5. SUMMARY AND CONCLUSIONS

*Pleurotus* mushroom is referred to as ‘Oyster Mushroom’ and is a lignocellulosic fungus growing naturally in the temperate and tropical regions on dead and decaying wooden logs or sometimes on drying trunks of deciduous or coniferous trees.

In the present study, a total of 31 isolates of *Pleurotus* were procured/collected from different sources. The present investigations were conducted on morphological aspects, strain improvement and genetic diversity. The results obtained are summarized as follows:

5.1 **Cultural studies:**

A temperature range of 25-28°C was recorded to be optimum for majority of the *Pleurotus* isolates. Mycelial pattern of all isolates was recorded.

5.2 **Morphological Studies:** The experimental fruiting trials of all the isolates revealed a faster spawn run and early pinning in isolate PO-3, which also gave maximum biological efficiency of 70.5%. Morphological studies including shape, size, and colour of the pileus showed considerable variations among all the isolates. The colour of the pileus varied from off-white, pale yellow, light brown and grayish brown. Colour of the spore print varied from creamish to white and size of basidiospores ranged from 6.5-9.5X3.0-4.5 to 8.0-11.0X3.0-6.0 µm showing no significant variation.

5.3 **Strain Improvement:** Strain improvement in three isolates of *P.ostreatus* viz. PO-2, PO-6 and PO-7 using physical (UV light) and chemical mutagens (EMS) resulted in mutants showing retarded mycelial growth in PO-7(U4), PO-2(E3) and PO-2(E4). Another mutant PO-7(U4) exhibited desirable white colour of the sporophore as compared to control. However, no significant change in sporulation was observed.
5.4 Genetic diversity:

PCR amplification based on domain specific markers for V6 and V9 domains of mitochondrial SSU rDNA of 12 test isolates belonging to 9 *Pleurotus* spp. resulted in single amplicon of approximately 170 and 300 bp size. Direct sequencing with both forward and reverse primers and sequence alignment revealed the variability between 149-178 and 285-362 bp for V6 and V9 domains of mitochondrial SSU rDNA. The sequence alignment of 12 isolates for V6 and V9 domains showed insertion/ deletion between various regions 94-149 and 149-231 bp respectively. The present study confirmed that the sequences of the two domains are species specific.

The phylogenetic analysis for both test and reference isolates characterized them into three major clusters i.e A, B  and C. The isolates under test were categorized into group A and B while none of the isolate was placed in group C. Besides eight species viz. *P. ostreatus*, *P. floridanus*, *P. columbinus*, *P. sapidus*, *P. sajor-caju*, *P. eryngii*, *P. pulmonarius* and *P. citrinopileatus* already reported in group B, an additional species *P. hypsizygus* has been included in the same group. Consensus tree generated after alignment of V6 and V9 domains of 12 isolates using both NJ and UPGMA method differentiated the isolates into two major groups.

Intra species variation among *Pleurotus ostreatus* isolates collected from diverse ecological niches of Himachal Pradesh was studied using SSR (Simple sequence repeats) markers. Five 18 -mer primers viz. GBPO025, GBPO064, GBPO149, GBPO157 and GBPO171 producing consistent polymorphic banding pattern were selected from a panel of 36 primers for SSR analysis of 15 isolates of *Pleurotus ostreatus*. The number of scorable and polymorphic bands ranged from 3 to 5. The scorable bands subjected to cluster analysis using UPGMA option of NTYSIS-pc package version 2.0 generated a dendrogram categorizing various isolates into 9 groups at a cut-off of 66% similarity coefficient. In the present study, SSR analysis showed a wide variation among various test isolates of *Pleurotus ostreatus*.

Amplification of 5’ portion of 26S ribosomal DNA of *Pleurotus* species/strains using pair primers LROR and LR7 yielded a fragment of 1460 bp
in all the 31 isolates of *Pleurotus*. PCR-RFLP analysis of the amplified product using eight restriction enzymes viz. *EcoR* I, *Hha* I, *Bsp* I, *Dra* I, *Taq* I, *Alu* I, *Msp* I, and *Hinf* I resulted in 3, 3, 4, 3, 7, 3, 2 bands, respectively. Cluster analysis of PCR-RFLP pattern divided 31 isolates of nine *Pleurotus* species into two major clusters at 75 per cent similarity as a cut-off point with six species in cluster I and three in cluster II. However, no inference was drawn with respect to division of various species isolates into a specific species group or clades.

**Conclusions:**

- A total of 31 isolates of *Pleurotus* were procured/ collected from different sources.
- A temperature range of 25-28°C was recorded to be optimum for majority of the *Pleurotus* isolates.
- Faster spawn run and early pinning in isolate PO-3, which also gave maximum biological efficiency of 70.5%
- Colour of the spore print varied from creamish to white and size of basidiospores ranged from 6.5-9.5X3.0-4.5 to 8.0-11.0X3.0-6.0 µm showing no significant variation.
- Strain improvement in three isolates of *P. ostreatus* viz. PO-2, PO-6 and PO-7 using physical (UV light) and chemical mutagens (EMS) resulted in mutants showing retarded mycelial growth in PO-7(U4), PO-2(E3) and PO-2(E4).
- Another mutant PO-7(U4) exhibited desirable white colour of the sporophore as compared to control. However, no significant change in sporulation was observed.
- PCR amplification based on domain specific markers for V6 and V9 domains of mitochondrial SSU rDNA of 12 test isolates belonging to 9 *Pleurotus* spp. resulted in single amplicon of approximately 170 and 300 bp size.
The sequence alignment of 12 isolates for V6 and V9 domains showed insertion/deletion between various regions 94-150 and 147-231 bp respectively.

Consensus tree generated after alignment of V6 and V9 domains of 12 isolates using both NJ and UPGMA method differentiated the isolates into two major groups.

SSR analysis using five 18-mer primers viz. GBPO025, GBPO064, GBPO149, GBPO157 and GBPO171 producing consistent polymorphic banding pattern showed a wide variation among various test isolates of *Pleurotus ostreatus*.

Amplification of 5' portion of 26S ribosomal DNA of *Pleurotus* species/strains using pair primers LROR and LR7 yielded a fragment of 1460 bp in all the 31 isolates of *Pleurotus*.