the centre of each lesion appeared lighter in colour and a grey bloom developed which result into heavy sporulation of the pathogen. Such infected caps became shrunken in blotched area, turned leathery, dry and shows cracks on fruits. Harelip symptoms were also observed in case of localized infection.

On stipe, infection manifested in the form of brown local lesions up to 2mm deep. Affected stipe showed abnormal swelling and downward peeling of the stem tissue result in to stipe blast. Infection at the pinhead stage resulted in masses of undifferentiated mushroom tissue called Sclermoid tissue or dry bubble up to 12-40 mm in diameter. Infected bubbles appeared dry and leathery with grey or pale in colour. The infected sporophore became shrunken, leathery and dirty white in colour, covered by mycelial weft of the pathogen.

4.3 Morphological studies

Observations on morphological characters were recorded from 15 days old culture grown on potato dextrose agar. On potato dextrose agar, the mycelial growth of the fungus was observed to be white, thick, circular, compact and slightly raised with yellowish colouration of the medium on reverse side of the plate. Mycelium was found irregularly branched, septate, measuring 1-3 µm in width. Conidiophores were erect, septate, verticillately branched and measured 10-200 µm × 1.5-5.0 µm. Conidia were observed single celled, hyaline, oblong to cylindrical in shape, occasionally fusoid with obtuse ends and were produced in gelatinous matrix and appeared like conidial balls. Conidiospores were measured 8-10 µm long by 1-3 µm wide and tapering to 0.5-1.0 µm at the tip.

When the host tissue were observed under high power the mycelium found inter and intra cellular; conidiophores were verticillately branched coming out of the host cell surface from the diseased spots. Light microscopy observations revealed the presence of appressoria on the host cell. The hyphal penetration of fungus and necrosis of the host cell was also observed in the infected cell.

4.4 Cultural studies

The growth characters of the fungus in various cultural media were studied and the results are presented in Table 3 and illustrated in Plate 3.

The cultural media evaluated for growth characters indicated that fungal radial diameter ranging from 52.33 to 71.33 mm.

The Richard's agar media significantly yielded maximum radial growth of the fungus to the extent of 71.33mm followed by Czapek-dox agar, Potato dextrose agar and Glucose-asparagine agar up to 66.00, 65.67 and 62.33mm respectively. However, excellent sporulation was observed in Richard's agar medium and Mushroom tissue agar.

In the present study among the synthetic media Richard's medium was found comparatively superior in supporting mycelial growth and sporulation than other media and hence it was used as a basal medium for further studies of the fungus. The results are graphically presented in Fig. 1.

Table 3. Cultural characters on different media

Sr. No.	Media	Mean colony dia.(mm)*	Sporulation
1	Compost agar	57.33	+ +
2	Corn meal agar	56.67	+ +
3	Czapek-Dox agar	66.00	+ + +
4	Glucose-asparagine agar	62.33	+ + +
5	Malt extract agar	55.00	+ + +
6	Mushroom tissue agar	52.33	+ + + +
7	Potato-dextrose agar	65.67	+ + +
8	Richard's agar	71.33	+ + + +
	S.E. ± 1	0.54	
	C.D. at 5 %	1.61	

* Average of three replications

+ + + + = Excellent sporulation.

+ + + = Good sporulation.

+ + = Moderate sporulation.

+ = Scanty sporulation.

- = No sporulation.

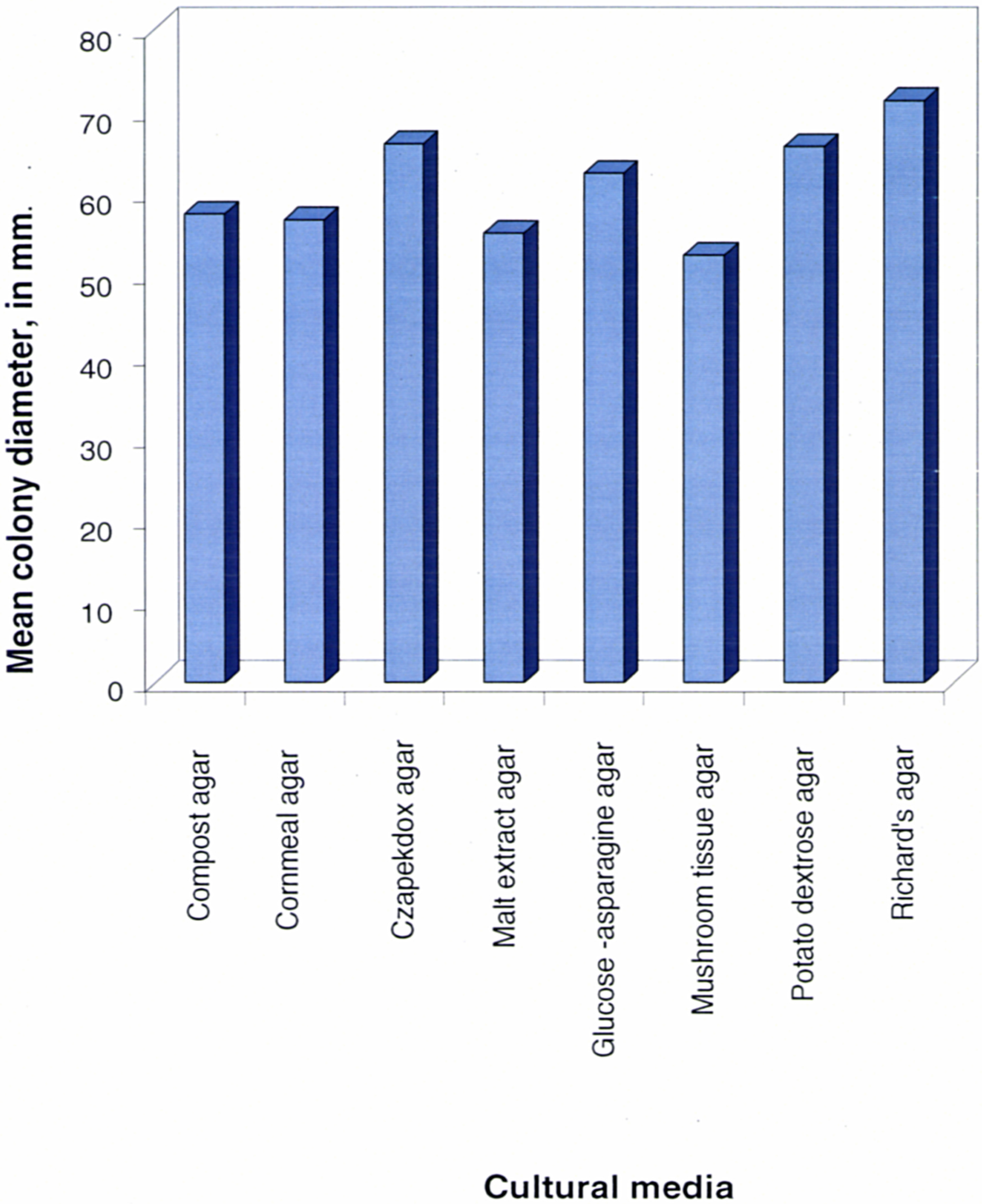

Fig.1. Growth of the fungus on different media

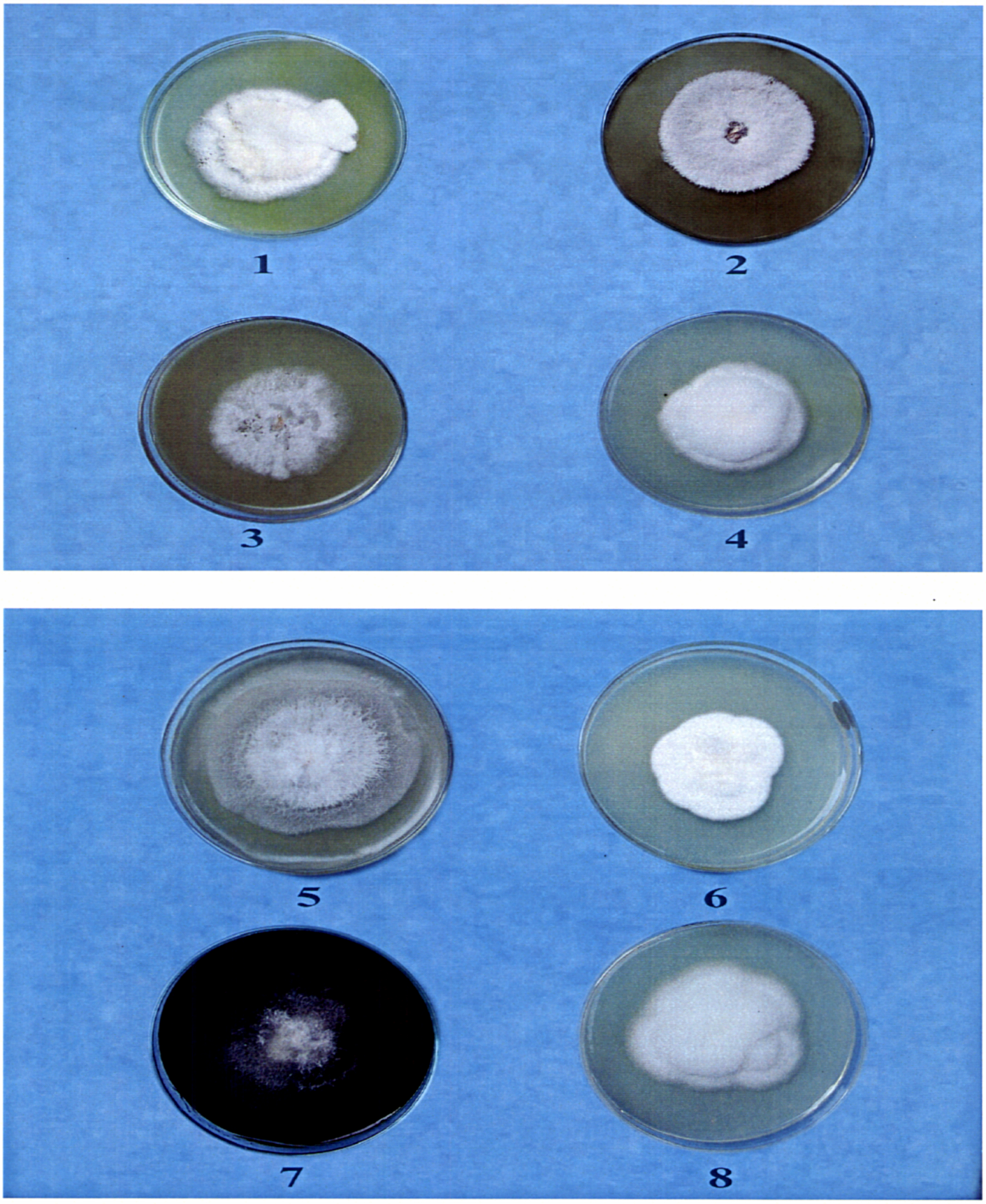

Plate 3. Growth characters of *Verticillium fungicola* on different media.

1. Glucose-asparagine agar.
2. Malt extract agar.
3. Corn meal agar.
4. Czapek-Dox agar.
5. Mushroom tissue agar.
6. Potato dextrose agar.
7. Compost agar.
8. Richard's agar.

4.5 Physiological Studies

4.5.1. Utilization of carbon compounds

The ability of the fungus to utilize different carbon compounds was studied to find out best carbon source used by the fungus under study. The observations recorded on dry mycelial weight after 25 days of incubation are presented in Table 4 and graphically presented in Fig.2.

From the results given in Table 4 and Fig.2, it is revealed that the fungus could luxuriantly utilize all the carbon compounds under study with the exception of D-xylose, sorbitol and citric acid. The carbon compounds *viz.,* sucrose, glucose, fructose, mannitol and starch were statistically superior and at par among themselves with the dry mycelial weight recorded to the extent of 1626.67 to 1376.33 mg. D-xylose and control yielded least mycelial dry weight. Further it was observed that carbon sources *viz.,* sucrose, glucose, starch and fructose induced good sporulation. Scanty sporulation was observed on sorbitol and D-xylose. Sporulation was not observed in citric acid and control treatments.

Table 4. Utilization of carbon compounds as reflected in growth and sporulation of the fungus

Sr. No	Carbon sources	*Mean mycelial dry weight (mg)	Sporulation
1	D-xylose	272.67	+
2	Mannitol	1402.33	++
3	D-fructose	1517.67	+++
4	Sorbitol	977.00	+
5	Citric acid	539.00	-
6	Sucrose	1626.67	+++
7	D-glucose	1605.33	+++
8	Starch	1376.33	+++
9	Control	64.00	-
	S.E. ± 1	88.45	
	C.D. at 5 %	262.42	

* Average of three replications

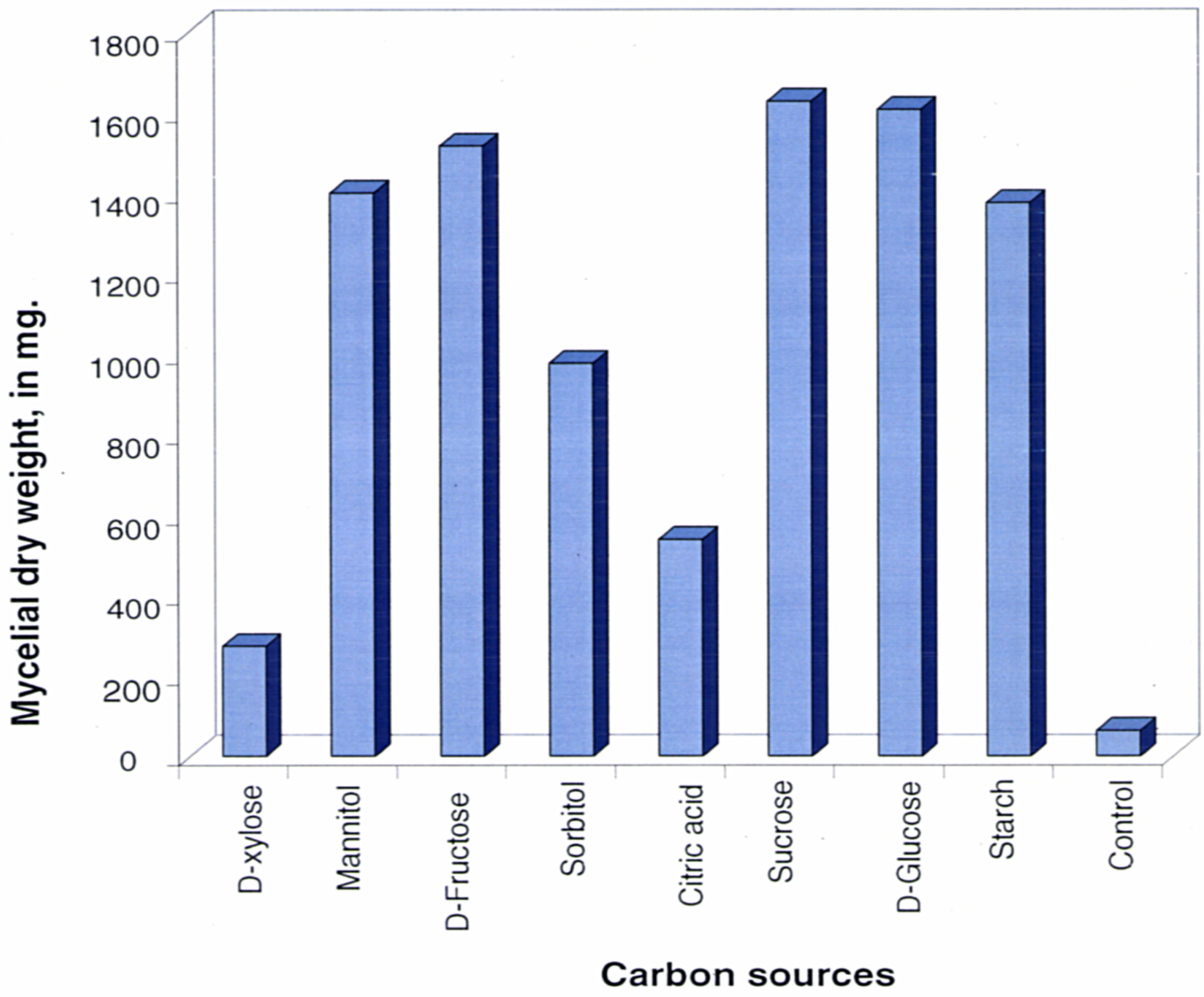

Fig.2. Effect of carbon compounds on growth of the fungus

4.5.2. Utilization of nitrogenous compounds

The mycelial dry weight in respect of various nitrogenous compounds is presented in Table 5 and also graphically shown in Fig.3.

It is evident from the results presented in Table 5 that the nitrogenous sources *viz.,* D-alanine, Urea, Ammonium phosphate, and L-asparagine significantly favoured the mycelial growth to the extent of 1588.67 to 1307.33 mg and were statistically superior to the rest of the compounds and at par among themselves. Poor mycelial growth was observed in sodium nitrite, D-methionine, Sodium nitrate and Potassium nitrate treatments.

Excellent sporulation was observed on D-methionine. Urea and D-alanine supported good sporulation of the fungus where as sporulation was scanty in case of sodium nitrate and L-asparagine. In Sodium nitrite and control treatments, no sporulation was observed.

Table 5.Utilization of nitrogenous compounds as reflected in growth and sporulation of the fungus

Sr. No	Nitrogen sources	*Mean mycelial dry weight (mg)	Sporulation
1	Sodium nitrate	682.67	+
2	Sodium nitrite	460.00	-
3	Potassium nitrate	887.67	++
4	Ammonium Phosphate	1332.33	++
5	D-alanine	1588.67	+++
6	L-asparagine	1307.33	+
7	D-methionine	627.67	++++
8	Urea	1419.00	+++
9	Control	207.67	-
	S.E. ± 1	133.41	
	C. D at 5 %	395.78	

* Average of three replications

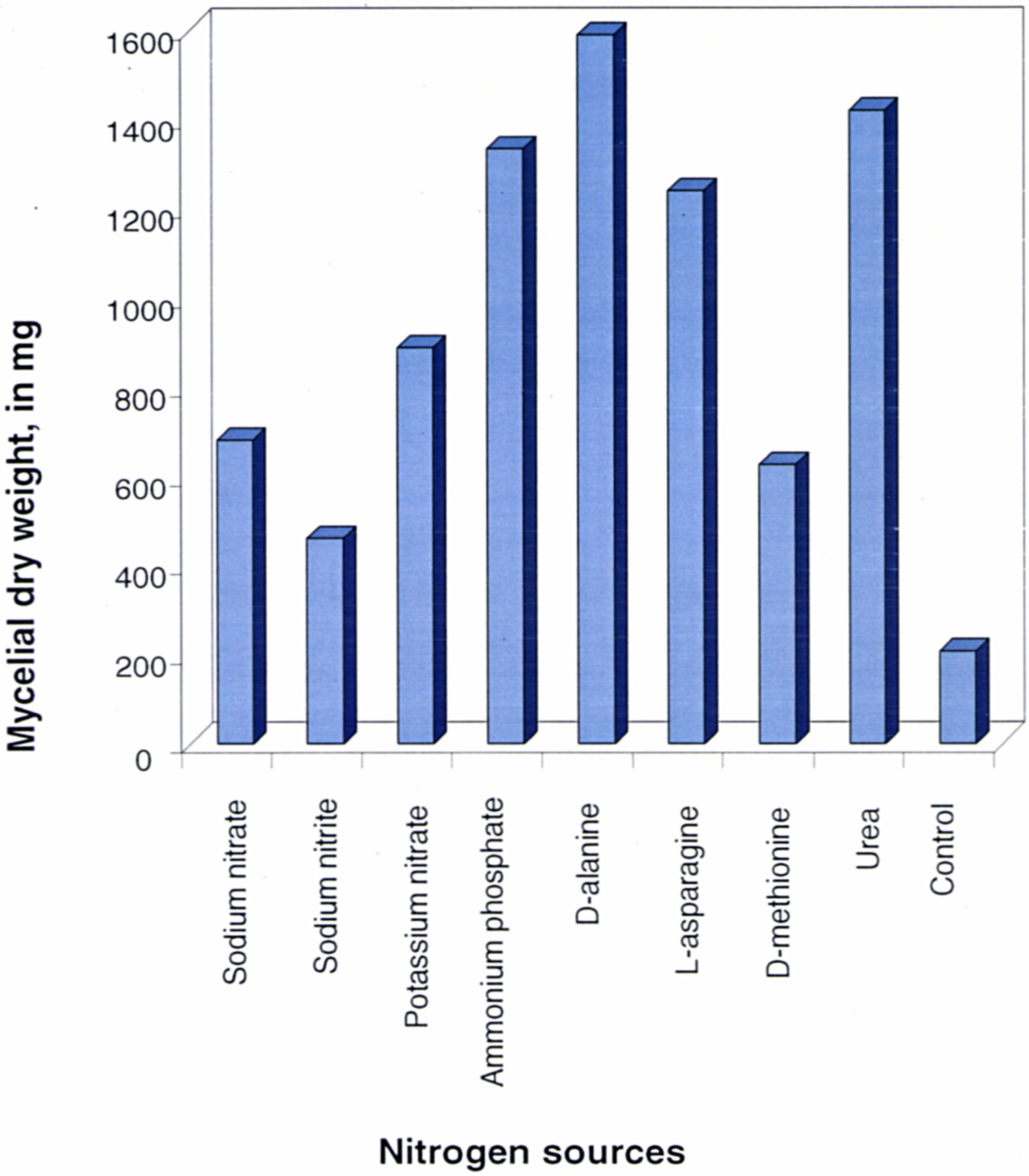

Fig.3. Effect of nitrogenous compounds on growth of the fungus

4.5.3 Effect of temperature

The studies were carried out to find out the maximum, optimum and minimum temperature requirement of the pathogen under study. The results of these studies are presented in Table 6.

Table 6. Effect of temperature on growth and sporulation

Sr. No.	Temperature (°C)	*Mean colony diameter (mm)	Sporulation
1	5	-	-
2	10	26.00	-
3	15	28.00	+
4	20	44.00	++
5	25	69.33	++++
6	30	34.00	++
7	35	11.67	-
8	40	-	-

* Average of three replications

It is revealed from the Table 6 that the fungus could grow between the temperatures 10 to 35^{0}C. However growth was not observed at 5 and 40^{0}C temperatures. Excellent growth (69.33mm) and sporulation was observed at 25^{0}C. The effect of temperature on growth is graphically presented in Fig. 4.

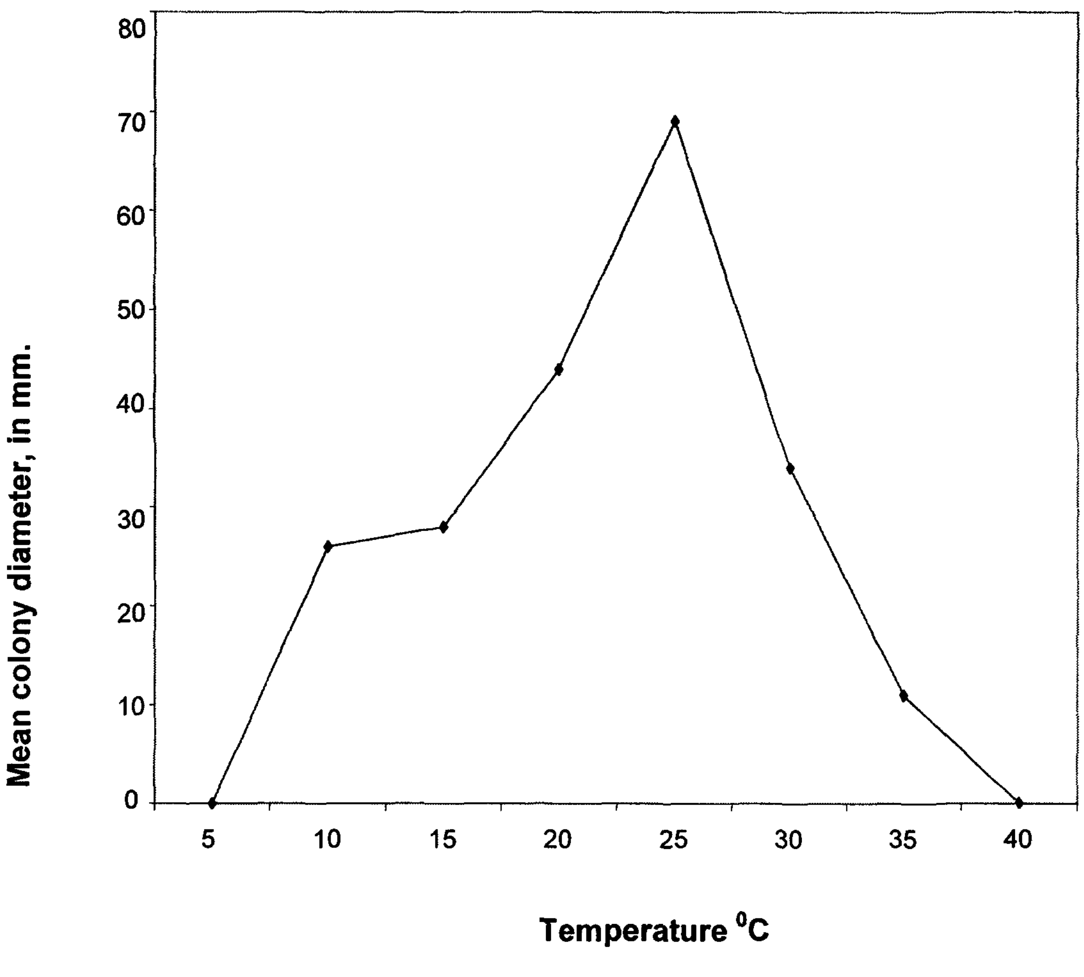

Fig.4. Effect of temperature on growth of the fungus

4.5.4 Effect of H-ion concentration

Results obtained from the present studies on effect of H-ion concentration on the growth and sporulation of fungus under study at different pH levels ranging from 2 to 8 are presented in Table 7 and shown in Fig.5.

A perusal of the Table 7 and Fig.5 revealed that the fungus could tolerate a wide range of pH 2 to 8. Significantly maximum mycelial growth (1140.33mg) was harvested at pH 6 followed by pH 5 with mean mycelial dry weight of 1112.00mg. The growth was retarded on either side of these optimum pH levels. Excellent sporulation was observed at pH 6. Good sporulation was supported by pH 5 , however sporulation was moderate in rest of the pH levels.

Table 7. Effect of H-ion concentration on growth and sporulation

Sr. No	pH	*Mean mycelial dry weight (mg)	Sporulation
1	2	140.00	+
2	3	252.33	+ +
3	4	644.67	+ +
4	5	1112.00	+ + +
5	6	1140.33	+ + + +
6	7	683.33	+ +
7	8	525.33	+ +
	S.E. ± 1	5.49	
	C.D. at 5 %	16.66	

* Average of three replications

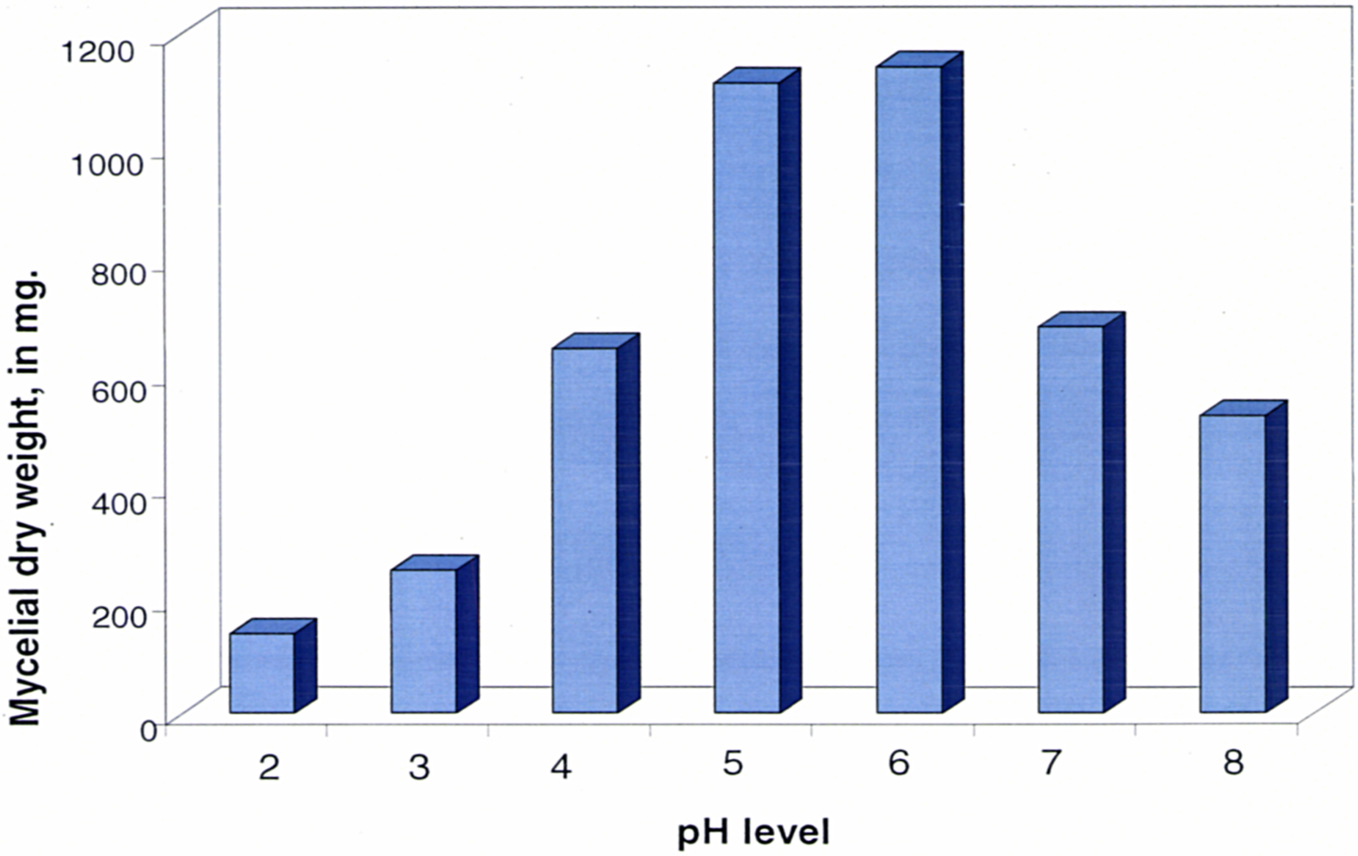

Fig.5. Effect of H-ion concentration on growth of the fungus

4.5.5 Spore germination

Observations on spore germination were recorded on seven different media at an interval of 2 hours up to 8 hours and the results are presented in Table 8.

It is evident from the data in the Table 8 that the spore germination initiated at two hours and maximum germination between 86 to 100 per cent was observed at 8 hours in almost all the substrates. The overall mean of spore germination percentage ranged from 63.00 to 85.50, maximum being in mushroom tissue extract (85.50 %) followed by sucrose solution (83.00 %) and potato dextrose broth (80.50 %).

Table 8. Effect of different substrates on spore germination

Sr. No.	Substrate	Per cent spore germination after				Mean
		2 hours	4 hours	6 hours	8 hours	
1	Distilled water	46	53	67	86	63.00
2	Sterilized water	49	67	83	90	72.25
3	Tap water	52	70	85	92	74.75
4	Sucrose solution (1 %)	61	79	92	100	83.00
5	Potato dextrose broth	55	77	90	100	80.50
6	Richard's solution	53	64	89	100	76.50
7	Mushroom tissue extract	65	81	96	100	85.50
8	Compost extract	63	74	84	100	80.25

4.5.6 Interaction studies

Button mushroom and the fungus under study were grown in dual culture to study the inhibitory effect of the pathogen on the host. The data obtained on growth and per cent inhibition of button mushroom and fungal mycelia are presented in the Table 9.

It is observed from the Table 9 that the fungus inhibited the mycelial growth of mushroom to the extent of 56.25 % at the end of 14 days of incubation. Where as mushroom fungus reduced the growth of pathogenic fungus by 4.58 per cent. Clear zone of inhibition was observed between the two fungi.

Table 9. Interaction studies on Host-Pathogen in dual culture

Incub. Period (Days)	*Mycelial growth of pathogenic fungus(mm)		*Mycelial growth of mushroom fungus (mm)		Per cent mycelial inhibition	
	Culture	Control	Culture	Control	Pathogen	Mushroom
2	13.50	13.50	8.50	8.50	-	-
4	23.50	23.50	10.00	10.00	-	-
6	36.00	36.25	11.25	13.37	0.68	15.85
8	45.00	46.25	12.00	21.50	2.70	44.18
10	47.00	48.50	12.37	23.50	3.09	47.36
12	50.12	52.00	13.50	29.25	3.61	53.84
14	52.00	54.50	14.00	32.00	4.58	56.25

* Average of four replications

4.6 Disease management studies

4.6.1 *In-vitro* evaluation of fungicides

In-vitro evaluation of four different fungicides and their combinations at different concentrations was undertaken in the laboratory to find out the effective fungicide and its efficient concentration against the dry bubble disease. It is revealed from the data presented in Table 10 that cent percent mycelial inhibition was observed in all treatments at their higher concentrations. However at lower concentrations of 0.15% for mancozeb and chlorothalonil and 0.075% for carbendazim and prochloraz, mancozeb + prochloraz treatment with 90.86% mycelial inhibition was statistically significant over other treatments. Next effective treatment was mancozeb + carbendazim with 88.32% inhibition. Chlorothalonil + prochloraz and chlorothalonil+carbendazim treatments were on par with each other with 86.29% and 85.79% inhibition respectively. Prochloraz spray was superior over carbendazim and mancozeb spray which differed significantly among themselves. Chlorothalonil with 77.66% mycelial inhibition was less effective among the treatments tried.

When concentrations were lowered to 0.10% for mancozeb and chlorothalonil spray and to 0.050% for carbendazim and prochloraz spray no significant variation was observed in behaviour of fungicides as compared to previous concentrations of 0.15 and 0.075% for contact and systemic fungicides used. Prochloraz + mancozeb spray was most effective with 84.77% mycelial inhibition and chlorothalonil was least effective with 60.91% inhibition. The *in-vitro* inhibition of the pathogen by various fungicides is illustrated in Plate 4.

Table 10. *In-vitro* effects of fungicides on growth inhibition

Sr. No	Fungicides	Concentration (%)	Mean Colony dia. (mm)	Per cent inhibition over control
1	Mancozeb	0.10	24.00	63.45
2	Mancozeb	0.15	13.67	79.18
3	Mancozeb	0.20	0.00	100.00
4	Chlorothalonil	0.10	25.67	60.91
5	Chlorothalonil	0.15	14.67	77.66
6	Chlorothalonil	0.20	0.00	100.00
7	Carbendazim	0.05	22.00	66.50
8	Carbendazim	0.075	12.67	80.70
9	Carbendazim	0.10	0.00	100.00
10	Prochloraz	0.05	18.00	72.59
11	Prochloraz	0.075	11.33	82.74
12	Prochloraz	0.10	0.00	100.00
13	Mancozeb+Carbendazim	0.10 + 0.050	13.00	80.20
14	Mancozeb+Carbendazim	0.15 + 0.075	7.67	88.32
15	Mancozeb+Carbendazim	0.20 + 0.10	0.00	100.00
16	Mancozeb+Prochloraz	0.10 + 0.05	10.00	84.77
17	Mancozeb+Prochloraz	0.15 + 0.075	6.00	90.86
18	Mancozeb+Prochloraz	0.20 + 0.10	0.00	100.00
19	Chlorothalonil+Carbendazim	0.10 + 0.05	15.00	77.15
20	Chlorothalonil+Carbendazim	0.15 + 0.0075	9.33	85.79
21	Chlorothalonil+Carbendazim	0.20 + 0.01	0.00	100.00
22	Chlorothalonil+Prochloraz	0.10 + 0.05	14.67	77.66
23	Chlorothalonil+Prochloraz	0.15 + 0.075	9.00	86.29
24	Chlorothalonil+Prochloraz	0.20 + 0.01	0.00	100.00
25	Control	-	65.67	0.00
	S.E.± 1		0.47	
	C.D. at 5 %		1.43	

4.6.2 *In -vivo* evaluation of fungicides

The fungicides and their concentrations found most effective under *in-vitro* conditions were evaluated for their efficacy under *in-vivo* conditions. The trial was laid out by giving three spray schedules of the fungicides at casing, 15 days and 30 days after casing. Per cent disease reduction and cumulative yield of healthy cut mushrooms were worked out as mentioned under materials and methods.

It is revealed from the data presented in Table 11 that disease incidence was significantly low (10.00%) in mancozeb + prochloraz treated bags. Mancozeb + carbendazim (13.89 %) and chlorothalonil + prochloraz (15.00 %) were next best treatments and were at par with each other. The other treatments resulted in varied disease incidence to the extent of 16.67 to 22.29 per cent. Chlorothalonil treatment was least effective with more disease incidence of 31.33 per cent. Correspondingly, the per cent reduction in disease incidence as worked out in comparison with control I (Inoculated-Unsprayed)was more with mancozeb + prochloraz (82.22 %) spray and less with chlorothalonil (44.30 %) spray.

Treatment with mancozeb + prochloraz spray significantly reduced disease incidence compared to treatment with mancozeb spray alone. No significant reduction in disease incidence was noticed between, mancozeb + carbendazim and mancozeb treatments. Chlorothalonil when sprayed alone recorded significantly more disease incidence as compared to its spray along with carebndazim and prochloraz.

Significant increase in yield of healthy mushrooms in different treatments was observed when compared with inoculated-unsprayed control. Mancozeb + prochloraz sprayed bags recorded maximum yield of 1616.67g and was statistically superior over other fungicide treatments. Next best treatments were mancozeb + carbendazim, chlorothalonil + prochloraz and prochloraz, which were at par with each other with yield to the extent of 1400.00-1333.33g. Chlorothalonil was less effective among the fungicides tried with yield of 1016.67g.

Per cent loss in yield of mushroom as worked out in comparison with control II i.e. un-inoculated un-sprayed check was low in mancozeb + prochloraz sprayed bags with percent yield loss 11.01 % and was statistically superior over rest of the treatments. Next best treatments were mancozeb + carbendazim (22.94 %) chlorothalonil + prochloraz (24.77 %), and prochloraz (26.61 %), which were at par with each other. The other fungicides treatments resulted in varying losses in yield ranging from 33.95 to 36.70 per cent. Chlorothalonil recorded highest yield loss of 44.03 per cent among various fungicides evaluated.

Table 11. *In-vivo* effect of fungicides on disease control and mushroom yield

Sr. No	Fungicides	Per cent disease incidence	Per cent disease reduction over control-I	Yield of healthy mushrooms in g/10 Kg Compost	Per cent loss in yield over control-II
1	Mancozeb	16.67 (24.04)*	70.36	1200.00	33.95
2	Chlorothalonil	31.33 (34.03)	44.30	1016.67	44.03
3	Carbendazim	22.29 (28.11)	60.37	1150.00	36.70
4	Prochloraz	21.42 (27.56)	61.92	1333.33	26.61
5	Mancozeb + Carbendazim	13.89 (21.83)	75.30	1400.00	22.94
6	Mancozeb + Prochloraz	10.00 (18.05)	82.22	1616.67	11.01
7	Chlorothalonil + Carbendazim	21.00 (27.25)	62.66	1166.67	35.78
8	Chlorothalonil + Prochloraz	15.00 (22.59)	73.33	1366.67	24.77
9	Control I (Untreated inoculated)	56.25 (48.60)		806.67	55.59
10	Control II (Untreated un-inoculated	-		1816.67	
	S. E. ± 1	1.39		40.16	
	C. D. at 5%	4.12		118.20	

*Arcsine transformed averages

DISCUSSION

5. DISCUSSION

Agaricus bisporus has been reported to be susceptible to various maladies caused by fungi, bacteria, nematode and viruses. Among them, dry bubble disease caused by *Verticillium fungicola* is very serious causing significant economic losses to mushroom growers. Dry bubble disease as originally described by Malthouse (1901) is one, which induces various degrees of button fruits discolouration and malformation. The yield loss due to this disease was recorded as much as 69.75 per cent (Bhatt and Singh, 2002).

In the present investigation detailed studies of the fungus in respect of survey and pathogenicity, its morphological, cultural, physiological characters and management were carried out and results obtained are discussed here under.

5.1 Survey

The incidence of the disease surveyed varied with the farms surveyed and mushroom strains used; it ranged between 2.5 to 13 percent. Maximum incidence of 13% was recorded in Nutan Mushrooms, Pune. The incidence of dry bubble in a survey report has been recorded to be 25-50 % at Solan and Kasauli and up to 15% at Shimla and Chail (Thapa, 1985). Bhatt and Singh (2002) have also reported 2.17 to 25.00 % incidence of *Verticillium fungicola* in North India.

5.2 Isolation, Pathogenicity and Symptomatology

The organism *Verticillium fungicola* (Preuss) Hassebr. was isolated on potato dextrose agar. Pathogenicity was established on *Agaricus bisporus* by inoculating the casing soil and by spraying spore / mycelial suspension on pinheads. The characteristic disease symptoms *viz.,* brown spot, stipe blast, dry bubble and harelip symptoms were produced in the inoculated bags. The fungus re-isolated from the infected portions was identical to that of the original isolate. Per-cent infection of fruiting bodies upon artificial inoculation was 46.23% in casing inoculation and was 32% when inoculated on pinheads. Similar type of symptoms and pathogenic nature of the organism were also observed by Ware (1933), Cross and Jacobs (1969) Holmes (1971) Seth *et al.* (1973), North and Wuest (1993), Cedeno and Carrero (1997).

5.3 Morphological studies

In the present investigation, morphological characters of the fungus such as size, shape, septation, colour of conidia, conidiophores and size of mycelium were studied as diagnostic characters for identification of causal organism under study. These characters are of primary importance in delimiting species of the genus *Verticillium.* The Mycelium of the fungus under study was found irregularly branched, septate, measuring 1-3 μm in width. Conidiophores were erect, septate, verticillately branched and measured 10-200 μm × 1.5-5.0 μm. Conidia were observed single celled, hyaline, oblong to cylindrical in shape, occasionally fusoid with obtuse ends and were produced in gelatinous matrix and appeared like conidial balls.

Conidiospores were measured 8-10 µm long by 1-3 µm wide and tapering to 0.5-1.0 µm at the tip. The morphological characters of the pathogen under study were more or less in agreement with the observations of Ware (1933), Treschow (1941) and Seth *et al.* (1973) who described the species from India. Thus, the pathogen under study was identified as *Verticillium fungicola* (Preuss) Hassebr. the causal agent of dry bubble disease of white button mushroom.

5.4 Cultural studies

Cultural behaviour of the fungus was studied on different media. Based on the radial expansion, it was observed that the fungus could prefer wide range of media for its growth. The fungus grew well on Richard's agar, Czapek-Dox agar, Potato dextrose agar and Glucose-asparagine agar in the order of merit. An ancillary observation on sporulating ability of the fungus in different media revealed that the media *viz.,* Richard's agar media and Mushroom tissue agar induced excellent sporulation, where as Potato-dextrose agar, Czapex-Dox agar, Malt-extract agar and Glucose asparagine agar supported good sporulation. The better growth and sporulation in Richard's agar may be attributed to high carbon nutrition as compared to other media. The results obtained in the present study are confirmed with the findings of Thapa (1985), the only earlier work available in the literature.

5.5 Physiological studies

5.5.1. Utilization of carbon compounds

Carbon compounds play an important role in the metabolism of fungi for their growth and reproduction in comparison with single sugar. Among eight different carbon sources tested, maximum growth was observed to be supported by sucrose followed by D-glucose, D-fructose, mannitol and starch in decreasing order, which did not differ significantly amongst each other. Least growth was favoured by D- xylose, sorbitol and citric acid. Excellent sporulation was noticed in sucrose. The results so reported are in conformity with the earlier reports of Coetze *et al.* (1991) and Thapa (1985).

5.5.2. Utilization of nitrogenous compounds

The dry bubble fungus was able to metabolize a variety of nitrogenous compounds when grown in a chemically defined medium in surface culture. The amount of growth varied with the nitrogen sources tested during present study. Among these D-alanine, urea, ammonium phosphate and L-asparagine produced excellent growth of the fungus. The fungus grew sparsely on sodium nitrite, D-methionine sodium nitrate, potassium nitrate and the basal medium lacking in nitrogen. D-methionine induced excellent sporulation. Good sporulation was supported by urea and D-alanine, where as sporulation was not observed in sodium nitrite and control treatments. While studying on physiological aspects of *V. fungicola*, Coetze *et al.* (1991) reported similar results and observed good growth in all nitrogenous sources.

5.5.3 Effect of temperature

In the investigations on effect of temperature on growth and sporulation of the pathogen under study, it was observed that it could grow between the temperature range of 10 to 35^{0} C. Maximum mycelial growth and sporulation was recorded at temperature 25^{0} C, indicating thereby the optimum temperature requirement of the fungus *Verticillium fungicola*. The results obtained were in the agreement with earlier reports of Jandaik and Guleria (2002) who recorded temperature of 23^{0} C as optimum for growth of *V. fungicola.* Coetzee *et al.* (1991) also reported 22-24^{0}C as optimum temperature.

5.5.4 Effect of H-ion concentration

The fungus under study tolerated a wide range of pH 2 to 8. The optimum pH range was found to be 5 to 6, since maximum fungal growth was recorded at this pH. Excellent sporulation was noticed at pH 6. Jandaik and Guleria (2002) observed pH 6 as optimum for growth and sporulation of the fungus. Thapa (1985) recorded maximum growth at pH 5.5 and heavy sporulation in pH range of 5.0 and 6.0. The results obtained in the present investigation are in agreement with the reports of above authors.

5.5.5 Spore germination

In the studies on spore germination of the fungus in different substrates it was observed that germination could initiate after 2 hours and 100% germination was noticed after 8 hours in almost all the substrates, except distilled water, sterile water and tap water. Maximum spore germination was recorded in mushroom tissue extract followed by sucrose solution and potato dextrose broth. These results are in resemblance with the findings of Wuest and Forer (1975) and Thapa (1985).

5.5.6 Interaction studies

Interaction of *Agaricus bisporus* and *Verticillium fungicola* in dual culture on Richard's agar medium revealed that *V. fungicola* growth was aggressive as compared to *A. bisporus*. The growth of *A. bisporus* was inhibited up to 56.25 % as compared to 4.58 % inhibition of *V. fungicola*. Clear zone of inhibition was observed between the two fungi. This indicate the release of toxins by the pathogen. Similar findings were also reported by Vijay and Gupta (1992) and Thapa (1985).

5.6 Disease management studies

Successful cultivation of crops necessitates the use of suitable varieties, supply of inputs, improved package of practices, efficient management and protecting the crop from the onslaught of pests and diseases that defer the cultivator from deriving benefits from the economically important crops, leading to economic losses. At present various fungicides are available in the market for plant disease control. The management of diseases of mushroom poses problems because both the host and the parasites are fungi. To overcome this problem there is a need to select those fungicides, which have selective toxicity against the pathogen and least effect on the growth of the mushroom. The *in-vitro* and *in-vivo* results of fungicides are discussed here under.

5.6.1 *In -vitro* evaluation of fungicides

In *in-vitro* efficacy studies four fungicides having different chemical base and their combinations were tested at three dose rates, based on available information relating to other crops. Present results showed that, cent per cent disease inhibition was observed in all treatments at their higher concentrations. Combination of Prochloraz + mancozeb treatment was observed to be superior over other treatments and showed pronounced inhibition of the pathogen to the extent of 84.77% and more at lower concentrations. Among the combinations of fungicides, evaluated mancozeb combinations were superior to chlorothalonil combinations while Chlorothalonil used alone was the least effective fungicide treatment. These results show a good correlation with earlier *in-vitro* studies of Samuels and Johnston (1980), Gea *et al.* (1997), Bhatt and Singh (2002) and Tokousbalides *et al.* (2006).

5.6.2 *In- vivo* evaluation of fungicides

Fungicidal evaluation studies on mushroom crops infected with *V. fungicola* were undertaken using the fungicides at concentrations found effective under laboratory studies. Four fungicides and their combinations were evaluated and prochloraz + mancozeb spray consistently gave the best results with 82.22% reduction in disease incidence and yield of 1616.67g. The fungicides spray in combinations had significant effect in reducing disease incidence and increase in yield as compared to their spray alone. Carbendazim and prochloraz in combination with gave significantly higher yields and disease reduction as compared to their combination with chlorothalonil. Chlorothalonil spray alone was the least effective treatment with only 44.30% reduction in disease. The above results are in conformity with the earlier reports of Zaayen (1979), Gandy and Spencer (1981), Russell (1984), Eicker (1987), Bonnen and Hopkins (1997), Coutinho *et al.* (2000), Grogan *et al.* (2000) and Sharma and Jandaik (2003).

SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

Studies on dry bubble disease of the cultivated button mushroom *Agaricus bisporus* (Lange.)Sing. incited by *Verticillium fungicola* (Preuss) Hassebr. were undertaken and results obtained are summarized as under.

The incidence of the disease varied with different farms and ranged between 2.5 to 13 per cent. Maximum incidence was recorded in Nutan Mushrooms, Pune on U-3 strain.

The fungus proved to be pathogenic on *Agaricus bisporus* and expressed the characteristic brown spot, stipe blast, harelip and dry bubble symptoms on mushrooms. Morphologically the pathogen was found to be identical to *Verticillium fungicola* (Preuss) Hassebr.

Among the eight culture media used, significantly good growth and sporulation was supported by Richard's agar followed by Czapek-Dox agar, Potato dextrose agar and Glucose-asparagine agar.

Among the carbon sources, sucrose, D-glucose, D-fructose, mannitol and starch, favoured maximum growth. The sources of nitrogen *viz.*, D-alanine, urea, ammonium phosphate and L-asparagine supported best growth of the fungus. Excellent sporulation was induced by D-methionine, urea and D-alanine.

The minimum, optimum and maximum temperature requirement of the fungus was found to be 10, 25 and 35^{0}C respectively. The fungus could tolerate pH 2 to 8 but optimum for growth being 5 to 6. Spore germination was favoured in different substrates tried but more preferential being in mushroom tissue extract (85.50 %) followed by 1% sucrose solution (83.00 %) and potato dextrose broth (80.50 %). In dual culture of *A. bisporus* and *V. fungicola*, growth of *A. bisporus* was inhibited by 56.25 % as compared to 4.58 % inhibition of *V. fungicola*.

When various fungicides with different concentrations were amended in the solid medium, cent percent inhibition was observed in all treatments at their higher concentrations. Prochloraz + mancozeb treatment was superior over other treatments and showed pronounced inhibition of the pathogen to the extent of 84.77% and more at lower concentrations. Among the combinations of fungicides, evaluated mancozeb combinations were superior to chlorothalonil combinations while Chlorothalonil used alone was the least effective fungicide treatment.

Out of four fungicides and their combinations evaluated under *in-vivo* conditions prochloraz + mancozeb spray consistently gave the best results with 82.22% reduction in disease incidence and yield of 1616.67g. The fungicides spray in combinations had significant effect in reducing disease incidence and increase in yield as compared to their spray alone. Carbendazim and prochloraz in combination with mancozeb gave significantly higher yields and disease reduction as compared to their combination with chlorothalonil. Chlorothalonil spray alone was the least effective treatment with only 44.30% reduction in disease.

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