

**CHEMICAL CONTROL OF 'BROWN SPOT'
DISEASE OF OYSTER MUSHROOM
CAUSED BY *Pseudomonas stutzeri*
(Lehmann and Neumann) Sijderius.**

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**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE**

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N. EARANNA B.Sc., (Agri.),

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CERTIFICATE

This is to certify that the thesis entitled "CHEMICAL CONTROL OF 'BROWN SPOT' DISEASE OF OYSTER MUSHROOM CAUSED BY Pseudomonas stutzeri (Lehmann and Neumann) Sijderius" submitted in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY to the University of Agricultural Sciences, Bangalore, is a record of bona-fide research work carried out by Mr. N.EARANNA under my supervision and guidance and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

BANGALORE,
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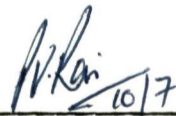
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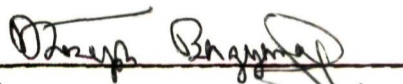
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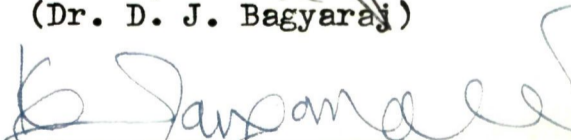
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GKVK, BANGALORE,

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(N. EARANNA)

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INTRODUCTION

I INTRODUCTION

India is the world's second most populous country with a population of 683.81 million as per the census of 1981. Due to population explosion the problem of protein hunger will become more and more acute. Animal protein is beyond the reach of low income group which forms a larger proportion of country's population. Under the prevailing circumstances all possible sources of protein production will have to be exploited to save the country from acute malnutrition and protein hunger. Mushrooms, yeasts and algal foods are frequently mentioned as alternative sources of food. Of these, mushrooms are the most preferred resources of food, its cultivation is acquiring greater importance both in developed and developing countries.

Mushrooms are epegiuous and hypogeous fleshy fruiting bodies of the fungi belonging to certain ascomycetes and basidiomycetes (Chang and Hayes, 1978). They are rich in protein (21-30% on dry weight basis), essential amino acids, vitamins, minerals, carbohydrates and fats (Sohi, 1986; 1988).

Of several cultivated mushroom varieties, oyster mushroom gains importance with a world production of 15,000 tonnes per annum (Shanmugam, 1986). Oyster mushroom can flourish successfully on a wide variety of undecomposed organic substrates like cereal stalks, banana leaves, saw dust and textile wastes that are inexpensive. The leading countries

producing oyster mushrooms are : Japan, Taiwan, Italy, France, Philippines, Thailand and India.

At present oyster mushroom is becoming popular in India, because of its simple production technology. This mushroom also faced with problems of pests and diseases causing economic yield loss in mushroom production. Important diseases reported on mushrooms are brown plaster mould, white mould, dry bubble, green mould, wet bubble, bacterial spot, bacterial blotch, mummy disease, etc. and of late brown spot of oyster mushroom substrate (Mallesha and Shivappa Shetty, 1988).

'Brown spot' disease of oyster mushroom caused by Pseudomonas stutzeri (Lehmann and Neumann) Sijderius was found to cause yield reduction upto 60 per cent. The pathogen was found resistant to pasteurization. However, autoclaving of the substrate was found to be effective to control the disease but considered uneconomical.

The chemical methods are always found priority over others to control most of the microbial diseases. The principles of chemotherapeutic control of human pathogens have been applied for all kinds of microbial pathogens including viruses (Rangaswami, 1954). Various antimicrobial chemicals like Formalin, Chlorine, Benzoic acid, Antibiotics, etc. were used for the control of many kinds of microbial diseases of plants including mushrooms (Van De Geijn, 1982; Lelley, 1984; Wong and Preece, 1985). Therefore, present investigation was

carried out by using antimicrobial chemicals like Antibiotics and Formalin with the following objectives :

1. To screen effective antimicrobial chemicals against "brown spot" disease.
2. To determine effective concentration of the chemical against brown spot disease.
3. To devise effective method of treatment of paddy straw to control the brown spot disease.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

1. Historical developments in mushroom cultivation

Naturally growing mushrooms are collected and used as vegetable by mankind from time immemorial. However, authentic record available about the cultivation of temperate mushroom (Agaricus bisporus), whose cultivation was started in Paris (France) region around 1650 by melon growers in open. Cultivation in green houses was started in England in the 18th century followed by cultivation in caves during middle of 19th century (Munjal, 1982).

In 1894, Costantin and Mathruchoth obtained pure culture from mushroom spore for the first time. Method of spore germination was standardized by Ferguson (1902). Tissue culture from stipe tissue of Pleurotus ostreatus and Agaricus bisporus on corn meal extract, potato and sugarbeat medium was obtained (Duggar, 1905). A method for compost preparation based on nitrogenous fertilizer and wheat straw was developed (Hutchinson and Richards, 1921). Lambert (1941) developed new indoor composting method which resulted in termination of outdoor composting.

The zonal system of indoor cultivation consisting of pasteurization, spawnrun, holding and composting rooms for indoor cultivation of mushrooms were introduced by Sinden (1947). Edwards (1954) developed a formula to grow mushrooms

by using dried blood. Later, application of superphosphate, gypsum, molasses and organic nitrogen was attempted (Bech and Rasmussen, 1968; Hayes and Randle, 1969).

2. Important cultivated varieties of mushrooms

There are several varieties of edible mushrooms that are reported from different parts of the world. Of which commercially important ones are, white button mushroom (Agaricus bisporus), black forest mushroom (Lentinus edodes), paddy straw mushroom (Volvariella volvaceae) and oyster mushroom (Pleurotus sp.) (Chang and Hayes, 1978).

3. Oyster mushroom cultivation

Oyster mushrooms are well known edible fungi and popularly known as 'Dhingri' in Hindi. The genus Pleurotus belonging to the family 'Tricholamataceae' under the order agaricales of the class basidiomycetes. Seven species of oyster mushrooms are presently being cultivated either on commercial scale or experimental scale (Zadrazil, 1978). The cultivated species of oyster mushrooms are Pleurotus ostreatus, P. florida, P. eryngii, P. cornucopiae, P. flabellatus, P. citrenopeleatus and P. sajor caju (Zadrazil, 1978).

In India, P. sajor caju (Fr.) Singer was first time reported by Bagchee in 1954 (Jandaik and Kapoor, 1975a). It was found growing on the dead wood of 'Sal trees' (Shorea robusta) in north and central Indian forests. Bagchee

described it as Lentinus sajorcaju Fr. but later Singer (1951) renamed it as Pleurotus sajorcaju. Jandaik and Kapoor (1975a) found Pleurotus sajorcaju growing on decaying plants Euphorbia roylena in foot hills of Himalayas. It was brought into pure culture by transferring a bit of trammal tissue on potato dextrose agar slants. It failed to grow on woody material, indicating that it was different from Bagohee's strain.

4. Growth on synthetic media

Oyster mushroom owing to the versatility in utilizing a wide range of carbon and nitrogen sources is easily cultured in laboratory conditions (Zadrazil, 1978).

Glucose, fructose, mannose and xylose produced greatest hyphal growth of Pleurotus sp. (Koch, 1958). Eger (1970) investigated the utilization of polysaccharides and effect of various amino acids and vitamins on growth of oyster mushroom. The effect of different lipids on oyster mushroom was studied by Kurtzman (1974). Superior growth of P. sajorcaju was observed on potato dextrose agar fortified with yeast extract (Jandaik and Kapoor, 1975b).

5. Spawn production

Spawn is a word used to denote mushroom seed material which is a medium impregnated with mycelium. Initially fermented manure and tobacco stems were used as substrates

for spawn production in case of Agaricus bisporus. In 1931 Sinden introduced cereal grains as substrate for spawn production (Kligman, 1950).

Go (1959) experimentally cultivated paddy straw mushroom by using coffee pulp spawn. A new type of spawn called 'Perlite spawn' was developed by Lemke (1971) and further in 1972 he proposed a formula for spawn production which is widely practised at present. This method emphasizes 50 per cent moisture content in the grain and a pH of 6.5 to 7.5 for better mycelial growth in the bottle. Application of gypsum and calcium carbonate separately was tried by Kumar et al. (1975). Krishnamoorthy (1981) reported the maintenance of 60 per cent moisture in jowar grains and autoclaving of spawn bottles at 15 PSI (121°C) for 30 minutes are required for successful spawn production.

6. Substrate and cultivation

Pleurotus species have been grown on tree stumps at the beginning of 20th century (Falck, 1917; 1919 and Passeeker, 1959). Various substrates are used for the cultivation of Pleurotus sp. Large scale Pleurotus on paddy straw was first suggested by Bano and Srivastava (1962). Jandaik and Kapoor (1976) used banana pseudo stem to cultivate oyster mushrooms. Nirwan and Upadyaya (1978) used mango saw dust, rice polish and rice husk in the proportion of 9:2:2 and

CaCO₃ at 0.3 per cent for P. ostreatus cultivation. Use of waste from sugar and dairy industry for cultivation of Pleurotus sp. was discussed by Morozova et al. (1978). Bukhalo et al. (1978) suggested the substrate like potato waste, molasses, whey and cotton waste for commercial cultivation of Pleurotus ostreatus. Pleurotus sajorcaju produced well developed fruiting body on pasteurized straw with amendments (Zadrazil, 1979). Kandaswamy and Sivaprakasam (1980) used various wastes like blackgram pod, hulled maize cob, paper, rice bran and wood shaving as substrates for cultivation. Lozovoi (1980) reported 20-30% increased yield under glass house condition on saw dust as a substrate. Harsha et al. (1981) reported the utilization of tea leaves and paper as substrates for cultivation of Pleurotus ostreatus. Higher yield of Pleurotus sajorcaju was obtained with paddy straw as a substrate (Chang et al., 1981; Prabhu Dessai, 1982; Krishnakumari, 1982). Bano and Rajarathnam (1982) obtained higher yield in rainy season by supplementation of protein from different sources. Combination of cotton straw and wheat straw at 1:1 proportion was used for the cultivation of Pleurotus sp. (Silanikove et al., 1988).

7. Factors influencing growth and fruit body formation

(a) Temperature

Zadrazil and Schneiderik (1972) worked out the temperature requirement of Pleurotus ostreatus, P. florida

and P. eryngii. Each species has specific optimum temperature requirement. Jandaik and Kapoor (1975b) reported the temperature requirement of P. sajorcaju between 20°C and 30°C and the optimum growth was observed at 25°C.

(b) pH

The effect of changes in pH on the mycelial growth and development of fruiting body of Pleurotus sp. was reported by Zadrazil and Schneiderik (1972). Jandaik and Kapoor (1975b) worked out the precise pH requirement for P. sajorcaju which grew in a range of 4-8, pH 5.6 being the optimum.

(c) Moisture

Moisture has a marked influence on the growth of mycelium and development of fruiting bodies. The substrate should have around 70 per cent moisture throughout the cropping period, whereas humidity during cropping should be between 80-90 per cent (Zadrazil, 1973 and Krishnamoorthy, 1981).

(d) Light

The effect of light was also considered important in the cultivation of oyster mushroom. In Pleurotus florida more number of primordia was found with increase in duration of intensity of light (Eger, 1970; Eger et al., 1974). Normal fruiting bodies were formed at light intensity of 400 lux and earlier fruitification with continuous white fluorescent light by about 400 lux and the temperature of 12°C, in

case of P. ostreatus on straw and wood (Poppe, 1973). Light being an initiating factor in the development of primordia, lighting is necessary atleast for 15 minutes per day for oyster mushroom (Zadrazil, 1975).

CO₂: Carbon dioxide requirement of bacteria, yeast and fungi has long been known (Valley and Rettger, 1927; Herman, 1963). Removal of CO₂ from the ecosystem can cause decrease in growth of microorganisms (Lafferty, 1963). Beneficial effect of CO₂ on P. florida, P. eryngii and P. ostreatus was observed by Zadrazil (1974). He concluded that upto 18 per cent of CO₂ in atmosphere had a stimulatory effect on mycelial growth of Pleurotus ostreatus. This was not true in case of Agaricus bisporus (Tschierpe, 1959; 1972). Reid et al.(1982) observed rapidity of lignin degradation by several white rot fungi in an atmospheric O₂ than in air.

8. Substrate microflora

Studies have been done on the beneficial and harmful effect of substrate associated with microflora on the growth and yield of cultivated mushrooms. The studies of Stanek (1969; 1972) showed that compost bacteria would synthesize vitamins, amino acids and produce polysaccharides which were responsible for better growth of Agaricus bisporus mycelium. Chanter and Spencer (1974) discussed the importance of thermophilic bacteria in compost fermentation of white button

mushroom. Quantitative and qualitative study of compost microflora was carried out and reappearance of actinomycetes groups at the final stages of composting and it could be used to diagnose the compost maturity (Dodyleya et al., 1976). Microbial associates and their products served as sources of nitrogen, carbon and minerals for button mushroom production (Fermor and Wood, 1981). Occurrence of higher microbial associates during early spawn run stage correlated with oyster mushroom yield (Krishnamoorthy, 1981). He identified majority of the beneficial associates belonged to Bacillus sp. and speculated a beneficial effect in oyster mushroom production. Prabhu Dessai (1982) also agreed with the speculation of Krishnamoorthy (1981) that higher the associative microbial population at an early spawn run stage gave increased yield in the case of paddy straw substrate.

Harmful effects of associated microflora on mushrooms have been reported at different stages of cultivation. The associates cause various types of losses in mushroom production either as pathogen or as associative competitors (Chang and Hayes, 1978).

9. Diseases, pests and competitors of mushrooms

Diseases and pests are the great maladies to mushroom industry. The term disease refers to any disturbance which interferes with normal structure, function or economic value of host. A summary of principal pests, pathogens and fungal competitors have been reported.

(a) Bacterial diseases

Kligman and Penny (1943) reviewed the details of mushroom diseases like mummy disease caused by Pseudomonas sp. and bacterial pit caused by Pseudomonas tolaasii in sporophore tissue of Agaricus bisporus. Schisler et al. (1968) isolated a species of Pseudomonas from mushroom affected by mummy disease and the inoculation of pure culture of this bacterium on healthy mushroom mycelium at spawning time, produced the disease. The toxin as well as killed cells of Pseudomonas tolaasii inhibited button mushroom mycelial growth at spawnrun stage (Nair and Fahy, 1973). Drippy gill, a bacterial disease caused by Pseudomonas sp. (Young, 1970; Oriordain, 1976).

Soil-borne bacterium belonging to genus Pseudomonas developed saprophytically and later parasitized on tissue of mushroom fungus (Vedder, 1978). The disease causing Pseudomonas attached themselves rapidly and firmly to Agaricus bisporus mushroom fungus compared to other bacteria (Preece and Wong, 1982). Yellowing of cultivated Pleurotus ostreatus and P. eryngii caused by Pseudomonas tolaasii frequently caused low yield (Ferri, 1985). Alan et al. (1985) reported that yellow blotch disease caused by Pseudomonas sp. and mushroom characterized with yellow droplets on the surface primordia further became stunted and deformed as the mushroom became matured. Mallesha (1987) studied the new brown spot disease of oyster mushroom which caused economical losses to the

mushroom farmers. The causal organism of the new brown spot disease was identified as Pseudomonas stutzeri (Mallesha and Shivappa Shetty, 1988).

(b) Fungal diseases and competitors

Verticillium malthousii caused severe losses of mushroom crops and appeared to be an endemic disease (Sinden, 1971). Hussey (1972) pointed out the spread of the disease from diseased mushroom through phorid flies. In button mushroom spawn production, fungal contaminants were found more serious (Bitner, 1973). Tatsuziro Ito (1978) reported disease causing organisms like Mycogone, Verticillium, Trichoderma, in paddy straw mushroom (Volvaria sp.). Dry bubble disease of oyster mushrooms caused by Verticillium fungicola was reported in California for the first time (Marlowe and Romaine, 1982; Thapa and Jandaik, 1984). Symptoms and measures against Mycogone sp., Verticillium sp. and Chaetomium sp. were discussed by Figueiredo and Mucci (1985).

Various fungal competitors like brown plaster mould (Myriococcum praeax), white plaster mould (Scopularopsis funicola), green mould (Trichoderma sp. and Penicillium sp.), olive green mould (Chaetomium olivaceum) and Coprinus sp. were reported by Rolf Singer (1961) and Van De Geijn, 1982).

(c) Viral diseases

Since the first description of an infectious diseases of cultivated mushrooms by Sinden and Hauser in

Pennsylvania, mushroom disorders have been observed and various names like LaFrance, brown disease and watery stipe, x-disease and die back disease were given to these disorders due to the variety of symptoms observed and the difficulty in determining their causes (Chang and Hayes, 1978). The observation of virus transmission through mushroom spores by Schisler et al.(1967) was a vital factor in understanding of the etiology of mushroom virus diseases (Chang and Hayes, 1978). Hill and Wright (1979) reported the relationship between mycelial growth rate and infection of mushroom virus four particles. Frost and Passmore (1980) produced evidence about the number of different viruses in mushrooms and suggested that virus like particles in mushroom may be similar to the killer particles in yeasts and smuts.

(d) Insect pests

Pests associated with mushroom crop such as phorids, sciarids, cecids, mites and nematodes were reported by Chang and Hayes(1978).

Wyatt (1959) reported cecid fly Mycophila speyri (Barnes) attaining pest proportions in mushroom beds more quickly than other cecid fly (Heteropoza pygmaea Winnertz.) because of shorter life cycle and larger family size. Lin and Ni (1974) described Heteropeza pygmaea to be a destructive pest of Agaricus bisporus in Taiwan. Shandilya et al.(1975) described phorids as one of the most important pests of mushrooms

in India. Sciarid fly (Bradysia tritici) was reported to cause 32.7 per cent infestation of the mushroom beds in Punjab (Sandu and Brar, 1980).

(e) Nematodes

Mushroom nematodes are posing a great threat to mushroom farms because they multiply rapidly under congenial conditions. Cayrol (1962) reported Ditylenchus myceliophagus, Aphelenchoids compositicola, A. lineberi, A. winchesi as parasites of mushroom crops in France. Bhardwaj et al. (1972) recorded heavy infestations of mushroom crop with Aphelenchoides and Ditylenchus species. Pleurotus sajorcaju was found resistant compared to the Agaricus bisporus against nematode infection (Thapa and Sharma, 1987).

(f) Rats

Rats are found eating spawn grains at early stages and at later stage they also damage the stipes and caps.

10. Management of diseases and pests in mushroom industry

Diseases and pests of mushrooms can be controlled by various methods such as cultural, mechanical, physical, biological and chemical methods.

(a) Cultural methods

Proper sanitation in mushroom house and its surroundings is prerequisite for avoiding pests and diseases. Therefore,

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maintenance of hygienic conditions, proper composting and rapid filling into beds, periodic washing of area with disinfectants are methods to minimize the fly infestations (Thomas, 1959; Morishita, 1960; Snetsinger, 1965; Kneeboie, 1968). Use of selective compost prepared by adding substrates like soybean, cotton seed and oat meal was reported to provide control (Binns, 1975).

(b) Mechanical methods

Preventive measures must be used by using air filters, mechanical traps and suitable sealing doors, and ventilators. The most successful method of controlling flies in crucial periods is the use of air filters. Moreton and John (1956) and Kneeboie (1968) described the traps consisting of two overlapping pans of glass with the inner pan coated with oil to avoid entering of flies.

(c) Physical methods

Physical methods like use of steam, heat, humidity and light are very common. Efficient steam pasteurization of compost and casing soil is necessary for effective control of pests and diseases (Thomas, 1959; Morishita, 1960 and Kneeboie, 1968). Wyatt (1970) devised an electrical apparatus using UV light which was found useful in attracting flies and lethal to pathogens.

(d) Biological methods

Hudson (1972) described a nematode Tetradomena pelicaus to adult flies and larvae. It can be inoculated in mushroom beds. Nair and Fahy (1976) reported the addition of peat containing bacterial antagonistic Pseudomonas fluorescens^{on}/blotch disease : causal agent (Pseudomonas tolaasii). To control Chaetomium (olive green mould) attempts were made by Tautarus and Townsley (1983). They found that a thermophillic Bacillus sp. showed dramatic activity to control mould when inoculated into beds.

(e) Chemical methods

The chemical methods are always found priority over others to control most of the pests and diseases of animals and plants including mushrooms. The principles of chemotherapeutic control of human pathogen have been applied to all kinds of microbial pathogens including viruses (Rangaswami, 1954). Various chemicals such as disinfectants, antibiotics, antiseptics, repellents, antifeedent, chemosterulents, etc. are used to control many pests and diseases of mushrooms (Stoller, 1978; Sandhu and Dulcha, 1982 and Van De Geijn, 1982).

In chemical control, chlorothalonil (as Daconil 2787 75% a.i. WP) used to control of dry bubble disease caused by Verticillium fungicola in Agaricus bisporus was tolerant to

certain Benzimidazole fungicides (Gandy and Spencer, 1976). Rutjens (1977) and Garcha (1978) reported the application of 10 per cent chlorinated water on Agaricus bisporus and A. bitorquis twice a week showed complete control of Pseudomonas tolaasii a causal agent of brown blotch disease. Three sprays of Dithane Z-78 (0.25-0.50%) were found better control of dry bubble as well as wet bubble diseases of white button mushroom (Garcha, 1978). Formalin at 3-4 per cent was used against brown plaster mould caused by Myriococcum sp. in button mushrooms (Seth and Shandilya, 1978). Niba Chakraborty and Purakayastha (1978) reported 10 per cent root extract of water hyacinth significantly reduced the growth of Coprinus, a contaminant of cultivated mushrooms. Soaking paddy straw for 18 hr in a carboxin suspension in water (5 mg/L) controlled the growth of Sclerotium rolfsii occurring as a natural contaminant during the cultivation of Pleurotus flabellatus (Rajaratnam et al., 1979). Application of 0.075% Feritrothion and Pirimiphosmethyl at 0.075% concentration (100 ml spray/100 cm² area) were found effective against larvae of sciarid fly (Bradysia tritici COQ) infested on mushroom (A. bisporus) beds (Sandhu and Dulcha, 1982). Van De Geijn (1982) suggested the use of 2 per cent Formalin as disinfectant in the casing soil for the control of diseases like dry bubble caused by Verticillium fungicola, wet bubble caused by Mycogone perniciosae cobweb caused by Cladobotrym dendroides, green mould caused by Penicillium, Trichoderma and Aspergillus spp.

in button mushroom cultivation. Use of systemic fungicides like Benlate, Topsin and Thiobendazole at 50 ppm gave good control of three species of Trichoderma which were parasitic on Agaricus bisporus as well as Pleurotus sajorcaju with no significant reduction in mushroom yield (Seth and Bhardwaj, 1984). Bronopol and CaCO_3 were used at 0.1% and 20% respectively to control the brown blotch caused by Pseudomonas tolaasi on button mushroom (Wong and Preece, 1985). Formalin in combination with Bavistin has been used for the chemical sterilization of the substrate in oyster mushroom cultivation (Vijay, 1986).

Antibiotics

Antibiotics are the biochemical substances produced by microorganisms and capable of inhibiting the growth or completely destroying other microorganisms at minute concentration. Antibiotics are of two types based on their activities : (a) broad spectrum antibiotics, e.g. Chloromycetin, Tetracyclines, etc. which are active against wide range of microorganisms and (b) narrow spectrum antibiotics which are effective against specific types of microorganisms, e.g. Penicillins, Streptomycin, etc. (Mishra, 1980).

Role of antibiotics in disease control

The antibiotics are commonly used as drugs for human and animal disease control. Lately the antibiotics have also found applications in agriculture and such antibiotics are called

non-pharmaceutical antibiotics or agricultural antibiotics (Subramanyam, 1958; Pramer, 1959; Goldberg, 1964; Jha, 1978; Dutta, 1978; Nene and Thapliyal, 1979). Gliotoxin was the first antibiotic used against Rhizoctonia solani in plant protection (Mishra, 1980).

Brown and Boyle (1954) noted that Penicillium was active against crown gall caused by Agrobacterium tumefaciens, Blackrot of cabbage caused by Xanthomonas campestris, bacterial blight of rice caused by X. oryzae and blackarm disease of cotton caused by X. malvacearum are successfully controlled by Streptocycline (MacKay and Friend, 1953; Thirumalachar, 1973; Shetty, 1968). Broad spectrum antibiotics like tetracyclines affect the growth of both gram positive and gram negative bacteria, rickettsiae, mycoplasma and large viruses (Bohonos et al., 1953 and Del-Love et al., 1954). Sandle spike disease caused by Mycoplasma group of organisms remained controversial since several decades and also showed susceptibility to tetracyclines and Benlate (Raychaudhuri et al., 1972 and Raychaudhuri, 1977). Spraying with terramycin (Oxytetracycline hydrochloride) before pinhead appearance gave an effective control of blotch disease caused by Pseudomonas sp. in Agaricus bisporus (Stoller, 1978). Antibiotics like HR 810 (2-amino-thiazolyl cephalosporin) and Azocillin were found effective against Pseudomonas stutzeri and P. mendaciana under in vitro condition (Husson et al., 1985). In vitro

action of BRL 36650, a new penicillin was found more active against Pseudomonas aeruginosa and other Pseudomonas sp. at 2 $\mu\text{g/ml}$ concentration (Van Landuyt et al., 1986). Thus the antibiotics are found promising chemicals to control many kinds of plant diseases including mushroom diseases. Therefore, the present investigation was carried out by using different antibiotics and formalin.

MATERIAL AND METHODS

III MATERIAL AND METHODS

Chemotherapeutic studies on the control of brown spot disease (Pseudomonas stutzeri) of oyster mushroom was taken up by using different antimicrobial chemicals like antibiotics and formalin. Cultivation of oyster mushroom was taken up at mushroom house, Department of Agricultural Microbiology, Main Research Station, University of Agricultural Sciences, Hebbal Campus, Bangalore 560024. Laboratory studies were carried out in the main laboratory of the Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra (G.K.V.K.) Campus, Bangalore 560065. The materials used and methods adopted during the present investigation are described hereunder :

1. Mushroom culture

Pure culture of oyster mushroom (Pleurotus sajorcaju (Fr.) Singer) was obtained from the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Bangalore 560065. The culture was maintained on potato dextrose agar fortified with yeast extract and preserved under refrigerated conditions and later it was used as stock culture for further studies.

2. Spawn production

Spawn is the mycelium impregnated with carrier materials like jowar, bajra, wheat, maize, compost, etc. In the

present study the production of spawn was the same as Lemke (1972) with slight modifications as suggested by Krishnamoorthy (1981).

Healthy jowar grains were washed clearly and boiled for 45 minutes. Then dried on a newspaper until the grains were well separated. Saline bottles of 500ml capacity were filled with 300 g of jowar grains and closed with cotton plugs. These bottles were autoclaved ^{for} 30 minutes at 20 lb pressure, then cooled and inoculated with stock culture of mushroom fungus. These bottles were incubated at room temperature for 15-18 days and during this period fungal mycelium developed over the grains. Such spawn bottles were used in the cultivation of present experiments.

3. Cultivation

Oyster mushroom cultivation was carried out by following the method of Shetty and Krishnamoorthy (1980). Freshly harvested dry paddy straw as a substrate and polythene bags (18" x 12", 150 guage) were used for mushroom production.

Paddy straw was chopped into 5-8 cm bits and the bits were soaked in water for 8-12 hours. The substrate was autoclaved at 15 lb pressure for 20 minutes. Then the substrate was cooled by spreading on the surface of sterilized platform. The substrate was filled into the polythene bag layer by layer with addition of spawn at the peripheri of the substrate

layer. After complete filling the substrate inside, it was pressed to compact and mouth was tied with rubber band. Finally bags were perforated all sides to create proper aeration and a few holes at the bottom of the each bag to drain off excess water. These bags were kept on the wooden racks provided in mushroom room for incubation. After 20th day of incubation, fungus mycelia covered all over the substrate. Then bags were opened and removed by using the blade. Tap water was sprayed preferably twice a day on opened bag to maintain required humidity. After 2-3 days, the bags were opened and seen that the mushroom buttons as small basidiocarp started developing. In another 2-3 days well developed mushroom heads formed which were ready for harvest.

Harvesting operations were carried out before sporulation. Harvesting was done by cutting stipe with sharp blade or other sharp implements. It can also be done by holding stipe firmly and twisting it.

4. Estimation of microbial population in different paddy straw substrate

This experiment was carried out to enumerate the microbial population for the healthy, diseased and fresh paddy straw substrate of oyster mushroom.

(a) Freshly harvested paddy straw was obtained from MRS, UAS, Hebbal, Bangalore, sun dried thoroughly, and the chopped straw of 10 g was used for microbial population by following

serial dilutions. One ml of suspension each from respective dilutions (10^3 for fungus, 10^5 for bacteria) was plated on respective medium (MRBA for fungi and nutrient agar for bacteria) by using sterilized pipettes of 1 ml capacity.

(b) Healthy substrate : 15 days old spawn running bag was selected for the purpose. The bag was opened on sterilized polythene sheet and the substrate in the bag was mixed thoroughly. Then a composite sample of 10 g was taken from the above substrate. Further procedure was followed as stated earlier.

(c) Diseased substrate : After 15 days of spawn running a severely brown spot infected bag was selected and used for sampling. The substrate was mixed thoroughly aseptically. A composite sample of 10 g was used from the above diseased sample and suspended in 100 ml sterilized water blank prepared in 250 ml conical flask. Further procedure was same as before.

5. Identification of brown spot disease

Development of brown spot disease on the surface of the substrate during the spawn running stage of the oyster mushroom was observed at mushroom house of the Department of Agricultural Microbiology, Main Research Station, UAS, Bangalore 560024.

The diseased samples were collected for the isolation of brown spot causal agent as per the procedure of Mallesha (1987) and Mallesha and Shivappa Shetty (1988).

6. Isolation

Isolation of brown spot causal agent was carried out by following general method for isolation of pathogen (Pelleroni, 1981). Eighteen days old brown spot infected bag was selected and disease sample was collected from well developed spots (Plate 1). The bacteria were isolated on nutrient agar medium by following serial dilution method. Each morphologically well distinguished colony was separately obtained, designated and subjected to further purification.

7. Purification

All bacterial isolates were purified by repeated streak plate method on nutrient agar dispensed plates. These isolates were separately maintained on nutrient agar slants as a stock culture for further studies. After purification, general characters like spore formation, gram reaction and colony colour for all the six isolates were recorded.

8. Pathogenity test

Pathogenity test was conducted by following Koch postulates at mushroom house of the Department of Agricultural Microbiology, MRS, Bangalore 560024.

Culture of 24 hours old from the isolate was collected (10^8 cells/ml) in physiological saline (0.85% NaCl) and inoculated to sterilized paddy straw substrate (10 ml/bag). From the substrate, bags were prepared using Pleurotus sajorcaju

Plate 1 : Mushroom bag infected
with brown spot disease
(18 days old)



Plate : 1

spawn. Then the bags were kept on wooden racks provided in the cropping room for incubation. After 15 days of incubation occurrence of brown spot on mushroom bags was recorded.

9. Characterization of the brown spot causal agent

Brown spot causal organism of oyster mushroom was characterized by following the methods of Pelleroni (1981).

(a) Morphology

To study the morphology of brown spot causal agent, simple staining was carried out by following the method of Crabtree and Hinsdill (1974). Young culture of 24 hours old pathogen was smeared on clear grease free glass slide, air dried and heat fixed. Then stained with crystal violet for one minute, washed with tap water, dried and observed under oil immersion objective.

(b) Motility

Motility test was carried out for the casual organism by using hanging drop technique as described by Crabtree and Hinsdill (1974). A drop of young culture of brown spot causal agent suspension was taken on cover slip and inverted it over cavity slide and observed under oil immersion objective for motility.

(c) Gram staining

Gram staining for the brown spot causal agent was carried out by following the procedure described by Crabtree and Hinsdill (1974). Brown spot causal agent culture of 18 hours old was

smear on clear glass slide, air dried and heat fixed. Then it was stained with crystal violet for one minute and washed with tap water. Smear was again flooded with Lugol's iodine solution for one minute and washed with tap water. Again the slide was dipped in 90% alcohol for 30 seconds and quickly rinsed with tap water. Finally counter stained with Safranin for 30 seconds, washed, air dried and observed under oil immersion objective.

(d) Endospore staining

Endospore staining was carried out by following the method of Crabtree and Hinsdill (1974). Bacterial culture of 24 hours old was smeared on clear glass slide, air dried, heat fixed and a piece of blotting paper was placed on the smear. It was flooded with melachite green stain and the slide was steamed over a water bath without drying the stain. After 5 minutes, it was washed with tap water, counter stained with safranin for 30 seconds, washed, air dried and observed under oil immersion objective.

(e) Growth on selective *Pseudomonas* medium

The test organism was streaked on the plates dispensed with acetamide-cetrimide glycerol mannitol medium (Mossel and Indacochea, 1971) and it was kept for incubation at room temperature. After 5th day of incubation growth was recorded.

(f) Growth on minimal medium

Brown spot causal agent was grown on minimal medium and the growth was compared with the growth on nutrient agar plates (Stolp and Gadkar, 1981).

(g) Gelatin hydrolysis

Gelatin hydrolysis test for brown spot causal agent was carried out by following the method of Sneath and Collins (1974). The test organism was streaked on nutrient agar supplied with 1% gelatin as a carbon source. After observing the bacterial growth, the plates were flooded with a solution of saturated ammonium sulfate in 1N sulphuric acid. Clear zone was observed for gelatin hydrolysis.

(h) Starch hydrolysis

Starch hydrolysis test was carried out by using starch medium. In this method, starch medium dispensed plates were streaked with test organism (Stolp and Gadkar, 1981). Then the plates were incubated at room temperature for 24 hours. After observing the bacterial growth, the plates were flooded with Lugol's iodene solution. Appearance of clear zone was observed for starch hydrolysis.

(i) Denitrification

Denitrification test was carried out by following the method described by Stolp and Gadkar (1981). For this purpose

10 ml of nitrate medium was dispensed in test tubes and sterilized. The test organism was inoculated into the medium. 3 ml of 1% Molten agar was deposited on top of the inoculated medium to create anaerobic condition. These tubes were incubated for 5 days. Presence of nitrate was tested by using reagents (1) and (2) (Appendix III).

(j) Fluorescence pigment production

Test for fluorescent pigment production was carried out by using indicator organism Pseudomonas fluorescens obtained from the Department of Agricultural Microbiology, G.K.V.K., Bangalore 560065. In this method, both indicator as well as brown spot causal organism were grown on King's A medium and King's B medium separately. Then plates with developed colonies of causal organism as well as Pseudomonas fluorescens were observed under ultraviolet light chamber for fluorescence (King et al., 1954).

(k) Accumulation of poly B-hydroxy butyrate (PHB)

The pathogen was grown on PHB medium and tested for PHB granules. Pathogen was stained with Sudan Black B for 5 to 7 minutes and washed with xylol. Then stained with safranin for 1-2 minutes, washed, air dried and observed under oil immersion objective (Hugh and Gilardi, 1974).

(l) Growth at 4°C and 41°C

Growth of the pathogen at 4°C and 41°C was tested by following the method described by Stolp and Gadkar (1981).

In this case pathogen was inoculated to peptone yeast extract broth in test tubes. Then one set at 4°C and another set at 41°C were incubated. Observation for growth was recorded after two days.

10. Chemotherapeutic agents used against Brown Spot causal organism:

Four antibiotics namely, Chloramphenicol, Streptomycin, and Tetracycline of Sarabai Chemical Company, and Streptocycline from Hindustan Antibiotic Ltd. were used. Similarly formalin from BDH company was used against Brown Spot causal agent of oyster mushroom.

(i) Antibiotics

Stock solutions of all the four antibiotics were prepared by using sterilized distilled water. The stock solutions were filter sterilized by using millipore filter unit of 0.45 μ size, maintained in refrigerated conditions for short time and used for further chemical screening with different concentrations.

(ii) Preparation of filter paper discs

Filter paper discs were prepared by using Whatman No.1 filter paper. Discs of equal size, each measuring 6 mm diameter, were prepared by using perforator. These discs were sterilized at 15 lb pressure for 15 minutes, oven dried aseptically in order to remove moisture from the disc and then

saturation point of filter paper discs were found out by using microsyringe. These discs were used for further antibiotic assay.

11. Antibiotic assay (Paper Disc Method)

Antibiotic sensitivity test was carried out for brown spot causal agent (Pseudomonas stutzeri) in the laboratory by using four antibiotics, mentioned earlier, at 10, 25, 50, 100 and 250^{ppm}/concentrations. A bacterial lawn was prepared on 15 ml each nutrient agar medium dispensed petriplates by spreading the P. stutzeri (10^4 cells/ml). Over the bacterial lawn sterilized filter paper discs saturated with antibiotics of respective concentrations were placed by using sterilized forceps. These plates were incubated at room temperature for 12-18 hours, and inhibition zone caused by different antibiotics were recorded.

12. Effect of streptomycin on Pseudomonas stutzeri in liquid medium

The experiment was conducted to determine antagonistic effect of streptomycin on Pseudomonas stutzeri at different intervals. Nutrient broth at the rate of 10 ml was taken in 20 ml sterile test tubes. The antibiotic streptomycin concentrations at 0, 10, 25, 50, 100 and 250 ppm were prepared. The tubes were inoculated with 1 ml of 24 hours old culture of P. stutzeri and plated on nutrient agar plates at different

intervals of 10, 30, 60 and 120 minutes. These plates were incubated at room temperature for 72 hours. After incubation, the survival of the brown spot causal agent was recorded.

13. Effect of streptocycline on *Pseudomonas stutzeri* in paddy straw substrate

In 250 ml conical flask, 10 g of chopped paddy straw was taken and sterilized. The paddy straw substrate sterilized was then inoculated with 1 ml of 24 hours old pathogen culture (10^6 cells/ml). These inoculated substrates were then treated with streptocycline solution at 0, 10, 25, 50, 100 and 250 ppm concentrations. Each sample of the above treatment was analysed for the survival of *P. stutzeri* pathogen at different intervals (10, 30, 60 and 120 minutes).

14. Effect of streptocycline on oyster mushroom fungus (*Pleurotus sajorcaju*)

Effect of antibiotic on the oyster mushroom fungus *Pleurotus sajorcaju* was determined by using potato dextrose agar solid medium plates. Antibiotic streptocycline was added at 0, 10, 25, 50, 100 and 250 ppm to each 20 ml medium at 45°C and plated into sterile petridishes. On such solid medium plates oyster mushroom fungal disc measuring 8 mm^{made}/øf from a week old culture disc was inoculated at the centre. These plates were incubated at room temperature for a week. After two days, observation on the radial growth of fungus was taken at day's interval for a week.

15. Effect of streptocycline on microbial population of fresh paddy straw

In this experiment effect of streptocycline on microbial population for fresh paddy straw substrate was determined. 10 g of chopped paddy straw was treated with streptocycline antibiotic at 0, 10, 25, 50, 100 and 250 ppm concentrations in 250 ml conical flask. These flasks were incubated under room temperature for one hour. After the incubation, each sample of paddy straw was suspended in 100 ml sterile distilled water and serial dilutions were made. Appropriate dilutions of each treatment were plated on nutrient agar for bacteria and MRBA for fungi. The plates were incubated at room temperature. The growth of bacteria after 3 days and fungus for 6 days was recorded.

16. Effect of streptocycline on mushroom yield and occurrence of brown spot

In this experiment, the fresh paddy straw was chopped into 5-6 cm pieces approximately, soaked for 8-12 hr and sterilized at 15 lb pressure for 20 minutes. Each sample of 1 kg substrate was separately inoculated with 10 ml of 24 hr old culture of Pseudomonas stutzeri using sterilized atomizer and thoroughly mixed. Further, streptocycline solution was prepared in 15 litre size plastic buckets at 0, 50, 100, 250 and 500 ppm concentrations by using tap water. Causal organism (P. stutzeri) inoculated substrate was immersed in the antibiotic solutions prepared for 1 hr. Then substrate was spread separately on hygienic plastic surface in order to drain excess

water. The substrate inoculated with oyster mushroom fungus (Pleurotus sajorcaju) was incubated at room temperature. When oyster mushrooms developed, influence of streptocycline on yield, bioefficiency and brown spot occurrence was recorded.

17. Effect of formalin on Pseudomonas stutzeri in liquid medium

In this experiment, 10 ml of nutrient broth was sterilized in different 20 ml test tubes. To each of the test tubes, 1 ml of 24 hr old young liquid culture of Pseudomonas stutzeri was inoculated aseptically and thoroughly mixed. Further, tubes were treated with formalin at 0, 5, 10, 25, 50 and 100 ppm concentrations. One ml each of the above treatments was serially diluted and plated on nutrient agar at different intervals (10, 30, 60 and 120 minutes). The plates were incubated at room temperature for 72 hrs and the growth of P. stutzeri was recorded in each of the above treatment. There were four replications.

18. Effect of formalin on Pseudomonas stutzeri in paddy straw substrate

Fresh paddy straw was chopped into 3-5 cm pieces approximately and soaked in tap water for 8-12 hr. Ten grammes of this was taken in 250 ml flask and the mouth of the flask was closed with cotton plug. These flasks were sterilized at 15 lb pressure for 20 minutes. The sterilized straw in each flask was separately treated with 1 ml of 24 hr old culture of Pseudomonas stutzeri (1×10^6 cells/ml) aseptically. The

substrate and the inoculum were thoroughly mixed by shaking for 5 minutes. Further, the substrate in the flask was treated with different concentrations of formalin (0, 5, 10, 25, 50 and 100 ppm) and mixed thoroughly. From these, further dilutions were made and appropriate dilution was plated on nutrient agar medium at 10, 30, 60 and 120 minutes intervals. These plates were incubated for 72 hrs. The growth of P. stutzeri in the plates as affected by formalin at different concentrations were recorded. There were four replications.

19. Effect of formalin on the growth of mushroom fungus

In this experiment, 50 ml of potato dextrose broth was taken in 100 ml conical flask, sterilized and inoculated with the fungal disc of 8 mm each. Further, the flasks were treated with formalin at 0, 5, 10, 25, 50 and 100 ppm concentrations. These flasks were incubated at room temperature for 15 days. After the incubation, the visual growth, fresh weight and dry weight were recorded. There were four replications.

20. Effect of formalin on the microbial population of fresh paddy straw

Fresh paddy straw was chopped into 5-8 cm pieces and soaked in water for 8-12 hr. Ten grammes of soaked straw was taken in 250 ml conical flask. It is treated with formalin at 0, 5, 10, 25, 50, 100 and 250 ppm concentrations. Then it was mixed thoroughly and incubated for one hour. Further, dilutions were made and microorganisms were plated on

respective medium using appropriate concentrations (10^5 dilution for bacteria on nutrient agar and 10^3 dilution for fungi on MRBA medium). There were four replications.

21. Effect of formalin bath of paddy straw on the oyster mushroom yield and occurrence of the disease

In this experiment, the fresh paddy straw was chopped into 5-8 cm pieces approximately. Chopped straw was soaked for 8-12 hr and sterilized at 15 lb pressure for 20 minutes. Each sample of 1 kg substrate was separately inoculated with 10 ml of 24 hrs old pathogen culture and mixed. Further, formalin solutions were prepared in 15 litre size plastic bucket at 0, 25, 50, 100, 250, 500 and 1000 ppm concentrations by using tap water. The sterilized paddy straw inoculated with Pseudomonas stutzeri was bathed with different concentrations of formalin and the buckets were closed for 30 minutes for fumigation. Then substrate was spread over hygienic plastic surface as thin layer for the evaporation and complete removal of formalin for 2 hrs. The substrate was then inoculated with mushroom spawn (Pleurotus sajorajju) separately used for mushroom production. When oyster mushroom developed, influence of formalin on the yield, bio-efficiency and brown spot occurrence were recorded.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

In the present study, chemicals like antibiotics and formalin were used to control the brown spot disease of oyster mushroom caused by Pseudomonas stutzeri. Effect of these chemicals on brown spot causal agent as well as the yield performance of oyster mushroom (Pleurotus sajorcaju) were determined at different concentrations. The experiments were carried out as per the procedure explained in the 'Material and Methods' and the results pertaining to the investigations are presented in this chapter.

1. Estimation of microbial population from different samples of oyster mushroom substrate

This experiment was carried out to assess the microbial population in different samples of paddy straw used as oyster mushroom substrate. Results are presented in Table 1.

Among the three samples used for the estimation of microbial population, the samples taken from brown spot infected substrate showed higher population of bacteria ($5.23 \times 10^6/\text{g}$) as well as the fungi ($37.33 \times 10^3/\text{g}$).

The sample obtained from healthy substrate showed minimum population of bacteria ($2.13 \times 10^6/\text{g}$) as well as fungi ($1.66 \times 10^3/\text{g}$) compared to other two. Fresh paddy straw sample showed slightly higher population of bacteria ($2.43 \times 10^6/\text{g}$) as well as fungi ($23.66 \times 10^3/\text{g}$) compared to healthy bag but the population was not as high as that of the sample obtained from disease substrate.

Table 1. Microbial population of oyster mushroom substrate

Substrates	Population per g on oven dry weight basis	
	Bacteria ($\times 10^6$)	Fungi ($\times 10^3$)
Fresh paddy straw	2.43	23.66
Diseased substrate	5.23	37.33
Healthy substrate	2.13	1.66
CD at 5%	1.61	13.45

Note : Each value given above represents an average of five replications.

However, in all the three samples used, the sample from healthy substrate showed the least fungal population ($1.66 \times 10^3/g$) compared to other two. The sample obtained from diseased substrate showed maximum population of bacteria as well as fungi followed by the sample taken from fresh paddy straw and healthy substrate sample, respectively.

2. Qualitative and quantitative determination of bacterial population of diseased substrate

Results pertaining to this experiment are presented in Table 2.

There were six types of bacterial isolate obtained (BSI₁, BSI₂, BSI₃, BSI₄, BSI₅ and BSI₆). Among these six brown spot isolates, the isolate BSI₆ was found to show the maximum population ($2.10 \times 10^6/g$), next to that was obtained BSI₃ ($1.05 \times 10^6/g$) and the least was observed in isolate BSI₁ ($0.27 \times 10^6/g$). Rest of the isolates showed a population varying from 0.32 to $0.62 \times 10^6/g$. Morphologically two out of six brown spot isolates were white to dull white in colour. However, isolates BSI₃, BSI₄ and BSI₅ appeared red, yellow and creamy yellow, respectively.

All isolates were rod shaped, non-spore former and gram negative in reaction except the isolate BSI₁ which was a gram positive spore former.

Table 2. Qualitative and quantitative determination of bacterial population of brown spot infected substrate

Isolates	*Population (x10 ⁶)	Colour	Shape	Spore	Gram reaction
BSI ₁	0.27	White	Rod	Yes	+
BSI ₂	0.62	White	Rod	No	-
BSI ₃	1.05	Red	Rod	No	-
BSI ₄	0.32	Yellow	Rod	No	-
BSI ₅	0.37	Creamy yellow	Rod	No	-
BSI ₆	2.10	Dull white	Rod	No	-

*Population expressed per g oven dry weight basis

BSI = Isolate obtained from brown spot infected substrate

+ = Gram positive

- = Gram negative

3. Pathogenity test

The results of pathogenity test are given in Table 3. Among the six isolates inoculated to sterilized paddy straw substrate, the isolate BSI₆ caused typical brown spot disease symptoms (Plate 2). Further, it gave least mushroom yield (53.33 g/bag). Rest of the isolates did not produce disease symptom of brown spot. However, the isolate BSI₅ gave slightly increased yield (251.33 g/bag) over the control (248.33 g/bag). Other isolates like BSI₁, BSI₂, BSI₃ and BSI₄ showed similar trend in yield performance (i.e. 243.33 g, 235.00 g, 236.66 g and 235.00 g/bag, respectively).

The per cent bioefficiency was observed ranging from 90.38 to 99.33 as influenced by other isolates except the isolate BSI₆ which caused brown spot observed least bio-efficiency (21.33%).

4. Identification of brown spot causal organism

Causal organism was identified by consulting the 8th Edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) and other biochemical tests were conducted by following methods explained by Pelleroni et al. (1970).

The causal organism of brown spot found to be gram negative, motile, small rods, did not produce endospore and fluorescent pigment. It grew well on minimal medium and selective Pseudomonas medium. It did not hydrolyse the

Table 3. Inoculation effect of brown spot isolates on mushroom yield and disease occurrence

Isolates	Yield (g/bag)	Bioefficiency (%)	Appearance of brown spot
BSI ₁	243.33	97.20	-
BSI ₂	235.00	94.00	-
BSI ₃	236.66	94.66	-
BSI ₄	235.00	90.38	-
BSI ₅	251.33	96.66	-
BSI ₆	53.33	21.33	+
Control	248.33	99.33	-

Note : Each value given above represents an average of three replications.

CD at 5% for yield = 59.83

BSI = Brown spot isolates

+ = Appearance of brown spot

- = No appearance of brown spot

$$\text{Bioefficiency} = \frac{\text{Total fresh yield of mushroom}}{\text{Total dry weight of substrate}} \times 100$$

Plate 2 : Mushroom bag inoculated with brown
spot isolate-6 (BSI₆) showing the
disease (Bag 1 : control;
Bag 2 : inoculated with BSI₆)



Plate : 2

Table 4. Characterization of brown spot causal organism

Tests conducted	Reaction of the organism
1. Gram reaction	-
Shape	Small rods
Motility	+
Endospore	-
2. Growth on	
(a) Selective Pseudomonads medium	+
(b) Minimal medium	+
3. Hydrolysis	
(a) Starch	+
(b) Gelatin	-
4. Accumulation of PBH	-
5. Fluorescent pigment	-
6. Denitrification	+
7. Growth at 4°C	-
41°C	+

Note : + = Positive for reaction

- = Negative for reaction

gelatin but hydrolysed the starch and caused denitrification. It grew well at 41°C but not at 4°C and did not accumulate poly-β-hydroxybutyrate (PBH).

5. Screening for different antimicrobial antibiotics against *Pseudomonas stutzeri*

The results of four antibiotics screened against *Pseudomonas stutzeri* are presented in Table 5. Of the four antibiotics used at five different concentrations, the antibiotics streptomycin and tetracycline gave maximum zone of inhibition (7 mm each) at 10 ppm concentration and chloramphenicol and streptomycin showed the least inhibition zone (6.33 mm each) at the same concentrations used.

However, similar trend was observed for streptomycin and tetracycline at 25 ppm and 50 ppm concentrations, whereas the antibiotic chloramphenicol showed least inhibition zone (6.66 and 7.33 mm, respectively) compared to rest of the antibiotics at the same concentrations. At 100 ppm and 250 ppm concentrations, maximum zone of inhibition was recorded for streptomycin (12.33 mm and 16.66 mm, respectively) followed by tetracycline (10.66 mm and 14.33 mm, respectively). Least zone of inhibition was observed for chloramphenicol (9.00 mm and 9.33 mm, respectively) at the same concentrations.

Finally among the four antibiotics used at five different levels, the streptomycin showed maximum zone of inhibition in all the concentrations (Plate 3), followed by tetracycline and streptomycin. Least zone of inhibition was recorded for chloramphenicol at all the five concentrations (Fig.1).

Table 5. Effect of antibiotics on Pseudomonas stutzeri

Antibiotics	Concentrations (ppm)				
	10	25	50	100	250
	(diameter of inhibition zone in mm)				
Chloramphenicol	6.33	6.66	7.33	9.00	9.33
Streptomycin	6.33	7.33	8.33	9.33	9.66
Streptocycline	7.00	8.33	9.33	12.33	16.66
Tetracycline	7.00	8.33	9.33	10.66	14.33

Note : Each value given above represents an average of four replications.

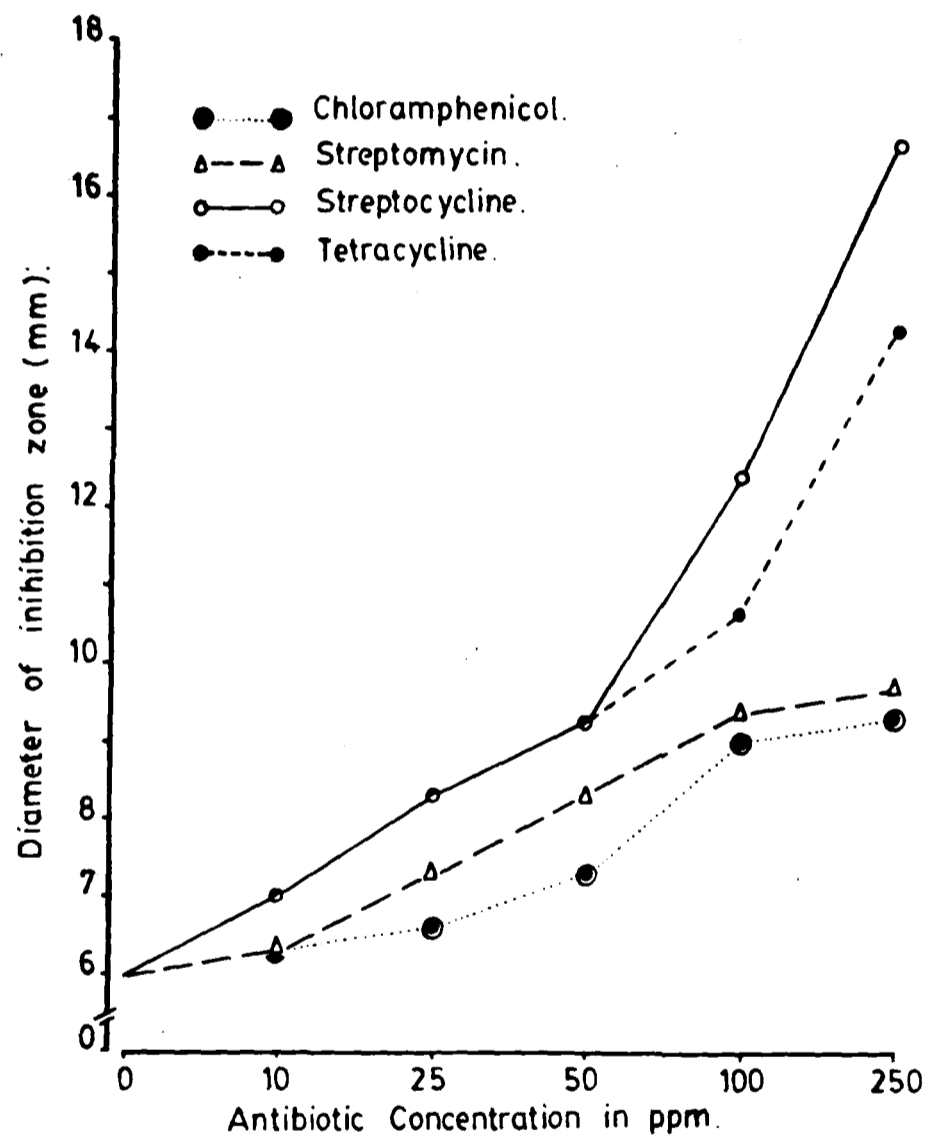


Fig.1: Effect of different antibiotics on Pseudomonas stutzeri.

Plate 3 : Streptocycline at different concentrations
(in ppm) showing inhibition of Pseudomonas
stutzeri (Paper disc assay)



Plate : 3

6. Effect of streptocycline on *Pseudomonas stutzeri* in liquid medium

The results pertaining to this experiment are presented in Table 6 (Plate 4A and 4B). At 10 minutes interval, maximum population ($10.60 \times 10^5/\text{ml}$) was obtained at 0 ppm (control). Rest of the concentrations (10, 25, 50, 100 and 250 ppm) showed decreased rate of population (8.13, 2.58, 0.79, 0.34, 0.28×10^5 per ml, respectively). No population was observed at 30, 60 and 120 minutes intervals at 100 ppm and 250 ppm. Rest of the concentrations such as 10, 25 and 50 ppm showed gradual decrease of population with increased time duration. However, none of the concentrations was found effective against the bacterium at 10 minutes interval, but after 30 minutes interval showed marked inhibition of the pathogen at 100 ppm and 250 ppm concentrations.

7. Effect of streptocycline on *Pseudomonas stutzeri* in paddy straw substrate

The causal organism *P. stutzeri* was treated with antibiotic streptocycline at different concentrations in paddy straw (sterilized). The results are given in Table 7.

Among the six concentrations used, the concentration at 250 ppm showed minimum record of population ($0.56 \times 10^5/\text{g}$) next to that of 100 ppm ($0.98 \times 10^5/\text{g}$) at 10 minutes interval. Rest of the concentrations like 10, 25 and 50 ppm showed population of $9.03 \times 10^5/\text{g}$, $1.41 \times 10^5/\text{g}$ and $1.27 \times 10^5/\text{g}$, respectively.

Table 6. Effect of streptomycin on Pseudomonas stutzeri in liquid medium

Streptomycin concentrations (ppm)	Population at different intervals in minutes ($\times 10^5$)			
	10	30	60	120
0	10.60	10.00	10.10	10.06
10	8.13	5.03	4.83	4.80
25	2.58	1.56	1.48	1.43
50	0.79	0.70	0.68	0.63
100	0.34	-	-	-
250	0.28	-	-	-

Note : Each value given above represents an average of four replications.
(initial population added 10^6 cells/ml)

Plate 4A : Different concentrations of streptomycin
(in ppm) showing inhibition of Pseudomonas
stutzeri in nutrient broth medium

Control: Uninoculated

Ino : Inoculated with pathogen

Plate 4B : Different concentrations of streptomycin
(in ppm) showing inhibition of P. stutzeri
plated from above (Plate 4A) treatments



Plate: 4A

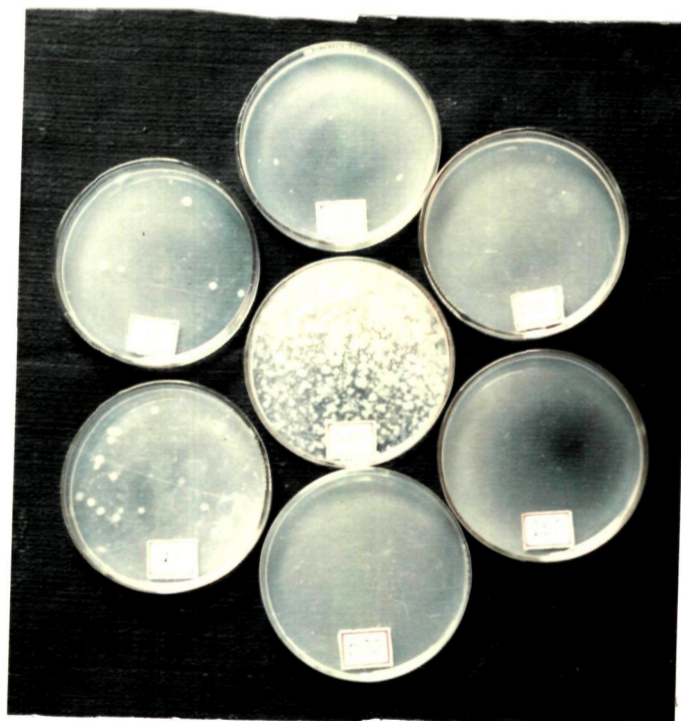


Plate: 4B

Table 7. Effect of streptocycline on Pseudomonas stutzeri in paddy straw substrate

Streptocycline concentrations (ppm)	Population/g at different intervals in minutes ($\times 10^5$)			
	10	30	60	120
0	9.93	9.93	9.96	9.90
10	9.03	6.50	5.49	5.41
25	1.41	1.35	1.29	1.27
50	1.27	0.23	0.14	0.13
100	0.98	-	-	-
250	0.56	-	-	-

Note : Each value given above represents an average of four replications.

At 30 minutes interval, no growth was observed at 100 ppm and 250 ppm concentrations. Least population ($0.23 \times 10^5/g$) was recorded at 50 ppm and the rest of the concentrations like 10 and 25 ppm showed $6.50 \times 10^5/g$ and $1.35 \times 10^5/g$, respectively. Almost similar trend was observed at 60 and 120 minutes intervals in all the concentrations. However, concentrations such as 100 ppm and 250 ppm were found effective against pathogen after 30 minutes intervals.

8. Effect of streptocycline on growth of mushroom fungus
(Pleurotus sajorcaju)

The results of the experiment on the effect of streptocycline at different concentrations under laboratory conditions are given in Table 8.

Good growth of the fungus was observed in all the concentrations used (Plate 5). On 3rd day interval maximum growth was observed at 250 ppm (30.9 mm) and minimum growth was recorded (28.7 mm) at 25 ppm. Rest of the concentrations like 0, 10, 50 and 100 ppm gave radial growth of 30.6 mm, 30.0 mm, 29.6 mm and 29.6 mm, respectively.

After four days maximum growth was observed at 100 ppm (39.3 mm) and least growth was observed at 25 ppm (35.8 mm). Remaining concentrations such as 0, 10, 50 and 250 ppm showed the growth of 36.2 mm, 37.7 mm, 38.6 mm and 38.9 mm, respectively.

Table 8. Effect of streptocycline on mushroom fungus (Pleurotus sajorcaju)

Streptocycline concentrations (ppm)	Radial growth in mm at different intervals in days			
	3	4	5	6
0	30.6	36.2	52.6	74.3
10	30.0	37.7	56.6	71.6
25	28.7	35.8	53.3	74.3
50	29.6	38.6	57.0	73.0
100	29.6	39.3	51.6	71.6
250	30.9	38.9	52.6	71.6
CD at 1%	NS	NS	NS	NS

Note : Value given above represents an average of the three replications.

NS = Not significant

Plate 5 : Growth of Pleurotus sajorcaju at
different concentrations (in ppm)
of streptocycline

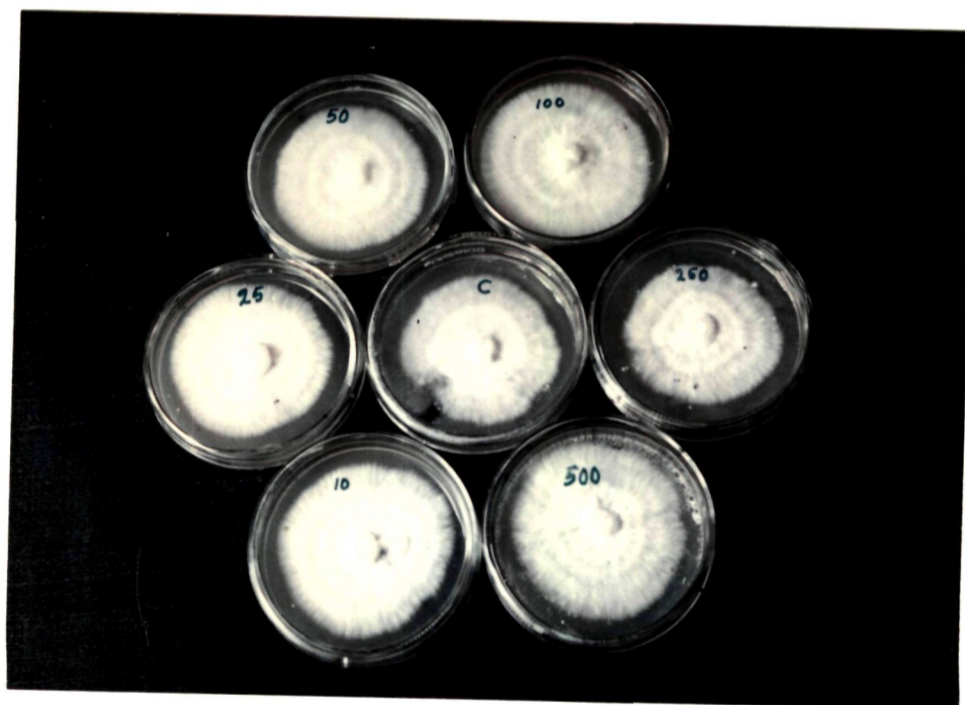


Plate : 5

After five days, maximum growth was observed at 50 ppm (57.0 mm) and the least was recorded at 100 ppm (51.6 mm). Rest of the concentrations like 0, 10, 25 and 250 ppm showed the growth of 52.6 mm, 56.6 mm, 53.3 mm and 52.6 mm, respectively.

During the sixth day observation, maximum radial growth was recorded at 0 ppm (control) and at 25 ppm (74.43 mm each). Rest of the concentrations showed growth varying from 71.6 mm to 73.0 mm. Nevertheless, none of the concentrations was found significantly adverse effect on the growth of mushroom fungus at all the intervals.

9. Effect of streptomycin on microbial population of fresh paddy straw

The experiment was carried out to find out the effect of streptomycin on microbial population of fresh paddy straw at different concentrations and the results of this experiment are presented in Table 9.

Paddy straw showed maximum bacterial population (2.31×10^5 /g) in control (0 ppm) which was not treated with antibiotic. Least bacterial population was observed (0.66×10^5 /g) at 250 ppm. The concentrations such as 10, 25, 50 and 100 ppm showed 2.03×10^5 , 1.76×10^5 , 1.56×10^5 and 0.76×10^5 per g respectively (Fig.2).

Fungal population also found maximum in control (2×10^3 /g) which was not treated with antibiotic and least fungal count was observed at 250 ppm (1.80×10^3 /g). Rest of the concentrations

Table 9. Effect of streptocycline on microbial population of fresh paddy straw

Streptocycline concentration (ppm)	Population per g on oven dry weight	
	Bacteria ($\times 10^5$)	Fungi ($\times 10^3$)
0	2.31	2.00
10	2.03	1.96
25	1.76	1.86
50	1.56	1.83
100	0.76	1.83
250	0.66	1.80
CD at 5%	0.98	1.14

Note : Value given above represents an average of the four replications.

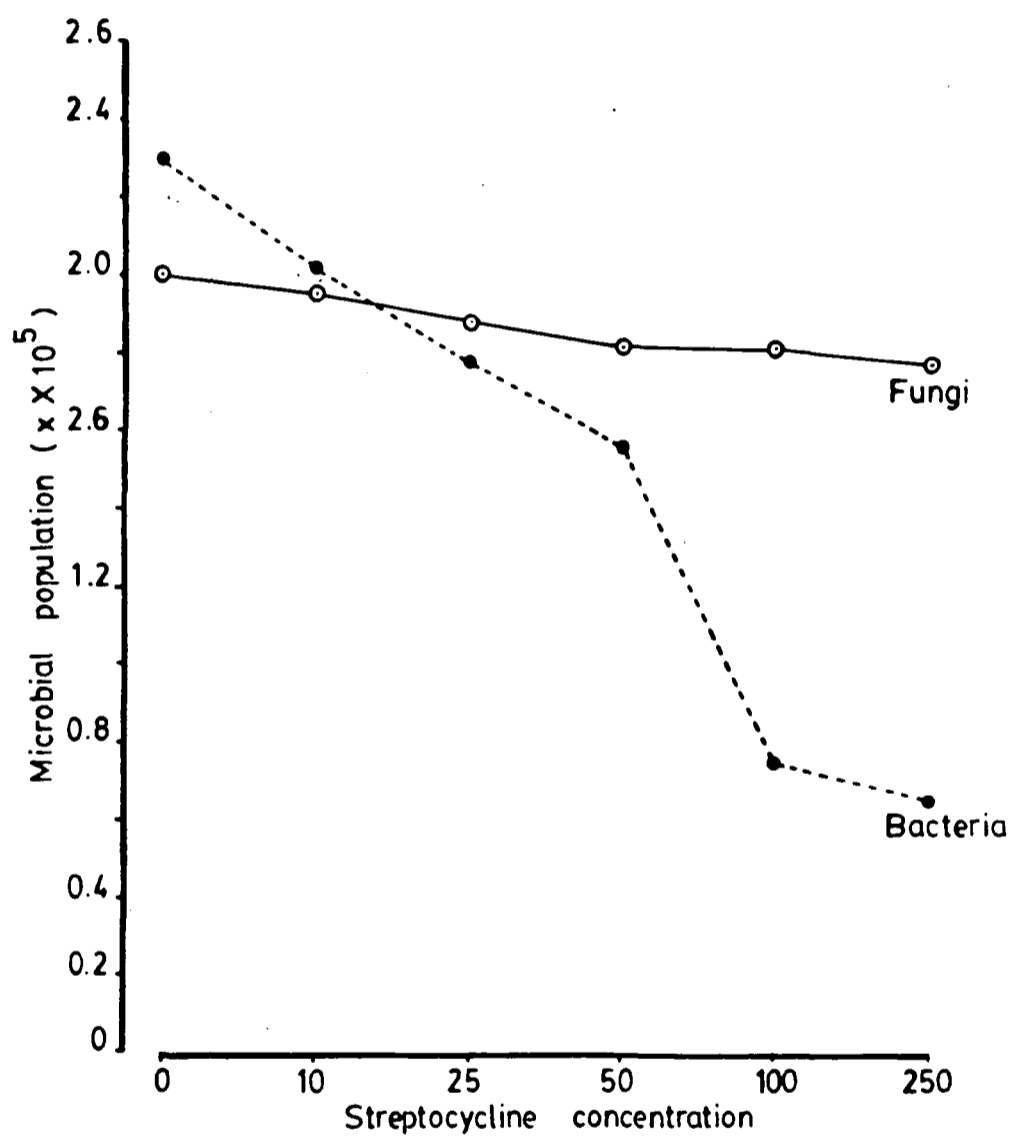


Fig.2: Effect of Streptocycline on microbial population of fresh paddy straw.

like 10, 25, 50 and 100 ppm showed reduced population count (1.96×10^3 , 1.86×10^3 , 1.83×10^3 and 1.83×10^3 per g respectively) with increased concentrations. However, none of the concentrations showed complete inhibition of the population (Fig.2).

10. Effect of streptocycline on oyster mushroom yield and occurrence of the disease

The present investigation was carried out to determine the effect of streptocycline on mushroom yield as well as the control of brown spot. Results pertaining to this experiment are presented in Table 10.

The control (T_1) which is neither treated with antibiotic nor with Pseudomonas stutzeri showed complete absence of brown spot whereas T_2 (treated with pathogen) expressed the disease symptoms. Complete absence of brown spot was observed in treatments T_4 , T_5 and T_6 which were treated with 100, 250 and 500 ppm of streptocycline respectively. However, T_3 (50 ppm) showed the symptom of brown spot disease.

Among the six treatments, T_6 (500 ppm) recorded the maximum yield (286.66 g/bag) with increased per cent bio-efficiency (114.66) compared to control (Fig.4). Next to that of T_5 (250 ppm) and T_4 (100 ppm) which have shown similar yield performance (285.00 g/bag and 276.66 g/bag respectively) as well as biological efficiency (114.00 % and 112.92 %, respectively).

Table 10. Effect of streptomycycline on mushroom yield and occurrence of the disease

Treatments	Yield (g/bag)	Bioefficiency (%)	Appearance of brown spot
T ₁ : No antibiotic	243.33	97.33	-
T ₂ : T ₁ + <u>P. stutzeri</u>	53.33	21.76	+
T ₃ : T ₂ + Streptomycycline 50 ppm	153.33	61.33	+
T ₄ : T ₂ + Streptomycycline 100 ppm	276.66	112.92	-
T ₅ : T ₂ + Streptomycycline 250 ppm	285.00	114.00	-
T ₆ : T ₂ + Streptomycycline 500 ppm	286.66	114.66	-
CD at 5%	66.80		

Note : Each value given above represents an average of three replications

+ = Appearance of brown spot

- = No appearance of brown spot

The lowest yield was observed in T₂ (53.33 g/bag) and T₃ (153.33 g/bag) with less per cent bioefficiency (21.76 and 61.33 respectively). However, the treatments with higher concentrations of antibiotics (100, 250 and 500 ppm) showed slightly increased yield as well as bioefficiency.

11. Effect of formalin on Pseudomonas stutzeri in liquid medium

Results pertaining to the experiment on the effect of formalin at different concentrations are presented in Table 11.

T₁ (control) showed slightly increased population with increased duration, whereas T₂ (5 ppm) and T₃ (10 ppm) recorded steady range of population in all the four intervals (10, 30, 60 and 120 minutes). Complete absence of pathogen observed for T₄ (25 ppm), T₅ (50 ppm) and T₆ (100 ppm) at all the four intervals.

12. Effect of formalin on Pseudomonas stutzeri in paddy straw substrate

Effect of formalin treated substrate on P. stutzeri was determined with four different intervals. Results are presented in Table 12.

T₁ (control) recorded slightly increased population (10.03×10^5 , 10.13×10^5 , 10.20×10^5 , 10.86×10^5 per g) at 10, 30, 60 and 120 minutes intervals, respectively. But T₂ (5 ppm) and T₃ (10 ppm) showed slightly reduced rate of

Table 11. Effect of formalin on Pseudomonas stutzeri in liquid medium

Treatments	Population/ml at different intervals (in minutes)			
	10	30	60	120
T ₁ : No formalin (0 ppm)	10.13	10.16	10.20	10.60
T ₂ : Formalin 5 ppm	9.76	9.83	9.83	9.80
T ₃ : Formalin 10 ppm	9.86	9.56	9.56	9.26
T ₄ : Formalin 25 ppm	-	-	-	-
T ₅ : Formalin 50 ppm	-	-	-	-
T ₆ : Formalin 100 ppm	-	-	-	-

Note : Each value given above represents an average of four replications.

- = No population

Plate 6 : Effect of different concentrations of formalin on Pseudomonas stutzeri in nutrient broth medium

UC = Uninoculated control

C = Inoculated control.

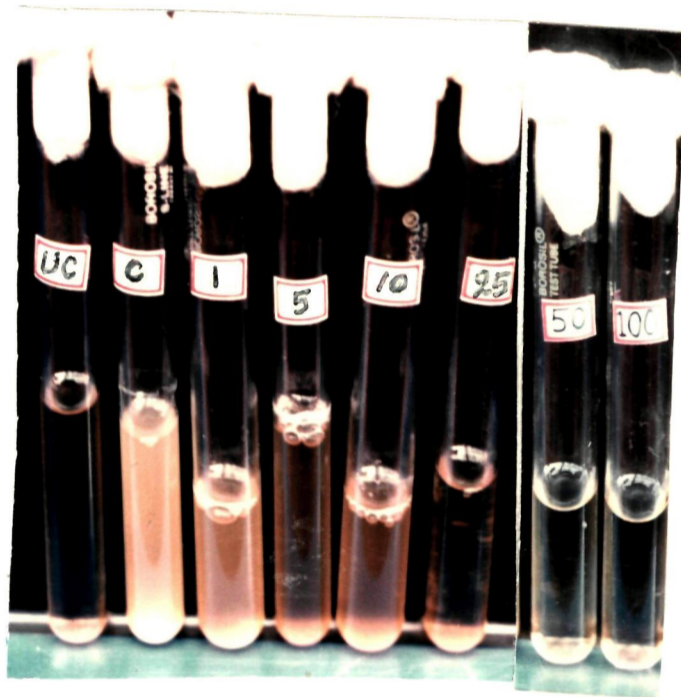


Plate : 6

Table 12. Effect of formalin on Pseudomonas stutzeri in paddy straw of substrate

Treatments	Population ($\times 10^5$) per g dry substrate			
	Interval (in minutes)			
	10	30	60	120
T ₁ : No formalin	10.03	10.13	10.20	10.86
T ₂ : 5 ppm formalin	9.86	9.83	9.66	9.66
T ₃ : 10 ppm formalin	9.60	9.43	9.53	9.00
T ₄ : 25 ppm formalin	-	-	-	-
T ₅ : 50 ppm formalin	-	-	-	-
T ₆ : 100 ppm formalin	-	-	-	-

Note : Each value given above represents an average of four replications.

population with increased time duration. The treatments such as T₄ (25 ppm), T₅ (50 ppm) and T₆ (100 ppm) revealed complete inhibition of the pathogen at all the four intervals.

13. Effect of formalin on the growth of mushroom fungus (Pleurotus sajorcaju) in liquid medium

The effect of formalin on mushroom fungus (P. sajorcaju) at different levels was determined. Results are presented in Table 13.

Good growth was observed at 0, 5, 10 and 25 ppm concentrations, whereas 50 and 100 ppm showed complete inhibition of the fungus. There is no marked reduction in fresh weight as well as dry weight observed at 0, 5, 10 and 25 ppm. In contrast, 50 ppm and 100 ppm showed complete inhibition of the mushroom fungus.

14. Effect of formalin on microbial population of fresh paddy straw

Results of the effect of formalin on microbial population of fresh paddy straw are presented in Table 14.

T₁ (0 ppm) showed maximum bacterial population ($3.50 \times 10^5/g$) and slightly decreased population was observed under T₂ ($2.83 \times 10^5/g$) and T₃ ($2.29 \times 10^5/g$) which was treated with 5 ppm and 10 ppm formalin respectively. Least bacterial population was recorded for T₄ ($0.98 \times 10^5/g$), T₅ ($0.51 \times 10^5/g$) and T₆ ($0.32 \times 10^5/g$) which contained 25, 50 and 100 ppm concentrations, respectively. No population was recorded for T₇ treated with 250 ppm formalin (Fig.3).

Table 13. Effect of formalin on growth of mushroom fungus in liquid medium

Formalin concentrations (ppm)	Growth	Fresh weight (g)	Dry weight (g)
0	+	3.16	0.30
5	+	3.13	0.29
10	+	3.06	0.30
25	+	3.06	0.30
50	-	-	-
100	-	-	-

Note : Each value given above represents an average of four replications.

+ = Growth

- = No growth

Table 14. Effect of formalin on microbial population of fresh paddy straw

Treatments	Population/g dry substrate	
	Bacteria ($\times 10^5$)	Fungi ($\times 10^3$)
T ₁ : No formalin	3.50	2.90
T ₂ : 5 ppm formalin	2.83	2.62
T ₃ : 10 ppm formalin	2.39	2.56
T ₄ : 25 ppm formalin	0.98	2.51
T ₅ : 50 ppm formalin	0.51	0.47
T ₆ : 100 ppm formalin	0.32	0.10
T ₇ : 250 ppm formalin	-	-

Note : Each value given above represents an average of four replications.

- = No growth of organism.

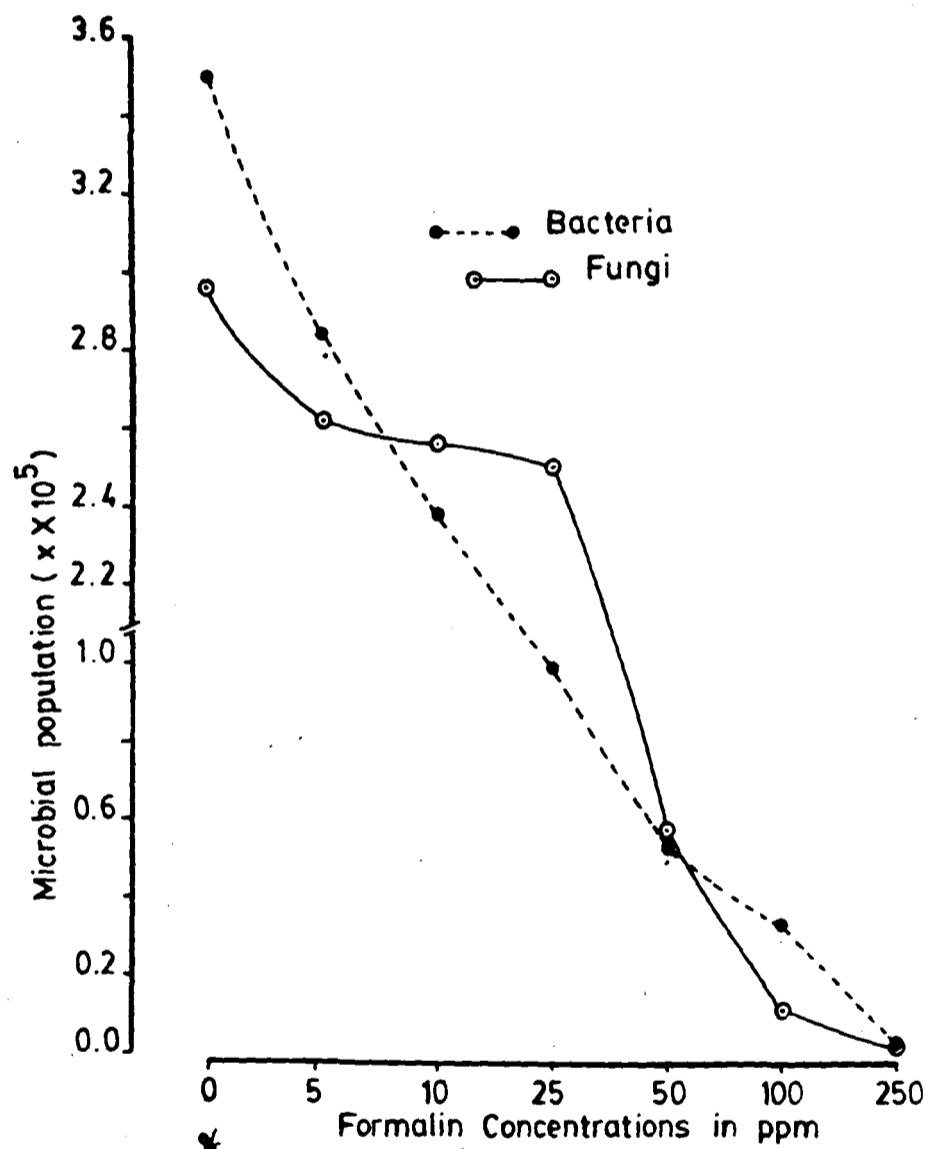


Fig.3 : Effect of formalin on microbial population of fresh paddy straw.

Maximum fungal population was recorded in T₁ ($2.9 \times 10^3/g$) whereas least fungal population ($0.1 \times 10^3/g$) was observed in T₆ (100 ppm) but complete inhibition of fungal population was found in T₇ (250 ppm). However, T₂ (5 ppm), T₃ (10 ppm), T₄ (25 ppm) and T₅ (50 ppm) showed population of 2.62×10^3 , 2.56×10^3 , 2.51×10^3 and $0.47 \times 10^3/g$, respectively (Fig.3).

15. Effect of formalin on mushroom yield and occurrence of brown spot

Effect of formalin on mushroom yield and occurrence of the disease was determined. Results pertaining to this experiment are presented in Table 15.

Among eight treatments with 3 replication each, T₂ treated only with pathogen) recorded the brown spot disease, whereas the rest of the treatments T₃ (25 ppm), T₄ (50 ppm), T₅ (100 ppm), T₆ (250 ppm), T₇ (500 ppm) and T₈ (1000 ppm) showed complete absence of the disease (Plate 7). No brown spot was observed in T₁ which was maintained as untreated control.

Maximum yield was recorded in T₇ (301.66 g/bag) with an increased bioefficiency (116.00 %) followed by T₆ (298.33 g/bag) with the bioefficiency of 114.70 per cent. The treatments T₃ (25 ppm), T₄ (50 ppm), T₅ (100 ppm) and T₈ (1000 ppm) showed the yield of 264.33 g, 290.00 g, 293.33 g and 296.66 g per bag with bioefficiency of 110.13, 111.53, 112.81 g and 115.30 per cent, respectively. Significantly reduced yield (78.33 g/bag) as well as bioefficiency (31.90%) were observed in T₂ treated with pathogen (Fig.4).

Table 15. Effect of formalin on the yield of oyster mushroom and occurrence of the disease

Treatments	Yield (g/bag)	Bioefficiency (%)	Occurrence of brown spot
T ₁ : Formalin 0 ppm	296.66	118.60	-
T ₂ : <u>P. stutzeri</u>	78.33	31.90	+
T ₃ : T ₂ + Formalin 25 ppm	264.33	110.13	-
T ₄ : T ₂ + Formalin 50 ppm	290.00	111.53	-
T ₅ : T ₂ + Formalin 100 ppm	293.33	112.81	-
T ₆ : T ₂ + Formalin 250 ppm	298.33	114.70	-
T ₇ : T ₂ + Formalin 500 ppm	301.66	116.00	-
T ₈ : T ₂ + Formalin 1000 ppm	296.66	115.30	-
CD at 5%	50.18		

Note : Each value given above represents an average of three replications

+ Appearance of brown spot

- No appearance of brown spot

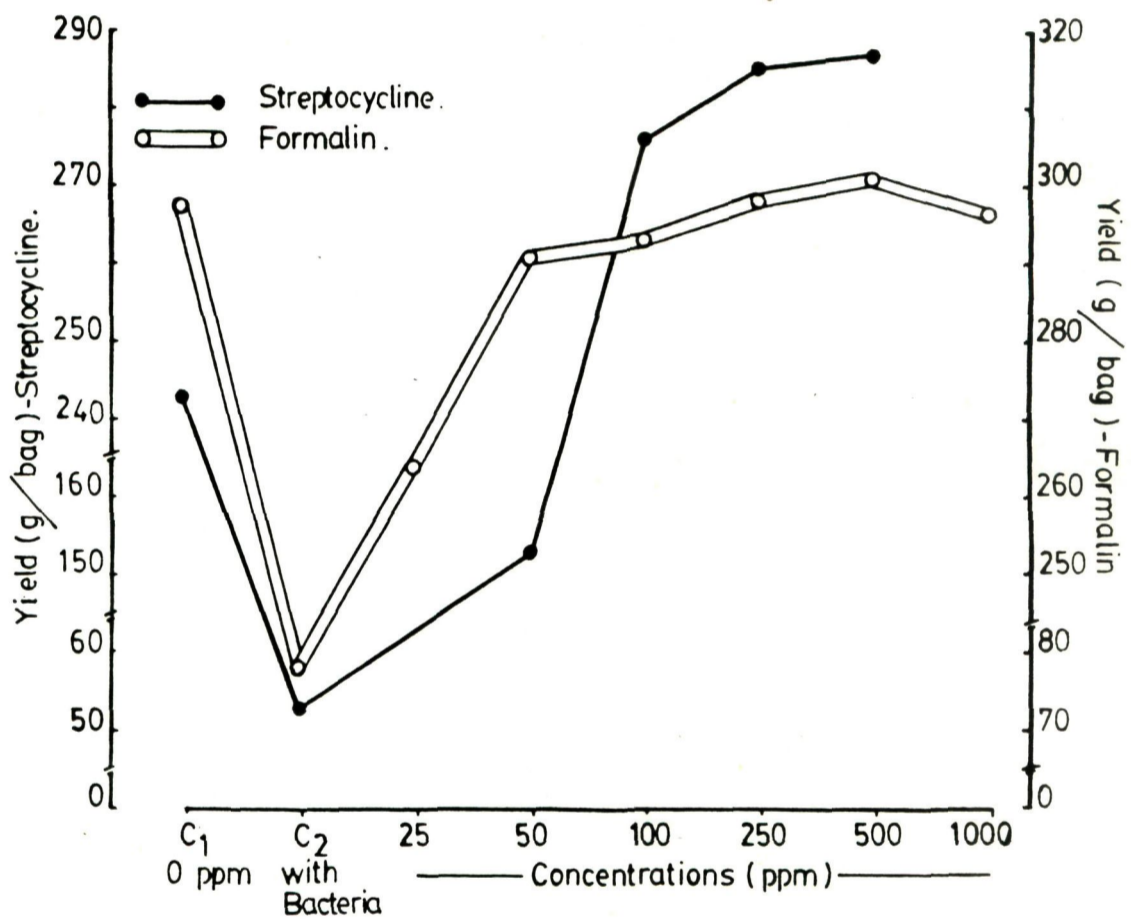


Fig. Effect of STREPTOCYCLINE and FORMALIN on yield of Oyster mushroom.

Plate 7 : Control of brown spot disease in formalin treated paddy straw at different concentrations (in ppm)

- 1 : Uninoculated control
- 2 : Inoculated with Pseudomonas stutzeri
- 3 : Pseudomonas stutzeri + 25 ppm formalin
- 4 : Pseudomonas stutzeri + 50 ppm formalin
- 5 : P. stutzeri + 100 ppm formalin
- 6 : P. stutzeri + 250 ppm formalin
- 7 : P. stutzeri + 500 ppm formalin
- 8 : P. stutzeri + 1000 ppm formalin



Plate : 7

DISCUSSION

V DISCUSSION

Among several diseases of mushroom, "Brown spot" of oyster mushroom caused by Pseudomonas stutzeri (Lehmann and Neumann) Sijderius is the most important one causing an economic yield loss upto 60 per cent to the mushroom industry. The disease was reported on mushroom bag during spawn run stage (Mallesha, 1987; Mallesha and Shivappa Shetty, 1988).

In the present study, since physical methods like pasteurization and heat treatment were found not successful in controlling the disease, emphasis was paid to the chemical method^{of}/control, using antimicrobial chemicals like antibiotics and formalin at various levels. The results of the investigation are discussed hereunder:

A preliminary study was carried out to assess the microbial population for the samples obtained from diseased, healthy and fresh paddy straw substrates. The results showed higher microbial population for the brown spot infected substrate compared^{to} the sample of fresh paddy straw and the sample selected from healthy bag (Table 1). These results are in agreement with the work of Mallesha (1987) who reported that the higher microbial population for brown spot infected substrate. Similar trend was also observed in oyster mushroom substrate by Shetty and Krishnamoorthy (1980) and Prabhu Dessai (1982).

The diseased mushroom paddy substrate was analysed qualitatively and quantitatively for bacterial population. The study indicated that the infected substrate contained six types of isolates grouped based on their colony morphology, namely, BSI₁, BSI₂, BSI₃, BSI₄, BSI₅ and BSI₆ (Table 2). Of these, BSI₁ was a spore forming gram positive rods. These results are comparable with the earlier workers (Krishnamoorthy, 1981; Prabhu Dessai, 1982) who reported that the bacterial population after pasteurization mainly consisted of Bacillus, Pseudomonas and Sarcina sp. However, further studies are required to elucidate the type of microflora associated.

Further, pathogenicity test was carried out by using six isolates obtained from infected paddy substrate. The bags inoculated with BSI₆ reduced the mushroom yield drastically (53.33 g/bag) so also the bioefficiency (21.33%) and brown spot symptom was also observed for the same (Plate 2). This indicated the effect of BSI₆ isolate on the mushroom yield. The rest of the isolates did not affect the yield and bioefficiency of mushroom to any significant level. However, the isolate BSI₅ gave slightly increased yield over the control (251.33 g/bag). It might be due to the stimulatory effect of certain group of associate bacteria. Similar observation was also recorded by Fermor and Wood (1981) who reported that the microbial associates and their products served as a source of nitrogen, carbon and mineral for button mushroom production. Krishnamoorthy (1981) and Prabhu

Dessai (1982) also reported the beneficial effect of associate microflora in oyster mushroom production.

The causal organism of brown spot was identified (Table 4). The bacterium was found to be gram negative, rod shaped, motile and did not form endospore. It could grow on minimal medium and selective Pseudomonas medium (Appendix II). The organism hydrolyzed starch but not gelatin, did not accumulate PBH, did not produce fluorescent pigment ~~but~~ denitrified the nitrate. It could grow at 41°C but not at 4°C. All these characters were found similar to that of Pseudomonas stutzeri (Pelleroni et al., 1970). These results are found in agreement with the work of Mallesha (1987) who followed the same procedure for identification of the pathogen. However, some of the strains of P. stutzeri were found closely related to other Pseudomonas sp. like P. stariari and P. mendociana (Pelleroni et al., 1970). Therefore, further studies are required to understand the clear taxonomy.

Since the causal organism was found resistant to pasteurization and heat treatment, the studies on chemical control methods were undertaken by using antimicrobial chemicals, antibiotics and a disinfectant formalin.

Control of brown spot disease by using antibiotics

Antibiotics are commonly called the 'miracle drugs'. They are the chemical substances produced by microorganisms and are capable of inhibiting the growth or of completely

destroying other organisms. The first antibiotic was discovered in Scotland from the fungus Penicillium notatum by a microbiologist, Alexander Fleming (1928) as quoted by Mishra (1980). Later many antibiotics have been discovered from all parts of the world one after another.

Antibiotics are of two types based on their activity (i) broad spectrum antibiotics which are active against higher group of microorganisms and (ii) narrow spectrum antibiotics which are effective against specific organisms (Mishra, 1980). Many antibiotics are now being successfully used to cure plant diseases (Waksman, 1963; Thirumalachar, 1968; Shetty and Rangaswami, 1968 and 1971; Stoller, 1978; Husson et al., 1985). In the present study, antibiotics used are chloramphenicol, streptomycin, streptocycline and tetracycline.

Chloramphenicol : Chloramphenicol, the first broad spectrum antibiotic, was isolated from an actinomycete, Streptomyces venezulae but now it is marketed as a synthetic product. The antibiotic is active against gram positive, gram negative bacteria, rickettsia and viruses. It inhibits the protein synthesis (Newton, 1965).

Streptomycin : It was discovered by Waksman in 1944, from an actinomycete, Streptomyces griseus. It is a polyhydroxy antibiotic, composed of three parts : Streptidine, streptose and N-methyl-L-glucosamine, jointed to one another by glycosidic linkages. The antibiotic inhibits the protein synthesis (Foster De Snell and Hilton, 1967).

Tetracyclines : Tetracyclines and its derivatives are a group of crystalline antibiotics, all possessing the hydroxynaphthalene ring. Antibiotics in this group include chlorocycline demethyl chlorotetracycline, methacycline, oxytetracycline and tetracycline. These groups are also known to inhibit several enzyme system and protein synthesis (Foster De Snell and Hilton, 1967).

Streptocycline : This antibiotic developed at Hindustan Antibiotic Limited, Poona, has proved active against many bacterial diseases caused by Pseudomonas and Xanthomonas sp. (Shetty and Rangaswami, 1968, 1971; Thirumalachar, 1973). It is a combined form of streptomycin and tetracycline hydrochloride. The mode of action on microorganisms is same as that of streptomycin and tetracycline.

When four antibiotics namely chloramphenicol, streptomycin, streptocycline and tetracycline were screened for antibiotics activity, they gave varying inhibitory responses. Generally, streptocycline, a combined antibiotic, gave maximum inhibitory response at all concentrations. The maximum inhibition was recorded at 250 ppm (Plate 3). Next best was tetracycline which is a broad spectrum antibiotic, its inhibition zone against Pseudomonas stutzeri ranged from 7.00 mm to 14.33 mm depending upon the concentrations. Similar results for streptocycline as well as other antibiotics on the control of bacterial pathogen were reported by many workers (Chakrabarty and Rangarajan, 1966; Chakrabarty et al., 1969; Thirumalachar,

1973; Shetty and Rangaswami, 1968 and 1971; Stoller, 1978; Husson et al., 1985; Van Landuyt et al., 1986). As the streptocycline was found the best (Fig.1) in chemical screening experiment, further studies with streptocycline were carried out in the forthcoming experiments.

The causal organism Pseudomonas stutzeri was checked in liquid medium containing streptocycline at different concentrations for its survivability at different intervals (Table 6, Plate 4A and 4B). The results reflected that the concentrations at 100 ppm and 250 ppm revealed the complete inhibition of the pathogen at 30, 60 and 120 minutes intervals, whereas at 10 minutes interval even though the population of pathogen decreased not completely inhibited. These results indicate the requirement of time and concentration of antibiotic to inhibit the pathogen. These results are in agreement with the findings of Shetty (1968) and Shetty and Rangaswami (1971) who provided the evidence that the higher concentrations of antibiotic have better persistence in vivo against Pseudomonas solanacearum. Similarly the experiment for pathogen treated paddy straw was carried out with different concentrations of streptocycline (Table 7). In this case also the results were found similar to that of previous experiment conducted in liquid medium indicating that the addition of paddy straw did not affect the effectiveness of the antibiotic.

Further study was carried out to know the effect of streptocycline on mushroom fungus (Pleurotus sajorajju) at

various levels (Table 8). The results showed positive effect on mushroom fungus at all the concentrations used (Plate 5). This envisaged that the concentrations used in this investigation will not be inhibitory to the mushroom fungus. Similar trend was also observed by Shetty and Rangaswami (1971) who reported that the higher concentration of antibiotic (i.e. 1000 ppm) controlled Pseudomonas solanacearum which resulted in better germination of potato. Antibiotics streptomycin and agrimycin were also used to control bacterial leaf blight of rice (Balasubramanian et al., 1972).

Microbial populations for non-sterilized paddy straw treated with streptomycin at different concentrations were assessed. Reduction in the population was found with increased antibiotic concentrations (Fig.2). This revealed that the higher concentrations were found effective depending on the type of microorganisms but none of the concentrations used was found inhibitory on all the organisms. This might be due to the following reasons : (i) The particular antibiotic concentrations used may not be effective for all group of organisms. (ii) The antibiotic might be inactivated by certain group of microorganisms. These observations are in agreement with the statement made by Freeman (1979) who reported that the antibiotic Penicillin inactivated by the enzyme Penicillinase produced by a wide variety of microorganism in soil.

The effect of streptomycin on P. stutzeri under in vivo condition was carried out. The results showed complete absence

of brown spot at 100, 250 and 500 ppm concentrations, confirming that the above concentrations were effective against the pathogen under in vivo condition. Normal yield was observed at 100, 250 and 500 ppm indicating that the streptocycline at higher concentrations significantly controlled the disease thereby restoring the mushroom yield. These results are in conformity with the results of earlier workers (Shetty and Rangaswami, 1968 and 1971; Balasubramanian et al., 1972; Stoller, 1978) who observed positive response of antibiotics on yield of various crop plants including mushrooms.

Control of brown spot disease by using formalin

Formaldehyde in aqueous solution is known as formalin and it contains 37 to 40 per cent formaldehyde. Vaporization of formaldehyde, either from formalin or paraformaldehyde, is used to sterilize an enclosed area. It is a strong reducing agent and inactivates enzymes and other cell constituents. It kills both vegetative cell and spore at sufficient concentrations (Smith et al., 1964). For the best results, a relative humidity of about 70 per cent and a temperature of about 22°C are required (Powar and Dagainawala, 1986).

Formalin reacts with practically all types of organic compounds including unsaturated hydrocarbons, aldehydes, ketones, esters, alcohols, carbohydrates, proteins, amines, amides and phenols. Particularly the action of formaldehyde against proteins makes it an effective embalming agent and also as a good soil fumigant.

Formalin has been used as a disinfectant in various agricultural fields. It was used as fumigant in soil, mushroom house, mushroom beds, mushroom substrates, etc. (Smith et al., 1964; Van De Geijn, 1982; Lelley, 1984; Vijay, 1986). It is also ^{used} in the disinfection of trays and ^apads in silk industry (Tripurari Sharan, 1984). Therefore formalin as a strong antimicrobial chemical can be used in the control of brown spot disease of oyster mushroom.

Formalin at different concentrations were tested on causal organisms in liquid medium. The results showed that the concentrations beyond 25 ppm were found inhibitory to the causal organism at 10 minutes interval (Table 1). Rest of the concentrations below 25 ppm were found non inhibitory even after 120 minutes of incubation in liquid medium (Plate 6). This suggests that minimum ^{con}centration required to inhibit the pathogen is 25 ppm. These results are found in conformity with Collins (1967) who worked out the inferior lethal coefficient (as the concentration and time required to kill the non-spore forming bacteria) against Staphylococcus aureus. Similar trend was observed when sterilized paddy straw inoculated with Pseudomonas stutzeri and then treated with varied concentrations of formalin (Table 12). It gave similar results as in liquid medium. This indicated that the formalin could be used as disinfectant against P. stutzeri in paddy straw which was used as substrate for oyster mushroom cultivation. These results are in consonance with the work of

Vijay (1986) who used the formalin in combination with Bavistin at various concentrations for the treatment of oyster mushroom substrate.

Effect of formalin on mushroom fungus^U was tested in liquid medium. The results revealed that the concentrations at 50 ppm and above permanently inhibited mushroom growth under in vitro condition (Table 13). It is obvious to say that particular concentrations of formalin killed even mushroom fungus. These results are in agreement with the statement by Smith et al. (1964) who claimed that the sufficient concentrations of formalin destroyed all the organisms. Rest of the concentrations below 25 ppm showed no effect on the mushroom fungus indicating that these concentrations were not inhibitory to the mushroom fungus under in vitro condition. Similar trend was also observed by Vijay (1986) who used formalin in combination with Blitox for the chemical sterilization of oyster mushroom substrate. These results suggested that the formalin bath could be developed in place of pasteurization for decontamination of paddy straw as oyster mushroom substrate.

Effect of formalin at 5, 10, 25, 50, 100 and 250 ppm on microbial population of non-sterilized paddy straw showed the reduction in population with increased formalin concentrations upto 100 ppm. The concentration at 250 ppm showed complete absence of microbial population (Fig.3). This envisaged that the higher concentrations of formalin were effective on all kinds of microorganisms. These results are in agreement with

the findings of the earlier workers (Smith et al., 1964; Rutjens, 1977, Van De Geijn, 1982; Anila Bhandari and Singh, 1982; Lelley, 1984; Wong and Preece, 1985; Marwan and Nagel, 1986; Gerrits, 1986; Ho, 1987) who tried formalin and other chemicals at various concentrations to control many fungal as well as bacterial diseases of mushroom.

Finally, formalin bath at 25, 50, 100, 250, 500 and 1000 ppm was used against the substrate infected with pathogen Pseudomonas stutzeri. The treatments ranging from 25 to 1000 ppm concentrations were free from disease symptom in the substrate during oyster mushroom production (Table 15). This indicates that formalin beyond 25 ppm is effective against brown spot causal agent P. stutzeri. Similar results were also observed with formalin and other chemicals by different workers (Van De Geijn, 1982; Anila Bhandari and Singh, 1982; Marwan and Nagel, 1986; Gerrits, 1986, Vijay, 1986) who used formalin in combination with other chemicals for the control of various fungal as well as bacterial diseases of mushrooms. Normal growth of mushroom was observed throughout the cropping period in all formalin treated bags even upto 1000 ppm (Plate 7). In vitro experiment (Table 13) indicated that the fungus was inhibited by formalin treatment at 50 ppm, where the mushroom fungus was in constant contact with the disinfectant chemical, whereas in the present experiment (Table 15) the formalin treated paddy straw was spread on clean polythene sheet for more than one hour until formalin effect was

completely removed due to evaporation which gave normal mushroom yield (Fig.4). However, further studies are required to understand the effect of this chemical on non-sterilized substrate and control of brown spot disease.

SUMMARY

VI SUMMARY

Causal organism of 'Brown Spot' disease of oyster mushroom Pseudomonas stutzeri was isolated, identified and its chemical control method was studied. Four antibiotics and formalin were studied as chemical control agents.

Screening of antibiotics showed that streptomycin was efficient among the four antibiotics in controlling the brown spot disease of oyster mushroom. Streptomycin at concentrations 100-250 ppm inhibited the Pseudomonas stutzeri,^a brown spot causing ^{organism} in liquid medium. Similarly, when the antibiotic treated to the substrate in the presence of the P. stutzeri gave similar results as in the case of liquid medium. Further, antibiotic streptomycin was tested on the mushroom fungus (Pleurotus sajorcaju) at different concentrations. The streptomycin even upto 250 ppm indicated that positive influence on the mushroom growth.

Pseudomonas stutzeri inoculated into the sterilized paddy substrate was further treated with streptomycin at 50, 100, 250 and 500 ppm concentrations. The result showed no disease occurrence in the antibiotic ranging from 100 to 500 ppm. The streptomycin even upto the 500 ppm restored the mushroom yield. Thus the results indicated the possibility of using antibiotic bath in the control of brown spot disease of oyster mushroom.

Formalin, an antimicrobial agent, was used for the control of brown spot disease causing organism in oyster mushroom cultivation. In this study, effect of formalin in different concentrations on Pseudomonas stutzeri in liquid medium was investigated. Formalin after 25 ppm inhibited the causal organism. Similarly, when the formalin treated to the substrate in the presence of P. stutzeri gave similar results as in the case of liquid medium. Further, effect of formalin at 5, 10, 25, 50 and 100 ppm concentrations on mushroom fungus was checked in liquid medium (PDB). The formalin after 50 ppm showed inhibitory effect on the mushroom fungus. Formalin effect on microbial population of non-sterilized paddy straw was investigated by using different concentrations (5, 10, 25, 50, 100 and 250 ppm). Formalin at 250 ppm inhibited all kinds of microorganism in 7 hrs. incubation period. Finally use of formalin dip on mushroom yield was studied. The causal organism (P. stutzeri) inoculated into the sterilized paddy straw was further treated with formalin at 25, 50, 100, 250, 500 and 1000 ppm concentrations. The results revealed non-occurrence of brown spot disease in the formalin range from 25 to 1000 ppm concentrations. The formalin even upto 1000 ppm did not affect the mushroom yield. Thus, the results of formalin dip of paddy straw on P. stutzeri and mushroom production strongly suggested the practical value to decontaminate brown spot causal organism from paddy straw.

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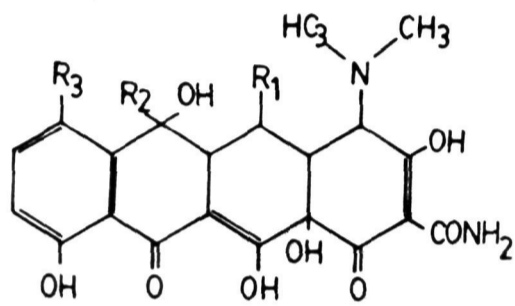
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APPENDICES

Appendix I. Properties of antibiotics used

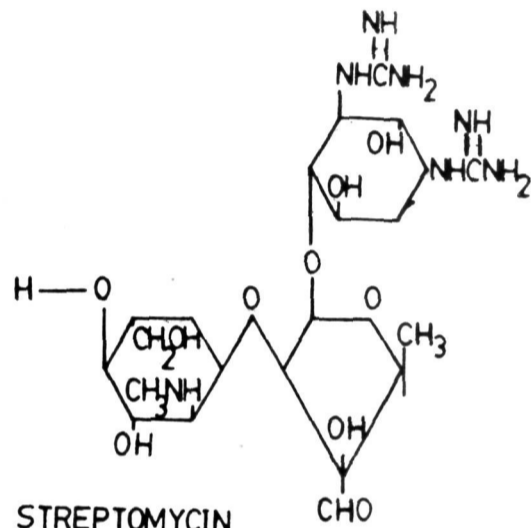
Antibiotics	Microbial source	Chemical type with formula	Spectrum	Inhibitory action
Chloramphenicol	<u>S. venezuelae</u>	Nitrobenzene derivative	Broad spectrum	PS, E : transpeptidation
Streptomycin	<u>S. griseus</u>	Aminoglycoside	Tuberculosis	PS, I, AA-tRNA coding, miscoding, f-met-tRNA-305 (50S) sub units
Tetracycline	<u>S. aureofaciens</u>	Polycyclic naphthone carboxide	Broad spectrum	PS, E : AA-RNA binding. 50S sub unit

S = Streptomycetes
 PS = Protein synthesis
 E = Elongation
 I = Initiation

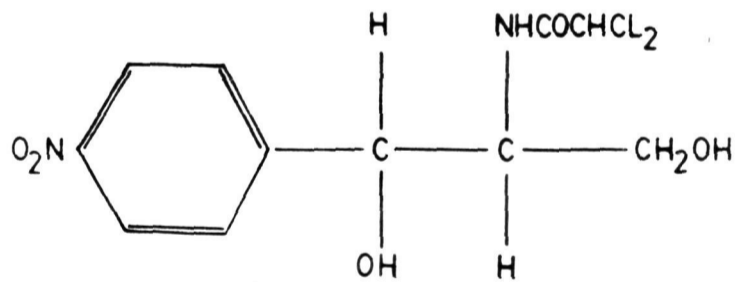


TETRACYCLINE

R ₁	R ₂	R ₃	
H	CH ₃	H	Tetracycline
OH	CH ₃	H	Oxytetracycline
H	CH ₃	Cl	Chlortetracycline
H	H	Cl	Dimethylchlortetracycline



STREPTOMYCIN



CHLORAMPHENICOL

Appendix II : Media used during investigations

I Potato dextrose agar fortified with yeast extract

Potato	.. 250 g (Boil and take the extract)
Glucose	.. 20 g
Yeast extract	.. 2 g
Agar	.. 15 g
Distilled water	.. 1000 ml
pH	.. 5.6

2. Nutrient Agar (NA)

Glucose	.. 5.0 g
Peptone	.. 5.0 g
Beef extract	.. 3.0 g
NaCl	.. 5.0 g
Agar	.. 15.0 g
Distilled water	.. 1000 ml
pH	.. 7.0

3. Martins Rose Bengal Agar (MRBA)

Dextrose	.. 10.0 g
Peptone	.. 5.0 g
KH_2PO_4	.. 1.0 g
Rose Bengal	.. 0.033 g
MgSO_4	.. 0.5 g
Streptomycin	.. 0.03 g
Agar	.. 15.0 g
Distilled water	.. 1000 ml
pH	.. 6.5-7.0

4. Starch Medium

Soluble starch	.. 3.0 g
Peptone	.. 10.0 g
Yeast extract	.. 10.0 g
K_2HPO_4	.. 5.0 g
Agar	.. 15.0 g
Distilled water	.. 1000 g
pH	.. 7.0

contd.

Appendix II (contd)

5. Minimal medium

Glucose	..	2 g
(NH ₄) ₂ SO ₄	..	1 g
K ₂ HPO ₄	..	7 g
MgSO ₄	..	0.5 g
Agar	..	15.0 g
Distilled water	..	1000 ml

6. Gelatin medium

Gelatin	..	5 g
Peptone	..	5 g
Beef extract	..	3 g
NaCl	..	5 g
Agar	..	15 g
Distilled water	..	1000 ml
pH	..	7.0

7. Nitrate medium

Tryptone	..	15 g
Yeast extract	..	3 g
KNO ₃	..	1 g
Distilled water	..	1000 ml

8. Acetamide-cetrimide-Glycerol-Mannitol selective medium for Pseudomonads (Mossel and Indacochea, 1971)

Peptone	..	0.2 g
K ₂ SO ₄	..	10.0 g
MgCl ₂ .6H ₂ O	..	1.4 g
Cetrimide	..	0.3 g
Glycerol	..	5.0 ml
D-Mannitol	..	5.0 g
Agar	..	15.0 g
Distilled water	..	900 ml
pH	..	7.0

When the sterilized medium has been cooled to 50°C a sterile (filtered) solution of 100 ml containing 10 g acetamide and 12 mg phenol red is added (pH 7.0)

contd.

Appendix II (contd)

9. King's A medium

Glycerine	.. 10 ml
Peptone	.. 20 g
K ₂ SO ₄	.. 10 g
MgCl ₂ ·6H ₂ O	.. 3.5 g
Agar	.. 15.0 g
Distilled water	.. 1000 ml
pH	.. 7.2

10. King's B medium

Glycerine	.. 10 ml
Peptone	.. 20 g
MgSO ₄ ·7H ₂ O	.. 1.5 g
K ₂ HPO ₄ ·3H ₂ O	.. 1.5 g
Distilled water	.. 1000 ml
pH	.. 7.2

11. Media for PHB productionPart A

K ₂ HPO ₄	.. 0.6 g
KH ₂ PO ₄	.. 0.2 g
MgSO ₄ ·7H ₂ O	.. 0.2 g
(NH ₄) ₂ SO ₄	.. 0.2 g
Distilled water	.. 900 ml

Part B

Glucose	.. 10 g
Distilled water	.. 100 ml
pH	.. 7.2

Part A and Part B are sterilized separately and then mixed under aseptic condition.

12. Pepton yeast extract medium

Peptone	.. 10 g
Yeast extract	.. 3 g
Distilled water	.. 1000 ml
pH	.. 7.2-7.4

Appendix III

Stains and Reagents

1. Gram's crystal violet, Hucker's modification

Solution A : Crystal violet (90% dye content) .. 2.0 g
Ethyl alcohol .. 20 ml

Solution B : Ammonium oxalate .. 0.8 g
Distilled water .. 80 ml

Mix solution A and B

2. Grams iodine (Gram's modification of Lugol's solution)

Iodine .. 1.0 g
KI .. 2.0 g
Distilled water .. 300 ml

Add iodine after KI is dissolved in water.

3. Gram's alcohol

95% ethyl alcohol .. 98 ml
Acetone .. 2 ml

4. Gram's safranin

Safranin O (2.5% solution in 95% ethyl alcohol 10 ml)
Distilled water : 100 ml

5. Malachite green:

Malachite green .. 5.0 g
Distilled water .. 95 ml
Filter before use

6. Reagents for testing nitrites

Reagent I : Sulfanilic acid : 2.0 g
5N acetic acid : 250 ml (1 part glacial
acetic acid to 2.5
parts water)

Mix the two reagents thoroughly, keep in dark coloured bottle.

Reagent II: Dimethyl-L-naphthylamine .. 1.5 ml
5N acetic acid .. 250 ml

Mix the two reagents thoroughly, keep in dark coloured bottle.

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