Chapter – 5

DISCUSSION

Mushrooms have been part of our human diet since time immemorial. The popularity of mushrooms is still based not on the nutrients that they contain but mostly on their exotic taste and their culinary properties. Mushrooms for medicinal purposes have been used for about 100 years. The cultivation of edible mushrooms has been accepted as a biotechnological process for conservation of various lignocellulosic, agricultural, industrial, forestry and horticultural wastes or their by-products into proteins.

Cultivation of mushrooms is popular in India in recent days. Among the cultivated mushrooms, *Pleurotus sajor-caju* has several advantages that can be easily and cheaply taken up by farmers and urbanites for cultivation. The species secrete an arsenal of enzymes specific for the digestion of lignocelluloses materials. Unfortunately like all other crops, mushrooms are also affected adversely by a large number of biotic and abiotic agents/factors. Among the fungal pathogens, *Fusarium oxysporum* is the most common and devastating fungi attacking the fruit bodies only, responsible for causing *Fusarium* rot in *Pleurotus* (Moore, 1959). The present investigations were carried out to study the integrated management of *Fusarium* rot through botanicals and biocontrol agents and to develop economically viable and ecofriendly management of this devastating disease of *Pleurotus* spp.

The results obtained in respect of above investigations are discussed in the following paragraphs.

The investigations on the management of *Fusarium* rot (*Fusarium oxysporum*) of Dhingri mushroom (*Pleurotus sajor-caju*) through botanicals and biocontrol agents were conducted in the Mushroom Research and Training centre (MRTC), Division of Plant Pathology and Division of Environmental Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir). The material (pathogen) for present investigation was collected from (MRTC), (SKUAST-Kashmir) Srinagar, from the diseased fruit bodies. The mycelium taken from *Fusarium* rot affected areas were placed on potato dextrose agar (PDA) and then subcultured on fresh PDA to have the pure culture of *Fusarium oxysporum*. The fungus initially produces white mycelium, which gradually turns to
violet or dark magneta colour producing a characteristic symptom of the disease. Morphological characters of _Fusarium oxysporum_ are in agreement with the results of the earlier workers, Nelson (1983), Gams and Nirenberg (1989), Booth (1971).

_Fusarium_ isolates collected from the fruit bodies were identified as two species viz., _Fusarium oxysporum_ and _Fusarium pallidiroseum_. _Fusarium oxysporum_ was the most common species found during the oyster mushroom production. On the basis of microscopic and morphological characteristics of the isolates fungus as compared with the authentic description together with its pathogenicity on _Pleurotus sajor-caju_, the fungus was identified as _Fusarium oxysporum_. _Fusarium_ spp. were compared with the description given by Synder and Hansen (1940) and were in agreement with their description.

Colony appearance, sporulation pattern, growth rate and micro morphological characteristics of _Fusarium oxysporum_ was examined on potato dextrose Agar (PDA). Macroscopic morphology varied significantly on different media and descriptions were based upon growth on PDA at 27°C. Colonies were initially white, becoming tinged with salamon and lavender at maturity. Lavender to purple reverse (Nelson _et al._, 1983) and (Sutton _et al._, 1998). The micro morphological characteristics of _Fusarium oxysporum_ were as follows. The hyphae were septate and hyaline. Conidiophores were short and simple (usually not branched). Macroconidia were usually produced abundantly, slightly sickle-shaped, thin walled, with an attenuated apical cell and a foot-shaped basal cell. They were 3 to 5-septate measuring 23-54 x 3-4.5 µm. Microconidia were abundant, mostly non-septate, ellipsoidal to cylindrical, slightly curved or straight, 5-12 x 2.3-2.5 µm occurring in false heads (a collection of conidia at the tip of the phialide) from short monophialides (Nelson _et al._, 1983).

Pathogenecity test was carried out according to Koch’s postulates. The symptoms of _Fusarium_ rot produced by _Fusarium oxysporum_ in _Pleurotus sajor-caju_ developed during the pathogenecity test, were characterized by browning of caps, especially towards the margins. Browning was followed by curling of margin of pileus, drying and shrinking of the fruiting bodies. Seth _et al._ (1978) studied that _Trichoderma_ spp., _Verticillium malthousei_ and _Fusarium semitectum_ were pathogenic among the fungi isolated from stored fruit-bodies of mushrooms. Zhang _et al._ (1992) reported isolates from wilted _P. ostreatus_, were identified as _F. semitectum_ (_F. pallidoroseum_), _F. monoliforme_ (_Gibberella fujikuroi_) and _F. graminearum_ (_G. zeae_). Field
observations and inoculations showed that Pleurotus wilt was caused mainly by *F. pallidoroseum* (frequency of occurrence 88%) followed by less pathogenic *G. Zeae* (6.7%) and *G. fujikuroi* (15.3%). Kim et al. (2007) observed the symptoms of water soaked lesions and soft rot in the stipes and pileus of cultivated *Pleurotus eryngii*. The interaction between *Pleurotus* and *Fusarium oxysporum* mycelia studied in dual culture revealed that hyphal collapse of *Pleurotus* at the point of contact with *Fusarium oxysporum* without any zone of inhibition, indicating thereby mycoparasitism between the two fungi. *Fusarium oxysporum* showed the maximum mycelial inhibition of mushroom mycelium (53.69%).

*In vitro* evaluation of Botanicals against both *Fusarium oxysporum* and *Pleurotus sajor-caju* revealed that all the botanicals more or less suppress the growth of *Fusarium oxysporum*. Among the ten botanical extracts tested, *Datura stramonium* expressed the strongest antifungal activity against *Fusarium oxysporum* but this botanical showed the strong inhibitory effect against *Pleurotus* mycelium also. Nidhi and Trivedi (2002) reported antifungal activity of *Datura stramonium* against *Fusarium oxysporum* f.sp. *cumini*. The best botanical that inhibits *Fusarium oxysporum* was *Allium sativum*, *Juglans regia*, *Metricaria*, *Azadiracta indica*, *Urtica dioeca* and *Mentha spicata*. These plant species may contain chemical compounds having antifungal properties. Leaf extracts of *Lycopersicon esculantum* and *Lantana camera* were less effective. Out of ten botanicals tested, only three botanicals, viz., *Metricaria*, *Artemisia indica* and *Azadiracta indica* were further evaluated against *Fusarium oxysporum* under mushroom house conditions. These botanicals displayed the maximum efficacy against *Fusarium oxysporum* and least adverse effects on *Pleurotus in vitro*. Sharma and Jarial (2000) evaluated Neem leaves and Neem cake against False Truffle (*Diehliomyces microspores*) disease of *Agaricus* spp. and recorded good results in controlling this disease *in vitro*. Shalini (2003) reported that complete inhibition of *Fusarium oxysporum* was obtained by *Allium sativum* (garlic) clove extract at 24 hr of incubation. Sharma and Rajesh (2005) observed that 10 percent Neem leaf extract was inhibiting the mycelial growth of *Sepedonium chrysospermum*, responsible for causing Yellow mold in button mushrooms. Saleh, et al. (2006) reported the antifungal property of *Artemesia herba alba* against *Penicillium citrinum* and *Mucora rouxii*.

*In vitro* evaluation of Bioagents against both *Fusarium oxysporum* and *Pleurotus sajor-caju* revealed that all the bioagents more or less suppress the growth of *Fusarium oxysporum*. Out of six bioagents, *Bacillus subtilis*-115 expressed the efficient antagonistic activity against
Fusarium oxysporum. In vitro evaluation of Bioagents against both Fusarium oxysporum and Pleurotus sajor-caju revealed that all the bioagents more or less suppress the growth of Fusarium oxysporum followed by P. flourescens-105, P. flourescens-104 and P. flourescens-103. Among these bioagents tested P. flourescens-103 revealed more inhibitory effect against pathogen but also inhibited the mycelial growth of Pleurotus sajor-caju strongly. The bioagents Bacillus subtilis-115, P. flourescens-105 and P. flourescens-104 revealed less antagonistic activity against P. sajor-caju but their inhibitory effect against the pathogen was very strong. Out of six bioagents tested in vitro, P. flourescens-105, Bacillus subtilis-115 and P. flourescens-104 were further evaluated for in vivo trail in mushroom house. The mode of antagonism observed with Bacillus spp. is antibiosis (Edward et al. 1994). Cho et al. (2002) reported the antagonistic activity of fluorescent Pseudomonas spp. against Pleurotus ostreatus (Bhanwar and Thakur, 2004) studied the effect of Azospirillum, Azatobacter, Bacillus Polymixa and Pseudomonas straita on vegetative growth and yield of oyster mushroom (P. sajor-caju).

This is supported by reports that most Bacillus spp. Produced many antibiotics such as bacillomycin, fengycin, mycosubtilin and zwittermicin, which are all effective in suppressing growth of pathogen both in vitro and in vivo (Pal and Gardener, 2006).

In vitro evaluation of fungicides against both Fusarium oxysporum and Pleurotus sajor-caju revealed that all the fungicides more or less suppress the growth of Fusarium oxysporum. Among the systemic fungicides tested by food poisoning technique, Carbendazim exhibited the maximum inhibition of pathogen (Fusarium oxysporum) followed by Hexaconazole. The least inhibition of mycelial growth of pathogen was expressed by Bitertanol. Systemic fungicides were tested against P. sajor-caju also and it was found that Carbendazim exhibit minimum efficacy against mushroom, indicating that P. sajor-caju is slightly resistant to this fungicide. Among the two non-systemic fungicides tested, Captan was found to be effective against Fusarium oxysporum. The minimum inhibition against Fusarium oxysporum was exhibited by Mancozeb and showed the maximum inhibition of mushroom mycelium. Out of five fungitoxicants tested in vitro, Carbendazim, Bitertanol and Captan were further evaluated for in vivo trail in mushroom house. The selected fungicides expressed the minimum inhibition of mushroom mycelium but strong inhibition of pathogen as compared to the other fungicides. Carbendazim was found to be most inhibitory against Fusarium oxysporum, but it expressed least inhibitory potential against P. sajor caju. This finding is in partial agreement of Rai and
Vijay (1992) who reported that Carbendazim stimulated the mycelial growth of *P. sajor-caju* at low concentration but inhibited it at higher concentration. Kang *et al.* (2002) reported that Hexaconazole gave the best inhibition of *Fusarium pallidoroseum* found on *Agaricus bisporus*. Parvez *et al.* (2009) evaluated Formalin, bavistin and combination of formalin and bavistin against mycoflora of oyster mushroom substrates. The combination of formalin and bavistin (500 ml + 75 ppm) was found to be the best in inhibiting the radial growth of all the identified fungi. Chakraborty *et al.* (2013) reported that bavistin at the dose of 0.5% provided highly significant inhibition to *Fusarium oxysporum* found to have associated with the fruit beds of *P. sajor-caju* and *Lentina edodes*.

*In vivo* evaluation of selected botanicals, viz., *Metricaria, Artemesia indica* and *Azadiracta indica* against *Fusarium oxysporum* was carried out in mushroom house. All the botanicals reduced the time taken for complete spawn colonizatrion as compared to control. A minimum mean average of (15.1 days) were taken by the treatment that receive *Metricaria*. It was followed by *Artemesia indica* and *Azadiracta indica* (15.2 days) and (15.5 days). Pin formation was observed (6.0, 6.3, 6.4 days) after complete spawn run in all the treatments which received different botanicals. The botanicals slightly reduced the time taken by pin head formation as compared to control (7.5 days). Similarly the maximum mean increase in yield (36.4%) over control was shown by the treatment which received *Metricaria*, followed by *Artemesia indica* (32.9%). Minimum mean increase in yield (27.7%) over control was recorded in the treatment which was amended with *Azadiracta indica*. It was further recorded that with the increase in concentration, the percent increase in yield over control also increased. Efficacy of botanicals against percent disease incidence of *Fusarium oxysporum* was also recorded, it was observed that all athe botanicals were effective in reducing the disease incidence when compared to control. *Metricaria* was most superior in reducing the incidence rate to (20.3%). It was followed by *Artemesia indica* (29.5%) and *Azadiracta indica* (51.8%). It was further observed that increase in concentrations of botanicals decreased the disease incidence of *Fusarium oxysporum*. Sharma and Janadik (1994) reported that leaves from *Azadiracta indica*, *Eucalyptus tereticornis* and *Eichhornia crassipes* and *Allium sativum* (colves) when incorporated in compost inoculated with various weed fungi, increased mushroom yield Grewal (1998) reported that incorporation of dried leaves of *Azadiracta indica* and *Eucalyptus* in mushroom compost eliminated *Fusarium* and *spendonium* sp. Raina (2004) reported that essential oil obtained from
exocarp of Citrus sinensis was effective against Curvularia, Fusarium oxysporum and Helminthosporium oryzae at 1000 ppm. Saleh et al. (2006) reported the antifungal property of Artemisia herba alba against Penecillium citrinum and Mucora rouxii. Inam-ul-Haq et al. (2010) investigated that certain active components of Eucalyptus camaldulensis, Azadiracta indica, Citrus lemon and Cymbopogon marginatus were capable of increasing mushroom yield and controls pathogenic microbes in oyster mushroom cultivation.

In vivo evaluation of selected bioagents, viz., P. flourescens-105, Bacillus subtilis-115 and P. flourescens-104 against Fusarium oxysporum was carried out in mushroom house. All the bioagents reduced the time taken for complete spawn colonizatrion as compared to control. The time taken for complete spawn-run in all treatments range from (14.5-15.0 days). It was further observed that the time taken for pin head formation was slightly reduced in treatments as compared to control. The average number of the days required for pin head formation in all treatments range from (5.3-5.7 days). Mandvi et al. (2000) reported that some isolates of Pseudomonas fluorescent reduced the time taken for pin head formation and increased the number of pin heads. Similarly the maximum mean increase in yield (25.3%) over control was shown by the treatment which received, P. flourescens- 105, followed by Bacillus subtilis-115 (21.9%). Minimum mean increase in yield (18.4%) over control was recorded in the treatment which was amended with P. flourescens-104. It was further recorded that with the increase in concentration, the percent increase in yield over control also increased. Bioagents were effective in controlling the Fusarium rot, by exhibiting the minimum disease incidence of Fusarium oxysporum as compared to control. P. flourescens-105 was the most effective antagonist exhibiting the minimum disease incidence (20.3%) followed by Bacillus subtilis-115 (27.7%) and P. flourescens-104 (37.0%) it was observed that with the increase in concentration of bioagents, the disease incidence was reduced. Ahlawat and Rai (1997) reported that Azatobacter and Phosphotika when mixed in compost at 1% w/w at the time of spawning stimulated the mycelial colonization of the compost. However, only phosphotika resulted in early pinning as well as early first harvest and a significant increase (32%) in yield. Cho et al. (2002) found that the inoculation of pure cultures of the mycelium with strains of fluorescent Pseudomonas spp. isolated from the mycelial plane of commercially produced mushrooms promoted the formation of primordia and enhanced the development of the basidiome of Pleurotus ostreatus. Bhanwar and Thakur (2004) reported that wheat straw supplemented with
Azobacter sp. and Azosprillim sp. at 2 and 4% required minimum time for spawn-run and produced more number of sporophores, while Bacillus polymixa (4%) and pseudomonas straita (2%) took maximum time for spawn run. The average weight of sporophore was maximum in B. polymixa and P.straita 2% while minimum weight was observed in P. straita (4%) and Azosprillim sp. (2%).

In vivo evaluation of selected fungicides, viz., Carbendazim, Bitertanol and Captan against Fusarium oxysporum was carried out in mushroom house. All the fungicides reduced the time taken for complete spawn colonizatrion as compared to control. It took (16.1 days) for spawn-run in the treatments of Carbendazim and Captan. It was followed by Bitertanol (16.2 days). Time taken for pin formation was also slightly reduced as compared to control, with (6.9 days) in Carbendazim followed by Captan (7.0 days) and Bitertanol (7.3 days). It was further recorded that with the increase in concentration, the number of days for pin head formation decreased. It was observed that fungicides had a significant effect on yield as well. Carbendazim was found to be superior in expressing the maximum increase in yield (37.4%) over control, followed by Captan (17.7%). The minimum increase in yield (6.6%) over control was shown by Bitertanol. It was further recorded that with the increase in concentration, the percent increase in yield over control also increased. Similarly fungicides were effective in reducing the incidence of Fusarium rot as compared to control. Carbendazim was the most effective antagonist exhibiting the minimum disease incidence (14.8%) followed by Bitertanol (20.4%) and Captan (27.7%). It was observed that with the increase in concentration of fungicides, the disease incidence was reduced. Kang et al. (2002) reported that Hexaconazole gave the best inhibition of Fusarium pallidoroseum found on Agaricus bisporus cultivated in paddy fields.

Integrated management of Fusarium rot was also tested in mushroom house. The most promising botanical, bioagent and fungicide were selected from previous in vivo treatments and were further evaluated against Fusarium oxysporum in mushroom house. Metricaria, P. flourescens-105 and Carbendazim were evaluated further in vivo. It was observed that a combination of (Carbendazim + P. flourescens-105 + Metricaria) and a treatment of P. flourescens-105 alone were superior in expressing the minimum number of days (13.6 days) for spawn-run. It was followed by Metricaria alone (14.6 days), followed by treatment showing the combination of (Carbendazim + P. flourescens-105) (15.3 days), (Carbendazim + Metricaria) (15.6 days), Carbendazim (15.6 days). Maximum time for spawn run (16.3 days) was shown by
the treatment (*P. flourescens* - 105 + *Metricaria*) but still was found to be lesser than control (18.0 days). In terms of pin head formation minimum time (5.0 days) was taken by the treatments inoculated with *P. flourescens*-105 alone and combination of (Carbendazim + *P. flourescens*-105 + *Metricaria*), followed by *Metricaria* alone (5.3 days), (Carbendazim + *P. flourescens*-105) (5.6 days), (Carbendazim + *Metricaria*) (6.0 days), (*P. flourescens*- 105 + *Metricaria* (6.3 days) and Carbendazim (6.6 days). It was further observed that Maximum increase in yield (29.8%) over control was recorded in treatment, showing the combination of (Carbendazim + *P. flourescens*- 105 + *Metricaria*), (Carbendazim + *Metricaria*) (27.6%), (Carbendazim + *P. flourescens*-105) (25.3%), Carbendazim alone (23.2%), *P. flourescens*-105 alone (22.7%), (*P. flourescens*- 105 + *Metricaria*) (22.0%). Minimum increase in yield (18.6%) was shown by *Metricaria* alone. Minimum disease incidence (5.5%) was recorded in (Carbendazim + *P. flourescens*- 105 + *Metricaria*), Carbendazim alone. It was followed by (11.11%) in (Carbendazim + *Metricaria*), (Carbendazim + *P. flourescens*- 105) and *P. flourescens*-105 alone. It was followed by (22.2%) in (*P. flourescens*-105 + *Metricaria*). Maximum disease incidence (44.4%) was recorded in the treatment of *Metricaria* alone but was lesser than the control (77.7%).

The problem of weed and competitor moulds is one of the limiting factors in successful cultivation of oyster mushrooms. Mushroom growers mostly rely on fungicides and pesticides for controlling mushroom diseases and pests but at the same time pesticides are less preferred in mushroom culture because of their limitations in health hazards. Moreover the use of pesticides leads to environmental pollution, residual toxicity, resistance in pathogen, microbial imbalance and reduced soil fertility. Now the researchers have focused their efforts on developing alternative inputs to control diseases. Botanicals and Biocontrol agents are more preferable than chemicals due to their lethal effects during consumption of mushrooms.

The current investigation provides an alternative to the chemical fungicides, minimizes the use of chemical load, minimizes the cost ratio, avoids the health hazards and is ecofriendly as well.