Chapter – 3

MATERIALS AND METHODS

The present investigation was conducted in Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Shalimar, Srinagar. All in vivo experiments were conducted in Mushroom Research and Training Centre (MRTC), Division of Plant Pathology, SKUAST-Kashmir. In vitro experimentation was carried out in Government Institute of Science, Aurangabad and Microbiology Laboratory, Division of Environmental Science, SKUAST-Kashmir.

3.1 General procedure followed

3.1.1 Glassware and cleaning

Borosil and corning glasswares were used throughout the experimentation. Glasswares were firstly rinsed with potassium dichromate solution (potassium dichromate 100g + sulphuric acid 500ml + distilled water 1000ml). These were then thoroughly cleaned with labolene liquid followed by rinsing several times in tap water and finally in distilled water.

3.1.2 Sterilization

Glasswares were sterilized by dry heat in hot air oven at 180°C for two hours. Media used for study were sterilized in an autoclave at 15lbs pressure/inch² for 15 minutes.

3.2 Isolation of the Fungus associated with Pleurotus Cajor-caju

Samples of Pleurotus sajor-caju showing various symptoms like Fusarium rot were collected from Mushroom Research Training Centre (MRTC), SKUAST-Kashmir, Srinagar. The sampling included the fruiting bodies of Pleurotus sajor-caju. For isolation of the fungus, pieces of mycelium taken from Fusarium rot affected areas were placed on potato dextrose agar (PDA) medium. The plates were incubated at room temperature (27°C) until fungal growth was visible. The fungi were then sub-cultured on fresh PDA medium for identification and characterization. Representative isolates from the samples were preserved for further studies.

3.3 Identification of the fungus

The isolated fungus were identified by preparing mounts in lactophenol-cottonblue and
comparing the morphology (size, shape, colour of conidia and conidiophores, mycelium and structures like acervulus) with standard descriptions of (Synder and Hansen, 1940).

The fungus causing *Fusarium* rot was identified as *Fusarium oxysporum*. Various isolates of *Fusarium* spp. were collected during Pleurotus mushroom cultivation in M.R.T.C-SKUAST-K. On the basis of microscopic examination, *Fusarium* isolates were classified into two species namely *Fusarium oxysporum* and *Fusarium pallidiroseum*.

### 3.4 Maintenance of the culture

The *Fusarium* isolates identified were subcultured on PDA slants and allowed to grow at 27 ± 1°C temperature for 6 days. The cultures so obtained were stored in refrigerator at 4°C and were maintained by periodic sub-culturing in PDA slants after every 15 days (Aneja, 2005).

### 3.5 Cultural and Morphological characteristics of *Fusarium oxysporum* and radial growth of *Fusarium oxysporum* on different media:

The characteristics and radial growth of *Fusarium oxysporum* were examined from cultures grown on five media at 28°C for 6-7 days.

#### 3.5.1 Potato dextrose agar (PDA)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peeled potato</td>
<td>200gm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20gm</td>
</tr>
<tr>
<td>Agar agar</td>
<td>20gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

#### 3.5.2 Czapek’s Dox Agar (CZDA)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>30gm</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>1gm</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.5gm</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>2gm</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.5gm</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.01gm</td>
</tr>
<tr>
<td>Agar agar</td>
<td>20gm</td>
</tr>
</tbody>
</table>
Distilled water : 1000ml

3.5.3 Corn meal dextrose agar (CMD)
Corn meal : 200gm
Agar agar : 20gm
Distilled water : 1000ml

3.5.4 Nutrient agar (NA)
Peptone : 5.0gm
Sodium chloride : 1.0gm
Beef extract : 1 gm
Yeast extract : 1.5 gm
Agar agar : 20 gm
Distilled water : 1000 ml

3.5.5 Sabourad dextrose agar (SDA)
Peptone : 5.0 gm
Dextrose : 20 gm
Agar agar : 17 gm
Distilled water : 1000 ml

Twenty ml of molten and cooled medium was poured aseptically into petriplates (9cm) and allowed to solidify. 5mm mycelial discs of seven days old culture of *Fusarium oxysporum* was used for inoculation. The plates were incubated at 27±1°C for five days. Radial growth was measured at 24 hourly intervals until colony covers the whole petriplate. Each treatment was replicated three times. The mean rate of growth was recorded and the data on radial growth was
analysed statistically.

3.6 Pathogenecity test

Pathogenecity test was done according to Koch’s postulates. The infected *Pleurotus sajor-caju* bags with fruiting bodies were collected from mushroom research and training centre. Infected areas of the crop causing *Fusarium* rot were placed aseptically on PDA. The plates were incubated at room temperature (27°C) until fungal growth was visible. The fungus was then subcultured on fresh PDA medium for identification. The fungus was then identified by studying its cultural and morphological characteristics by following standard description in the literature of (Synder and Hansen, 1940).

The pure culture of the fungus was made. The isolated fungus was inoculated on *Pleurotus* mycelium, growing on paddy straw, showing some pin formation, by pouring 3ml spore suspension @ 1x 10^5 spores/ml on the top of bed. A control treatment was maintained without any inoculums of weed fungus. Both inoculated and un-inoculated paddy straw bags were kept in cropping room at a temperature of 25°C. After 7-8 days of incubation period, symptoms of the disease were developed on the fruiting bodies. The same symptoms were also observed from the disease samples, collected from MRTC. This gives the positive pathogenecity test for *Fusarium oxysporum*.

3.7 Dual culture of *Pleurotus sajor-caju* and *Fusarium* spp.

To determine the effect of *Fusarium* spp. on mycelial growth of *Pleurotus* mycelium, a dual culture of both *Fusarium* spp. and *Pleurotus sajor caju* was performed .The pure culture of *Pleurotus sajor-caju* was procured from (MRTC) SKUAST-K, Shalimar, Srinagar .The culture discs of 5mm size of each *Pleurotus sajor-caju* and *Fusarium oxysporum* taken from the margins of vigorously growing 7 days old cultures were aseptically transferred to solidified PDA on the opposite side (5cm apart) in petriplates (9cm) .The petriplates containing culture bits of *Fusarium oxysporum* and *Pleurotus sajor-caju* alone served as control. The colony diameter of both *Fusarium* spp. and *Pleurotus sajor-caju* were measured after 7 days, when control plates showed the maximum mycelial growth and expressed as percent inhibition, after comparison with control Vincent (1947).

\[
\% \text{ inhibition} = \frac{D_1 - D_2}{D_1} \times 100
\]
\[ D_1 = \text{Colony diameter in the control.} \]
\[ D_2 = \text{Colony diameter in treated.} \]

3.8 Management of *Fusarium rot* disease

3.8.1 *In vitro* evaluation

3.8.1.1 *In vitro* evaluation of botanicals against both *Pleurotus sajor-caju* and *Fusarium oxysporum*:

In this study, ethanol extracts of 10 botanicals were evaluated for their antifungal activity against both *Pleurotus sajor-caju* and pathogen (*Fusarium oxysporum* the most frequent contaminant), causing *Fusarium* rot of mushroom. The botanicals and their parts used are given in the Table-1.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Local name</th>
<th>Botanical name</th>
<th>Plant part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metricaria</td>
<td>Metricaria</td>
<td><em>Metricaria</em></td>
<td>Foliage</td>
</tr>
<tr>
<td>Artemesia</td>
<td>Tethwan</td>
<td><em>Artemesia indica</em></td>
<td>Foliage</td>
</tr>
<tr>
<td>Lantana camera</td>
<td>Lantana camera</td>
<td><em>Lantana camera</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>Nettle</td>
<td>Soi</td>
<td><em>Urtica dioec a L.</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>Tomato</td>
<td>Ruvangun</td>
<td><em>Lycopersicon esculentum</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>Neem</td>
<td>Neem</td>
<td><em>Azadiracta indica</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>Mint</td>
<td>Pudina</td>
<td><em>Mentha spicata L.</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>Walnut</td>
<td>Doon</td>
<td><em>Juglans regia L.</em></td>
<td>Hull</td>
</tr>
<tr>
<td>Garlic</td>
<td>Rohann</td>
<td><em>Allium sativum L.</em></td>
<td>Cloves</td>
</tr>
<tr>
<td>Datura</td>
<td>Daturr</td>
<td><em>Datura stramonium L.</em></td>
<td>Leaves</td>
</tr>
</tbody>
</table>

3.8.1.1.1 Preparation of extracts

The extracts of different plant parts as mentioned above were prepared in ethanol.
Extraction was done at room temperature by simple extraction method (Deshpande et al., 2004). Plant parts were first washed with tap water and then with sterile distilled water and then shade dried. The cloves of garlic were not dried. They were grinded in an electric grinder. The shade dried plant parts were ground with the help of electric grinder to obtain the fine powder of each botanical. The dried powder was then kept in plastic bags for further use.

Dried powder of plant parts (20 g) was mixed with 200 ml solvent (70% ethanol) in 250 ml conical flasks. The garlic cloves (20 g) were crushed in electric grinder and then mixed with 200 ml solvent (70 % ethanol) in 250 ml conical flasks. All the flasks were plugged tightly with sterile cotton bungs and the mouth of conical flasks were wrapped with transparent polythene. All the conical flasks were kept on shaker for 36 hrs. Then the flasks were allowed to stand for 6 hr, in order to settle the plant material. Thereafter it was filtered and centrifuged at 500 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 40-50° C to make the final volume one fifth of the original volume. Heating the plant extracts at 40-50° C avoids the contamination. The extracts were stored at 4° C in air tight bottles for further use.

The plant extracts were evaluated in vitro through poison food technique (Nene and Thapliyal, 2000). The filtered was considered as standard extract solution (100%). Test concentrations of 0.5, 1, 2, 4 and 8 per cent were obtained by adding appropriate amount of sterile distilled water. 2 ml of each test extract (0.5, 1, 2, 4, 8%) was dispensed in petriplates (9 cm) with the help of sterilized pipettes. After pipetting the extract solution in petri plates, 20 ml of molten PDA was poured in petri plates containing the extract solution. The plates were gently rotated to ensure uniform distribution of extract in the media. After solidification, inoculations were done with 5 mm dia mycelial discs cut from 6 days old cultures of both Fusarium oxysporum and Pleurotus cajor-caju separately. Three replications were maintained for each treatment. The media without the extract served as check. The plates were incubated at 27±1° C till the complete growth was observed in control plates. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1947).

\[
\text{Mycelial inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100
\]

### 3.8.1.2 In vitro evaluation of bioagents against both Pleurotus cajor-caju and Fusarium oxysporum
Various bioagents were evaluated against *Pleurotus sajor-caju* and *Fusarium oxysporum*. The antagonists in this study were *Pseudomonas flourescens* isolate-103, *Pseudomonas flourescens* isolate-104, *Pseudomonas flourescens* isolate-105, *Bacillus subtilis* isolate-115, *Bacillus subtilis* isolate-116 and *Azospirillum*. Pure cultures of all these above mentioned microorganisms were procured from department of MRTC, SKUAST-Kashmir, Srinagar. All these bioagents were then tested for their antagonistic activity against both *Pleurotus sajor-caju* and *Fusarium oxysporum*. The culture discs of 5 mm size of each of the antagonists and the pathogen (*Fusarium oxysporum*) and *Pleurotus sajor-caju* taken from the margins of 7 days old cultures were aseptically transferred to solidified PDA on the opposite side (5 cm) apart in petriplates (9 cm). The petriplates having pathogen (*Fusarium oxysporum*) and *Pleurotus sajor-caju* separately served as control. The petriplates were incubated at 26 ± 1° C, till the complete growth was observed in control plates. Colony diameter of the *Fusarium oxysporum* and *Pleurotus sajor-caju* were recorded and percent growth inhibition over control was calculated according to the formula given by Vincent (1947).

\[
\text{Mycelial inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100
\]

3.8.1.3 *In vitro* evaluation of fungitoxicants against both *Pleurotus cajor-caju* and *Fusarium oxysporum*

In this study, five fungicides, 3 systemic: Carbendazim, Bitertanol and Hexaconazole each at 4o, 80, 100, 250, 500 µg/ml concentrations and 2 non systemic fungicides Captan and Mancozeb, each at 250, 500, 1000, 1500, 2000 µg/ml concentrations were evaluated to see their efficacy on the growth of *Fusarium oxysporum* and *Pleurotus sajor-caju*. 
Table-2: Fungicides used in the study

<table>
<thead>
<tr>
<th>Common name</th>
<th>Trade name</th>
<th>Chemical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>Bavistin</td>
<td>Methyl benzimidazole-2-yl carbamate.</td>
</tr>
<tr>
<td>Bitertanol</td>
<td>Baycor</td>
<td>1-(biphenyl-4-yloxy)-3, 3-dimethyl-1 (1h-1, 2, 4-triazol-1 yl) butan-2-ol</td>
</tr>
<tr>
<td>Hexaconazole</td>
<td>Anvil</td>
<td>(RS)-2- (2, 4-dichlorophenyl)-1-(1 h -1, 2,4 triazol-1- yl) hexan-2-ol.</td>
</tr>
<tr>
<td>Captan</td>
<td>Captain</td>
<td>N-trichloromethylthio-4-cyclohexane-1, 2- dicarboximide</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>Diathane M-45</td>
<td>Manganese ethylene bisdithio carbamate</td>
</tr>
</tbody>
</table>

The poisoned food technique was adopted for in vitro testing of fungicides (Nene and Thapliyal, 2000). Firstly, the required amount of distilled water was poured in conical flasks (100 ml). The flasks were plugged and then autoclaved at 15 lbs pressure/inch² for 15 minutes. The calculated quantities of fungicides were aseptically added to the cooled sterile distilled water in conical flasks, so as to get the desired concentration of active ingredient of each fungicide separately. The flasks were shaken gently to ensure the proper mixing of chemicals in distilled water. 2 ml from each flask was dispensed in petriplates with the help of sterilized pipettes. 20 ml of molten and cooled PDA was poured in each petriplate. The petriplates were rotated gently to ensure the uniform distribution of fungicide solution in the media. After solidification of media, mycelial discs (5 mm) were cut from the edges of 6 days old culture of both pathogen (Fusarium oxysporum) and Pleurotus sajor-caju with the help of sterile cork borer. Each disc of both fungi was transferred aseptically to the centre of each petriplate separately. Three replications per treatment were maintained. The PDA plates without fungicide were also inoculated with the discs and maintained as controls. The plates were incubated at 27 ± 1°C until the petriplate in control treatments were fully covered with mycelia growth. The radial colony growth was measured and the efficacy of fungicides was expressed as per inhibition of mycelia growth over control, calculated by using formula suggested by Vincent (1947).

\[
\text{Mycelial inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100
\]
3.8.2 *In vivo* evaluation

In this study, the botanicals, bioagents and fungicides which displayed least adverse effects on the growth of *Pleurotus sajor-caju* were evaluated to determine their inhibitory effect against the pathogen (*Fusarium oxysporum*) during its cultivation.

Studies were conducted in Mushroom Research and Training centre (MRTC), division of plant pathology, SKUAST-Kashmir, Shalimar, Srinagar.

3.8.2.1 Procurement and maintenance of culture

The pure culture of *Pleurotus sajor-caju* used in present investigation was procured from MRTC, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar. The culture was multiplied further in tubes containing potato dextrose agar medium.

3.8.2.2 Preparation of master grain spawn

Healthy, fresh and unbroken wheat grains were used for spawn preparation and procedure as described by Munjal (1973) was adopted. Wheat grains were thoroughly washed, 2-3 times with tap water and boiled for about 20 minutes in a steel pot till water was taken up by the grains. The excess water was drained out by spreading the grains over a wire mesh. Calcium carbonate and calcium sulphate (1:4 ratio) was mixed thoroughly with softened and cooled grains at the rate of 30 gms and 120 gms in 9 kg of boiled grain. The grain mixture was subsequently filled in empty, well cleaned, wide mouth milk bottles upto ¾ of their capacity.

These bottles were then plugged tightly with non absorbent cotton and sterilized at 1.58 kg pressure per square cm for one and half hour at 126.5°C.

The sterilized bottles were taken out of autoclave and shaken to prevent clumping of grains and shifted to inoculation chamber. The bottles were inoculated under aseptic conditions, with pure culture of *Pleurotus sajor-caju* and incubated at 23±2°C or 15-20 days till complete colonization of grains occurred. The bottles were gently shaken after 6 and 12 days of incubation to induce quick and uniform mycelial growth. Any contaminated bottles were discarded. The spawn bottles free from any contamination were used as master culture.

3.8.2.3 Substrate: processing and preparation

During the present investigation locally available cheap agricultural residue, paddy straw
was used as base material for cultivation of *Pleurotus sajor-caju*.

### 3.8.2.4 Substrate pre-treatment

The chopping of paddy straw was done manually into bits of 3-5 cm in length and were cleaned thoroughly 2-3 times with tap water and then soaked in water for 12 hours. These were then dipped in boiling water for 30 minutes, taken out, cooled in wooden basket and kept there till excess water was drained off. The desired moisture content of the straw was tested by squeezing the straw in between the palms and seeing that droplets of water do not trickle out from the straw.

### 3.8.2.5 Experimental design

The substrate (paddy straw) at the rate of 1 kg/bag was filled according to completely randomized block design (CRBD).

The spawned paddy straw with different concentrations of most promising botanicals, bioagents and fungitoxicants were filled into polythene bag @ 1 kg/bag separately to test their efficacy against the pathogen, *Fusarium oxysporum*.

### 3.8.2.6 In vivo evaluation of Botanicals against both *Pleurotus sajor-caju* and *Fusarium oxysporum*

This study was conducted to check the efficacy of botanicals *in vivo*. Botanicals with maximum *in vitro* efficacy against the pathogen and least inhibition effect on *Pleurotus sajor-caju* were evaluated *in vivo*. The most promising botanicals, *Metricaria, Artemesia indica* and *Azadiracta indica* were used for the study. Paddy straw after washing was drained properly. The dried powder @ the rate of 1, 2 and 3% (w/w) of selected plant materials was incorporated separately in the paddy straw and then filled in polythene bags at the rate of 1 kg dry substrate. The untreated bags (devoid of botanicals) were kept as control. All the treatments including control were replicated six times in CRBD. Spawn of *Pleurotus sajor-caju* was added at the rate of 1% on dry weight basis of substrate. Filling of spawn was done in layers. The bags were tied properly and 10-15 pin holes were made over the entire surface of the bag, to allow the proper aeration. The bags were then incubated inside the cropping room where temperature (28°C max. and 25°C min.) and relative humidity (80%) was maintained. Room having spawn running bags were kept in dark for 10-15 days till complete colonization of the compost with fungal mycelium (El-Kattan and El-Hadded, 1998).
The polythene bags were cut open when the substrates were completely colonized with mycelium. The colonized substrate blocks were kept on the stall shelves at a distance of 25 x 25 cm from one another in the production room. The blocks were then inoculated with 3 ml acrospore suspension of *Fusarium oxysporum* with a spore load of (1x 10³ spores/ml) in the middle, with the help of syringe. The untreated bags (devoid of botanicals) with the same inoculums load were kept as control. All the treatments including control were replicated six times in CRBD.

### 3.8.2.7 *In vivo* evaluation of bioagents against both *Pleurotus sajor-caju* and *Fusarium oxysporum*

The bioagents *Pseudomonas flourescens*-105, *Bacillus subtilis*-115 and *Pseudomonas flourescens*- 104 were used for *in vivo* study. Mass culture of bacterial antagonists was made on lignite. Bacterial and suspension was added in sterilized lignite powder @ 1:2 v/w and mixed well under sterile conditions. The lignite powder was thoroughly mixed with the pre treated paddy straw substrate at 1, 2 and 3% (w/w) on dry weight basis just before spawning. The substrate was filled in the polythene bags at the rate of 1 kg dry substrate per bag and the method of spawning was adopted using 1% spawn on dry weight basis of the substrate. Filling of substrate in the polythene bags was done in layers. Bags devoid of biofertilizers served as control. The filled bags were tied properly and 10-15 pin holes were made over the entire surface of the bag, to allow the proper aeration. The bags were then incubated inside the cropping room where temperature (28° C max. and 25° C min.) and relative humidity (80%) was maintained. Room having spawn running bags were kept in dark for 10-15 days till complete colonization of the compost with fungal mycelium.

After complete colonization, the poly bags were cut open. The blocks amended with biofertilizers were inoculated with 3 ml acrospore suspension of *Fusarium oxysporum* with a spore load of (1 x 10³ spores/ml) in the middle, with the help of syringe.

### 3.8.2.8 *In vivo* evaluation of fungicides against both *Pleurotus sajor-caju* and *Fusarium oxysporum*

The three fungicides, Carbendazim, Bitrrtanol and Captan were used for *in vivo* study.
The three fungicide concentrations were tested: 250, 500, 1000 µg/ml. The calculated quantities of fungicides were weighed and added to the water in plastic tubs. The chemicals were properly mixed with water in tubs. The paddy straw after proper washing and draining was soaked in different aqueous fungicides solutions in tubs. The straw was kept in solutions for 5 hours. After that, straw was drained properly keeping moisture content of 60%. The treated straw was then filled in poly bags at the rate of 1 kg dry substrate per bag. The bags were inoculated with the spawn of *Pleurotus cajor-caju* at the rate of 1% per bag, in layers. Un-treated (no fungicide), but spawned bags were kept as control. After spawning, the mouth of the bags were closed properly and 10-15 pin holes were made all over the bags for proper aeration. All the treatments including control were replicated six times in CRBD. The bags were kept for spawn running in the dark cropping room for 10-15 days till complete colonization of compost by mushroom mycelium. The temperature (28°C max. and 25°C min.) and relative humidity (80%) was maintained in cropping room. After the mycelia had completely covered the substrate, the poly bags were cut and prepared doses of inocula of 3 ml spore suspension of *Fusarium oxysporum* @ 1× 10³ spores/ml, was inoculated in the middle of the substrate block, with the help of syringe.

3.8.2.9 Fruiting and harvest:

After the completion of spawn running in all treated and untreated bags, the temperature was dropped to 15-17°C, relative humidity was raised to 90-95%. Fresh air wash was given to growing room by exhaust fan to lower the carbon dioxide, for 3-4 hours. Pin heads appeared and mushroom harvesting was done at maturity, the three mushroom flushes were picked for one month.

While carrying out the above three experiments *in vivo*, the observations on days to spawn-run, ist pin formation, percent increase in yield over control and (%) disease incidence were recorded. Yield of oyster mushroom was expressed as gram fresh fruiting body/bag of compost (1 kg) in three flushes of mushroom.

Per cent increase in yield in treated bags over control (un-treated) was calculated using the formula:

\[
\text{Per cent increase in yield over control} = \left( \frac{\text{Yield treatment} - \text{Yield control}}{\text{Yield treatment}} \right) \times 100
\]

Disease incidence was scored on a 0-5 scale with 0= disease free, 1=1-20% area
covered by disease, 2=21-40% area covered by disease, 3=41-60% area covered by disease, 4=61-80% area covered by disease and 5=81-100% area covered by the disease (Bhardwaj, et al. 1998). Percent disease incidence (PDI) was calculated as follows:

\[
PDI = \frac{\text{Sum of numerical values x Grades}}{\text{Total number of observations x Maximum grade}} \times 100
\]

3.8.2.10 Integration of management components

In this study, botanical, bacterial antagonists and fungitoxicant with maximum efficacy were evaluated in vivo together in different combinations for the development of integrated management strategy.

The selected botanical, bioagent and fungicide which displayed maximum efficacy against the pathogen in the previous trials were Metricaria, Pseudomonas flourescens-105 and Carbendazim. All these treatments were evaluated in vivo in cultivation trial of Pleurotus cajor-caju. The trail was laid during 2011. Following combinations were made for developing an integrated management strategy.

1) Fungitoxicants (F)
2) Biocontrol agent (BA)
3) Botanical (BO)
4) F x BA
5) F x BO
6) BA x BO
7) F x BA x BO

Control (un-treated straw)

A single trail was laid for carrying out this study. During this investigation, bag method of cultivation was adopted.

In first treatment, carbendazim at the rate of 0.1% was evaluated against the pathogen inoculated with the paddy straw. Paddy straw bits after rinsed twice, were drenched in carbendazim solution at the rate of 0.1% for 5 hours. The straw was drained properly and then filled in poly bags. The spawn of Pleurotus cajor-caju was added to the straw layer wise.
In the second treatment, the efficacy of *Pseudomonas flourescens*-105 was checked *in vivo*. The lignite powder containing *Pseudomonas* 105 inoculants was added to paddy straw at the rate of 3% (w/w) on dry weight basis just before spawning. This paddy straw amended with the bacterial inoculants was then filled in bags and spawn was inoculated layerwise.

In third treatment, the best botanical i.e., *Metricaria* was evaluated *in vivo*. Drained paddy straw was mixed with dried powder of *Metricaria* at the rate of 3% (w/w). Paddy straw amended with the *Metricaria* powder was filled in bags and spawn was added to it layerwise.

In fourth treatment, a combination of Carbendazim and *Pseudomonas flourescens*-105 was evaluated *in vivo*. The straw was soaked in 0.1% of Carbendazim for 5 hours. After the substrate was drained properly. Lignite powder containing *P. flourescens*-105 inoculants was incorporated in the paddy straw by mixing, at the rate of 3% on dry weight basis before spawning. The paddy straw was then filled in bags and the spawn was added to it.

In fifth treatment, a combination of Carbendazim and *Metricaria* was evaluated *in vivo*. The straw was soaked in 0.1% aqueous solution of carbendazim for five hours. The straw was then drained properly. Dried powder of *Metricaria* at the rate of 3% was incorporated in the substrate before filling in bags. Spawn at the rate of 1% was added layerwise.

In the next treatment, a combination of *Pseudomonas* 105 and *Metricaria* was tested *in vivo*. Paddy straw was washed and then drained properly. Dried powder of *Metricaria* and lignite with *Pseudomonas* 105 inoculants was added to the straw at the rate of 3% on dry weight basis. The straw amended with *Metricaria* and *Pseudomonas* 105 inoculants was then filled in poly bags. Spawn was added to it layerwise.

In the last treatment, a combination of Carbendazim, *P. flourescens*-105 and *Metricaria* was evaluated *in vivo*. Firstly paddy straw was drenched in 0.1% aqueous solution of Carbendazim for 5 hours. The straw was drained and then mixed with fine powder of *Metricaria* and lignite containing *flourescens*-105 inoculants at the rate of 3%. This treated straw along with spawn (1%) was filled in bags. All the treatments along the check (un-treated) were replicated six times in CRBD. The bags were transferred to cropping room, where temperature (30°C max. and 26°C min.) and relative humidity of 80% was maintained. Cropping room having spawned bags was kept in dark for 10-15 days till complete colonization of the substrate with mushroom mycelium. All the treated bags including control (un-treated) were inoculated with 3 ml spore
suspension of *Fusarium oxysporum* at the rate of $1 \times 10^3$ spores/ml.

After complete colonization, the temperature was dropped to 15-17°C and relative humidity was increased to 90-95%. Cropping room was aerated with the help of exhaust fan for 3-4 hours. Three mushroom flushes were picked for one month.

The observations on days for spawn run, 1st pin formation, percent (%) increase in yield over control and percent (%) disease incidence were recorded. Percent increase in yield in treated bags over control (un-treated) was calculated using the formula:

\[
\text{Percent increase in yield over control} = \frac{\text{Yield treatment} - \text{Yield control}}{\text{Yield treatment}} \times 100
\]