CHAPTER 4
RESULTS

4.1. Study on mycelial growth pattern of oyster mushroom

Different media has been used to evaluate the growth rate of the fungal mycelia and it was observed that all the four fungal species grow fast in Malt extract agar when incubated at 25-27^0 C in BOD incubator. Increased growth rate was also observed in case of Potato dextrose agar but very slow growth was observed in case of water agar (Table 6). At first, large amount of hyaline areal mycelium was observed which become whitish after some time (Figure 4and 5). In case of *Pleurotus djamor*, the colour of mycelia was white in early stage but it becomes light pink at maturity. The mycelia cover the media with regular wavy mat with distinct margins. On the other hand, the mycelia of *P. florida* growing irregular with thin margin. Growth of *P. sajor-caju* and *P. ostreatus* was white cottony mat growing in concentric manner. Optimum temperature for mycelial growth was recorded at 25^0 C. With maturity distinct scanted smell was observed in all the culture plates (Table 8).

Table 8: Mycelial growth pattern of *Pleurotus* species in different media

Species	Media	Growth initiation (h.)	Growth rate (cm/day)	Colour	Texture
P. ostreatus	Water agar	36	0.50±0.023	White	Cottony
	Potato Dextrose agar	24	1.35±0.020		
	Malt extract agar	24	1.60±0.078		
P. sajor-caju	Water agar	38	0.40±0.040	White	Cottony with distinct weave
	Potato Dextrose agar	22.	1.25±0.026		
	Malt extract agar	20	1.40±0.012		
P. djamor	Water agar	40	0.30±0.008	Initial white become light pink after maturity	Regular with distinct weave
	Potato Dextrose agar	25.	1.45±0.032		
	Malt extract agar	22	1.70±0.052		
P. florida	Water agar	39	0.30±0.008	White	Irregular with thin margins
	Potato Dextrose agar	23	1.20±0.020		
	Malt extract agar	20	1.85±0.064		

(±) means Standard Error, calculated using 5 replicates of each plates

Figure 4: Mycelial growth pattern of *Pleurotus* species on potato dextrose agar (A) *P ostreatus*, (B) *P sajor-caju*, (C) *P djamor* and (D) *P florida*

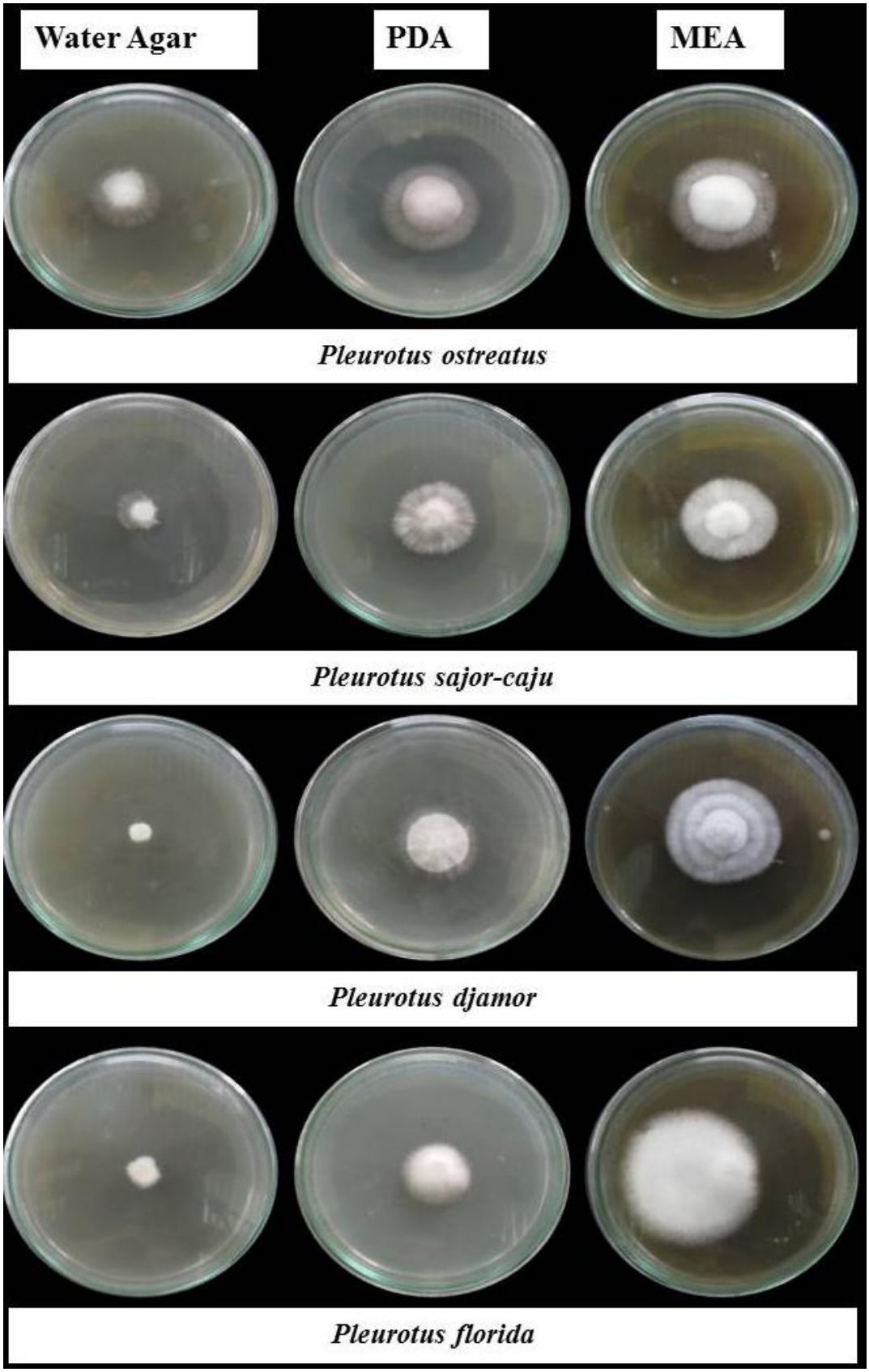


Figure 5: Mycelial growth rate of *Pleurotus* species on different media (incubation period 48h., temperature 28 $\pm 2^0$ C)

Table 9: Morphological Characteristics of different species of oyster mushroom

Organism	Shape	Size	Colour	Optimum Temperature	Characteristics	Spore characteristics
Pleurotus ostreatus (Jacq.Fr.) (Black Oyster)	The fruit body looks like a horse shoe	Diameter of the pileus is about 2-3 inches and the whole fruit body is about 4-5 inches	Dark black at young stage but become lighter at maturity	18-22^0 C is favourable but can grow up to 28^0 C	The mushroom grows in acropetal bunches. It is very popular for its fleshy velvety texture with distinct aroma. Gills are decurrent on lower side.	Spores are long, oval to kidney shaped, spores attached with basidium at middle portion of the base, about 2-4µm in length
Pleurotus djamor (Pink Oyster)	Fruiting body wide, wavy outer edge with no particular shape	3-4 cm diameter. Stipe is absent or present in very little length. Thickness is about 3-4mm outer edge.	Dark pink in young stage but become light pink on mature stage	Primordia initiated at lower temperature about 18-20^0 C.	Primordia are thin, leathery in texture with distinct aroma. Gills are also pink in lower side. Limited amount of water is required during fruiting.	Spores are oval shaped short, often kidney shaped and attached at the base with basidium. Spore length 1.8-3.5 µm
Pleurotus sajor-caju Fr. (Grey Oyster)	Fruiting body is fan shaped	Pileus diameter upto 4-5 inches	Grey at young stage and at maturity the colour become light grey	It grows at higher temperature about 25-30^0 C	Thick wide fruiting body With long stipe.	Spores are long, oval shaped with sharp edges and attached with basidium in one corner of the base, size about 2.5-5 µm
Pleurotus florida (Eger) (Milky white Oyster)	Pileus is like a disc on a long stipe	3-5 inch pileus with 2-3 inch of stipe	Bright white colour at pinhead as well as in mature stage	18-20^0 C is favourable but it can be grown in 28^0 C also.	Pileus is graciously white with delicate flesh which is turgid in texture with decurrent gills. Stipe is thick and extended upto the base of the pileus	Spores are short, single chambered attached in a corner of the base with basidium, size 2.6-4 µm

4.2. Morphological and histopathological study of oyster mushroom

Pleurotus ostreatus is one of the oyster mushroom widely cultivated in North Bengal for its shape, texture, taste and the environmental condition is very much favourable for its cultivation (Figure 6). Fruiting body initiated in 18-22^0 C with 80-85% relative humidity which is very common in this region. Pileus is thick, fleshy with a very short stipe. Pinhead appears light blackish thus it is popularly known as Black oyster mushroom (Table 9). Diameter of the pileus is about 2-3cm and the whole part of the fruiting body edible. Basidiospores are long, oval to kidney shaped about 2-4µm long and four spores attached with the basidium. On the other hand, *Pleurotus sajor-caju* is one of the major oyster mushroom cultivated in North Bengal in a very large scale. This mushroom is very popular for its large size which increases the production rate. This species can be cultivated throughout the year for its wide range of environmental requirement (Table 9). The fan shaped pileus diameter is very large (4-5cm) with a ling stipe (Figure 7). The pileus is fleshy, prominent edges with distinguish grey colour. Anatomical study reveals that the basidiospores are long, oval shaped with quite prominent edges. Spores are attached with the basidium by the corner of the basal part of the spores. Cultivation of oyster mushroom is very common practice in North Bengal and the environmental condition is very much suitable for the cultivation. There are two species mainly cultivated in North Bengal. Pink oyster mushroom is very commonly cultivated in the north western part of India. *Pleurotus djamor* is a new introduction of oyster mushroom in North Bengal. It looks very gracious on bed; pileus is pink in colour with very small stipe. Sometimes stipes is absent. Pileus diameter is about 2-3.5 cm, fleshy with light aroma, gills are decurrent (Figure 8). *P. djamor* had been cultivated during the autumn to spring as it requires lower temperature (18-20^0 C) with 75-85% relative humidity. Basidiospores are oval to kidney shaped and four spores attached with the basidium. *Pleurotus florida* also cultivated widely in this area and about 18-20^0 C requires for fruiting initiation. Pileus is bright white, fleshy, thick and about 2-3cm diameter. Stipe is long with decurrent gills. Fruiting initiation occurs in a cluster (Figure 9). Basidiospores are long oval shaped attached with basidium. Spores were about 2-3.5µm attached with basidium. *P. florida* is being cultivated very commonly during the winter season as it requires very low temperature for its fruiting body initiation with very low amount of relative humidity.

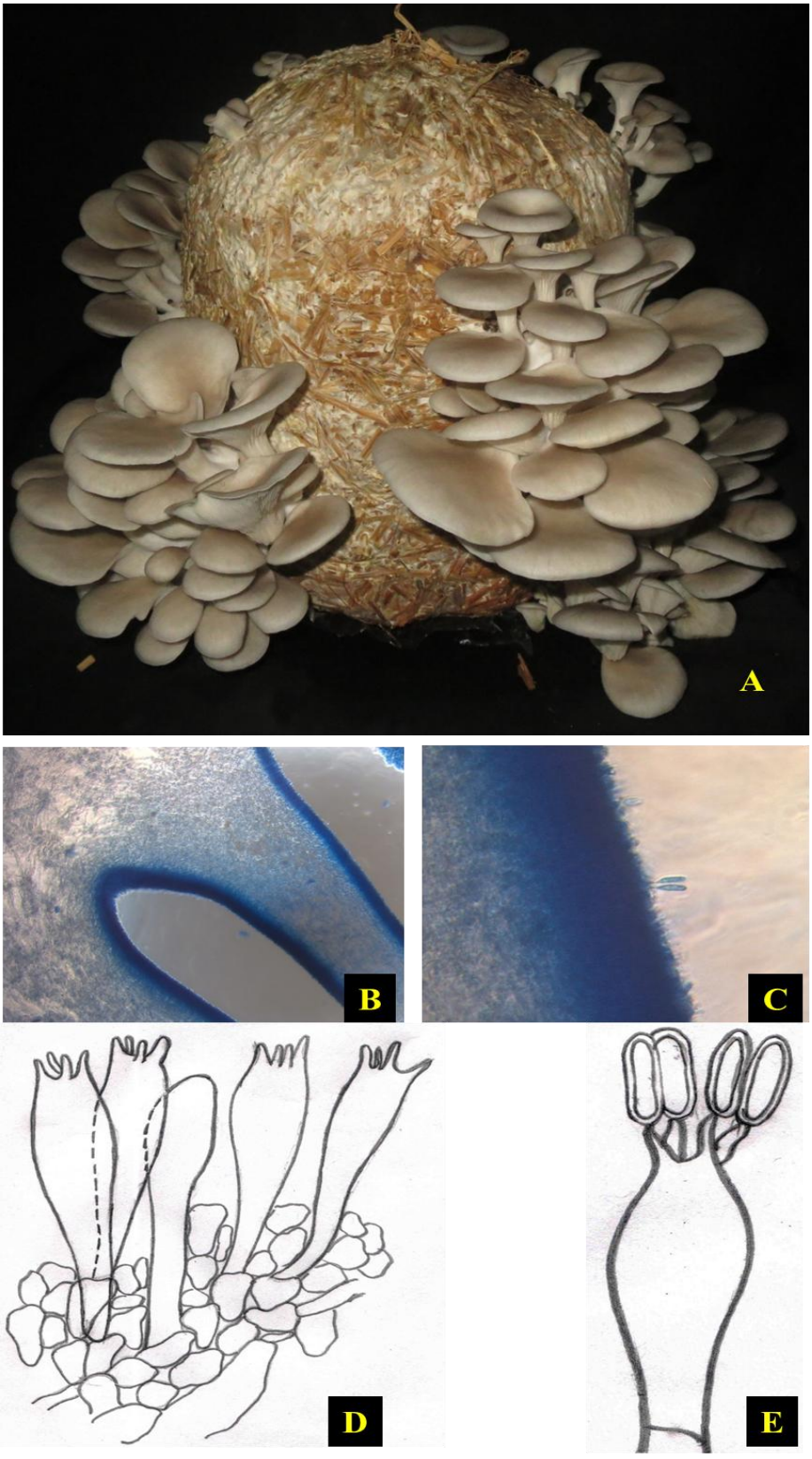


Figure 6: Morphological and anatomical features of *P ostreatus* (A) showing the fruiting body, (B) T.S of gill (10X); (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

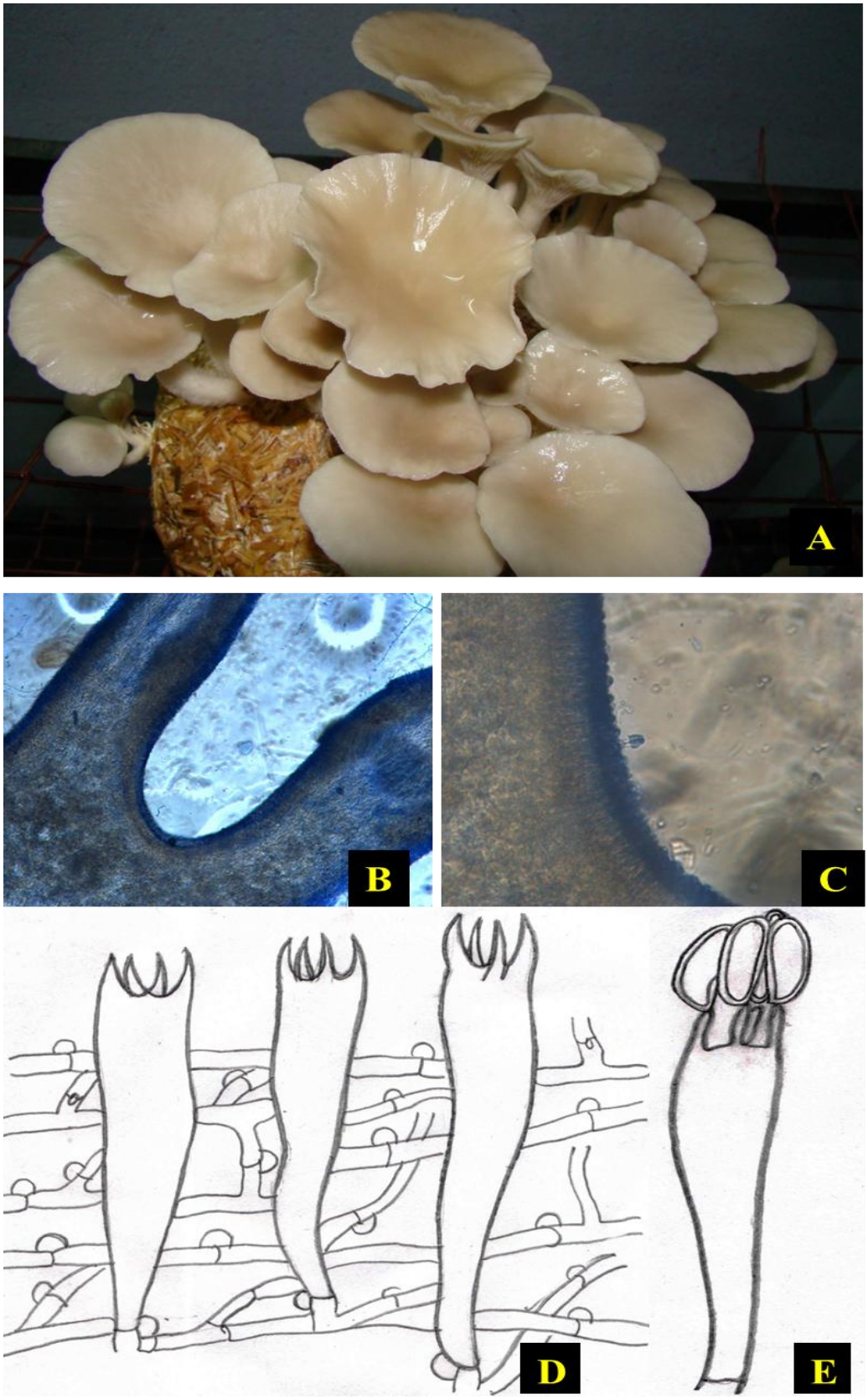


Figure 7: Morphological and anatomical features of *P sajor-caju* (A) showing the fruiting body, (B) T.S of gill (10X); (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

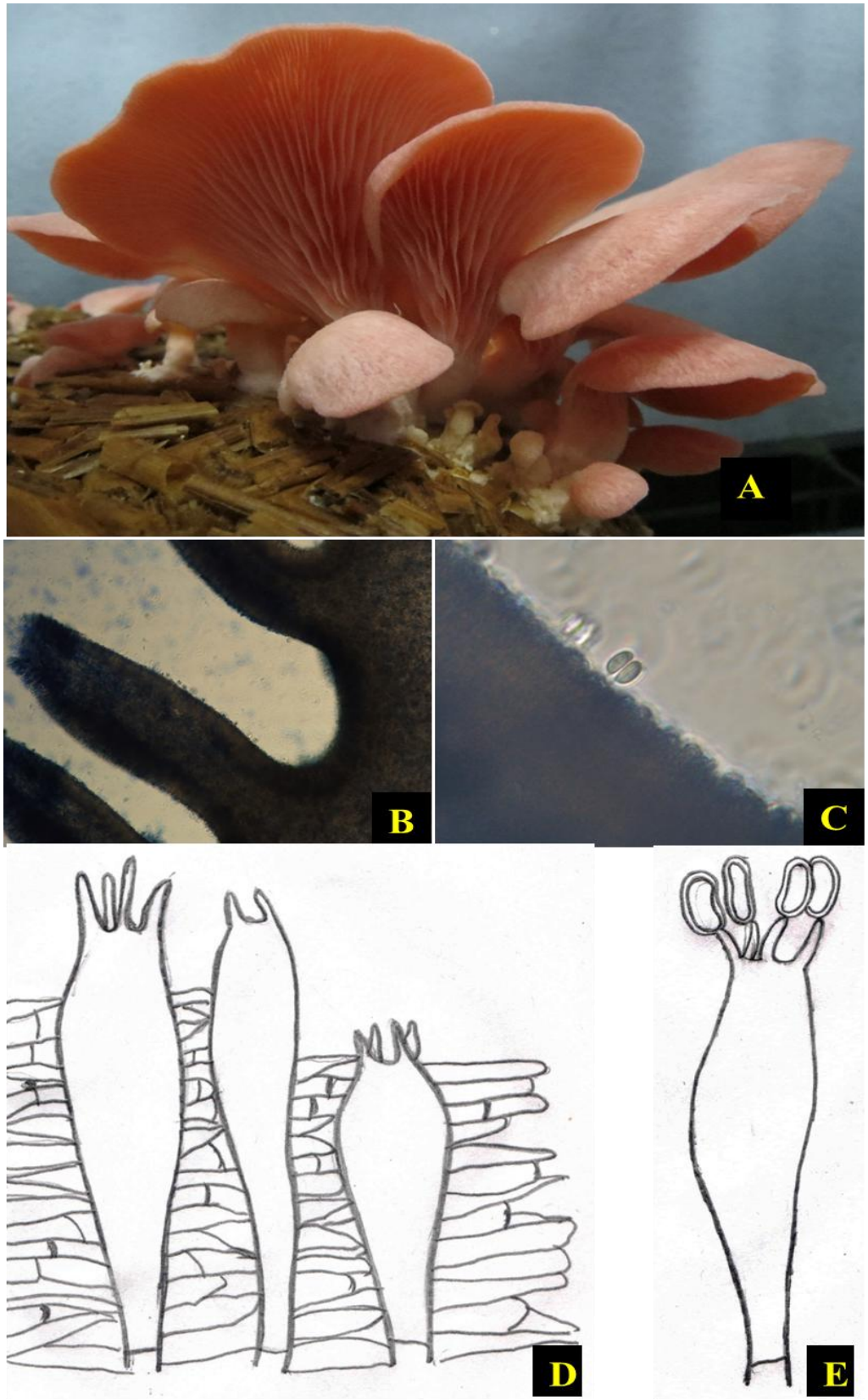


Figure 8: Morphological and anatomical features of *P. djamor* (A) showing the fruiting body, (B) T.S of gill 10X; (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

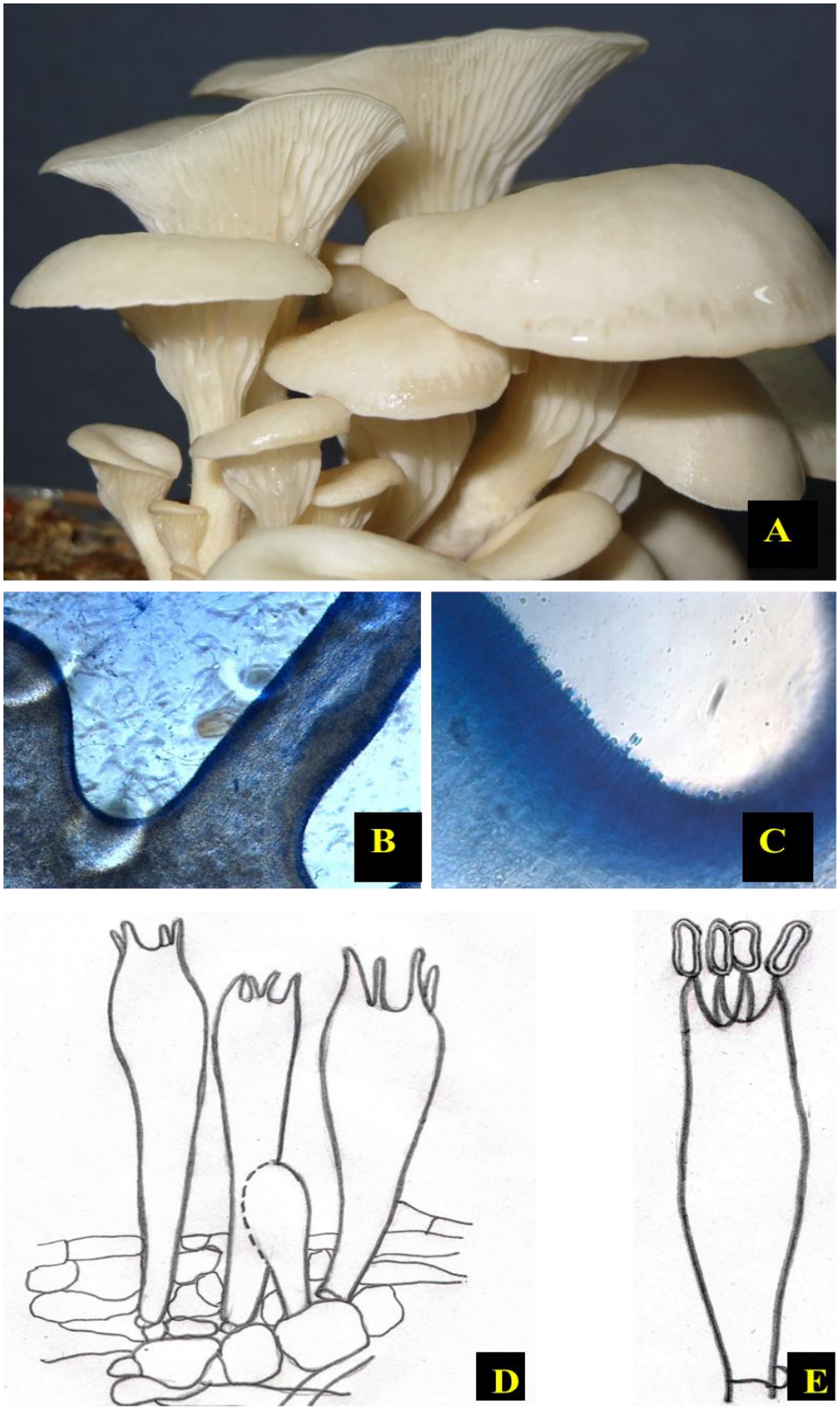


Figure 9: Morphological and anatomical features of *P. florida* (A) showing the fruiting body, (B) T.S of gill 10X; (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

4.3. Molecular Characterization of *Pleurotus ostreatus*

4.3.1. 18S rDNA sequence and phylogenetic analysis of *P.ostreatus*

Genomic DNA of *Pleurotus ostreatus* (IPL/MC/PO-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Agarose gel electrophoresis of genomic DNA revealed that they were RNA free. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS1 and T/ITS4 primers where the amplified product of 700 base pair size was produced by both the primers.

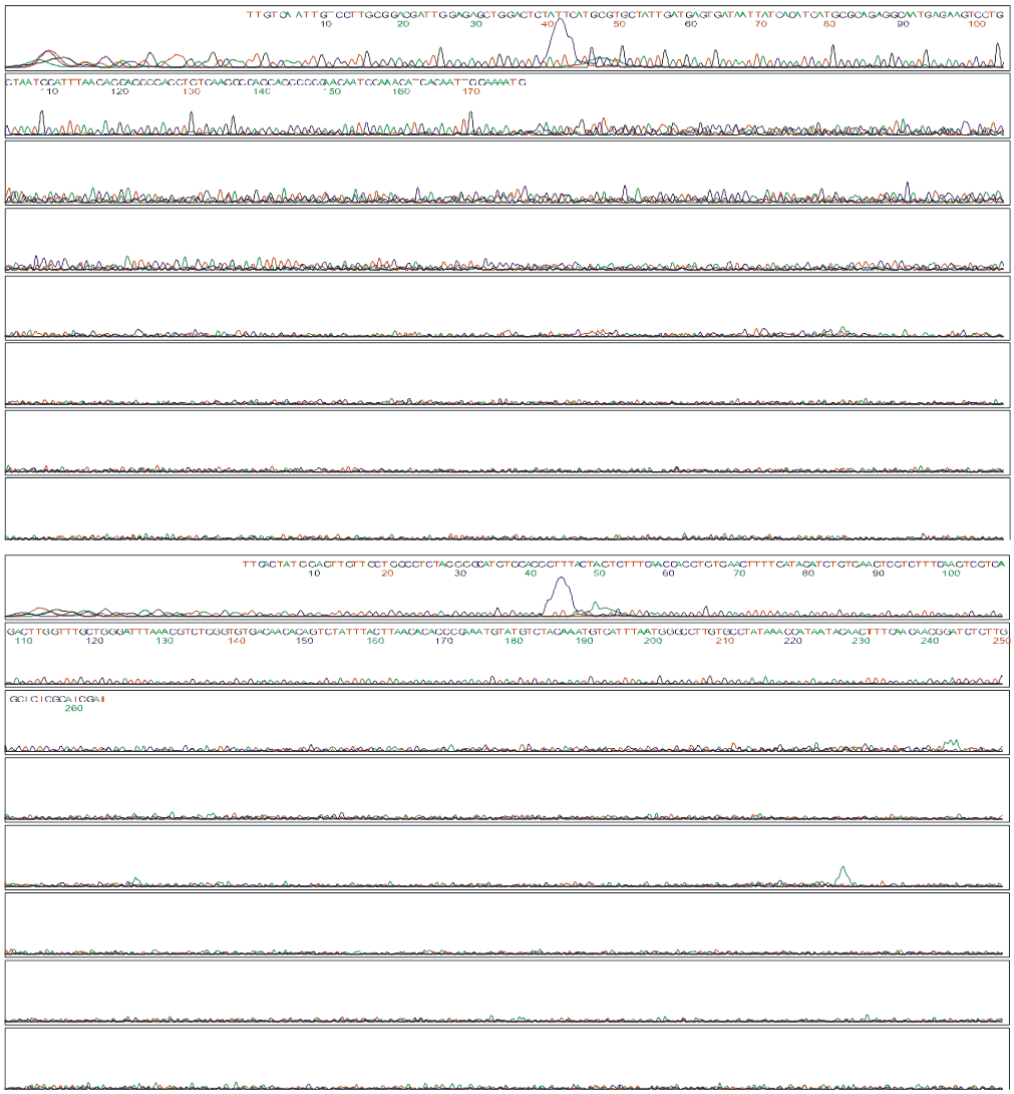
4.3.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS1 and T/ITS4 (for *Pleurotus ostreatus*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT768095. The sequence chromatograms have been represented in Figure 10.

4.3.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *Pleurotus ostreatus* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1software. The use of CLUSTAL-W determines that, once a gap is inserted, it can only be removed by editing. Therefore, final alignment adjustments were made manually in order to remove artificial gaps. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 11). Phylogenetic analyses were completed using the MEGA package (version 4.01; Institute of Molecular Evolutionary Genetics, University Park, PA). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. ostreatus* (KT768095) (Table 10).

Chromatogram



Sequence Deposited NCBI

Accession No. KT768095
DNA Linear 470 BP
Strain No: IPL/MC/PO-1

Title: *Pleurotus ostreatus* strain (IPL/MC/PO-1) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

Partial sequence of 18S ribosomal RNA gene

GAGGACGGACATCTACCTGATTGAGGTCAATTGTCAATTGTCCTTGCGGACGATTGGAG
AGCTGGACTCTATTCATGCGTGCTATTGATGAGTGATAATTATCACATCATGCGCAGAGG
CAATGAGAAGTCCTGCTAATGCATTTAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCC
CCAACAATCCAAACATCACAATTGGAAAATGGCAAAGGGCGTTTGTGTCTTCTACCCCC
TCTGCTGCGCAAGTCCCTCATATTACAACAAAGCTCATCTAGAATACTATGACCTGATCA
TCCAGCTCCTTATTGTGTATTCATCCTGACTTTTCATCCAGGAATCCACCATCACGGCAA
TTGAATCAAACGCCTTCCGCCCGCAGATTATGCTCTGCAAGGTGACCATCTCCCCGCAC
ATACCCCCCCATCACAAGATTTCCTGATGTACTGCATATTTTTTTGCAGAACATT

Figure 10: Chromatogram and sequence of 18S rDNA region *P ostreatus* (IPL/MC/PO-1) deposited in NCBI Genbank

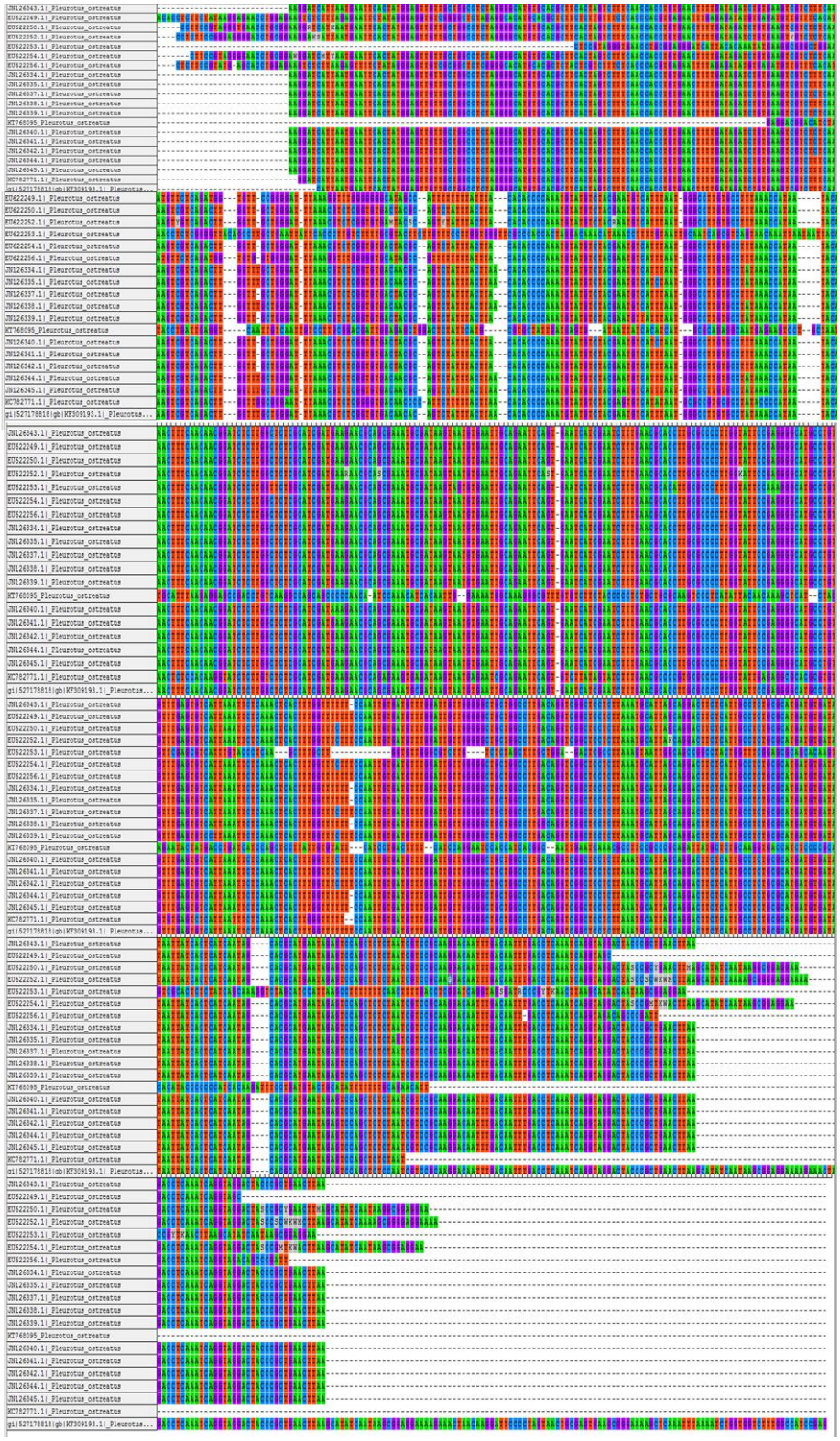


Figure 11: 18S r DNA sequence alignments of *P ostreatus* (IPL/MC/PO-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 10: Genbank accession numbers of the Ex-Type strains of *Pleurotus ostreatus* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	DQ077888	PHZAU2	639	China
2	HM998809	LGMACC 850404	630	Hungary
3	AY450345	6689	1551	Austria
4	EU622256	NW446	652	China
5	EU622249	NW423	648	China
6	GQ249947	PU001	563	India
7	HM067973	COIR PTK	635	India
8	HM138675	PAK1	635	India
9	KC782771	PLO6	575	Brazil
10	KT968336	PoVF8	677	Korea
11	KT968340	PoVF18	677	Korea
12	KJ020935	ST	632	Italy
13	KT818506	IPL/MC/PS-1	656	India
14	KT956122	EB1001	698	Thailand
15	KT768095	IPL/MC/PO-1	204	India

4.3.4. Phylogenetic analysis of *P. ostreatus*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 55.72288571 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 203 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 12).

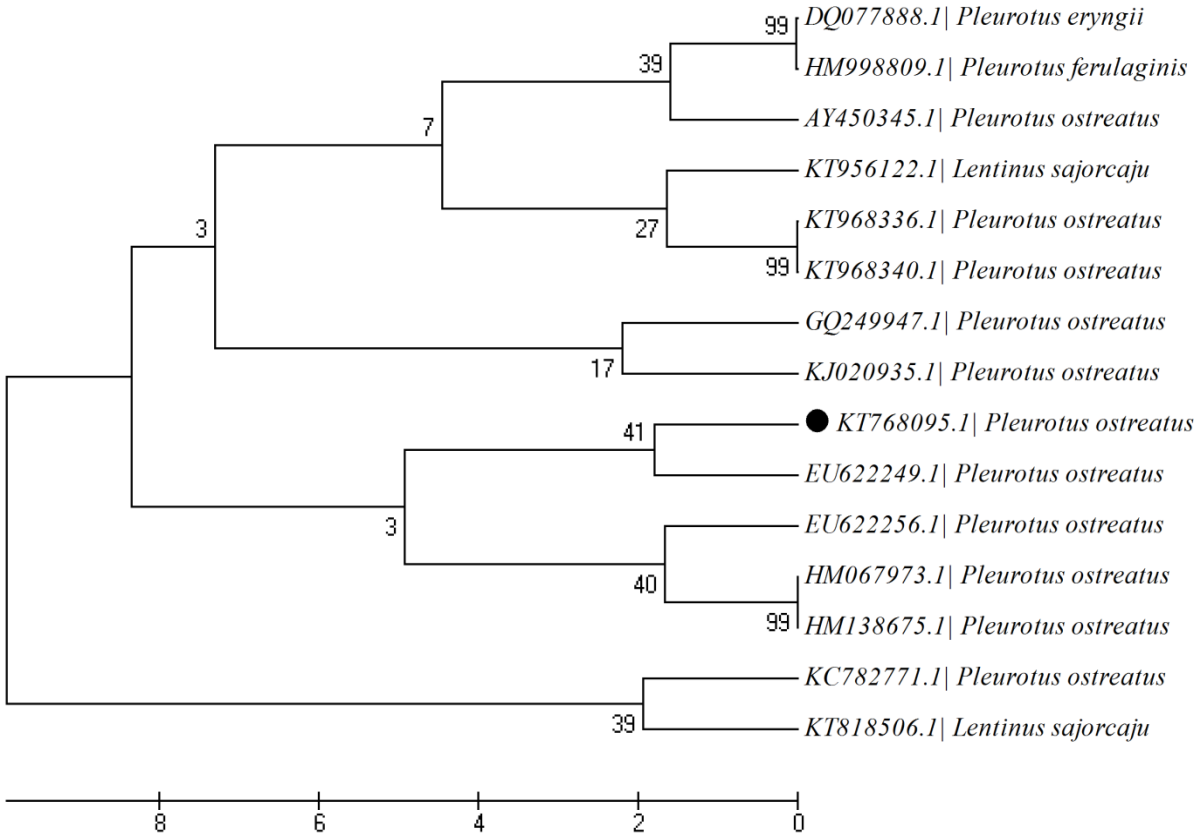


Figure 12: Phylogenetic placement of *P ostreatus* (IPL/MC/PO-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.4. Molecular characterization of *Pleurotus sajor-caju*

4.4.1. 18S rDNA sequence and phylogenetic analysis of *P. sajor-caju*

Genomic DNA of *Pleurotus sajor-caju* (IPL/MC/PS-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Agarose gel electrophoresis of genomic DNA revealed that they were RNA free. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 700 base pair size was produced by both the primers.

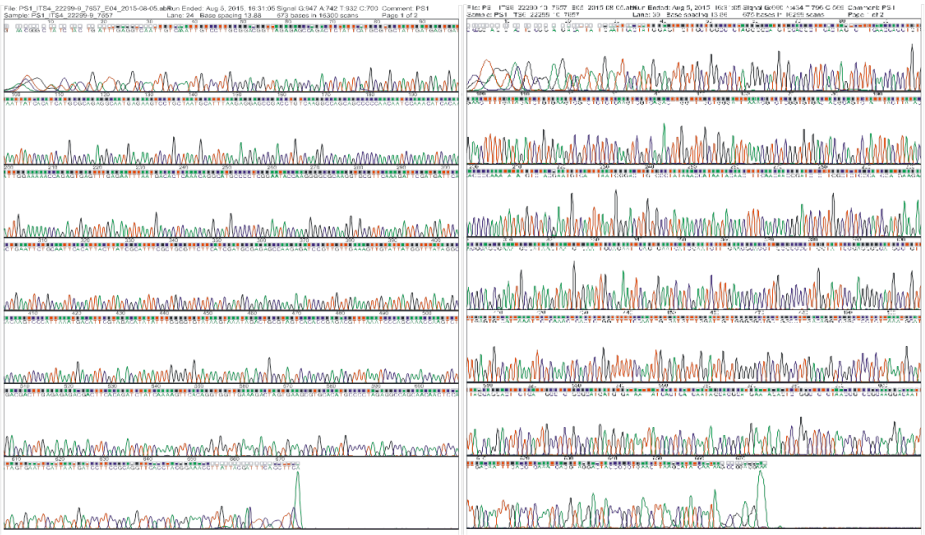
4.4.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *Pleurotus sajor-caju*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT818506. The sequence chromatograms have been represented in figure13.

4.4.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. sajor-caju* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 13 and 14). Phylogenetic analysis was carried out with ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. sajor-caju* (KT818506) (Table 11).

Chromatogram



Sequence Deposited NCBI

Accession No. KT818506
DNA Linear 656BP
Strain No: IPL/MC/PS-1

Title: *Lentinus sajor-caju* (*Pleurotus sajor-caju*) isolate IPL/MC/PS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGGAAGGATCATTAATGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCA
GTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACTTTTGATAGATCTGTGAAGT
CGTCCTTCAAGTCGTCAGACTTGGTTTGCTGGGATTTAAACGTCTCGGTGTGACA
ACGCAGTCTATTTACTTAACACACCCCAAATGTATGTCTACGAATGTCATTTAATGG
GCCTTGTGCCTATAAACCATAATACAACTTTCAACAACGGATCTCTTGGCTCTCGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG
AATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCT
GTTTGAGTGTCATTAAATTCTCAAACTCACATTTATTTGTGATGTTTGGATTGTTGG
GGGTTGCTGGCTGTAACAAGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCAT
TGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTC
CAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATCAGGTAGG
ACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

Figure 13: Chromatogram and sequence of 18S rDNA region *P sajorcaju* (IPL/MC/PS-1) deposited in NCBI Genbank

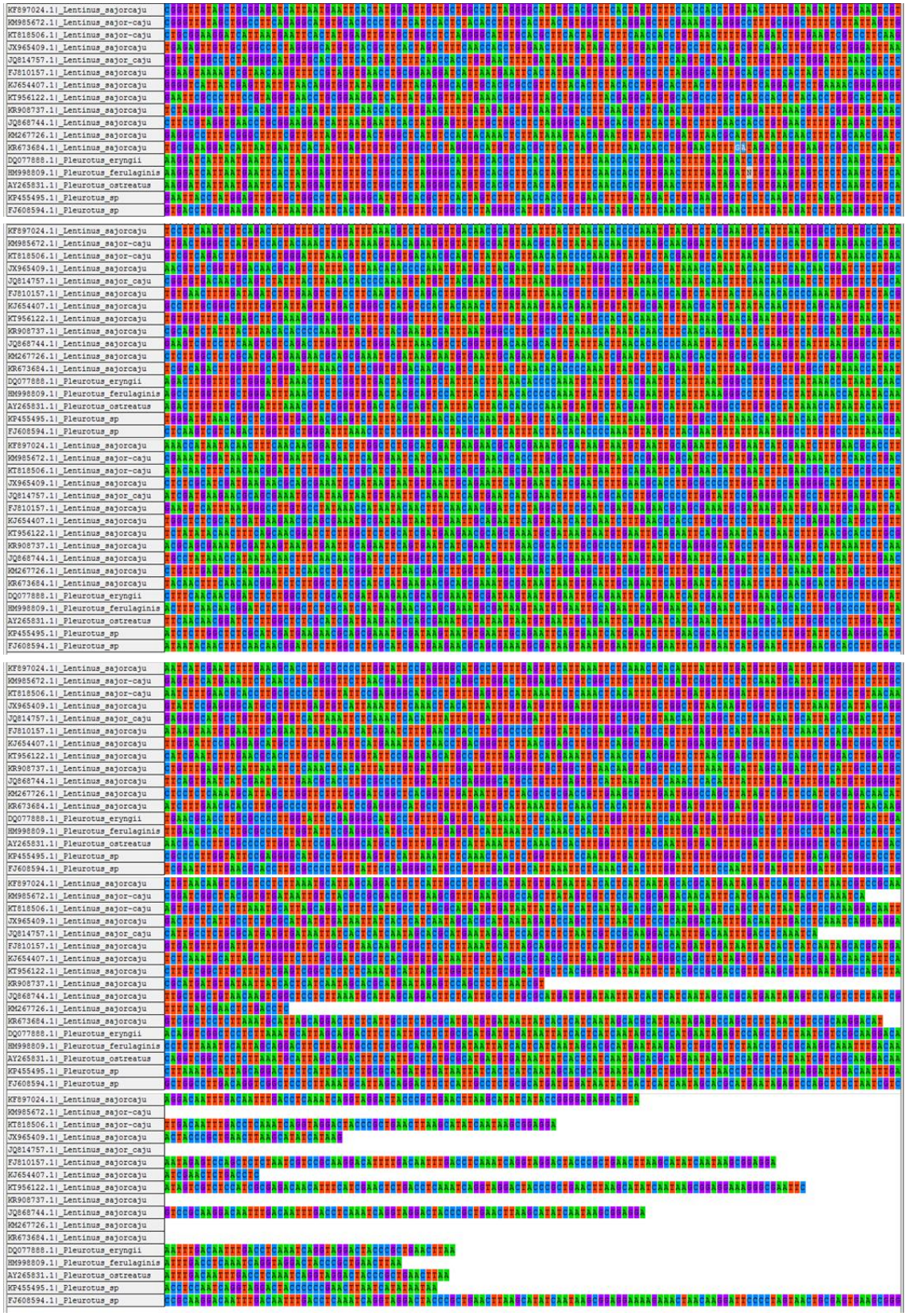


Figure 14. 18S r DNA sequence alignments of *P. sajor-caju* (IPL/MC/PS-1) with other extype isolates. The conserved regions of the gene are demonstrated in different colours

Table 11: Genbank accession numbers of the Ex-Type strains of *Pleurotus sajor-caju* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	KM985672	BPSM35	585	India
2	JX965409	pau3	620	India
3	JQ814757	CS-32	577	Russia
4	KJ654407	E882B	606	Australia
5	KT956122	EB1001	698	Thailand
6	KR908737	NCIM 1133"	531	India
7	KM267726	JMH36	488	Tanzania
8	KR673684	KA13-1213	588	South Korea
9	DQ077888	PHZAU2	639	China
10	HM998809	LGMACC 850404	630	Hungary
11	AY265831	ASI 2016	638	Korea
12	KP455495	DL501	636	India
13	FJ608594	AG X	848	Czech Republic
14	KT818506	IPL/MC/PS-1	656	India

4.4.4. Phylogenetic analysis of *P. sajor-caju*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 31.00795241 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 487 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 15).

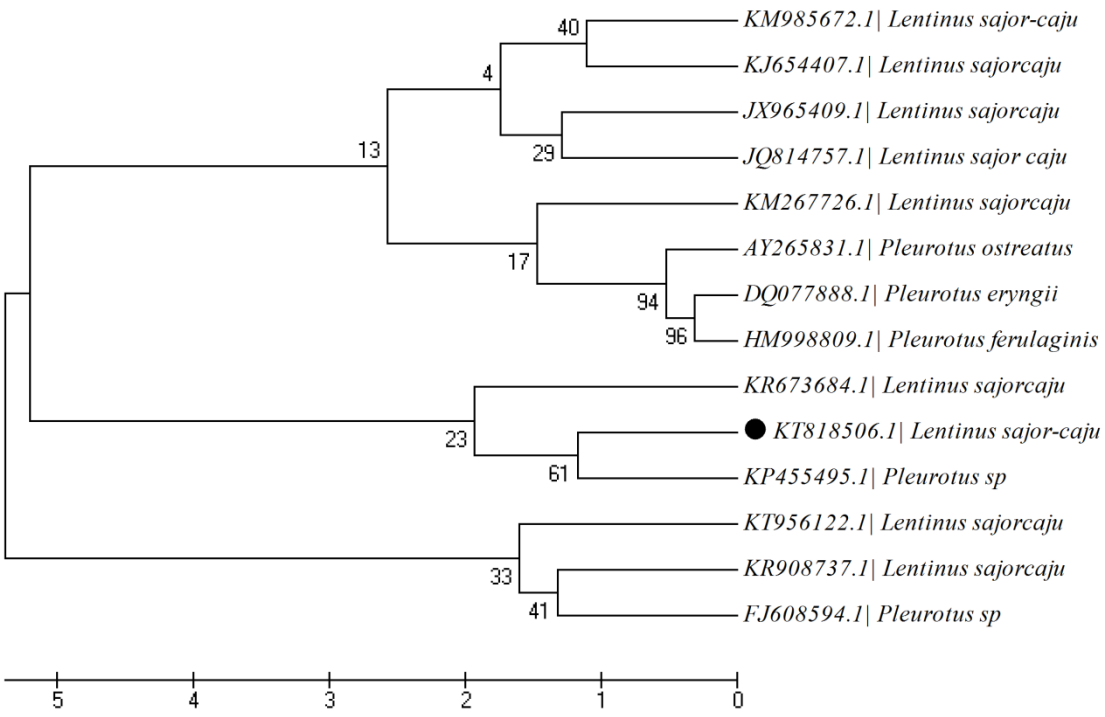


Figure 15: Phylogenetic placement of *P sajor-caju* (IPL/MC/PS-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.5. Molecular Characterization of *Pleurotus djamor*

4.5.1. 18S rDNA sequence and phylogenetic analysis of *P. djamor*

Genomic DNA of *Pleurotus djamor* (IPL/MC/PD-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 700 base pair size was produced by both the primers.

4.5.2. Chromatogram

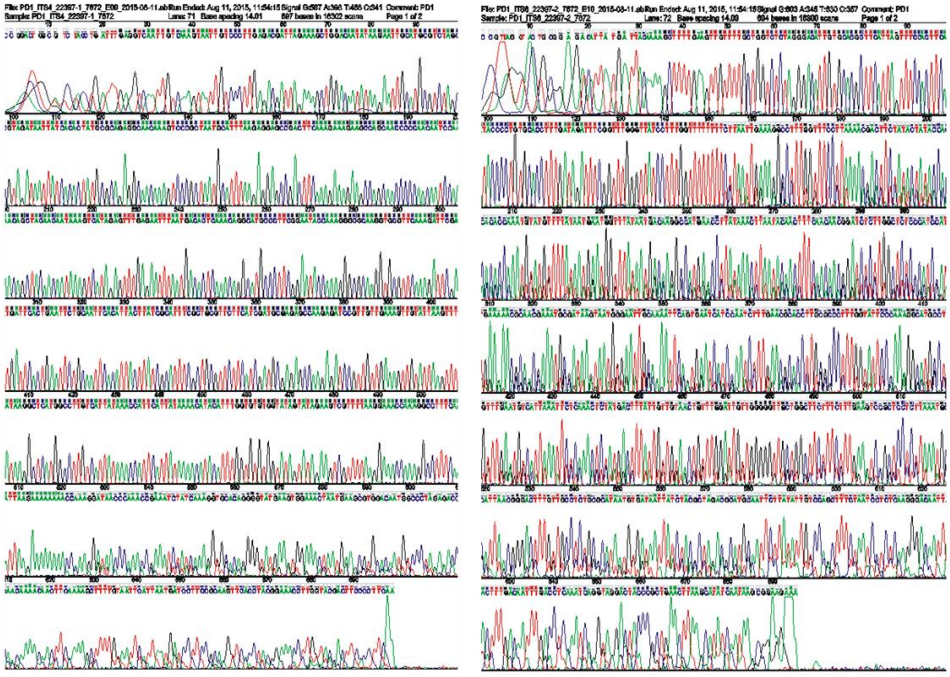
The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *P. djamor*) against GenBank database confirmed their identity. The sequences have been deposited in NCBI, GenBank database under the accession no. KT768094. The sequence chromatograms have been represented in figure 16 and 17.

4.5.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. djamor* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a

general purpose multiple sequence alignment program for DNA of MEGA4 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 16 and 17). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. djamor* (KT768094) (Table12).

Chromatogram



Sequence Deposited NCBI

Accession No. KT768094
DNA Linear 655BP
Strain No: IPL/MC/PD-1

Title: *Pleurotus djamor* isolate IPL/MC/PD1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGAAGGATCATTAATAATTACAAAAGCTTTTGAAGTTGTTTTGCTGGTCTCTA
GGGACATTGTGCACGCTTCATTAGTTTCCACTTCATACCCCTGTGCACCTTTGATA
GATTTCGGTTTGGGTTATCCTTTGGTTTTTTTTTCTTAATTGAAAGGCCTTTGGTTT
CCTTAAAACGACTTCTATACTATACCACACACCAAATGTATGTTTTATAATGAATGG
TTTATAATGACAAGGCCATGACCTTATAAACTTAATACAACTTTCAACAACGGATCT
CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATTCAGTGAATCATCGATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGA
AGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCTATGACTTTATTGTTGTAG
CTGTTTGGATTGTTGGGGGTTGCTGGCTTCTTTCTTTGAAGTCGGCTCCTCTTAAA
TGCATTAGCGGGACTTTGTTGCCTCTGCGCATAGTGTGATAATTATCTACGCTAGAC
GCATGCAATTCTTATATTGTCCAGCTTTCTAATCGTCTCAAGGGACAATTACTTTGA
CAATTTGACCTCAAATCAGGTAGGACTACCCGCT

Figure 16: Chromatogram and sequence of 18S rDNA region *P djamor* (IPL/MC/PD-1) deposited in NCBI Genbank

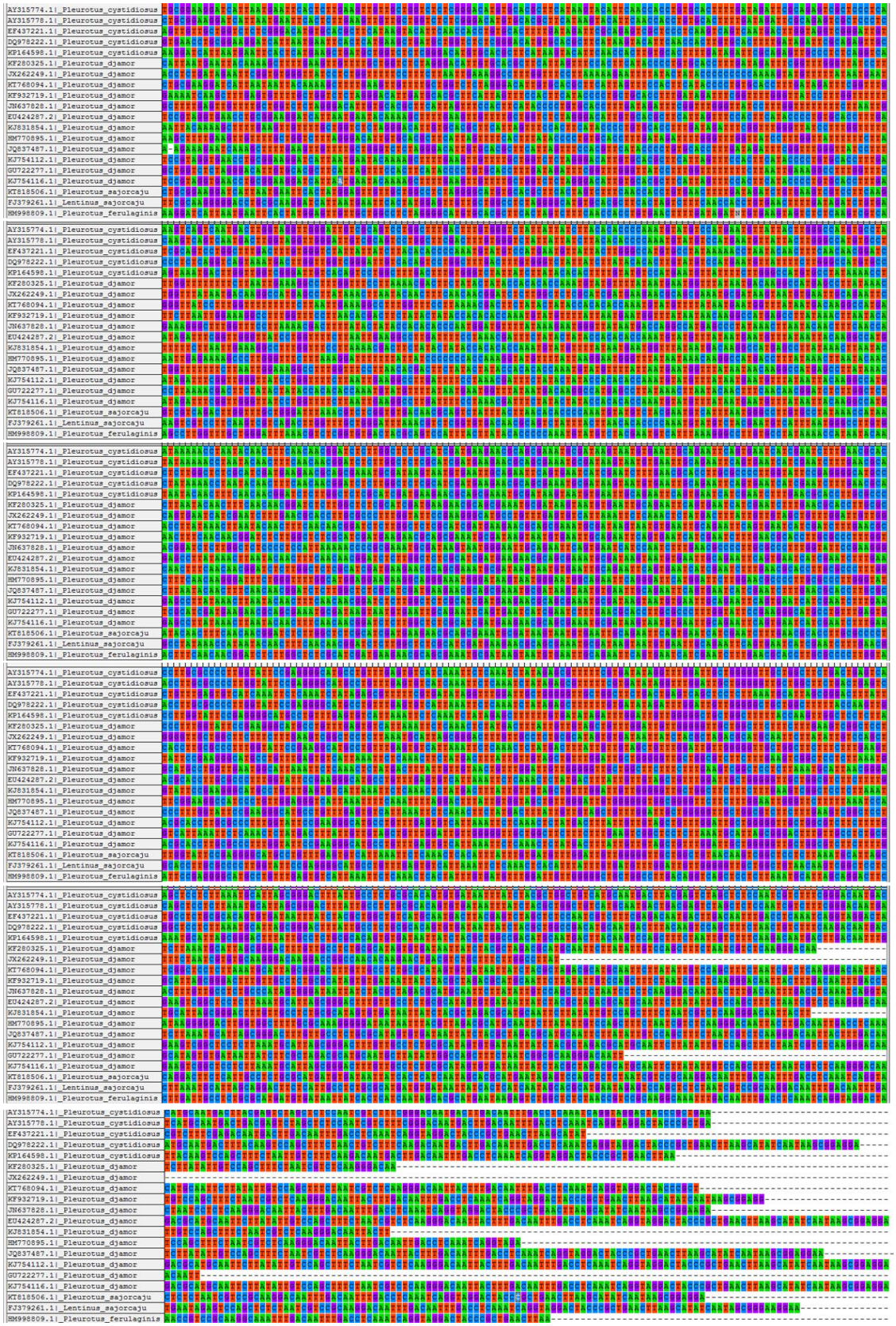


Figure 17: 18S r DNA sequence alignments of *P djamor* (IPL/MC/PD-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 12: Genbank accession numbers of the Ex-Type strains of *P. djamor* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	EF437221	P-19"	636	India
2	DQ978222	X 652	682	India
3	KP164598	ZYB 2013	651	China
4	KF280325	MBsn	604	Brazil
5	JX262249	CBE 11	560	India
6	KF932719	1526	666	Russia
7	JN637828	B-36	657	Cuba
8	EU424287	CBS 100134	687	China
9	KJ831854	IB36	603	Peru
10	HM770895	IUM1794	625	South Korea
11	JQ837487	Z1	675	Russia
12	KJ754112	7	687	Kenya
13	GU722277	ECS-01130	571	Mexico
14	KT818506	IPL/MC/PS-1	656	India
15	HM998809	LGMACC 850404	630	Hungary
16	KT768094	IPL/MC/PD1	655	India

4.5.4. Phylogenetic analysis of *P. djamor*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 97.46225950 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004)and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 559 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 18).

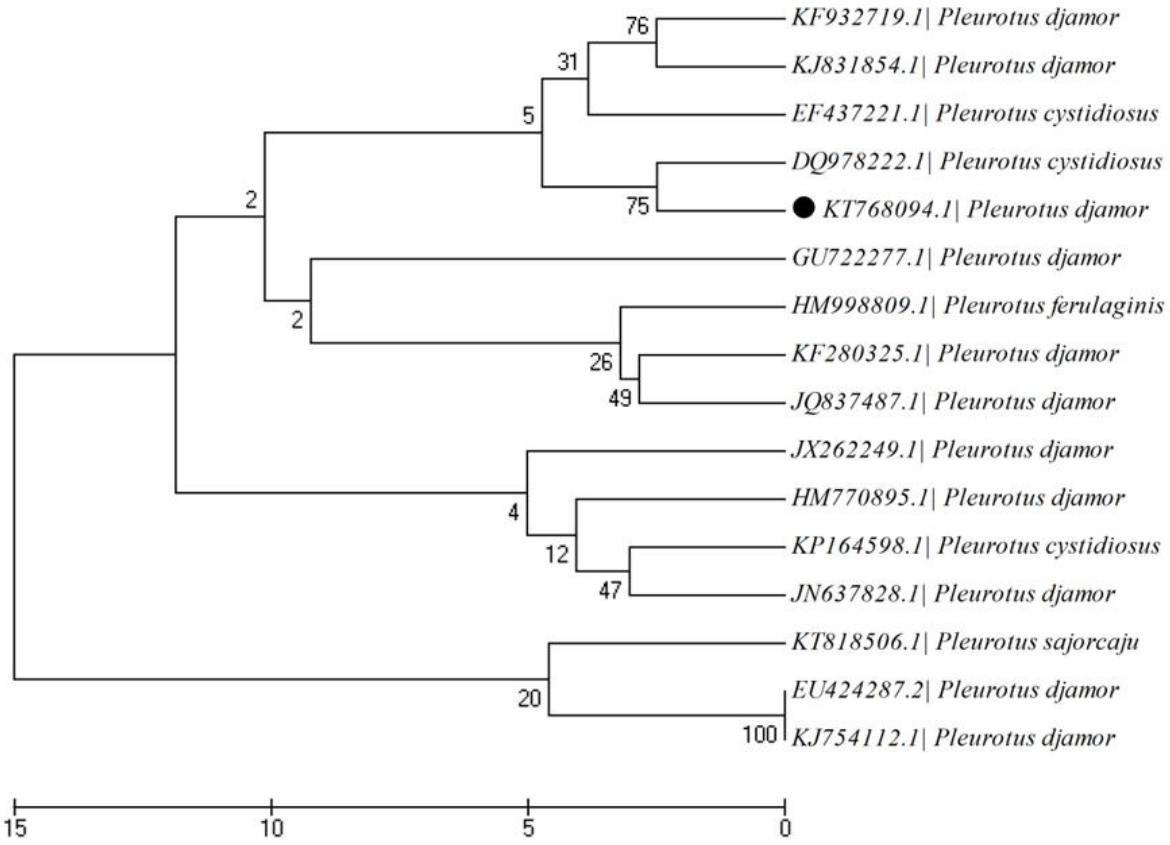


Figure 18: Phylogenetic placement of *P. djamor* (IPL/MC/PD-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.6. Molecular Characterization of *Pleurotus florida*

4.6.1. 18S rDNA sequence and phylogenetic analysis of *P. florida*

Genomic DNA of *P. florida* (IPL/MC/PF-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 665 base pair size was produced by both the primers.

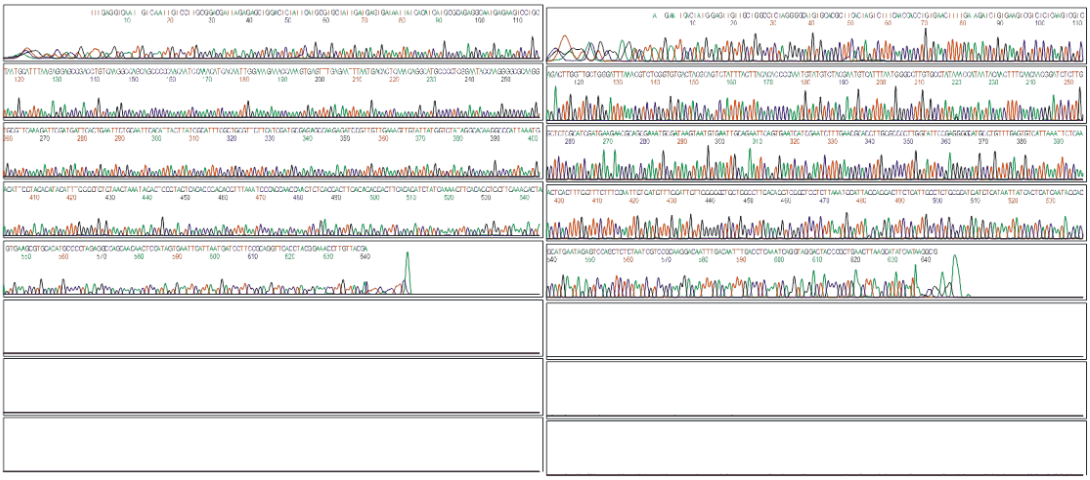
4.13.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *P. florida*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT826605. The sequence chromatograms have been represented in figure 19.

4.6.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. florida* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA4 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (figure 20). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. florida* (KT826605) (Table 13).

Chromatogram



Sequence Deposited NCBI
Accession No. KT826605
DNA Linear 665BP
Strain No: IPL/MC/PF-1

Title: *Pleurotus floridanus* isolate IPL/MC/PF 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGGAAGGATCATTATGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCAC
GCTTCACTAGTCTTTCAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTC
GTCAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACAC
ACCCCAAATGTATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAAACCATAATACAAC
TTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTC
CGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTG
TGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGC
AGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAAT
AGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATCAGGTAGG
ACTACCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAA

Figure 19: Chromatogram and sequence of 18S rDNA region *P. florida* (IPL/MC/PF-1) deposited in NCBI Genbank

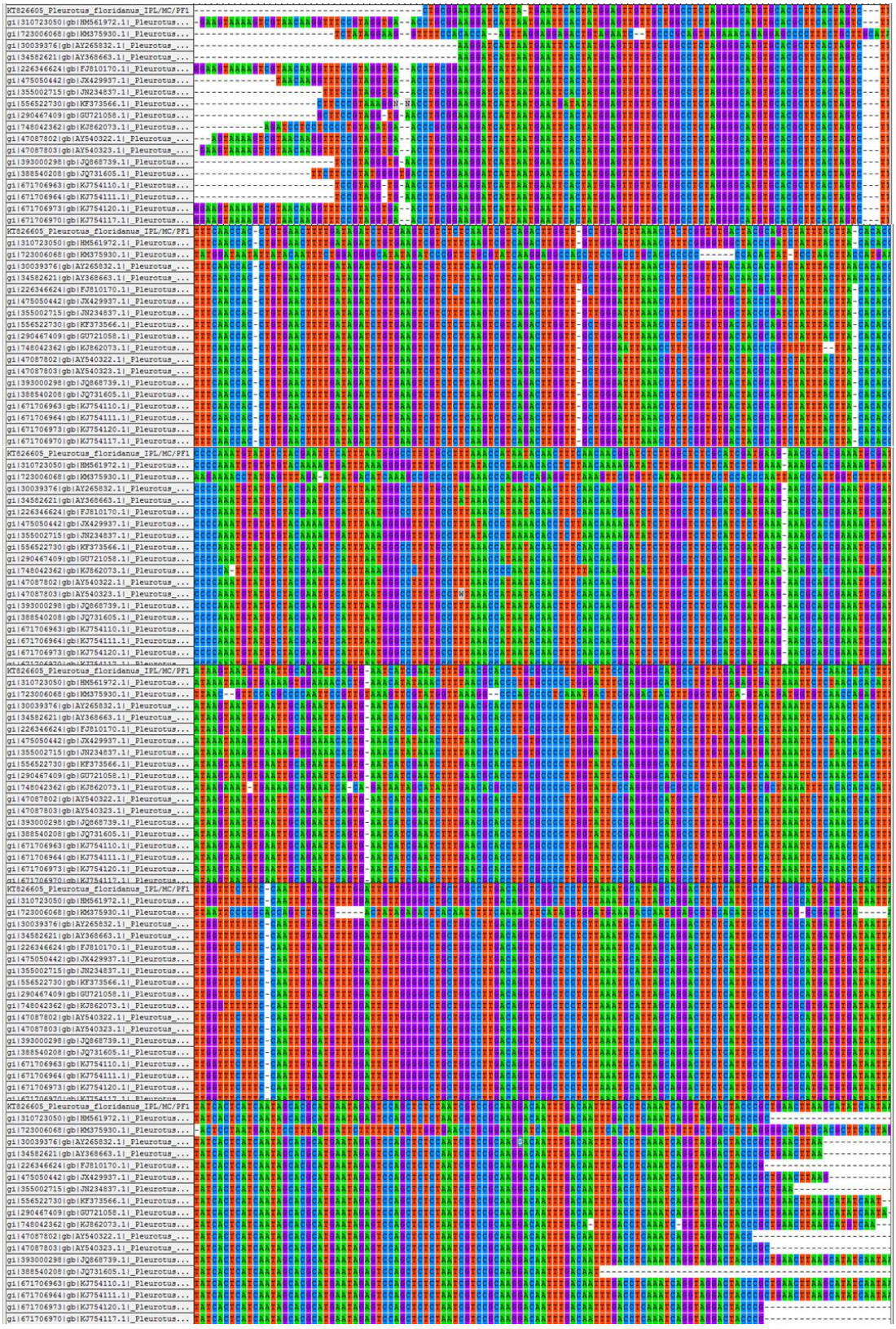


Figure 20: 18S r DNA sequence alignments of *P florida* (IPL/MC/PF-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 13: Genbank accession numbers of the Ex-Type strains of *P. florida* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	JQ731605	VKESR1	625	India
2	JX429937	FPFMK	668	Malaysia
3	JN234837	FTCW1 (PFW1)	654	Malaysia
4	KF373566	LCJ 155	683	India
5	GU721058	PF101	671	India
6	HM998809	LGMACC 850404	630	Hungary
7	DQ978222	X 652	682	India
8	KM375930	AAU-SAP	1835	India
9	KT968336	FLO-01	652	Korea
10	KT968340	Flo-01	667	Korea
11	KJ020935	Pfu-652	432	China
12	KT826605	IPL/MC/PF-1	665	India

4.6.4. Phylogenetic analysis of *P. florida*

The evolutionary history was inferred using the UPGMA method (Sneath PHA and Sokal; 1973). The optimal tree with the sum of branch length = 1886.85763040 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamur, Nei and Kumar; 2004)and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 622 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 21).

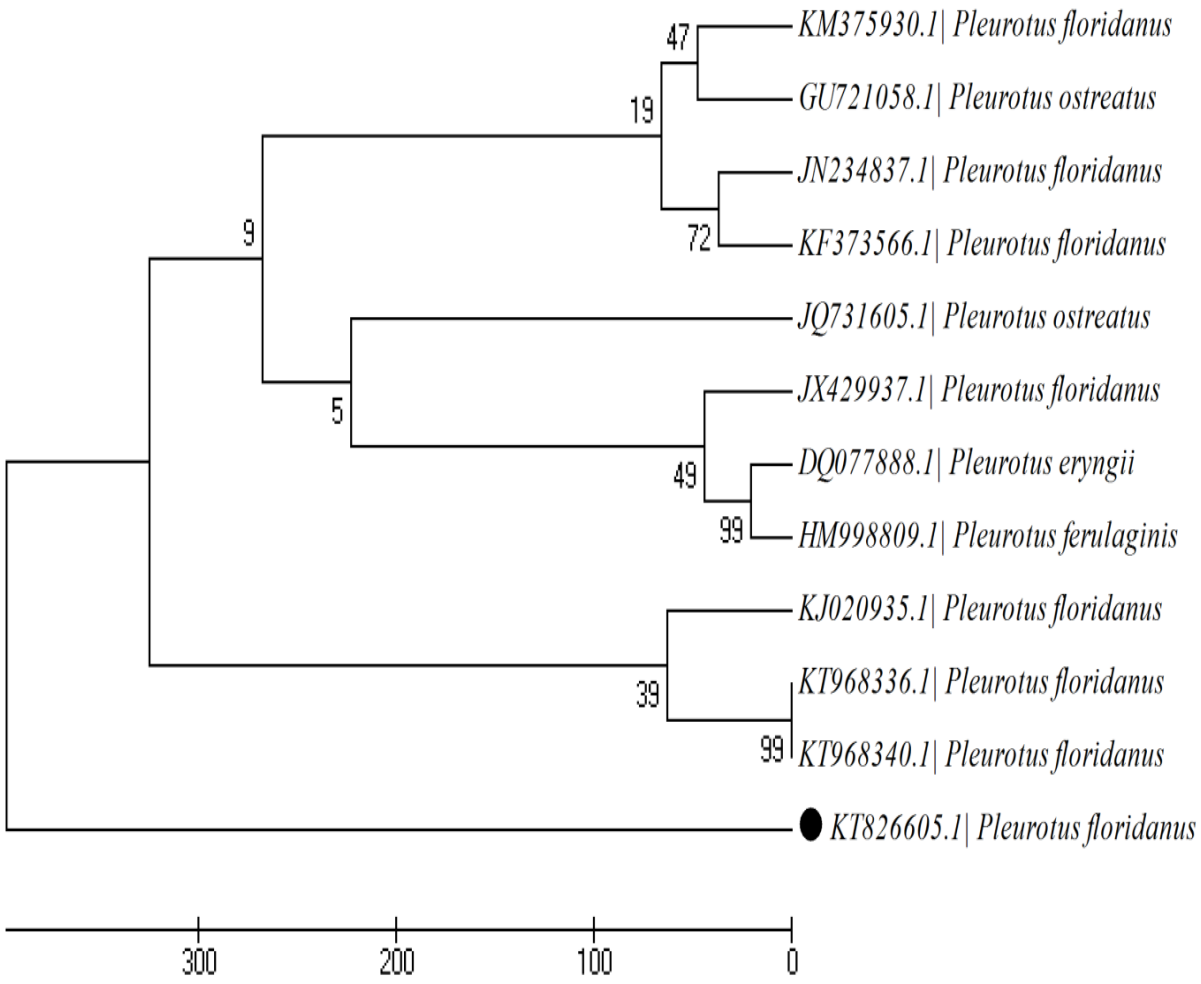


Figure 21: Phylogenetic analysis of *P florida* (IPL/MC/PF-1) with other ex-type strain sequences obtained from NCBI Genbank Database

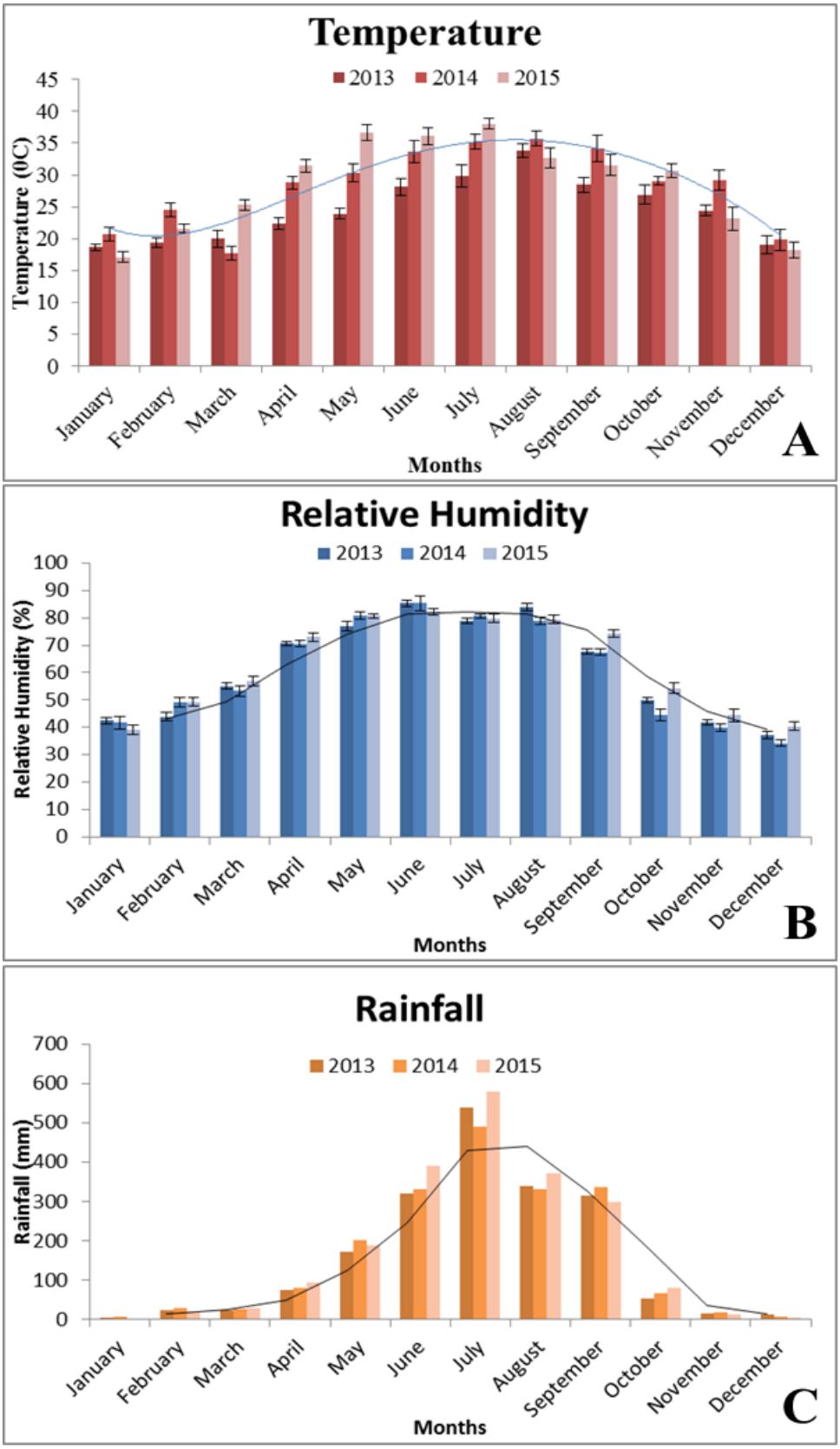


Figure 22: Weather condition of North Bengal during 2013-2015 (A) temperature, (B) relative humidity and (C) rainfall throughout the year

4.7. Cultivation of *P. ostreatus*

4.7.1. Growth in different substrates

Cultivation of *P. ostreatus* is very common in North Bengal. Paddy straw is one of the major substrate commonly used in this region. Paddy straw is largely used in cultivation throughout the year. Cultivation of *P. ostreatus* was done throughout the year in different agro climatic conditions and it was observed that the production was increased during the summer and rainy season when the temperature was about 21-32^0 C with 50-87% relative humidity (Figure 22). It was also observed that the production rate was very high during June to July. The above study reveals that paddy straw can be used as a major substrate for cultivation of *P. ostreatus*. Wheat is one of the major cereals cultivated throughout the world and India is one of the large producers of wheat. The straw after harvesting of the wheat was used as substrate for mushroom cultivation. Efficacy of wheat straw for the production of *P ostreatus* was evaluated and the results showed that the use of paddy straw for *P. ostreatus* cultivation enhances the production during July to September as the temperature was in between (24-30^0 C) with relative humidity (78-90%). It was also observed that fruiting body initiation was delayed during the winter season but it grows faster during the rainy season. From the above results, it can be said that the use of wheat straw as the substrate for mushroom cultivation showed very promising result (Figure 23 24). Evaluation of saw dust as a substrate has been done and it was observed that the growth of mycelia over the substrate has been increased and also the period of fruiting body initiation also decreased when the substrate was supplemented with saw dust (figure 25).

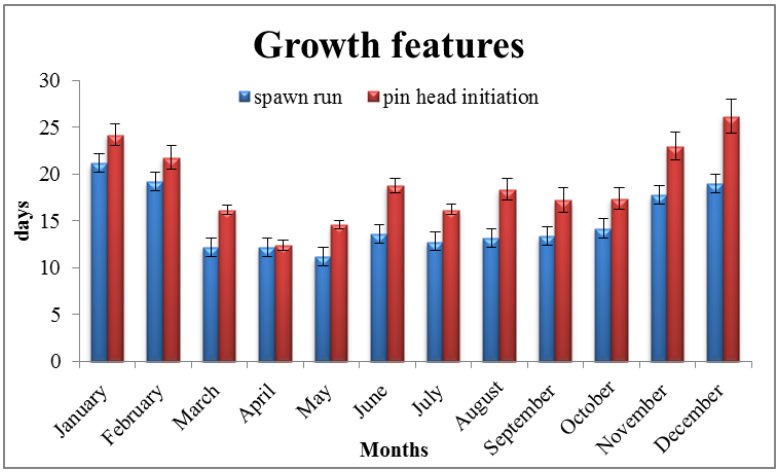


Figure 23: Assessment of spawn run period for pin head initiation during cultivation of *P. ostreatus* throughout the year

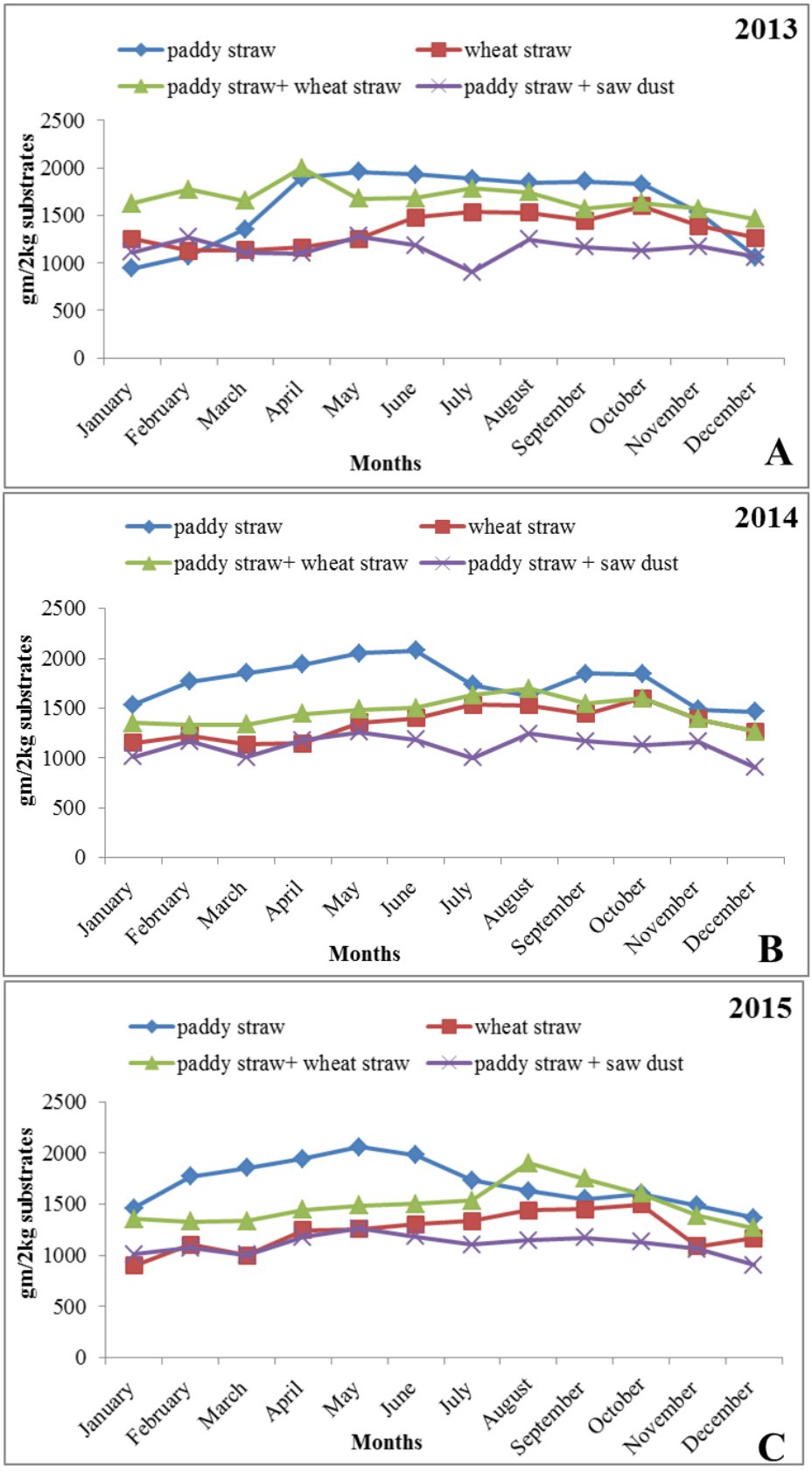


Figure 24: Comparative study on yield of *P. ostreatus* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

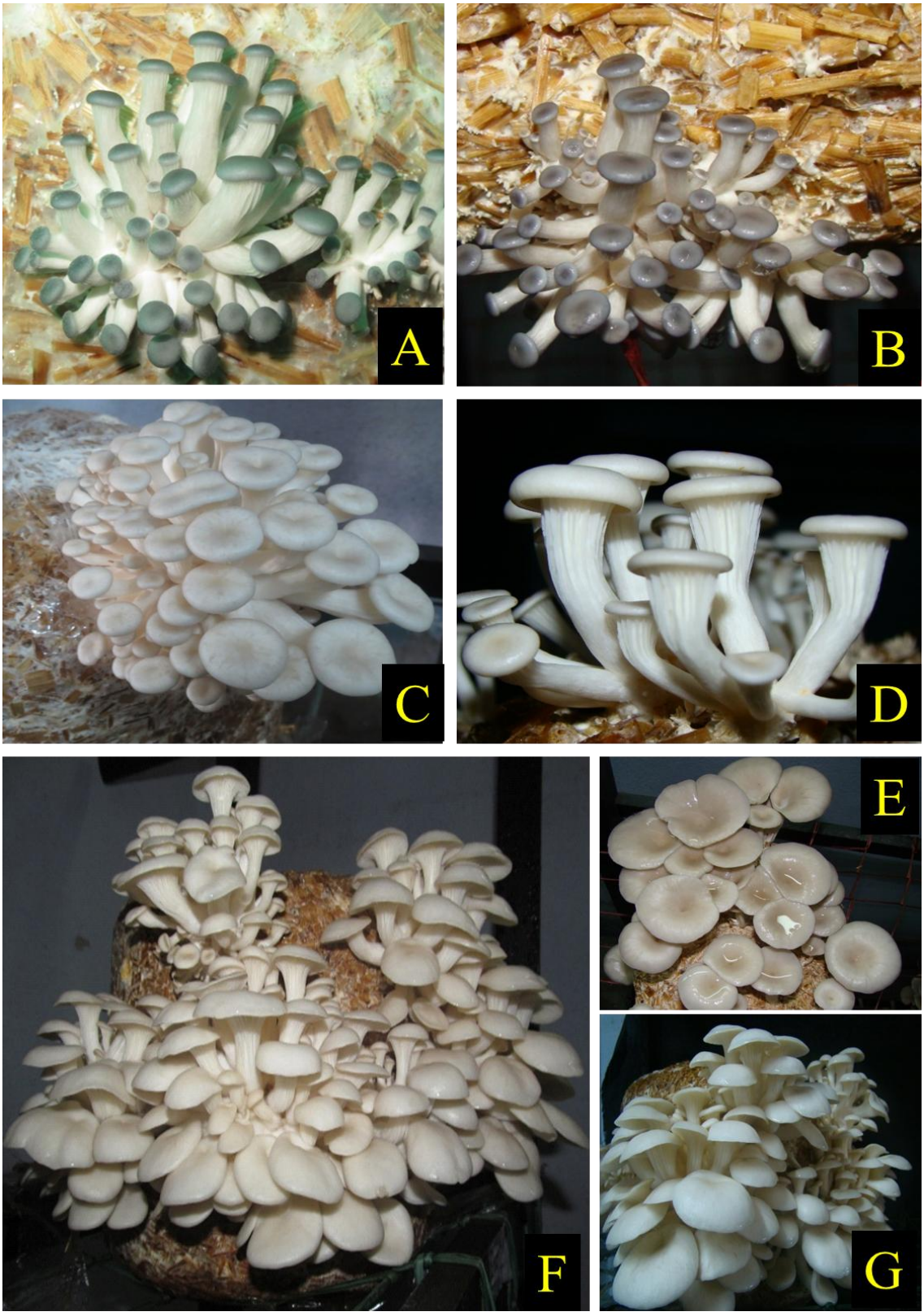


Figure 25: (A-G) Cultivation of *P ostreatus* in polypropylene bags.
(A-D) pinhead stage, (E) early mature stage and (F & G) mature stage.

Production of *P. ostreatus* also enhanced during September and October when the temperature was about 21-24^0 C with 40-42% relative humidity and with an average rainfall and it was also observed that during early summer, growth of mycelia and initiation of fruiting body period decreased. Paddy straw and wheat straw was singly used as substrate for the cultivation of *P. ostreatus*. To evaluate the efficacy of both the substrate in combination, the above results revealed that the period of mycelial run was very much less during August to October when cultivated in paddy straw wheat straw combined substrate (1:1). It was also observed that the rate of production was higher during the rainy season and during September the production per bag seems to be highest than the other months.

Table 14: Cultivation of *P. ostreatus* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Average Days of colonization	Fruiting Initiation	Production (g/2kg substrate)
Summer	Polypropylene bags	45X30	16	18	1250
	Bottles	35X5(dia.)	12	15	575
	Box	80X40X30	13	16	545
Rainy	Polypropylene bags	45X30	15	21	590
	Bottles	35X5(dia.)	10	13	615
	Box	80X40X30	14	17	585
Winter	Polypropylene bags	45X30	15	20	425
	Bottles	35X5(dia.)	12	16	430
	Box	80X40X30	14	19	365

Mean value of three replicates of each case

4.7.2. Growth in different containers

Mushroom cultivation practiced in polypropylene bags is one of the most used traditional methods of cultivation. Cultivation of *P. ostreatus* was also practiced in different types of containers to evaluate their potentiality in cultivation. Polypropylene bags were also compared with the other containers like bottles and paper box (Figure 26). Experiment was conducted using the paddy straw as substrate and equal amount of substrate was used and the results revealed that the waste bottles were very much efficient in cultivation. During the summer and rainy season, *P. ostreatus* can be cultivated and it was observed that the mycelia run over substrate and initiation of

fruiting body also enhances. Production was also differs from polypropylene bags and in bottles and boxes yield was quite higher than the polypropylene bags. Bottles and boxes also reduce the full cropping period (Table 14) than that of the bags.

Figure 26: (A-H) Cultivation of *P. ostreatus* in different containers.
(A-B): Polypropylene bags; (C-E) plastic bottles (F) broken glass goods; and (G-H) box made up of aluminium sheet.

4.8. Cultivation of *Pleurotus sajor-caju*

4.8.1. Growth in different substrates

Oyster mushroom is one of the major mushrooms cultivated in North Bengal and in this region *Pleurotus sajor-caju* is widely cultivated out of other species for its large fruiting body and wide range of favourable cultivation period. Paddy straw mostly used a substrate as it is commonly found in this region. It was observed that the period of initiation of fruiting body is very less during the rainy season. Above results showed that the production increase during April to November and the cropping time also very lesser during this period (Figure 27). It was observed that the production increased during the August to October (about 510-560gm/kg substrate) when the temperature lies between 21-27^{0}C. Different lignocellulosic components are used to cultivate the oyster mushroom and it was observed that the cultivation period and spawn run period very less than the paddy straw. Results revealed that the *Pleurotus sajor-caju* can be cultivated throughout the year but the production rate high during march to November and the production increased around 610 gm/kg straw when the temperature lies between 21-30^{0}C with a relative humidity about 42-88. Saw dust is one of the very popular lignocellulosic components found in this region. Saw dust was used as supplement to increase the productivity and it was observed that during September and October, production ratio was very high (620-635 g/ kg substrate) (Figure 28). It also enhances the growth rate of the mycelia resulted in shortening the cropping period. It was observed that *P. sajor-caju* grows faster in wheat straw combined with paddy straw and fruiting body initiation and yield per kg substrate rate was also higher than the single substrates.

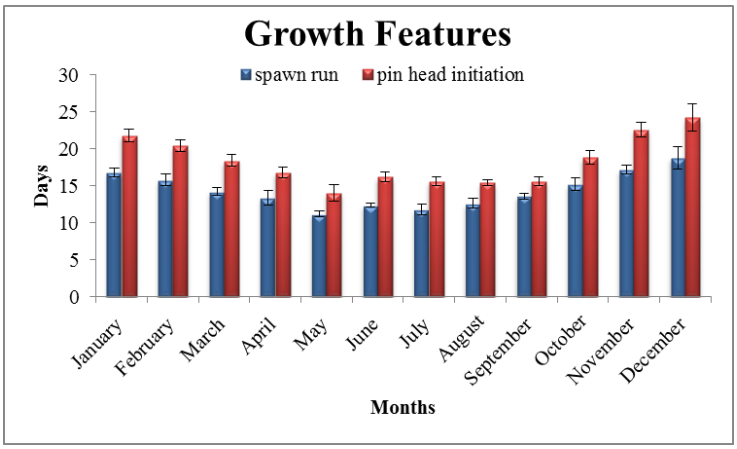


Figure 27: Assessment of spawn run period for pin head initiation during cultivation of *P. sajor-caju* throughout the year

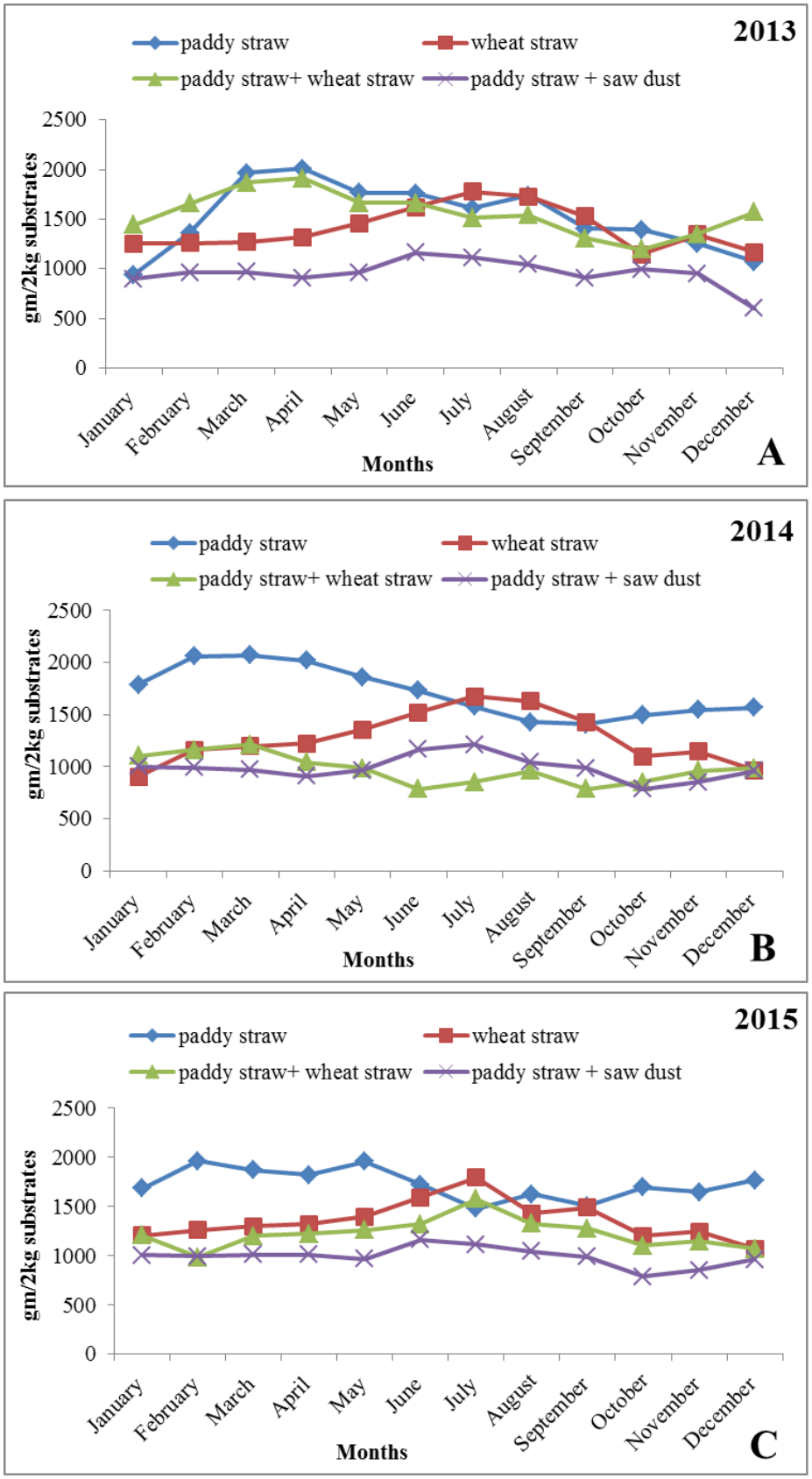


Figure 28: Comparative study on yield of *P. sajor-caju* grownin paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

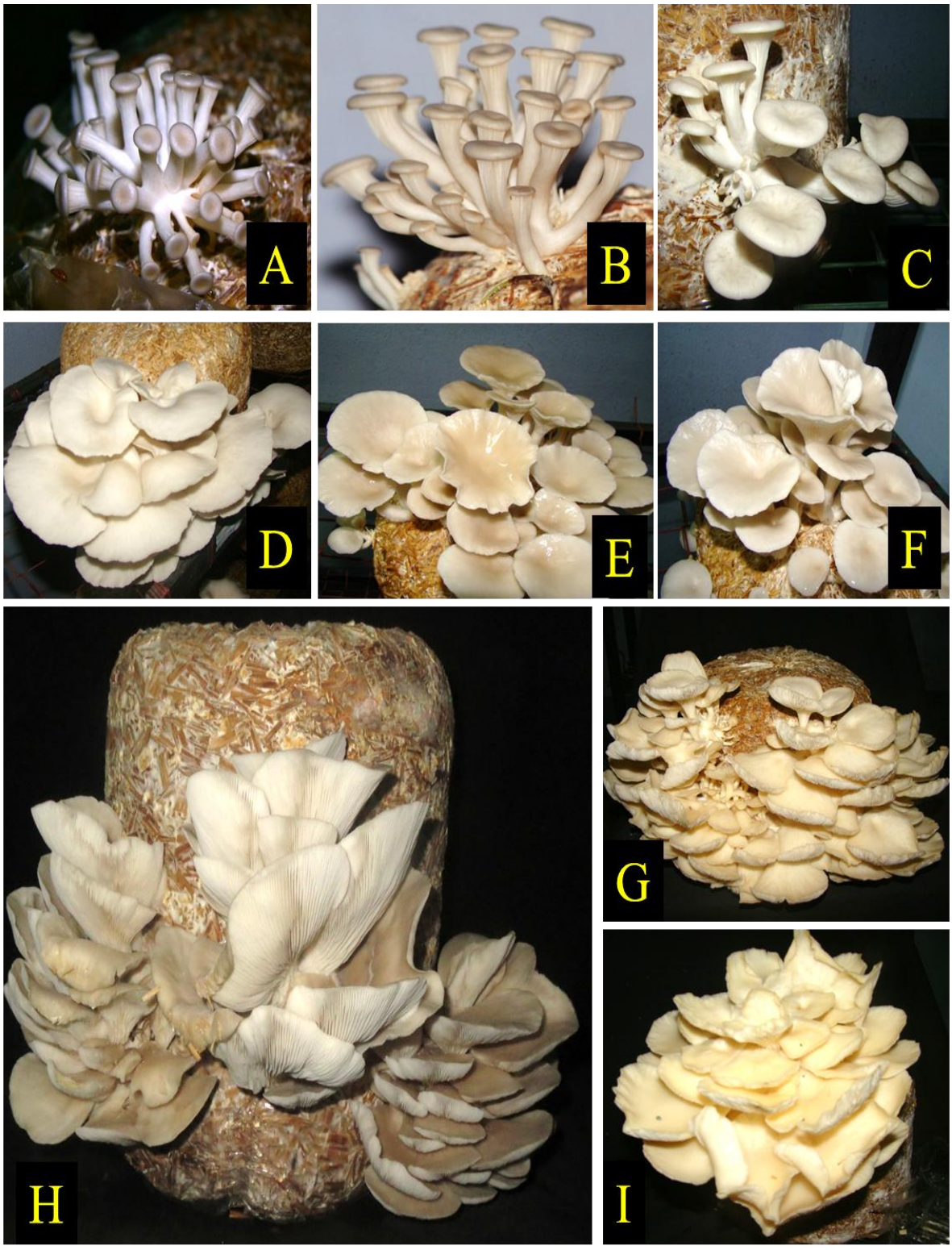


Figure 29: (A-I) Cultivation of *P sajor-caju*
(A-C) pinhead, (D-F) early mature stage and (G-I) showing the mature fruiting body.

Table 15: Cultivation of *P. sajor-caju* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	15	20	426.6±27.28
	Bottles	35X5(dia.)	11	16	491.6±10.13
	Box	80X40X30	15	20	411.6±11.66
Rainy	Polypropylene bags	45X30	14	17	545±25.65
	Bottles	35X5(dia.)	13	14	600±13.22
	Box	80X40X30	15	20	561.6±10.13
Winter	Polypropylene bags	45X30	15	23	366.6±18.55
	Bottles	35X5(dia.)	15	19	366.6±24.55
	Box	80X40X30	18	23	301.6±13.64

(±) standard error, mean value of 3 replicates

4.8.2. Growth in different containers

Pleurotus sajor-caju is widely cultivated in this region for its large fruiting body, ability to grow throughout the year and taste. Large number of people cultivating the species but due to proper cultivation technology knowhow most of the farmers are unable to cultivate successfully. Containers play an important role in the mycelial growth as well as production of oyster mushrooms. Different containers were used to evaluate their ability to grow as well as yield potential bottles and boxes were used. It was observed that mycelial matt covers the substrate much more early than in case of bottle cultivation which results in reduction in the full cropping period. On the other hand, it was also observed that the yield potential was higher in boxes than the polypropylene bags (Table 15). Waste plastic bottles results in higher yield and lower cropping period as well as recycling the containers. Using the plastic bottles also does not require large production unit (Figure 30)

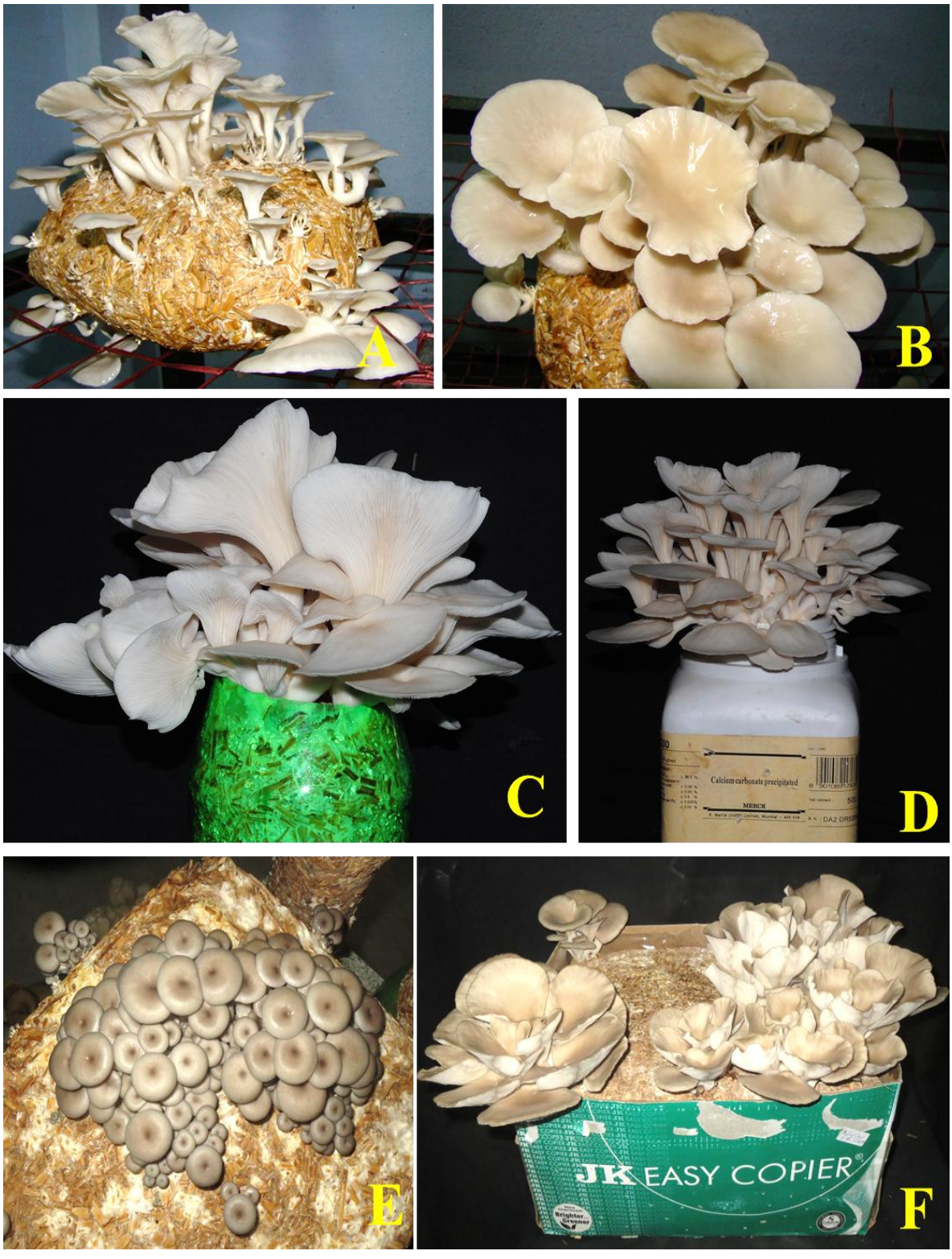


Figure 30: (A-F) Cultivation practices of *P. sajor-caju* using different containers, (A-B) polypropylene bags; (C) plastic bottles (D) waste bottles and (E-F) paper box.

4.9. Cultivation of *Pleurotus djamor*

4.9.1. Growth in different substrates

Different species of oyster mushroom cultivated in North Bengal. But *P. djamor* pink oyster mushroom is a new introduction in this region. Cultivation of pink oyster mushroom was carried out using different substrate. Paddy straw was used to evaluate the cultivation efficiency and it was observed that *Pleurotus djamor* gives better yield during winter season when the temperature lies between 18-22^{0}C with a very low range of relative humidity (50-80%). The results also revealed that the period of mycelial run as well as fruiting body initiation was very less during the winter (Figure 31). *Pleurotus djamor* commonly known as pink oyster mushroom can be cultivated a wide range of lignocellulosic substrate and it requires optimum temperature for its better growth and yield. Wheat straw is one of the major crop widely cultivated in India. *P. djamor* was cultivated using wheat straw as substrate and the result showed that the mycelial run period much lower during December to March when the temperature was quite lower than the summer. Final yield of *P. djamor* was also increased during this period which proves that the cultivation period of *P djamor* is much more favourable during the December to March in this region. It was also observed that fruiting body initiation require very lower amount of relative humidity (40-70%). Different substrate was used to evaluate their efficacy in cultivation of different species of oyster mushroom and *P djamor* was also cultivated using the paddy straw in combined with wheat straw (1:1). It was observed that the mycelial run period reduces in during the winter season (December to February) and also during the early summer season (Figure 32). The results revealed that using the paddy straw and wheat straw in combined form was more efficient and it was also extends the cropping time up to early summer season.

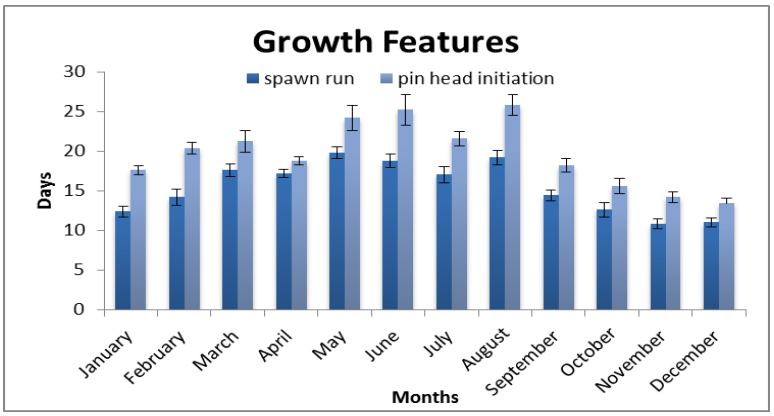


Figure 31: Assessment of spawn run period for pin head initiation during cultivation of *P. djamor* throughout the year

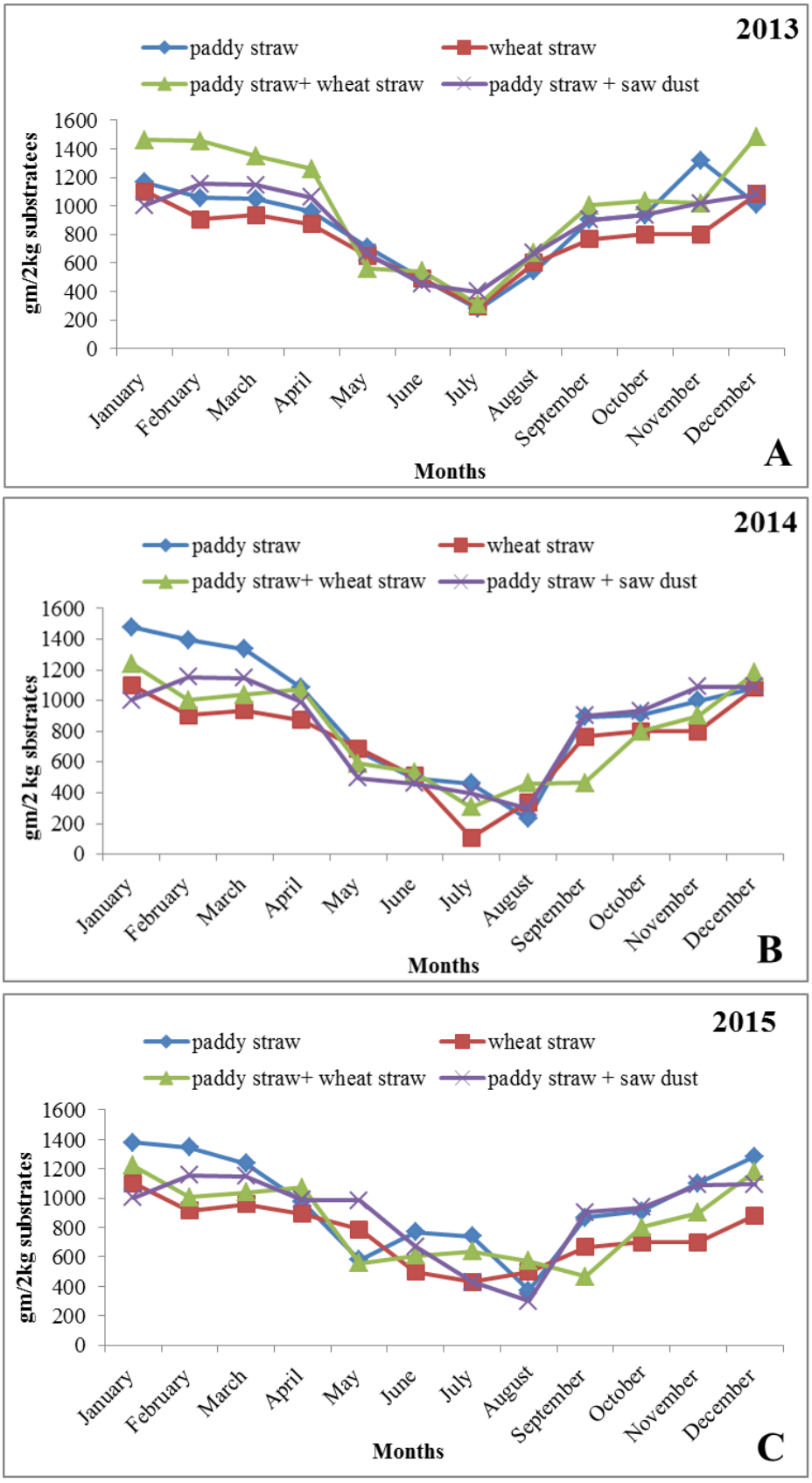


Figure 32: Comparative study on yield of *P. djamor* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

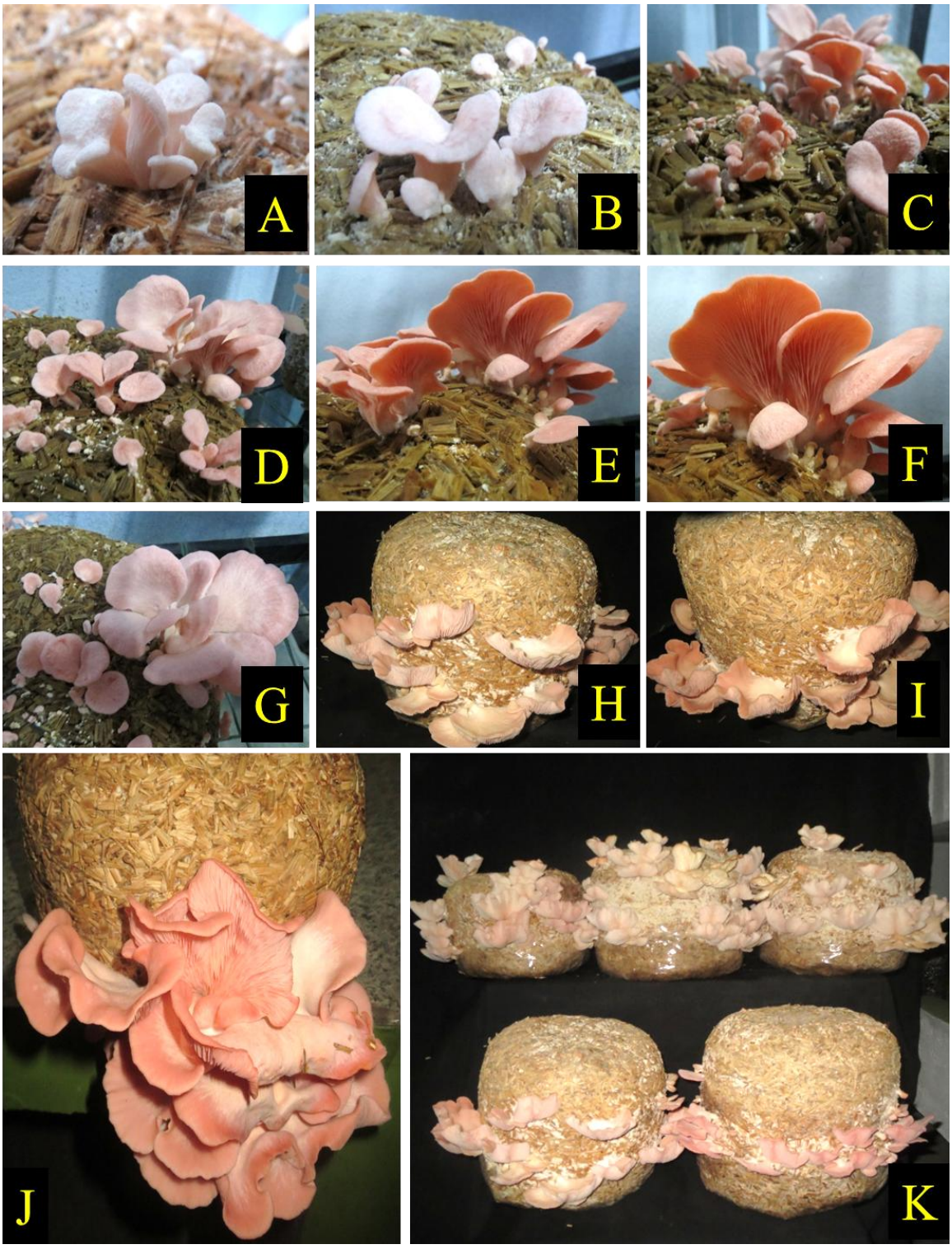


Figure 33: (A-K) Cultivation of *P djamor* (pink oyster mushroom); (A-C) pinhead stage, (D-G) early mature stage and (H-K) mature stage ready for harvesting.

Table 16: Cultivation of *Pleurotus djamor* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of containers	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	19	23	408.3±8.81
	Bottles	35X5(dia.)	18	22	410±13.22
Rainy	Polypropylene bags	45X30	21	25	308.3±15.89
	Bottles	35X5(dia.)	16	19	335±12.58
Winter	Polypropylene bags	45X30	13	18	591.6±14.24
	Bottles	35X5(dia.)	12	16	613.3±17.40

Mean value of five replicate containers, (±) standard error

4.9.1. Growth in different containers

Pleurotus djamor popularly known as pink oyster mushroom was cultivated in this region. Various substrates were tested for better cultivation and it was observed that paddy straw supplemented with saw dust and paddy straw mixed with wheat straw showed better result for its cultivation. Pink oyster mushroom is very much popular for its colour, texture as well as taste. Pink oyster mushroom very recently introduced in this region and the cultivation of this mushroom is now very popular. Pink oyster mushroom was cultivated using the plastic waste bottles in compare to polypropylene bags. Results revealed that the plastic bottles were very much useful to cultivate. Mycelial run period was very less than the polypropylene bags as the structure was much more compact that the bags (Table 14). It was also observed that yield was also increased. Plastic bottles were recycled to cultivate and for the large scale production, waste plastic bottles were much more efficient. The above result showed that the use of waste bottles can also be used for the cultivation as the colonization period, full cropping time reduces and production increases (Figure 34).

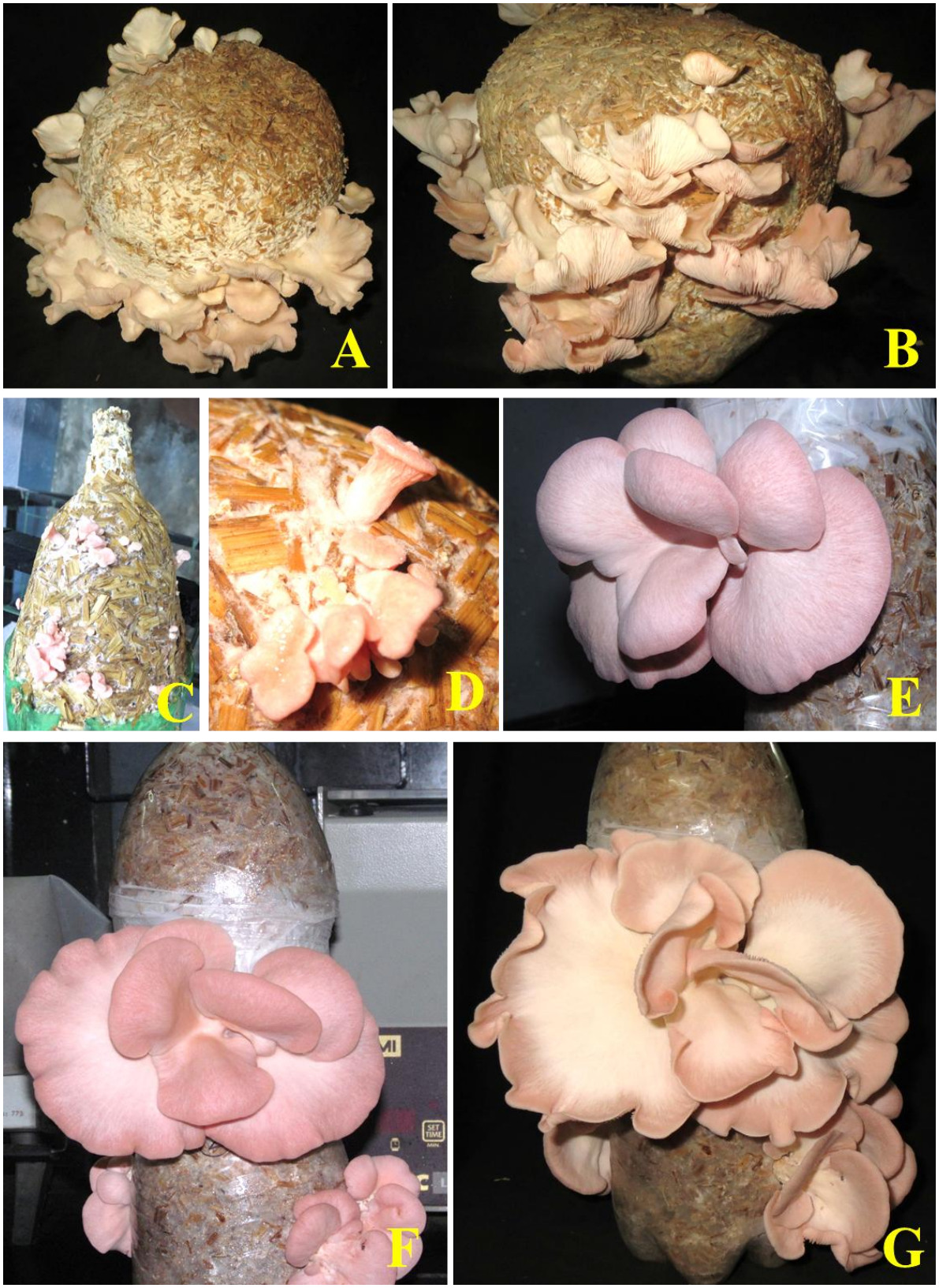


Figure 34: (A-G) Growth and development of fruitbody during cultivation of *P. djamor* in different containers; (A-B) polypropylene bags; (C-G) using waste plastic bottles

4.10. Cultivation of *Pleurotus florida*

4.10.1. Growth in different substrates

Oyster mushroom is one of the major mushrooms cultivating widely in North Bengal and different species of oyster mushroom are now being cultivated by a large number of farmers in this region. *P. florida* commonly known as white oyster mushroom largely cultivated for its bright white fruiting body, texture and great taste. In North Bengal, paddy straw is commonly used for the cultivation as it is the most available substrate in this region. The results showed that the mycelial run period was about 14 days during the early winter season to early summer (Figure 35). It was observed that the spawn run period reduces during November to April. Cultivation also depends on the environmental conditions and the results the production increased during December to April and the complete cropping period also decreased during this period. Wheat straw was used for the cultivation of *P. florida* and it was observed that the spawn run period decreased during the winter (January and February). Production rate was also high during this period (Figure 36). Results also revealed that for fruiting body initiation, lower temperature required with very low relative humidity. Wheat straw was successfully used for the cultivation and it requires less time period for full cropping as well as increase amount of production. *P florida* was also cultivated using the paddy straw in combined with wheat straw (1:1). It was observed that the mycelial run period reduces in during the winter season (December to March) and also during the early summer season. The results revealed that using the paddy straw and wheat straw in combined form was more efficient and it was also extends the cropping time up to early summer season. A large number of industries are producing timber and thus north Bengal is a very renowned source of saw dust. Saw dust is very hard and thus it was used as supplement with paddy straw (Figure 37).

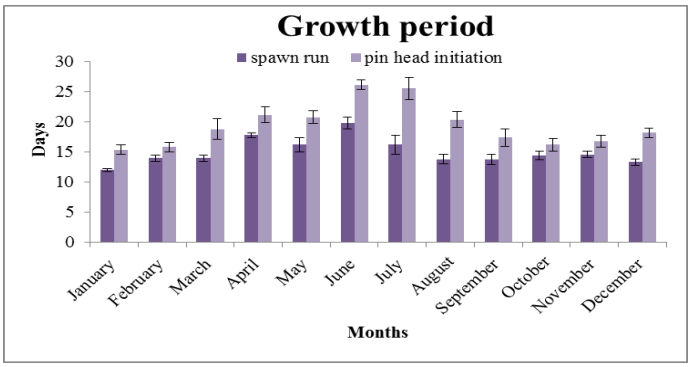


Figure 35: Assessment of spawn run period for pin head initiation during cultivation of *P. florida* throughout the year

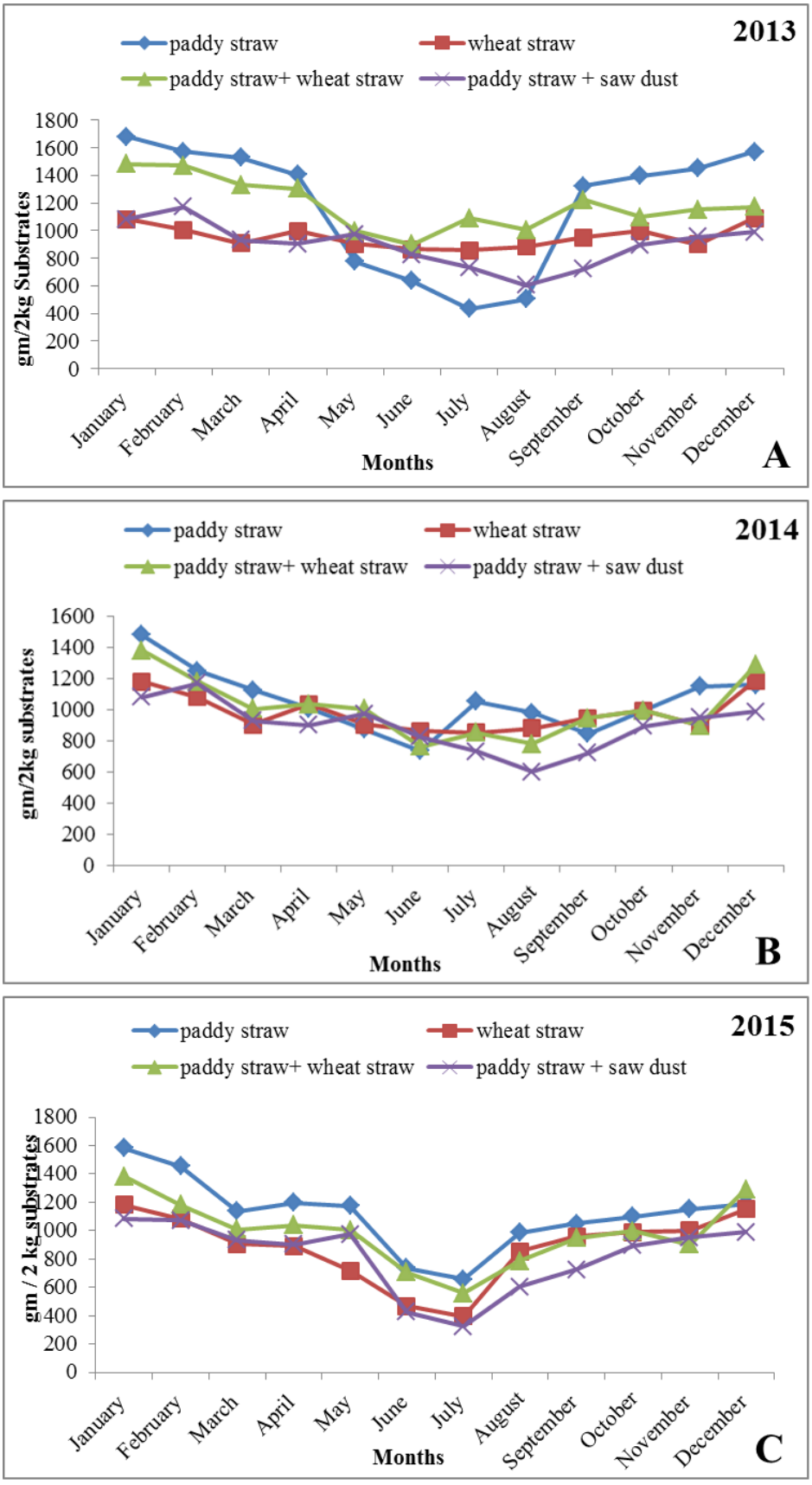


Figure 36: Comparative study on yield of *P. djamor* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

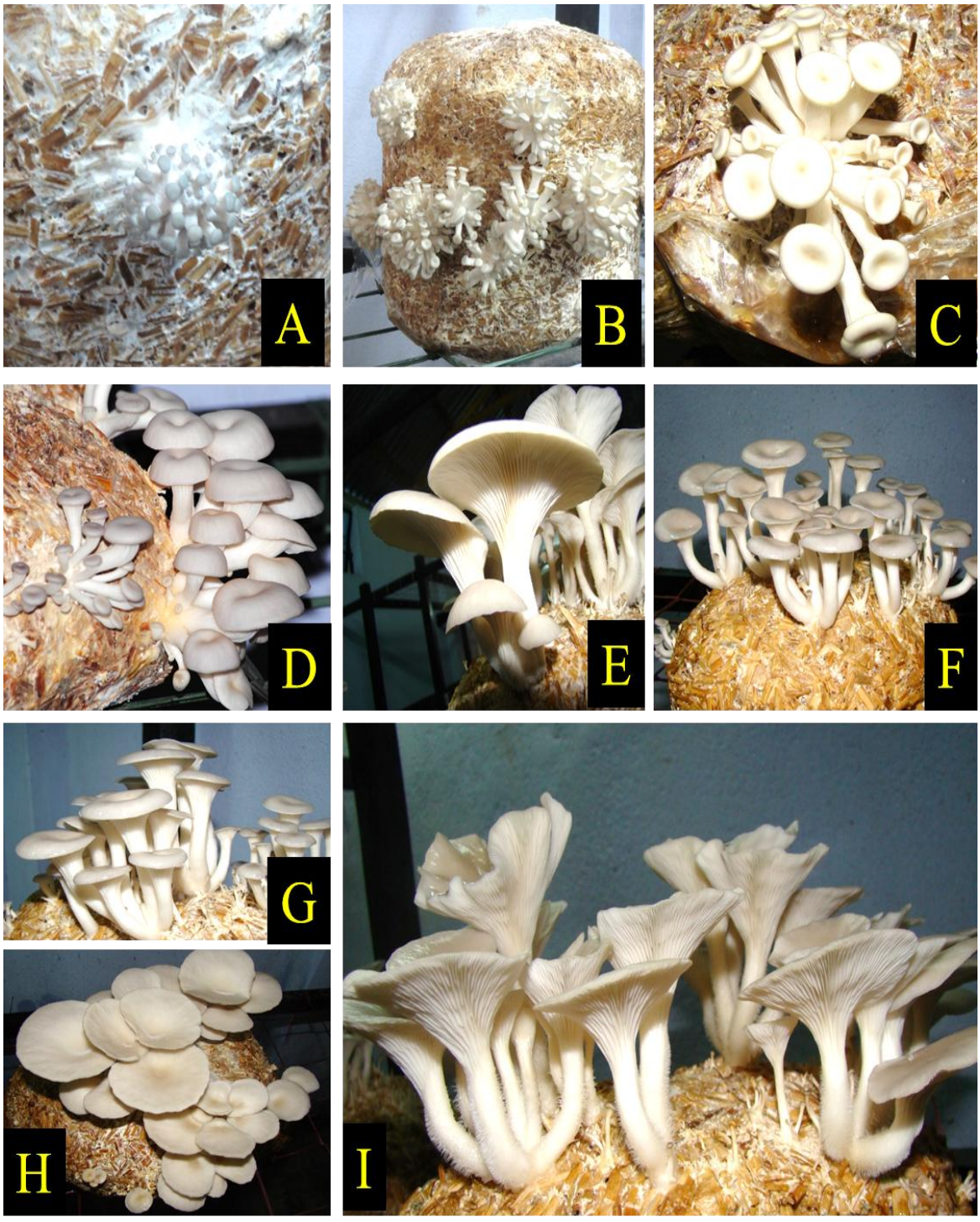


Figure 37: (A-I) Cultivation of *P. florida* (A-C) pinhead stage, (D-G) early mature stage and (H&I) showing the mature stage.

Table 17: Cultivation of *P. florida* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	25	26.33±1.45	480±8.66
	Bottles	35X5(dia.)	20	22	495±14.43
	Box	80X40X30	23	27	465±16.07
Rainy	Polypropylene bags	45X30	24	27	390±11.54
	Bottles	35X5(dia.)	20	23	406.6±9.27
	Box	80X40X30	25	27	363.3±14.8
Winter	Polypropylene bags	45X30	18	22	558.3±10.13
	Bottles	35X5(dia.)	14	17	575±16.07
	Box	80X40X30	18	23	530±8.66

Mean value of three replicates, (±) standard error

4.10.2. Growth in different containers

Pleurotus florida is one of the major oyster species cultivated in this region for its large fruiting body, ability to grow throughout the year and taste. Large number of growers of North Bengal region cultivating *P. florida*. For the cultivation different containers were used to evaluate their ability to grow as well as yield potential. Results revealed that the mycelial run period is quite lower in case of bottles as well as polypropylene bags while it took more time in case of boxes for colonization (Table 17). On the other hand, it was also observed that the yield potential was higher in boxes than the polypropylene bags. Waste plastic bottles results in yield, lower cropping period as well as recycling the containers (Figure 38). Using plastic bottles helps in lowering the cropping time as well as it was also observed that bottle cultivation dose not required such a large production unit.

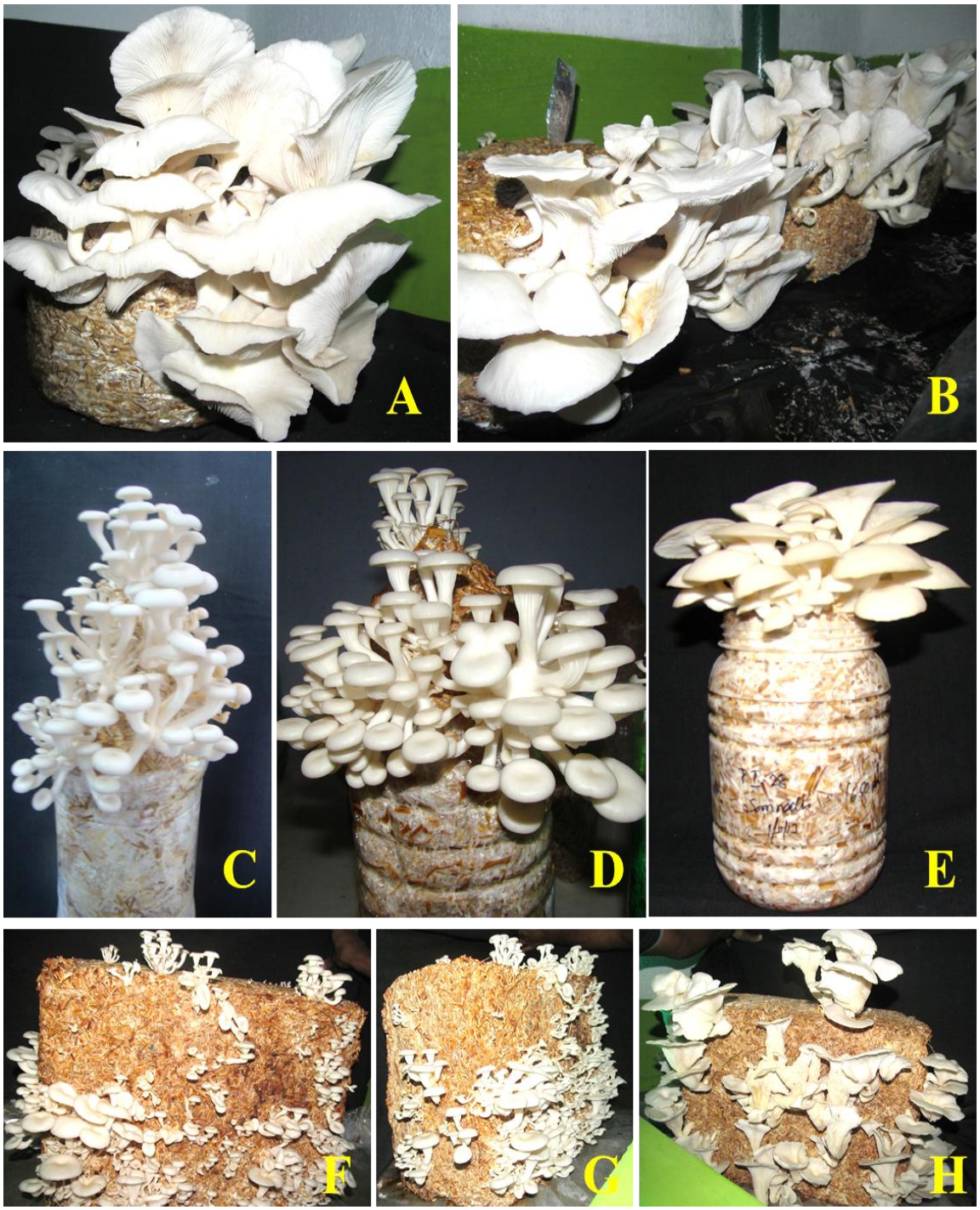


Figure 38: (A-H) Cultivation practice of *P. florida* using different types of containers (A-B) polypropylene bags; (C-E) waste plastic bottles and (F-H) in paper box.

4.11. Determination of moisture content of cultivated oyster mushroom (*P. ostreatus, P sajor-caju, P djamor* and *P florida*)

Different species of oyster mushroom was cultivated using different substrates such as paddy straw, wheat straw and saw dust in single as well as in combination with others. Mushroom fruiting body consists a high amount of water content and it was observed that substrate effects on moisture content. Results revealed that in case of *Pleurotus ostreatus* cultivated on paddy straw and wheat straw single as well as combination showed highest moisture content than the other substrates (Figure 39A). The moisture content of *Pleurotus sajor-caju* was found to be about 45-90% cultivated on different substrates (Figure 39B) highest moisture content was found in mature pileus cultivated on paddy straw followed by paddy straw combined with saw dust. It was also observed that the moisture content of *P. djamor* was higher in both young pileus as well as in mature pileus and pileus of paddy straw and wheat straw showed highest (80-90%) amount of moisture content (Figure 39C). In case of *P. florida,* moisture content was found to be higher in both young pileus as well as in mature pileus. Mature pileus cultivated on paddy straw and wheat straw showed very high (80-93%) amount of moisture content (Figure 39D).

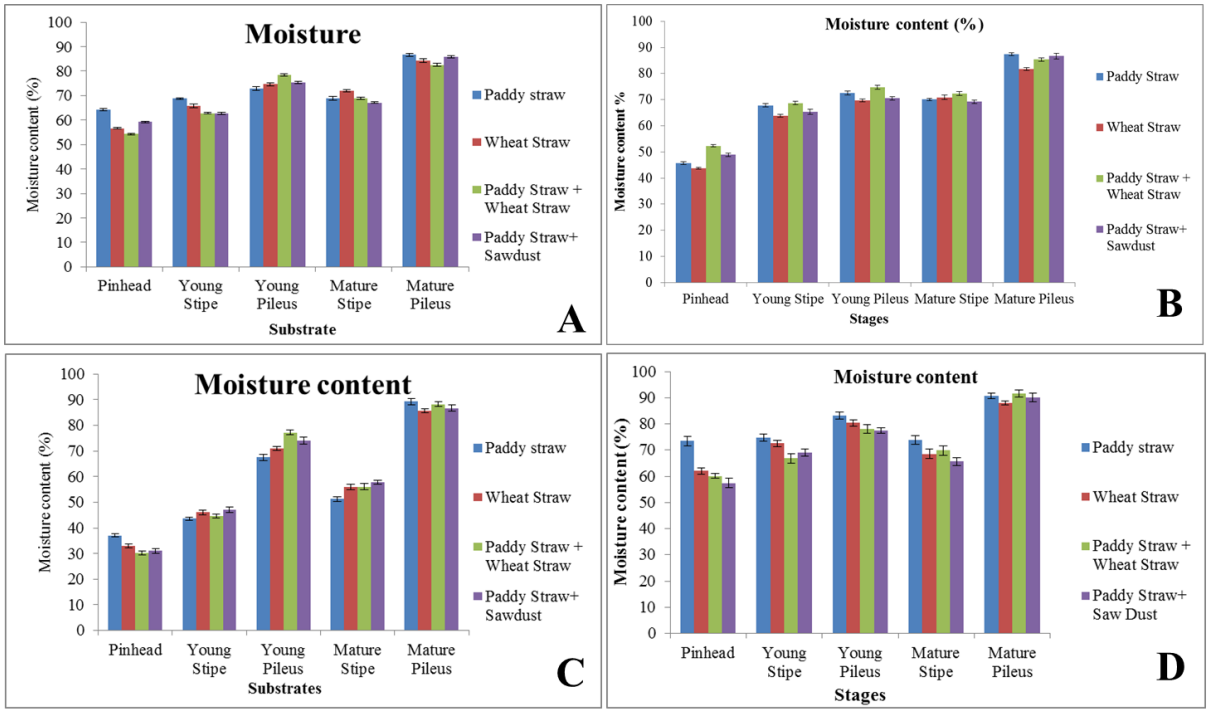


Figure 39: Comperative study of moisture content of (A) *P. ostreatus,* (B) *P. sajor-caju,* (C) *P djamor* and (D) *P florida*

4.12. Estimation of protein, sugar and lipid content of oyster mushroom grown on different substrates

4.12.1. *Pleurotus ostreatus*

Major compounds of mushroom are protein and carbohydrates. *Pleurotus ostreatus* was cultivated using different substrates which also effects on the nutritional composition. Total sugar and reducing sugar estimation were performed and it was found that mature pileus consists about 280-310 mg/gm tissue total sugar and 42-50 mg/gm tissue reducing sugar (Figure 40 A&B). The results also revealed that the reducing sugar significantly higher in case of mature pileus as well as young pileus cultivated in paddy straw. Different stages were taken into consideration for the analysis of protein, sugar and it was found that the substrates also affect the protein and carbohydrate content. In case of paddy straw and wheat straw mature pileus found to be consisting high amount of total sugar content as well as reducing sugar content in compare to other two substrates. Oyster mushroom is known to be a very good source of protein and such proteins was due to the presence of total free amino acid. Protein content was found to be very high in different stages of growth. In compare to carbohydrates, protein content was very high (Figure 41 A). The results showed that *P. ostreatus* containing about 250-400 mg/gm tissue protein. Oyster mushroom is also very popular as it contains lower amount of lipid content and it was observed that about 6-8mg/gm tissue lipid content (figure 41B)was found and highest lipid content was observed in case of paddy straw and wheat straw combined substrate.

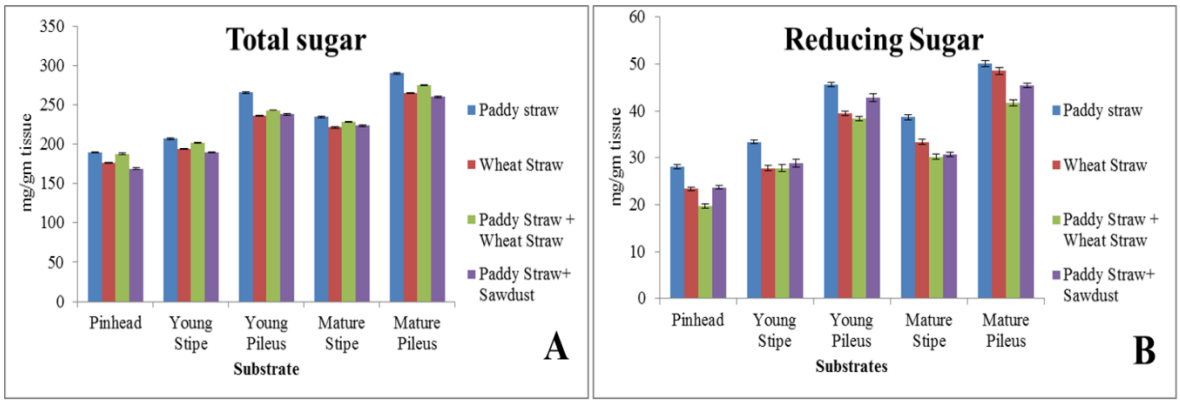


Figure 40: Total sugar (A), reducing sugar (B) of *P. ostreatus* in different stages of its growth

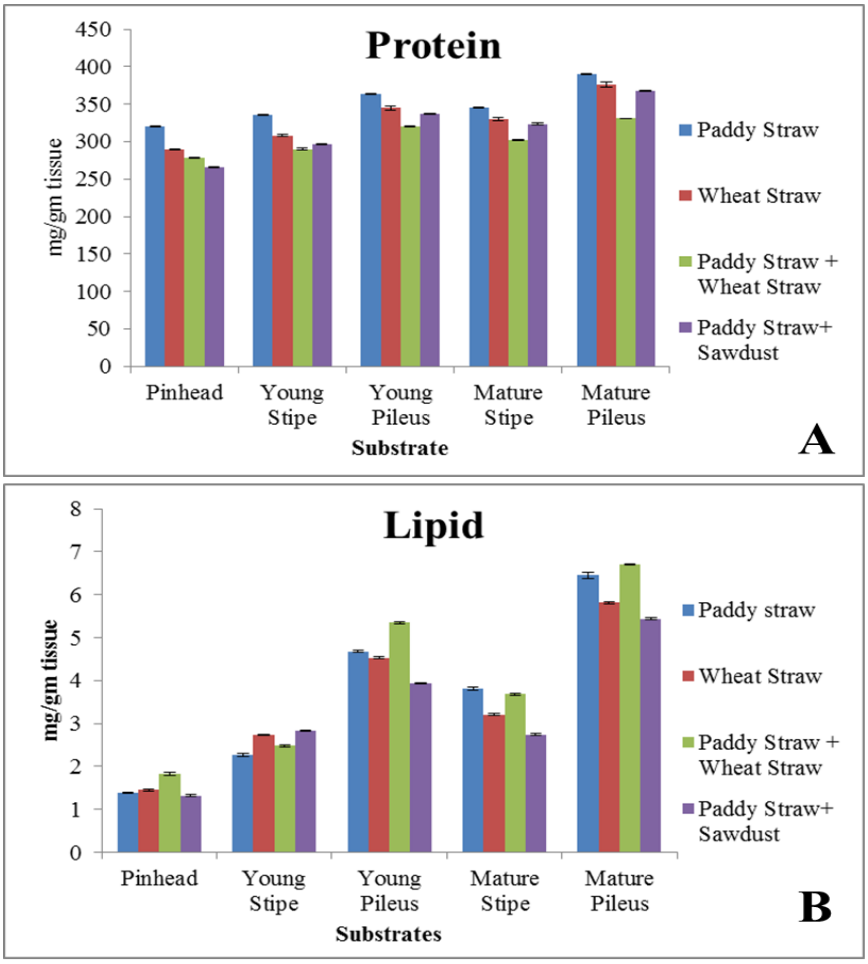


Figure 41: Total soluble protein (A) and total lipid content (B) of *P. ostreatus* in different stages of its growth

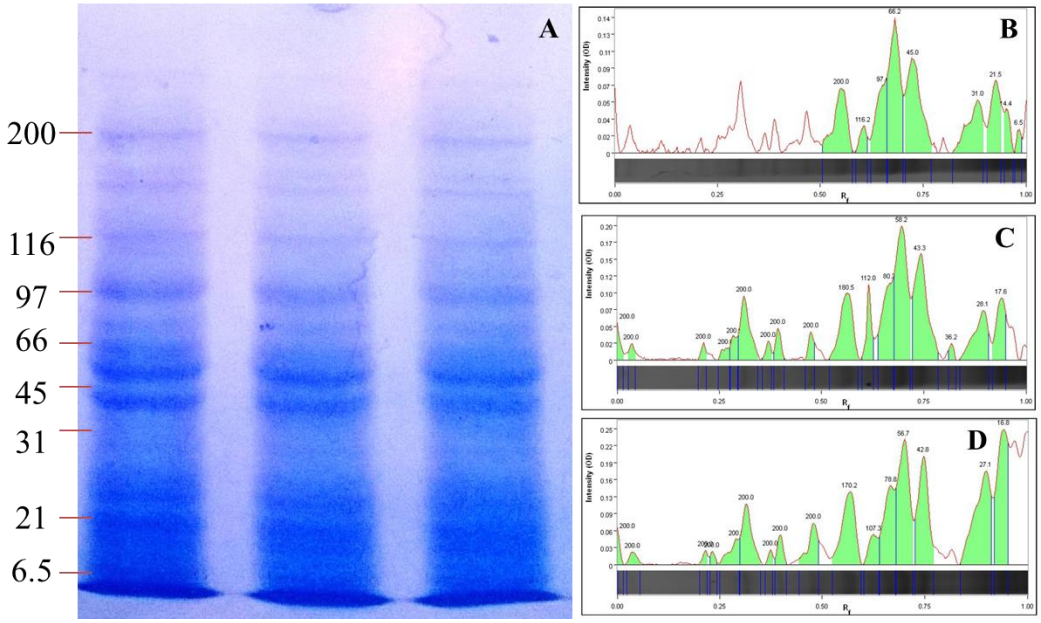


Figure 42: SDS PAGE analysis of soluble protein of *P. ostreatus* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D).

Table 18: Study of band profileof SDS PAGE analysis of *P. ostreatus*

Lane	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig 42 A)	1	200.00	0.013189	12.44674	0.4008	0.337419
	2	200.00	0.081535	20.85496	0.671555	0.565358
	3	96.74	0.184652	97.44839	3.137954	2.641734
	4	69.61	0.302158	15.64255	0.503709	0.424055
	5	47.99	0.338129	64.47263	2.076096	1.747792
	6	32.61	0.364508	62.60395	2.015922	1.697134
	7	19.82	0.468825	92.97852	2.994019	2.52056
	8	16.41	0.546763	267.3797	8.609945	7.248411
	9	13.66	0.622302	500.562	16.11869	13.56976
	10	12.74	0.651079	264.3306	8.511762	7.165754
	11	11.88	0.679856	102.6751	3.306259	2.783424
	12	6.50	0.973621	992.6242	31.96369	26.90911
Lane 2 (Fig 42 B)	1	200.00	0.019185	11.02235	0.35936	0.302164
	2	166.91	0.148681	64.38208	2.099036	1.764952
	3	101.80	0.176259	12.69237	0.413807	0.347945
	4	93.86	0.195444	90.98459	2.966352	2.494226
	5	68.92	0.305755	52.19265	1.701627	1.430795
	6	40.65	0.348921	77.52654	2.527582	2.125291
	7	30.00	0.374101	74.18512	2.418643	2.03369
	8	19.82	0.468825	31.41851	1.024331	0.861298
	9	14.44	0.59952	124.1616	4.048015	3.40373
	10	13.34	0.631894	418.7857	13.65359	11.48048
	11	11.53	0.691847	91.66617	2.988574	2.512911
	12	6.50	0.983213	903.6602	29.46185	24.77269
Lane3 (Fig 42 C)	1	200.00	0.121103	28.72748	1.051931	0.828372
	2	133.13	0.154676	47.49951	1.739317	1.369674
	3	93.23	0.197842	171.5727	6.282577	4.947389
	4	69.38	0.303357	36.91237	1.351642	1.064388
	5	36.10	0.357314	60.86674	2.228793	1.755125
	6	28.47	0.383693	140.6357	5.149741	4.055306
	7	20.76	0.44964	22.42603	0.821187	0.646666
	8	18.86	0.489209	52.44514	1.920414	1.512283
	11	13.15	0.63789	508.7912	18.6307	14.67126
	12	12.16	0.670264	247.3634	9.057849	7.132854
	13	11.40	0.696643	135.378	4.957216	3.903697
	16	6.50	0.990408	593.5545	21.73453	17.11546

4.12.2. *Pleurotus sajor-caju*

Nutritional composition of *Pleurotus sajor-caju* was evaluated of its different stages of growth. Total sugar and reducing sugar also estimated and it was found that mature pileus of paddy straw consists about 310 mg/gm tissue total sugar while mature pileus of paddy straw combined with wheat straw consists about 270 mg/gm tissue total sugar. Reducing sugar was also estimated and mature pileus of paddy straw combined with saw dust possess highest amount (58 mg/gm tissue) among the other substrates (Figure 43 A&B). *Pleurotus sajor-caju* is also known to possess high amount of protein content and it was observed that protein content of *P. sajor-caju* ranges from 320-370mg/gm tissue. Paddy straw showed significantly high amount of protein content followed by paddy straw combined with wheat straw (Figure 44A). The results showed that *P. sajor-caju* contains lower amount of lipid content and it was observed that about 6-8 mg/gm tissue lipid content was found and highest lipid content was wheat straw substrate (figure 44B).

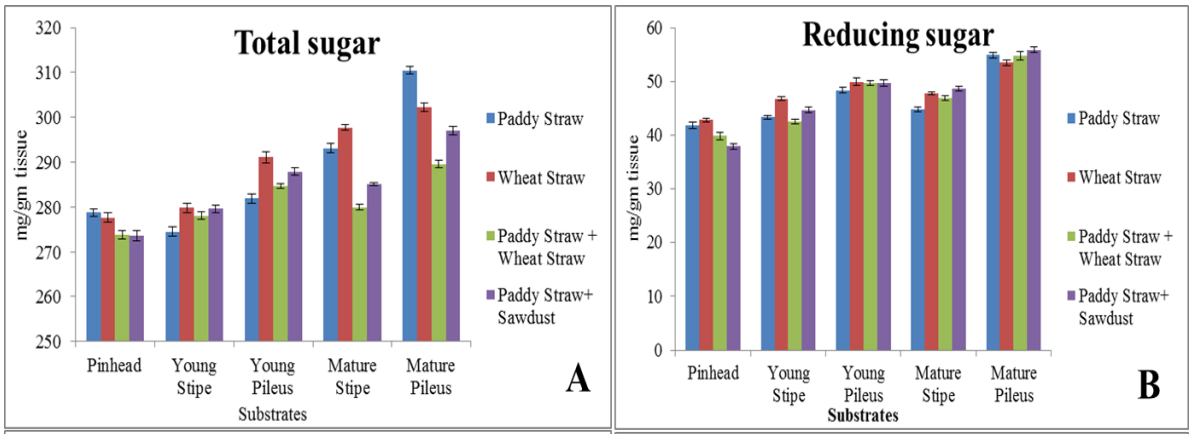


Figure 43: Total sugar (A) and reducing sugar (B) of *P. sajor-caju* in different stages of its growth

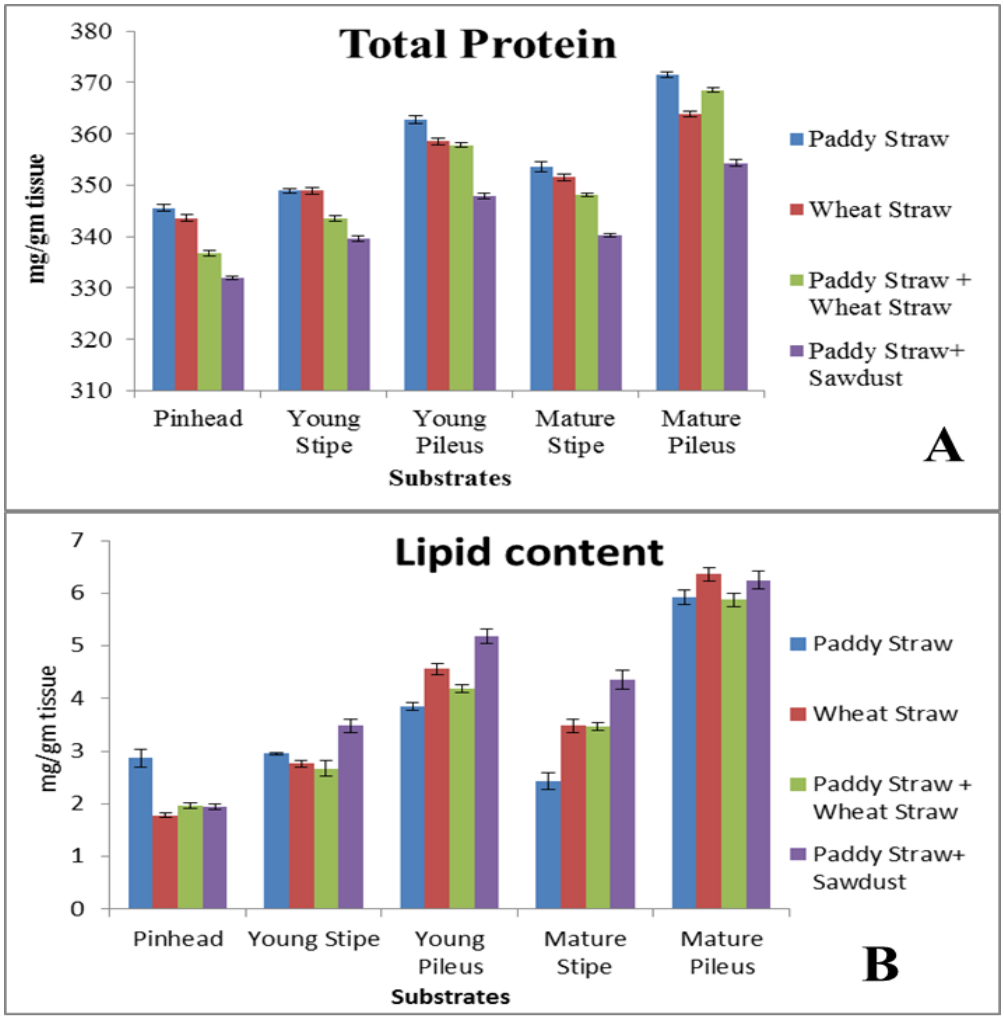


Figure 44: Total soluble protein (A) and lipid content (B) of *P. sajor-caju* in different stages of its growth

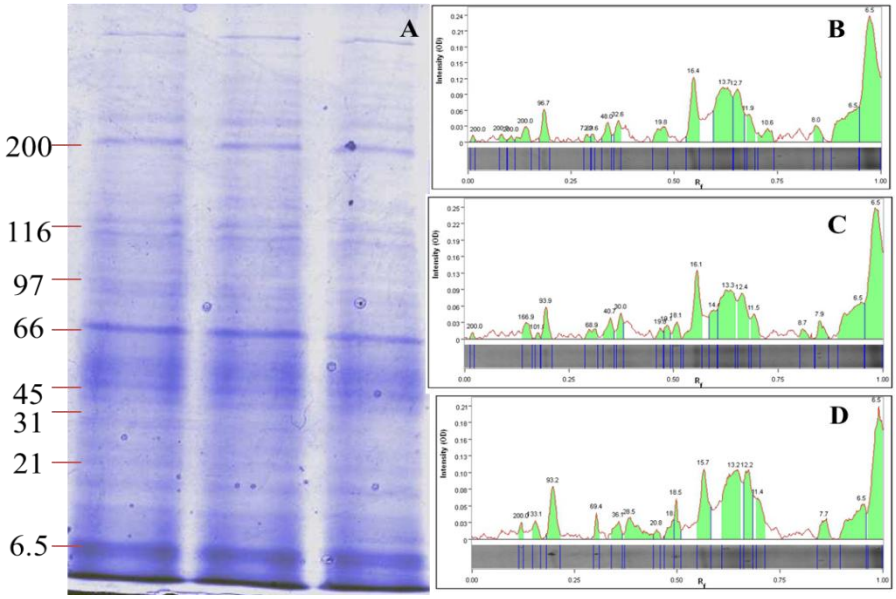


Figure 45: SDS PAGE analysis of soluble protein of *P. sajor-caju* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 19: Study of band profile of SDS PAGE analysis of *P. sajor-caju*

Sample	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig.45 A)	1	200	0.098321	91.5938	0.90804	0.859197
	2	200	0.129496	321.1632	3.18394	3.012675
	3	114.2114	0.160671	368.7475	3.65568	3.45904
	4	91.38015	0.205036	341.6145	3.386689	3.204518
	55	79.89527	0.252998	522.7445	5.182371	4.903611
	6	67.09514	0.315348	683.6541	6.777592	6.413024
	7	29.42106	0.377698	1,193.62	11.83331	11.1968
	8	22.80235	0.42446	2,222.84	22.03675	20.85139
	9	15.75675	0.563549	642.4049	6.368657	6.026086
	10	12.85324	0.647482	1,073.29	10.64036	10.06802
	11	7.809665	0.852518	126.2965	1.252075	1.184725
	12	6.614631	0.920863	454.0175	4.501027	4.258916
Lane 2 (Fig.45 B)	1	200	0.105516	111.6134	0.880936	0.727138
	2	200	0.134293	330.7872	2.610818	2.15501
	3	108.3077	0.167866	428.4581	3.38171	2.791316
	4	20.70332	0.450839	980.2002	7.736468	6.385802
	5	15.52957	0.569544	798.7853	6.304607	5.203922
	6	12.96608	0.643885	1,334.20	10.53048	8.692023
	7	9.410589	0.775779	437.7055	3.454697	2.851561
	8	8.673335	0.809353	291.9919	2.304617	1.902267
	9	7.832452	0.851319	157.1769	1.240557	1.023975
	10	7.389076	0.8753	71.73798	0.566209	0.467358
	11	6.5	0.979616	161.836	1.27733	1.054328
Lane 3 (Fig.45 C)	1	200	0.001199	45.44377	0.466087	0.419898
	2	200	0.134293	264.4839	2.712635	2.443818
	3	108.3077	0.167866	480.7018	4.930239	4.441661
	4	89.25734	0.213429	381.3492	3.911246	3.523648
	5	78.30174	0.260192	602.678	6.181268	5.568715
	6	67.09514	0.315348	418.6321	4.293631	3.86814
	7	20.88451	0.447242	735.9051	7.54769	6.799727
	8	15.71105	0.564748	692.2629	7.100081	6.396475
	9	12.92836	0.645084	1,252.72	12.84837	11.57512
	10	11.5061	0.693046	990.3778	10.15765	9.151043
	11	10.54306	0.729017	104.1724	1.068428	0.962548
	12	9.548689	0.769784	340.0886	3.488063	3.142402
	13	6.770624	0.911271	566.3581	5.808758	5.233121
	14	6.5	0.970024	108.0794	1.108499	0.998648

4.12.3. *Pleurotus djamor*

Pleurotus djamor is very commonly cultivated in north western part of India and very recently this species is introduced in North Bengal. Nutritional composition of was evaluated of its different stages of growth. Morphologically *P. djamor* possess very short stipe and thus the total sugar and reducing sugar was higher in pileus than that of stipe. Results revealed that young pileus and mature pileus possess highest amount of total sugar (180-230 mg/gm tissue) and maximum activity found in case of wheat straw than the other substrates. Reducing sugar was also estimated and it was observed that reducing sugar high in case of young pileus cultivated on paddy straw (45mg/gm tissue) supplemented by saw dust while in case of mature pileus, paddy straw showed highest (60mg/gm tissue) activity of reducing sugar (Figure 46). *Pleurotus djamor* is very well known to possess higher amount of protein content and it was observed that protein content ranges from 200-290 mg/gm tissue. In case of young pileus of all substrates showed significantly similar amount of protein content while in case of mature pileus, paddy straw showed highest amount of protein content followed by paddy straw supplemented with saw dust. The results showed that *P. djamor* possess low amount of lipid content (5-7 mg/gm tissue) it was also found that young pileus of paddy straw combined with wheat straw and mature pileus of wheat straw showed highest lipid content (Figure47B).

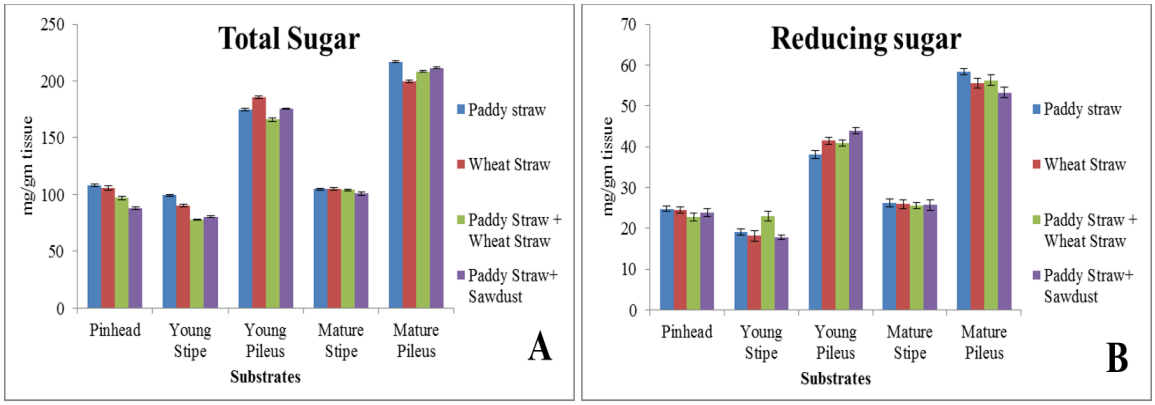


Figure 46: Total sugar (A) and reducing sugar (B) of *P. djamor* in different stages of its growth

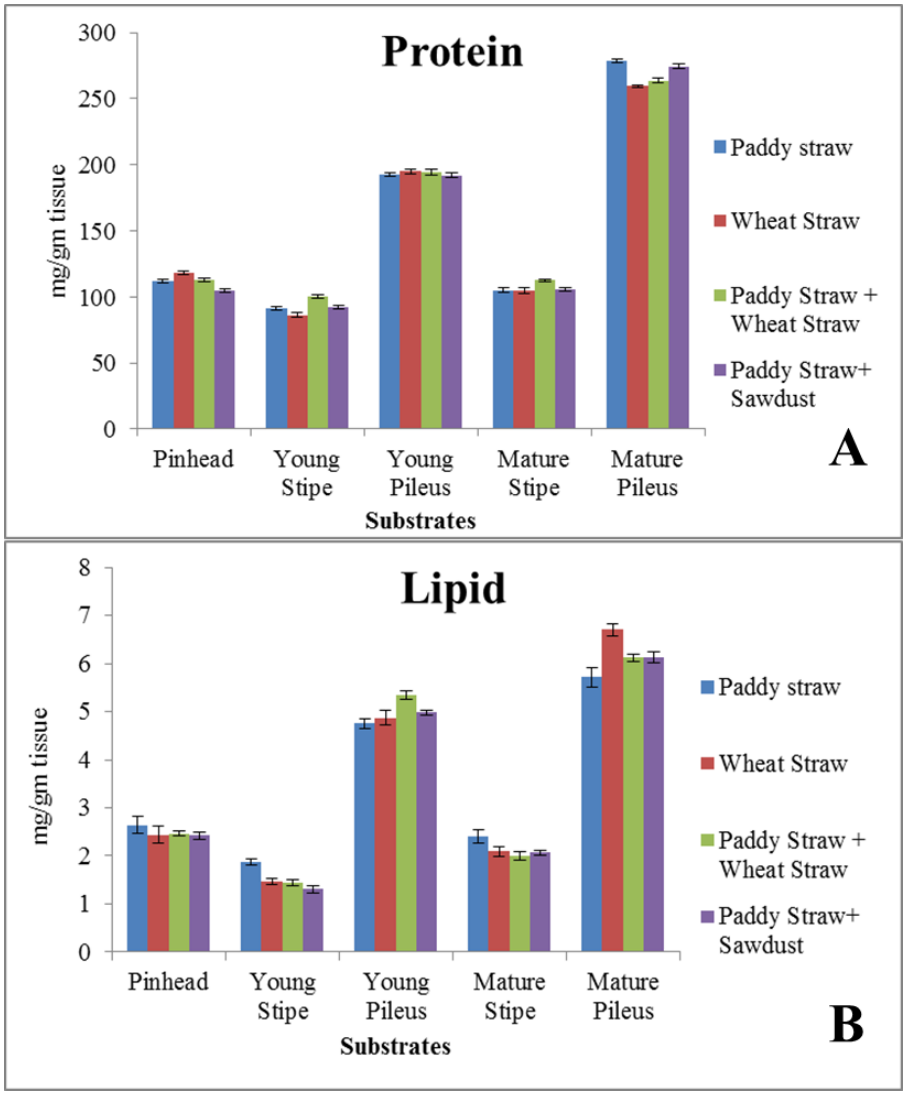


Figure 47: Total soluble protein (A) and lipid content (B) of *P. djamor* in different stages of its growth

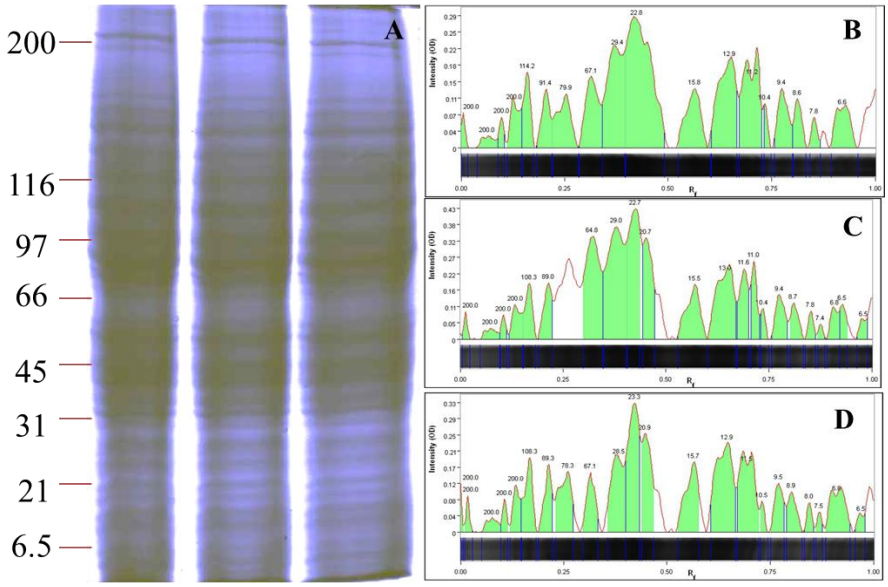


Figure 48: SDS PAGE analysis of the protein of *P. djamor* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 20: Study of band profile of SDS PAGE analysis of *P. djamor*

Lane	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig.48 A)	1	200	0.044386	279.82	3.62	3.18
	2	200	0.087467	43.52	0.56	0.49
	3	149.74	0.562663	449.21	5.82	5.10
	4	106.73	0.617493	140.88	1.82	1.60
	5	97.4	0.656658	458.32	5.94	5.21
	6	73.92	0.682768	640.69	8.30	7.28
	7	60.59	0.725849	525.85	6.81	5.97
	8	35.38	0.861619	683.04	8.85	7.76
	9	21.20	0.902089	656.49	8.50	7.46
	10	16.05	0.955614	473.84	6.14	5.38
Lane 2 (Fig 48 B)	1	200	0.056136	335.1687	3.598444	3.402808
	2	200	0.099217	61.59143	0.661259	0.625308
	3	200	0.25718	72.8148	0.781756	0.739254
	4	136.7993	0.569191	630.7203	6.77155	6.403402
	5	105.4391	0.622715	184.32	1.9789	1.871313
	6	93.45247	0.660574	515.0943	5.530164	5.229506
	7	69.95445	0.68799	729.0304	7.827028	7.401497
	8	60.16323	0.72846	586.4365	6.296109	5.953809
	9	35.38283	0.861619	654.2344	7.024003	6.64213
	10	20.36268	0.909922	1,378.88	14.80399	13.99914
	11	14.4	0.993473	3,179.31	34.13377	32.27802
Lane 3 (Fig 48 C)	1	200	0.074413	320.5739	4.08886	3.856258
	2	200	0.266319	26.06545	0.33246	0.313547
	3	200	0.5	141.1019	1.799728	1.697347
	4	122.7317	0.577023	376.3368	4.800105	4.527043
	5	103.527	0.630548	80.68259	1.029091	0.97055
	6	86.031	0.668407	414.2325	5.283457	4.982899
	7	65.96597	0.694517	610.1875	7.782826	7.340087
	8	59.52741	0.732376	517.3527	6.598736	6.223356
	9	32.92052	0.869452	725.2917	9.250958	8.724702
	10	19.68261	0.916449	1,321.33	16.85332	15.89459
	11	14.4	0.992167	2,999.93	38.26359	36.0869

4.12.4. *Pleurotus florida*

Pleurotus florida commonly known as white oyster mushroom is very rich in nutritional constituents. Reducing sugar was higher in pileus than that of stipe. Results revealed that young pileus and mature pileus possess highest amount of total sugar (210-300 mg/gm tissue) and maximum activity was found in case of paddy straw and wheat straw combined substrates than the other substrates. Reducing sugar was also estimated and it was observed that reducing sugar very much high in case of mature pileus than that of the young pileus (60-65 mg/gm tissue) (Figure 49). *Pleurotus florida* is also very popular to possess higher amount of protein content and it was observed that protein content ranges from 160-280 mg/gm tissue (Figure 50 A and 51). In case of young pileus and mature pileus of all substrates showed significantly similar amount of protein content. The results showed that *P. florida* possess low amount of lipid content (2-6.5 mg/gm tissue) it was also found that mature pileus of wheat straw showed highest lipid content (figure 50B).

Figure 49: Total sugar (A) and reducing sugar (B) of *P. florida* in different stages of its growth

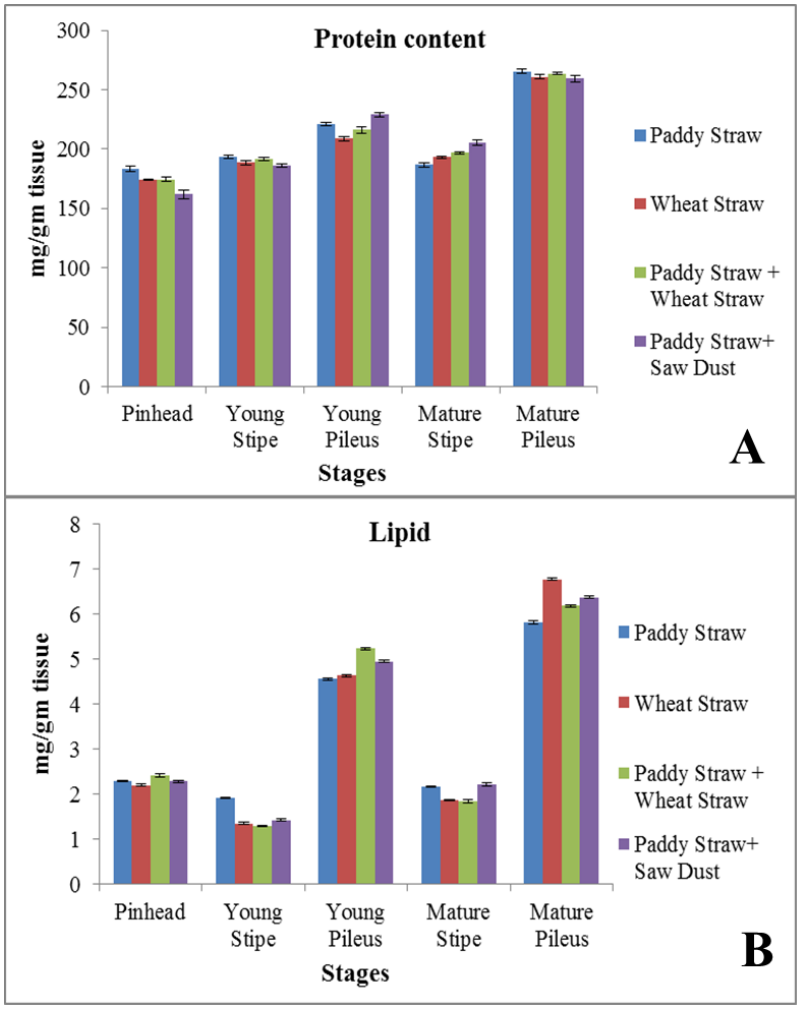


Figure 50: Total soluble protein (A) and lipid content (B) of *P. florida* in different stages of its growth

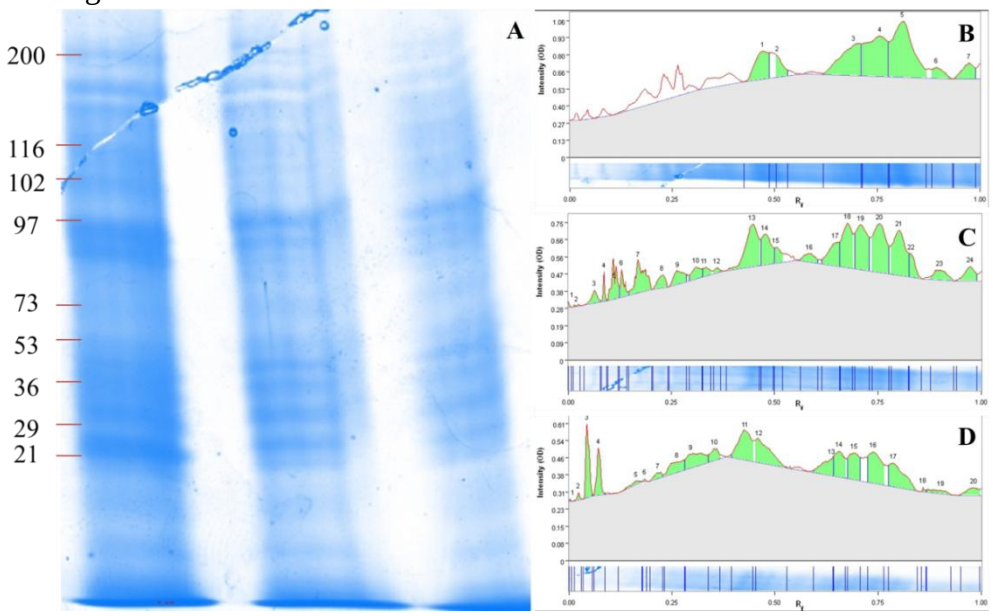


Figure 51: SDS PAGE analysis of the protein of *P. florida* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 21: Study of band profile of SDS PAGE analysis of *P. florida*

	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig. 51A)	1	200	0.360265	34.653	0.520023	0.486758
	2	200	0.446358	709.9122	10.65336	9.97188
	3	183.9832	0.476821	376.6394	5.652069	5.290517
	4	121.2045	0.503311	137.7503	2.067161	1.934928
	5	107.955	0.584106	131.539	1.97395	1.84768
	6	101.6451	0.647682	346.7105	5.20294	4.870117
	7	98.87763	0.676821	569.8318	8.551227	8.00422
	8	88.43693	0.708609	692.2633	10.38851	9.723972
	9	67.27373	0.753642	831.5951	12.4794	11.68111
	10	48.06567	0.801325	742.4813	11.14211	10.42937
	11	41.17908	0.830464	188.7193	2.832031	2.650871
	12	30.25289	0.899338	180.8631	2.714137	2.540519
	13	21.5	0.97351	192.1468	2.883467	2.699017
Lane 2 (Fig. 51B)	1	200	0.429139	426.8258	11.42745	10.35526
	2	200	0.460927	383.0376	10.2551	9.292911
	3	102.6705	0.637086	208.8628	5.591905	5.067241
	4	100.8827	0.655629	266.2467	7.128248	6.459436
	5	97.4	0.692715	313.3303	8.388823	7.601737
	6	73.49824	0.739073	442.685	11.85205	10.74002
	7	53.31053	0.786755	400.7581	10.72954	9.722831
	8	36.36858	0.858278	10.42855	0.279205	0.253008
	9	29.88611	0.901987	64.00752	1.71368	1.552893
	10	21.5	0.981457	101.1085	2.706987	2.453002
Lane 3 (Fig. 51C)	1	200	0.074413	320.5739	4.08886	3.856258
	2	200	0.266319	26.06545	0.33246	0.313547
	3	200	0.5	141.1019	1.799728	1.697347
	4	122.7317	0.577023	376.3368	4.800105	4.527043
	5	103.527	0.630548	80.68259	1.029091	0.97055
	6	86.031	0.668407	414.2325	5.283457	4.982899
	7	65.96597	0.694517	610.1875	7.782826	7.340087
	8	59.52741	0.732376	517.3527	6.598736	6.223356
	9	32.92052	0.869452	725.2917	9.250958	8.724702
	10	19.68261	0.916449	1,321.33	16.85332	15.89459
	11	14.4	0.992167	2,999.93	38.26359	36.0869

4.13. Utilization of dry tea leaves following pruning practice in tea estates in North Bengal as alternative substrate for cultivation of *P. ostreatus* and *P. sajor-caju*

Tea is one of the major economic crops in North Bengal and Darjeeling is world famous for tea. Large numbers of tea gardens are situated in this region and every year pruning is commonly practiced in this region. After pruning, generally tealeaves are used as fuel for the common people. Efficacy of tea leaves in cultivation was done and it was observed that tea leaves were very much efficient in cultivating the *P. ostreatus*. Tea leaves were collected and the dried for 7-10 days. Spawning was done in two different combinations such as (A) tea leaves in combination with paddy straw (1:1) and (B) tea leaves alone and it was observed that the mycelia colonize rapidly over the tea leaves. Similar growth was also observed in case of combined substrate. Results also revealed that tea leaves in combined with paddy straw helps to colonize mycelia rapidly (table 22). Number of pinhead was higher in case of combined substrate than that of the single substrate. Development of fruiting body was rapid in case of tea leaves alone substrate. Production of *P. ostreatus* was also very high in case of combined substrate and tea leaves single substrate. It was observed that the production rate was higher in case of combined substrate than that of the single tea leaves substrates (figure 52&53). In compare to *P. ostreatus*, *P sajor-caju* was also cultivated using the tea waste as substrate (figure 54). Using pruned dry tea leaves helps is rapid growth and higher yield of *P sajor-caju*. The results revealed that the mycelial run period become very less in case of tea leaves in compare to combine substrate of paddy straw along with tea leaves. Along with the short growth period, it also helped in increase number of pinhead per bags. Yield of *P sajor-caju* also varies and the results revealed that the use of combined substrate helps in increasing the yield performance.

Table 22: Cultivation of *P. ostreatus* and *P sajor-caju* using pruned tea leaves along with paddy straw

Substrate	Initial wt. of bags (gm)	Days of colonization	No. of Pinhead	Yield (gms)			Total production (gm)
				1st flush	2nd flush	3rd flush	
Pleurotus ostreatus							
Tea leaves + Paddy straw (1:1)	450	18	115	200	120	90	410
	500	19	135	350	120	50	520
	1200	21	143	500	210	115	825
	1000	21	136	350	100	60	510
	850	21	157	250	100	45	395
	1200	20	123	375	120	70	570
Tea Leaves	350	17	30	280	135	65	480
	950	20	94	390	145	80	615
	900	18	102	300	160	95	555
	650	21	110	265	105	55	425
	350	20	98	130	75	20	225
	400	19	84	210	105	45	360
Pleurotus sajor-caju							
Tea leaves + paddy straw	350	17	75	290	100	35	425
	490	19	87	200	120	50	370
	450	19	98	170	90	30	290
	600	21	117	310	100	40	450
	650	16	93	180	75	30	285
	500	18	107	275	100	90	465
Tea leaves	350	17	83	210	90	45	345
	390	19	76	185	80	50	315
	490	15	89	240	110	45	395
	560	21	94	275	90	25	390

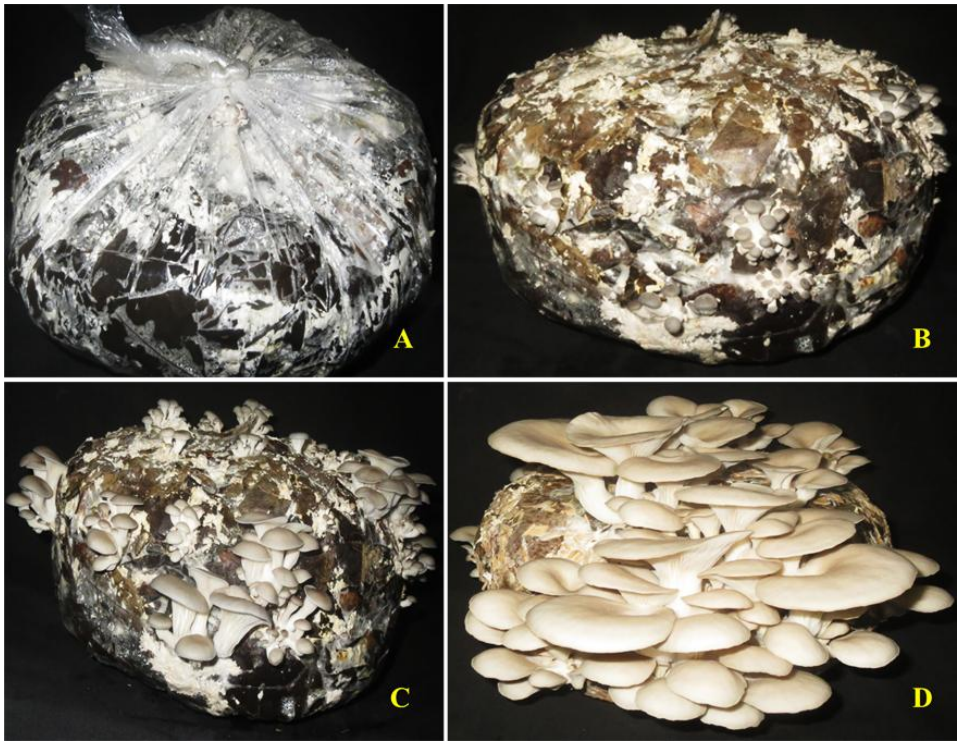


Figure 52: Cultivation of P. ostreatus using pruned tea leaves; (A)fully colonized bag, (B) pinhead formation, (C) young stage and (D) mature fruiting body

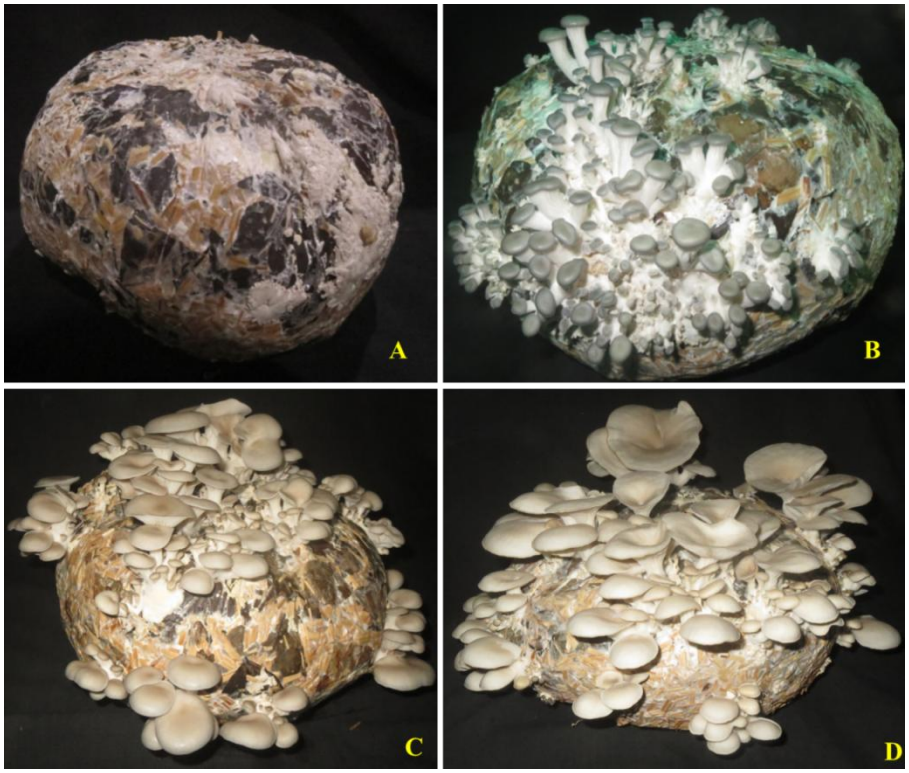


Figure 53: (A-D) Cultivation of *P. ostreatus* on tea waste substrate in combination with paddy straw (A) fully colonized, (B) pinhead stage, (C) young fruiting body and (D) mature fruiting body

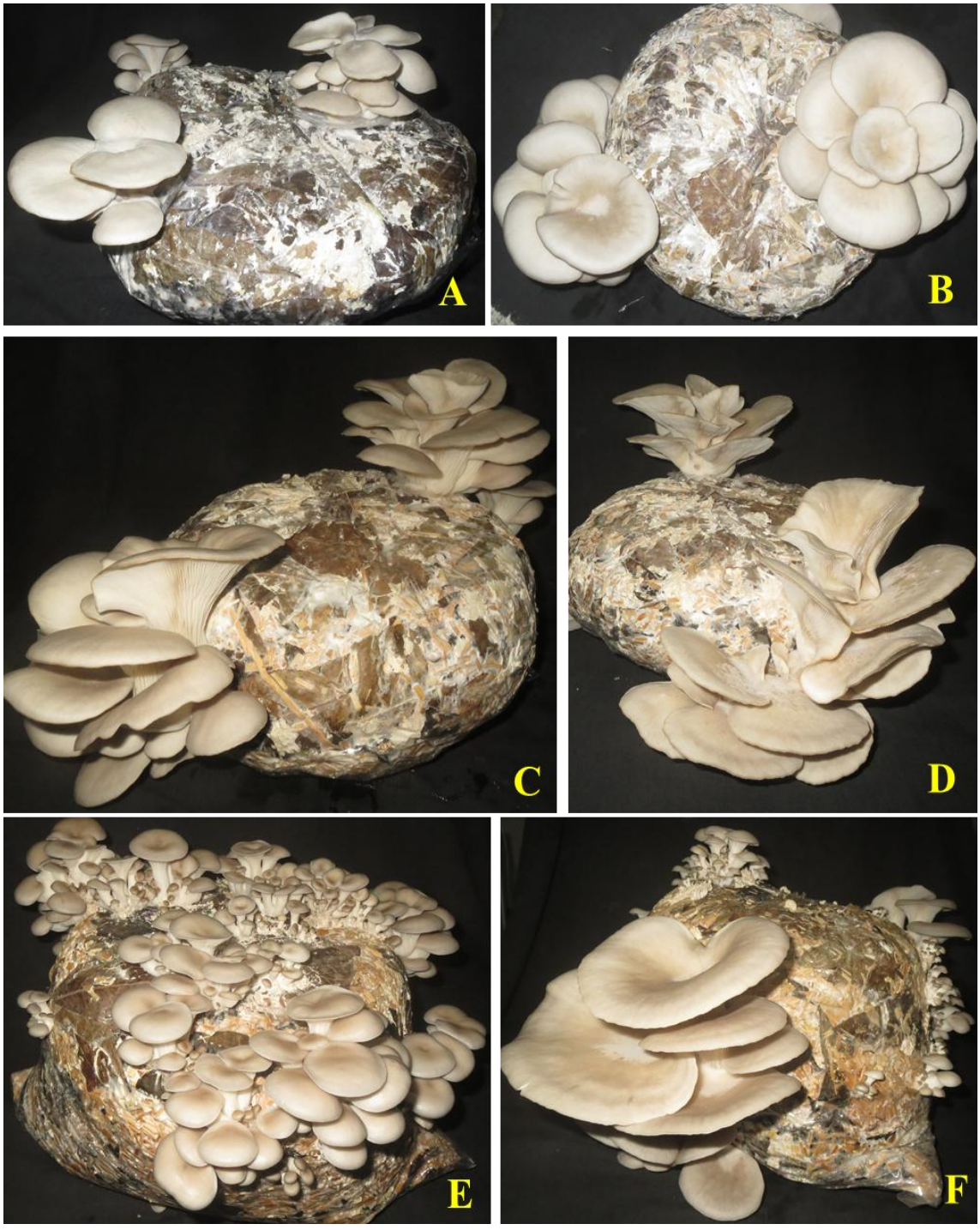


Figure 54: Cultivation of *Pleurotus sajor-caju* on pruned tea leaves (A-D) and in combine with paddy straw (E and F)

4.14. Biochemical characterization of fruiting body cultivated on tea waste

Waste tea leaves were used as substrates singly or in combination with paddy straw to evaluate the effect of waste tea leaves as an alternative substrate for the cultivation of oyster mushroom. The growth and production was significantly higher in case of the waste tea leaves used singly. Further the biochemical characterization of the harvested fruiting body was estimated to evaluate the effect of tea leaves on their food value. Results revealed that the moisture content was higher in case of tea waste in compare to combined substrates (figure 55). Mature pileus of tea waste substrates showed highest moisture content. Protein content of the fruiting body cultivated on tea waste was estimate and it was observed that the mature pileus of tea waste was significantly high in compare to the combined substrates. Protein content of the young pileus was also high but pinhead stage and stipe was very much similar in compare to combined substrates (Figure 56C). Total sugar and reducing sugar content was also evaluated and it was also estimated and the results revealed that the total sugar as well as the reducing sugar was significantly enhanced in compare to the combined substrates (figure 56A&B). It was also observed that the total sugar in young pileus enhanced more and the results revealed that the total sugar more or less similar to the mature pileus.

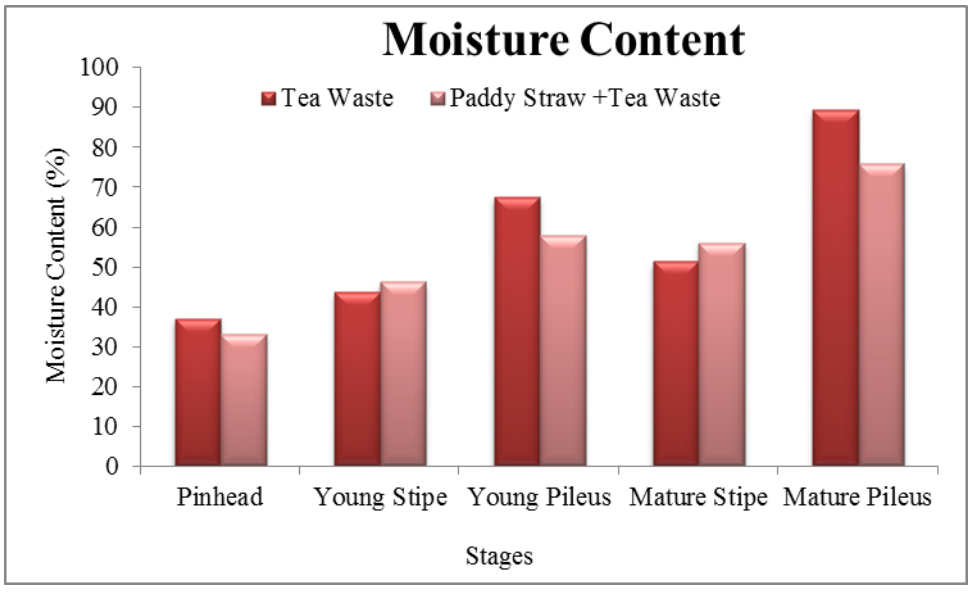


Figure 55: Moisture content of *P. ostreatus* grown on tea waste

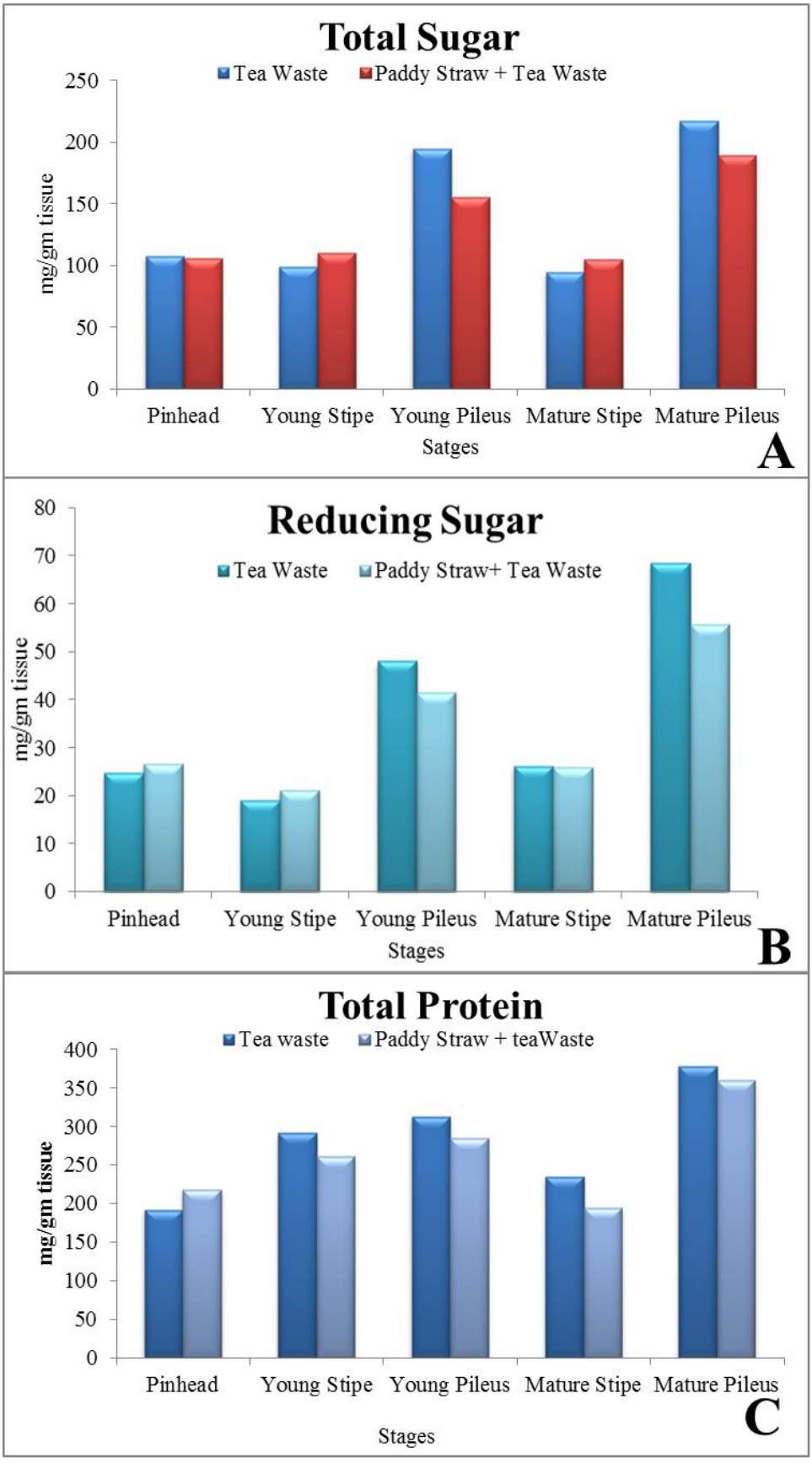


Figure 56: Total sugar (A) reducing sugar (B) and total protein (C) content of *P. ostreatus* grown on tea waste

4.15. Antioxidant activity of different species of oyster mushrooms

The extracts of different species of oyster mushroom showed positive antioxidant activity by fading the violet colour of DPPH solution to yellow to pale violet colour. Results revealed that the scavenging activity of DPPH were directly proportional with the concentration of the samples used. As the concentration of the sample was increased, the scavenging activity of towards DPPH radicles also elevated (Figure 57A). Different concentrations were used for the evaluation of DPPH scavenging activity and it was observed that *P. djamor* showed maximum activity among the other species in respect to all the concentrations. It was found that *Pleurotus djamor* showed about 88% DPPH scavenging activity in 20mg/ml concentration while *P. florida* showed lowest scavenging activity (77%) among the other species.

All the mushroom species showed appreciable reducing power activity in different concentrations (5-20mg/ml). Highest amount of reducing power ability was observed in case of *Pleurotus djamor* at 20 mg/ml concentration while *P. ostreatus* and *P. sajor-caju* showed lowest amount of reducing power activity at 20 mg/gm tissue concentration. Free reducing power activity was estimated using the different concentrations of four species of oyster mushroom and the highest activity was observed in case of *P. djamor* whereas in case of *P. ostreatus* and *P. sajor-caju* the activity is quite lower than that of the others (Figure 57B). Antioxidant is an important parameter and mushroom is one of the major sources of antioxidant compounds.

Total flavonoid content and carotenoid content is also an important compound showing antioxidant activity. Total flavonoid content of four different species was assessed and the results revealed that all the species were showing significant amount of flavonoid content. Different concentrations were taken into consideration and it was observed that *Pleurotus djamor* and *P. florida* showed highest amount of flavonoid in compare to other two species (Figure 57C). The results were also revealed that the higher concentration of the sample helps in increasing the flavonoid content and thus highest flavonoid content activity was found in case of *P. djamor* and *P florida* in 20mg/ml concentration in compare to 5gm/ml and 10mg/ml concentration.

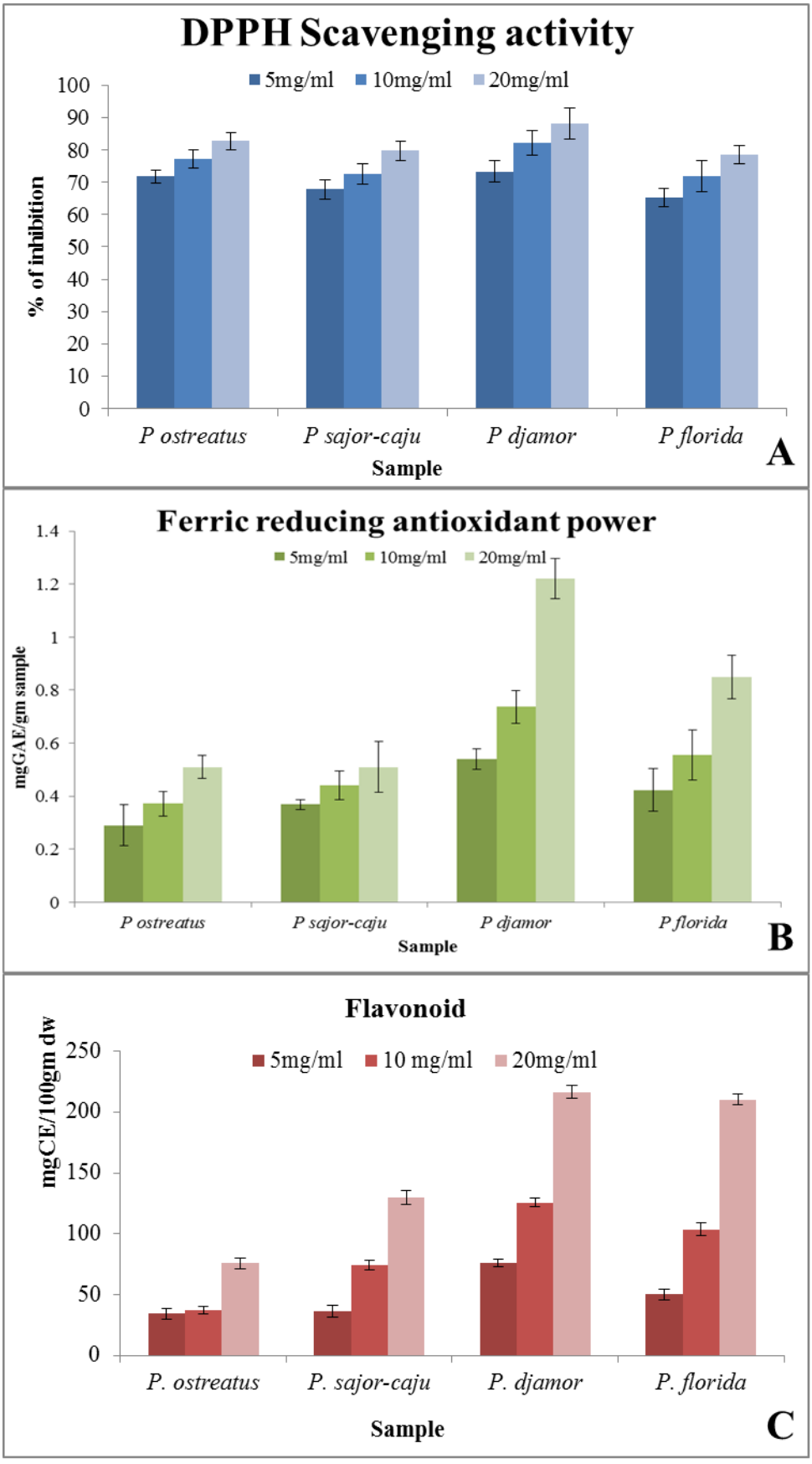


Figure 57: Antioxidant activity of *Pleurotus* species; (A) DPPH scavenging activity (B) Free radicle antioxidant power assay and (C) flavonoid activity

4.16. Antidiabetic activity of different species of oyster mushroom

Diabetes mellitus is a disease that affects millions of people throughout the world. A growing number of people each day are diagnosed with diabetes mellitus, due to the rise in obesity and sedentary lifestyle. In many places throughout the world, diabetes is kept under control by the use of different treatments. Healthy white albino rats were taken for the induction of blood sugar (Figure 58). After induction, body weight were measured and it was observed that in case of control groups, there was no significant changes in case of normal control group, but in case of negative control body weight drastically decreased and gradually the rats were very sick. On the other hand, initially the body weight of the positive control rats was decreased but gradually the rats were gained their body weight and become healthy again (Figure 59A). In case of mushroom treated rats, it was observed that initially after the induction the body weight decreases which generally happened in diabetic patients. But by the treatment, it was observed that the rats were able to regain their weight. The results revealed that the treatment of *P djamor* helps rapidly for gaining the body weight followed by *P. sajor-caju, P. florida* and *P. ostreatus*.

It was also observed that the blood glucose level increases after the induction of Streptozotocin. In case of normal control, blood glucose level was similar throughout the experiment. In case of negative control, the blood glucose level increases drastically and the experimental rats were become very sick while in positive control, initially the blood glucose level was high but after continuous treatment with metformin, the blood glucose level become normal. In case of sample treated groups, similar trends were observed that the blood glucose level initially increases after the induction of Streptozotocin and the sample treatment helps in lowering the blood glucose level (Figure 59B). *Pleurotus djamor* and *P. florida* showed high antidiabetic activity in compare to the other two species. The results revealed that the sample treatment significantly helps in controlling the blood glucose level in compare to control sets.

Figure 58: Induction of hyperglycaemia in white albino rats; (A &B) selected healthy rats of same size and weight kept in grouped, (C) induction of hyperglycaemia using STZ intraperitoneally, (D) oral treatment of mushroom, (E) treatment after taking blood from tail vein.

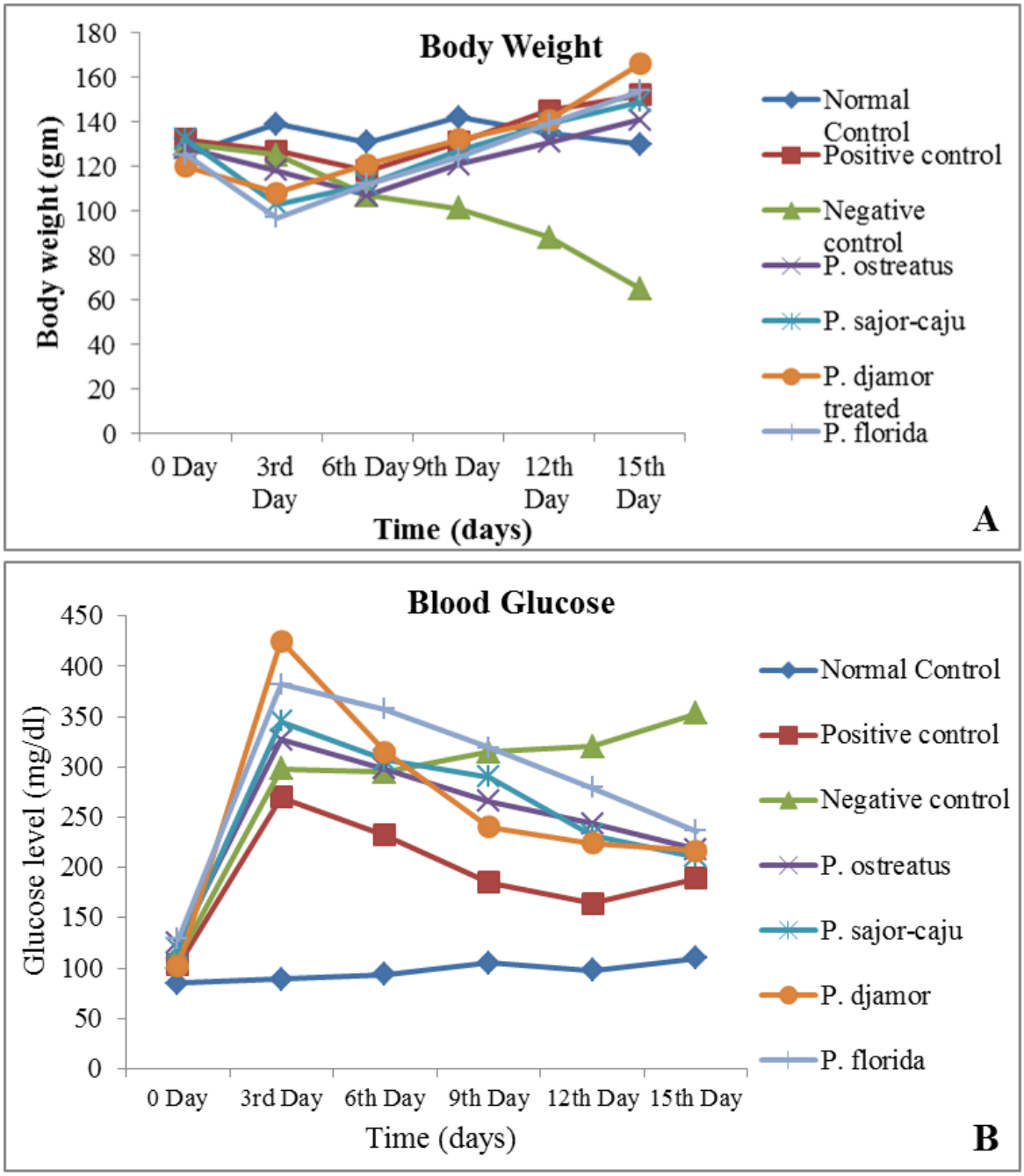


Figure 59: Evaluation of antihyperglycemic activity of *Pleurotus* species on induced rats (A) effect on body weight and (B) blood glucose level following induction

4.16.1. Effect of oyster mushroom on kidney function test of STZ induced diabetic rats

Creatinine is a waste product formed in the muscle from the high energy storage compound creatinine phosphate and it is an important indicator of renal function. Creatinine level is also increasing in proportionate to blood glucose level. The results revealed that the blood creatinine level was increased after induction of all the groups of animals. In case of negative control group, blood creatinine level was very high which affects the animal health but in case of positive control and mushroom treated rats, the creatinine level was less in compare to negative control (Figure 60). It was also observed that the effect was much less in case of *P. djamor* and *P. ostreatus* while compared with the positive control. *P. sajor-caju* and *P. florida* also successfully reduced the amount of creatinine level in compare to control sets.

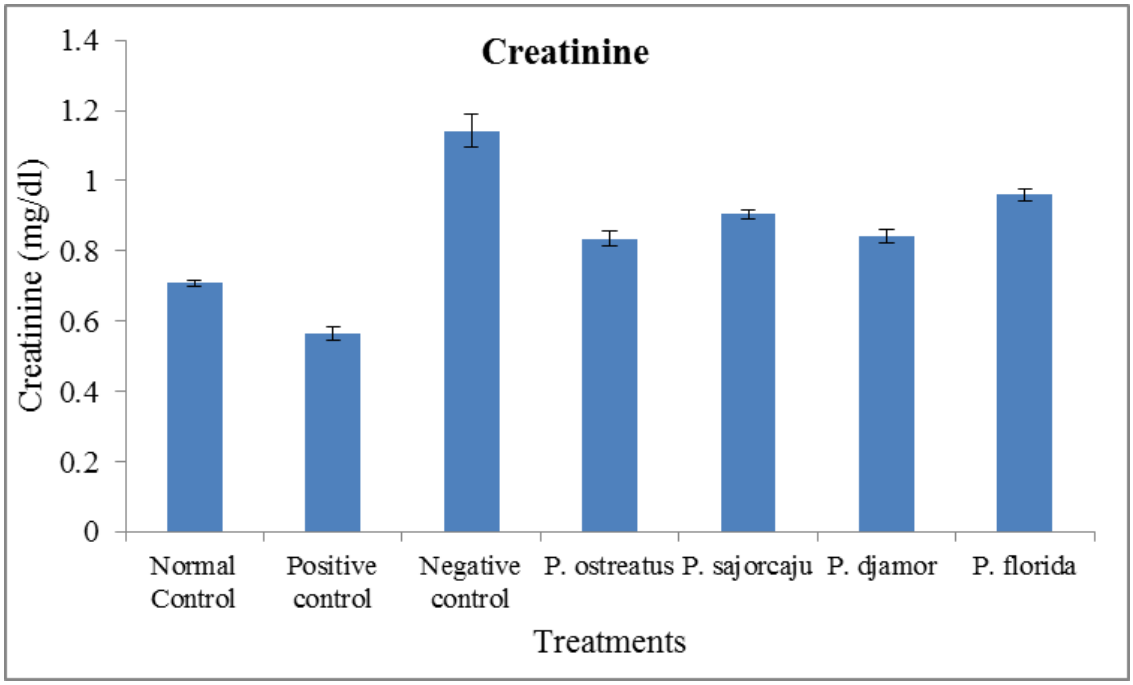


Figure 60: Changes in creatinine level following oral treatment of *Pleurotus* species in hyperglycaemic rats

On the other hand, blood urea level was also estimated which regulates the liver disease as well as kidney function. Urea level of diabetic patients increased which results in different renal diseases and also affects in liver function. The results showed that the induction of Streptozotocin treated negative control possesses highest amount of blood urea level but in case of positive control, it was observed that the medicine helps in reducing the blood urea level and become closer to normal range. Blood urea level of the mushroom powder treated groups were also analysed and it was observed that *P. djamor* showed highest activity in lowering the blood urea level in followed by *P. ostreatus, P florida* and *P. sajor-caju* (Figure 61B). Results also prove that the use of mushroom powder of different species of oyster mushroom significantly reduces the blood urea level in compare to positive control group as well as negative control groups.

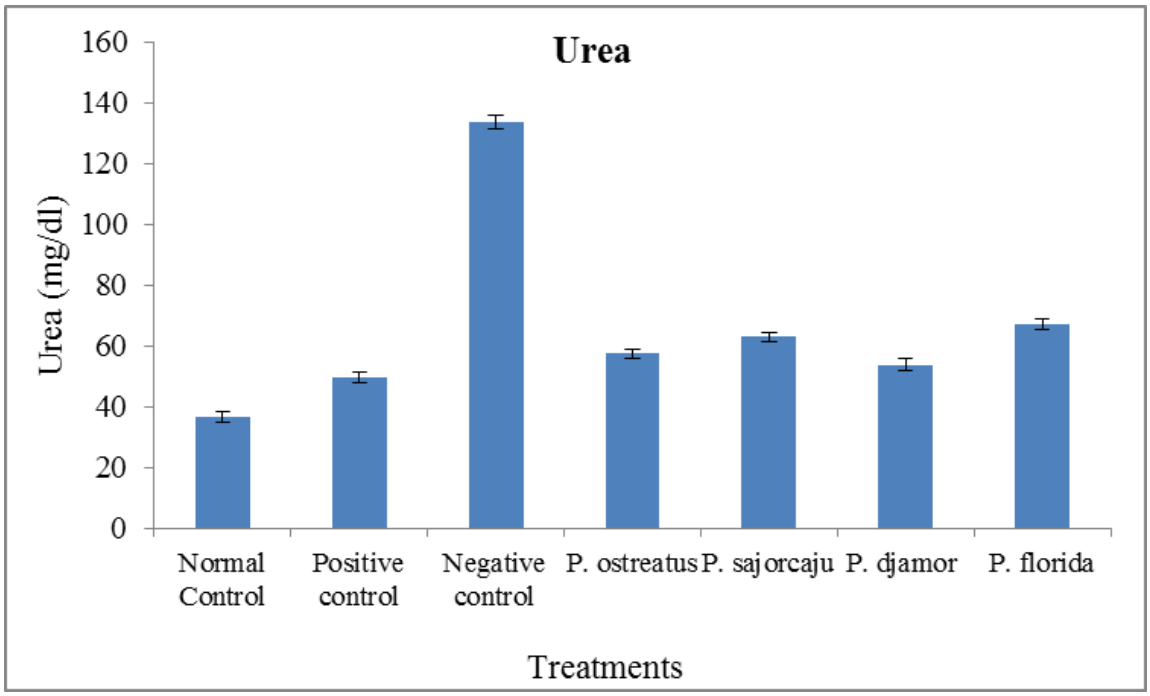


Figure 61: Changes in blood urea level following oral treatment of *Pleurotus* species in hyperglycaemic rats

4.16.2. Effect of oyster mushroom on liver enzymes of STZ induced diabetic rats

Diabetes is one of the major diseases spread throughout the world. Different age groups are now suffering from diabetes. Diabetes also effects on the liver function and thus liver induced to secrete some liver enzymes. Serum Glutamic Pyruvate Transaminase (SGPT) is one of the important enzymes secreted by the liver. The results revealed that SGPT enzyme was controlled by the normal control about 52 IU/L. similar results was found in case standard drug treated rats but in case of negative control, it increased (Figure 62). Significant result found in case of sample treated rats where it reduced to normal. Among the sample treated rats, it was clearly observed that *Pleurotus djamor* showed highest activity in lowering the SGPT level followed by *P. florida, P. ostreatus* and *P. sajor-caju*.

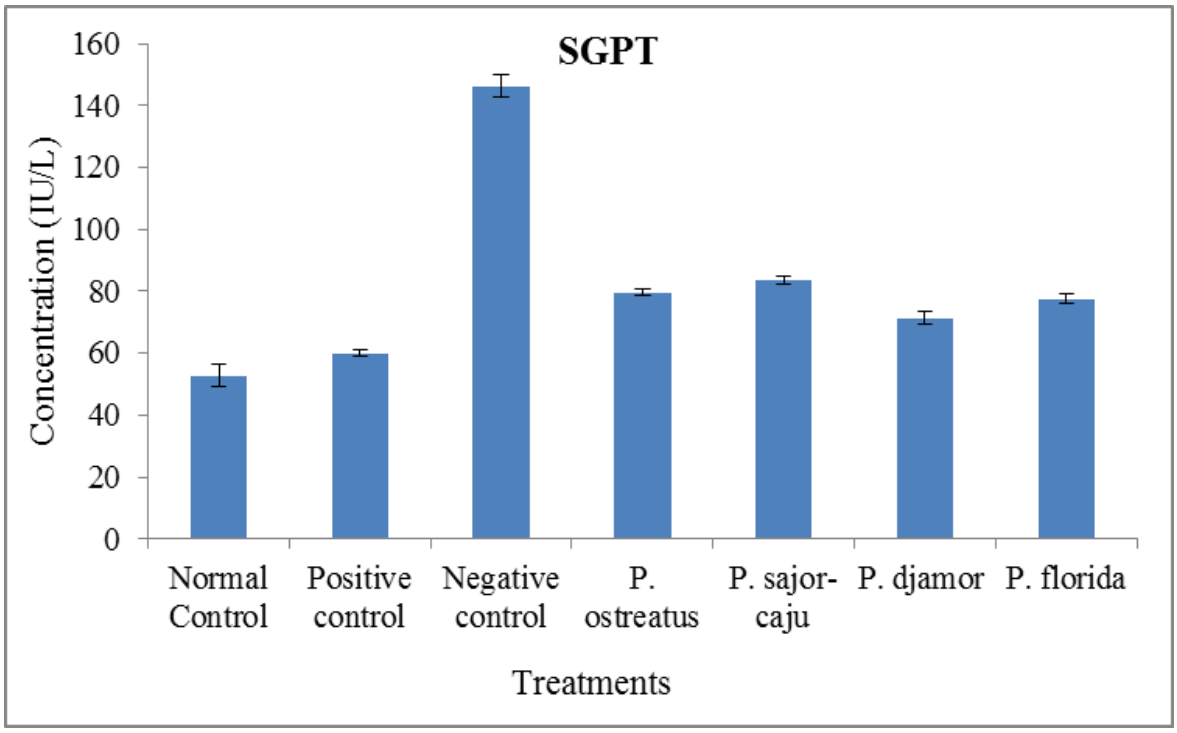


Figure 62: Liver enzyme serum glutamic pyruvate transaminase (SGPT) activity of STZ treated diabetic rats following oral treatment of *Pleurotus* species in relation to control

Serum glutamic oxaloacetic transaminase is also another liver enzyme secreted by the liver due to high diabetic level. Results revealed that SGOT level increase in case of negative control but in case of standard drug treated rats, the SGOT level decreases to normal level. It was also observed that the sample treatment significantly reduced the SGOT activity. *Pleurotus ostreatus* and *P. djamor* decreases more than the *P. sajor-caju* and *P. florida* (Figure 63). Results revealed that the samples were capable of lowering the SGOT secretion significantly in compare to the control sets.

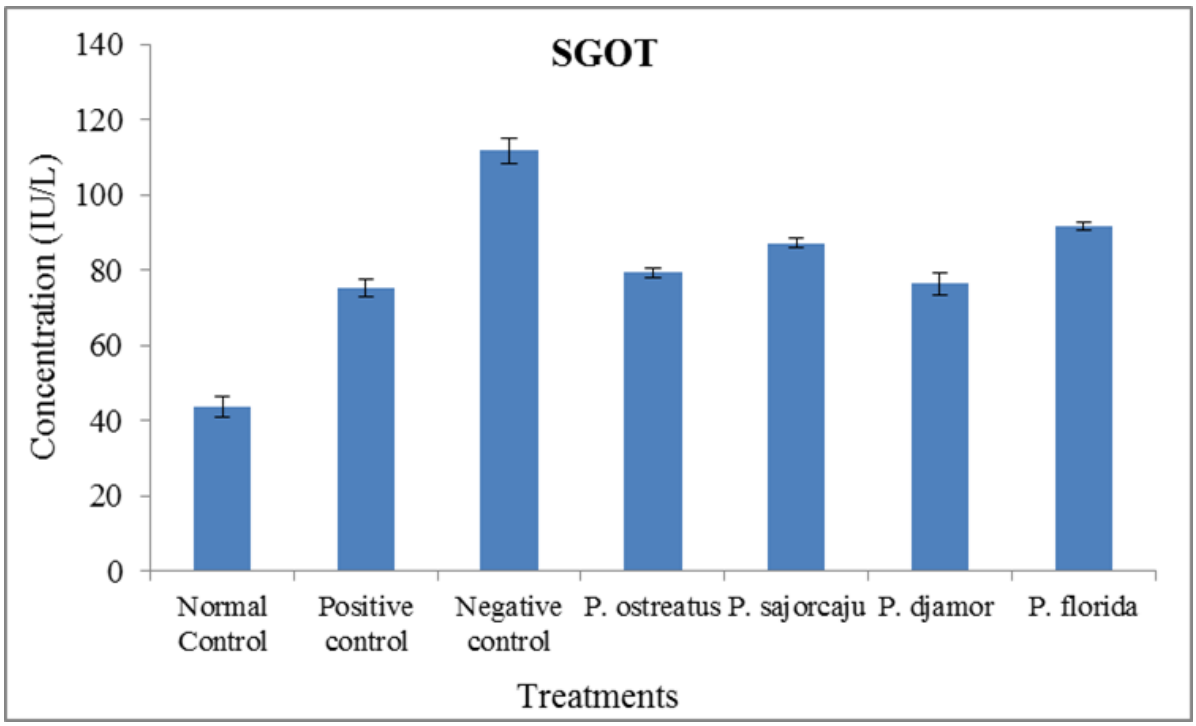


Figure 63: Liver enzyme serum glutamic oxaloacetic transaminase (SGOT) activity of STZ treated diabetic rats following oral treatment of *Pleurotus* species in relation to control

4.16.3. Effect of oyster mushroom on serum cholesterol level of STZ induced diabetic rats

Cholesterol is one of the major biochemical constituents which regulate the coronary arterial occlusion, myocardial infraction, liver function, thyroid function and adrenal disease. Diabetes severely affects the cholesterol level of serum. Results revealed that the cholesterol level higher in case of negative control but in case of positive control rats, lower cholesterol activity indicates that the drug able to control the cholesterol and triglyceride level. Treatment of mushroom samples also showed significant result in

lowering the blood cholesterol and triglyceride level (Table 23). All the four mushroom species showed lowering activity but *P. djamor* and *P. ostreatus* showed more activity than the *P. florida* and *P. sajor-caju*.

Table 23: Effect of oral treatment of *Pleurotus* species on cholesterol and triglyceride levels of diabetic rats

Groups	Triglycerides	Cholesterol
Normal Control	80.89±2.69	122.09±1.74
Positive control	170.40±1.37	146.30±1.00
Negative control	226.83±3.23	206.13±1.15
P. ostreatus	193.64±1.83	168.13±0.82
P. sajor-caju	201.96±1.21	183.26±1.14
P. djamor	184.9±1.31	156.63±1.38
P. florida	200.95±1.34	190.98±0.98

'±' standard error of three replicates of each group

4.17. Effect of spent mushroom substrate on plant growth and yield

4.17.1. *Capsicum chinense*

Spent mushroom substrate of oyster mushroom was tested of their effect on growth promotion of *C. chinense* in potted condition. Spent mushroom were used directly in the soil at 250gm/kg soil ratio and the growth promotion in terms of height, number of leaves, leaf size and yield were evaluated. The results revealed that all the treated plants showed significant increase in height in comparison to control after 7 days of planting (Figure 64). After 49 days of interval, the height, number of leaves was showed significant result. It was observed that flowering started after 25 days in case of treated plants while it started 35 days in case of control plants. Size of the leaves also showed very wide variation in comparison to the control plants. In case of treated plants, leaf length ranges from 17 - 20cm while it is ranges from 7-12.5cm in case of control plants. Leaf diameter also increased in case of treated plants (7.5-9.5cm) while it is lower in control plants (2.5-4.5cm). Final yield also was determined and it was found that both the size and number of fruits was higher in the treated plants (Table 24).

Table 24: Comparison of growth of *C. chinense* grown in spent substrate of *P. ostreatus* and in untreated soil.

Treatment	Height (cm)	No of leaf	Average Leaf size (cm)		Flowering (days)	Yield/plant (gm)
			Length	Diameter		
Paddy straw	64.0	35	18.0	8.8	48	30
Paddy straw + Saw dust	64.5	37	20.0	9.0	45	37
Wheat straw	56.0	30	17.5	9.0	54	45
Paddy straw + wheat straw	60.0	35	17.0	7.5	51	42
Control (soil)	21.0	12	7.0	4.5	57	20

Average of 5 replicate plants of each treatment

Figure 64: Effect of spent substrate of oyster mushroom on growth of *Capsicum chinense*;(A) control plants and (B) plants grown in spent substrate amended soil [after 15 days]; (C) untreated control and (D) plants grown spent substrates amended soil [after 45 days]; (E) flower, (F) developmental stages of fruit.

4.17.2. *Capsicum annuum*

Spent mushroom substrate of oyster mushroom and compost of button mushroom were tested for their effect on growth promotion of *C. annuum* L.in potted conditions. Spent mushroom substrates were used directly as well as in leached form and weathered compost was also applied either singly or in combination. After this growth promotion in terms of height, number of branches and root-shoot biomass were evaluated at several intervals. Final yield was also estimated by harvesting the capsicum according to their treatment. The results revealed that all the treated plants showed significant increase of height after 35days out of which, those treated with spent substrate of fresh oyster mushroom, button mushroom leachate and weathered compost of button mushroom showed highest increment in growth (Figure 65). On the other hand, it was observed that the number of branches significantly increased in oyster mushroom leachate, button mushroom weathered and button mushroom fresh compost (Table 26). In case of yield highest yield was obtained by treatment with SMS of oyster mushroom leachate followed by oyster mushroom weathered SMS. It has been reported that the PGPR also stimulate the beneficial plant fungal symbiosis involving both AM fungi and ectomycorrhizae. Results revealed that the spent oyster mushroom leachate, fresh oyster mushroom substrate and button mushroom leachate showed better yield. It was also reported that the ectomycorrhizal treatment influences the growth of plants.

Table 25: Effect of spent mushroom substrate on growth of *C. annum*

Treatment	Height of Plants (cm)				
	7days	14days	21days	28days	35days
Control	18.7±0.3	19.3±0.3	28.33±0.2	37.67±0.3	41.17±0.4
Fresh oyster SMS	21.9±0.4	24.33±0.4	30.66±0.2	49.3±0.6	64.3±0.3
Oyster SMS leachate	20.5±0.4	25.1±0.6	31.0±0.1	45.7±0.3	60.3±0.7
Oyster Weathered SMS	19.5±0.1	22.3±0.2	27.75±0.1	39.4±0.1	53.5±0.6
Button SMC leachate	22.3±0.5	25.3±0.2	33.67±0.6	55.8±1.1	60.9±0.6
Button weathered Compost	22.57±0.4	27.7±0.4	39.7±0.4	63.3±0.6	72.1±0.5
Fresh SMC of Button	19.7±0.3	23.7±0.4	30.3±0.5	64.03±0.6	71.9±0.3
Oyster SMS + Button SMC	19.3±0.4	21.0±0.2	32.0±0.6	56.73±0.2	69.16±0.3

'±' standard error of 5 replicate plants of each treatments;

Table 26: Effect of spent mushroom substrate on number of branches

Treatment	21 days	28 days	35 days
Control	4.28±0.1	6.27±0.1	7.55±0.1
Fresh oyster SMS	6.39±0.2	8.39±0.2	11.39±0.5
Oyster SMS leachate	7.39±0.2	11.16±0.6	12.33±0.6
Oyster Weathered SMS	8.68±0.4	9.39±0.2	10.72±0.1
Button SMC leachate	4.68±0.0	7.61±0.2	11.22 ±0.3
Button weathered Compost	14.04±0.1	7.52±0.0	12.61±0.4
Fresh SMC of Button	4.63±0.1	7.5±0.28	11.53±0.5
Oyster SMS + Button SMC	4.53±0.3	7.3±0.1	9.56±0.1

'±' standard error of 5 replicate plants of each treatments;

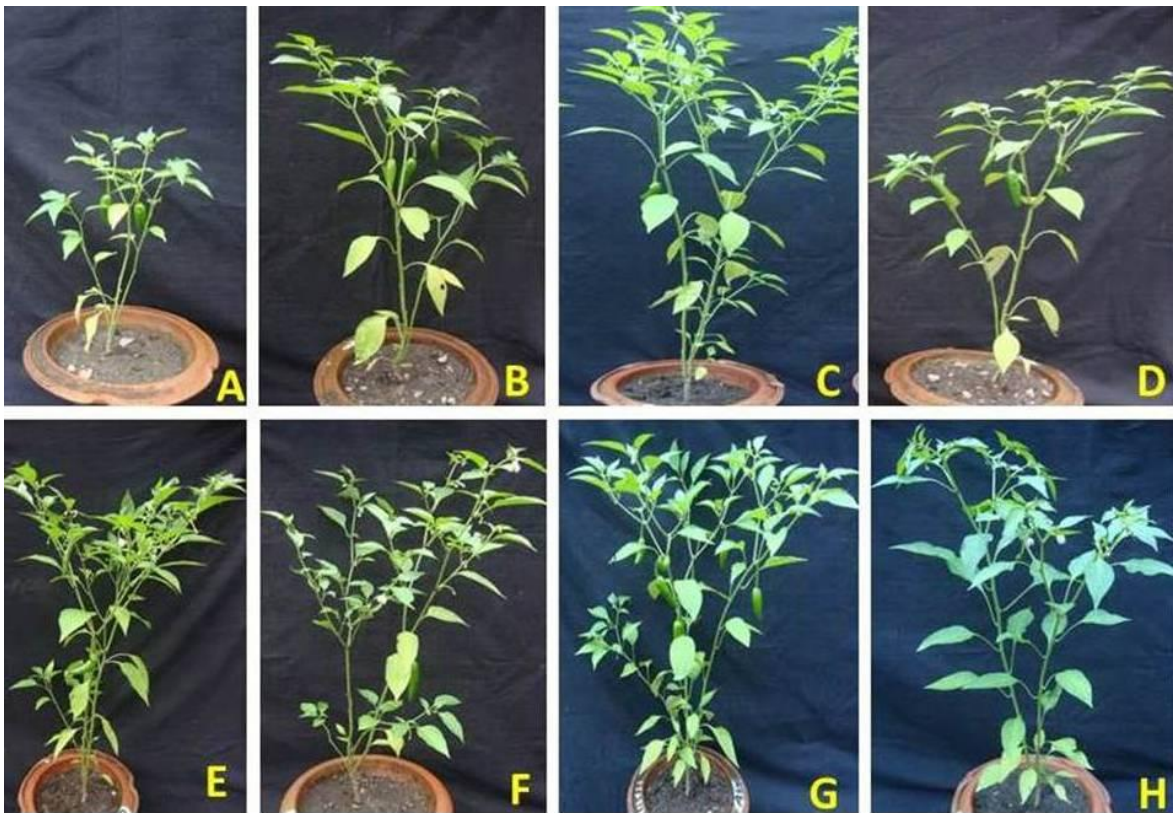


Figure 65: Effect of spent mushroom substrates on the growth of *Capsicum annuum* L. (A) control; (B) fresh spent oyster mushroom substrate; (C) spent oyster mushroom substrate leachate; (D) spent weathered oyster mushroom substrate; (E) fresh button mushroom compost; (F) spent button mushroom compost leachate; (G) spent weathered button mushroom compost; (H) combined treatment of spent oyster mushroom and button mushroom substrate

4.17.3. *Solanum lycopersicum* and *Amaranthus* sp

Tomato and *Amaranthus* are very important vegetable widely cultivated and consumed by the world. Spent mushroom substrate of Oyster mushroom was tested for their effect on growth promotion of Tomato and *Amaranthus* in field condition (Figure 66). 10 days old seedlings of tomato were sowed in pre-treated plots and seed of *Amaranthus* was also sowed. After this growth promotion in terms of height, number of branches was evaluated. The results revealed that all the treated plants showed significant increase of height after 35 days out of in treated plants in compare to untreated control plants (Table 30). Germination period was also lesser in case of *Amaranthus* plant SMS treated in compare to control plots. On the other hand, it was observed that the number of branches significantly increased in SMS treated plants than the untreated plots. In case of yield highest yield was obtained by treatment with SMS of oyster mushroom followed by the control plants (Table 31).

Table 27: Effect of spent mushroom substrate on height of tomato *and Amaranthus* in different intervals

Plant	Treatment	Height of plants (cm)			
		7days	14days	21days	28days
Solanum lycopersicum	Control	12.7±0.3	15.3±0.3	23.33±0.2	27.67±0.3
	SMS	17.9±0.4	21.33±0.4	29.66±0.2	39.3±0.6
Amaranthus sp.	Control	2.5±0.4	5.1±0.6	12.0±0.1	19.7±0.3
	SMS	9.5±0.1	16.3±0.2	21.75±0.1	33.4±0.1

'±' standard error;

Table 28: Effect of spent mushroom substrate on number of branches of tomato *and Amaranthus* in different intervals

Plant	Treatment	Branches (cm)		
		21 days	28 days	35 days
Solanum lycopersicum	Control	7.28±0.1	9.53±0.1	13.55±0.1
	SMS	12.39±0.2	18.39±0.2	21.39±0.5
Amaranthus sp.	Control	3.43±0.2	7.16±0.6	15.33±0.6
	SMS	8.68±0.4	17.32±0.2	19.72±0.1

'±' standard error;

Figure 66: Effect of spent mushroom substrates of oyster mushroom on *Solanum lycopersicum* (A& D) untreated control and (B &C) SMS treated and *Amaranthus* (C) Untreated control and (D) SMS treated

4.18.1. Effect of spent mushroom substrates on biochemical changes of crop plants

4.18.1. *Capsicum chinense*

Mobilization of soil phosphate after treatment were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the control sets. The uptake of phosphate content was significantly increased in spent mushroom substrate treated plants while it shows lower uptake of soil phosphate to the root and leaf in case of control plants (Table 25). Spent mushroom substrate improves the soil quality by having a direct influence on the uptake of phosphate content and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus. Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates affects in the total chlorophyll content of the plant leaves. Chlorophyll content (mg/g tissue) includes the chlorophyll a and chlorophyll b which significantly increased in the plants treated with spent mushroom substrate of oyster mushroom (Figure 67A).

Table 29: Effect of spent mushroom substrate on total phosphate content of soil, root and leaf of *C. chinense* after 15 days of seedling transfer.

Treatment	Total Phosphate Content (µg/gm)		
	Soil	Root	Leaf
Control	51.30±2.3	9.12±1.3	7.67±0.8
Paddy straw	44.51±1.3	11.53± 0.7	8.47± 1.2
Paddy straw + Saw dust	42.50±3.4	15.45±1.3	13.35±1.4
Wheat straw	45.65 ± 1.1	14.25± 1.4	10.44± 1.2
Paddy straw + wheat straw	40.35± 0.8	11.75± 1.2	9.33± 1.1

± Standard error,

Carotenoid is one of the most important compounds which show antioxidant activity and this was also estimated to evaluate the effect of spent oyster mushroom substrate on the enhancement of antioxidant compound. The results revealed that the application of spent substrate enhances the carotenoid as the leaf and fruit of the treated plants showed significant higher amount of carotenoid in comparison to the control plants. The results also shows that the effect different substrates influence the level of carotenoid compound and it was observed that the combined treatment of spent mushroom substrate of saw dust and paddy straw showed maximum enhancement of carotenoid compound followed by the spent mushroom substrate of paddy straw (Figure 67B).

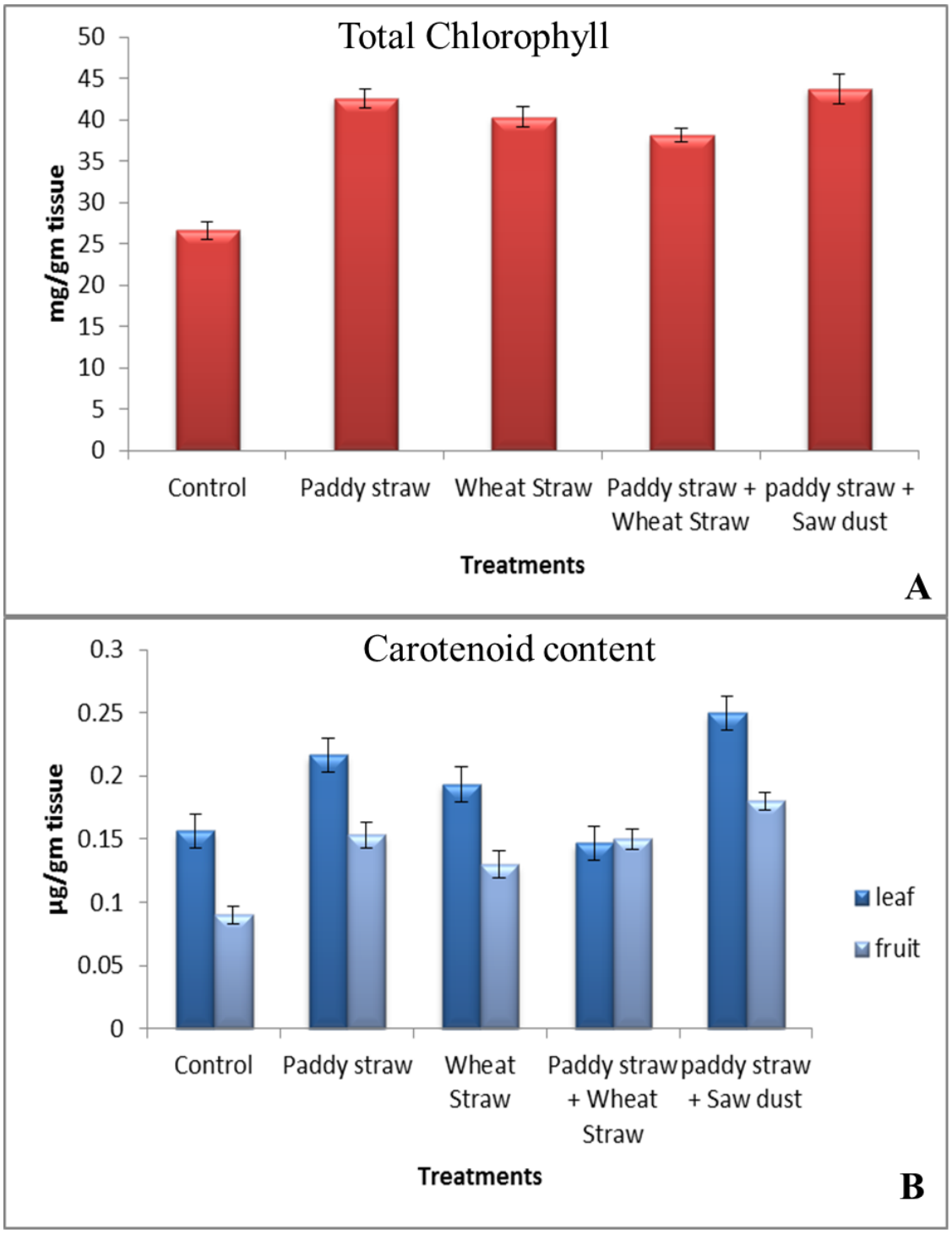


Figure 67: Effect of spent mushroom substrate on total chlorophyll (A) and carotenoid content (B) of *C. chinense*.

4.18.2. *Capsicum annuum*

Apart from this, mobilization of soil phosphate by these treatments were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the untreated control sets. The uptake of phosphate content was

significantly increased in fresh oyster mushroom substrate and in button mushroom weathered compost (Table 28). SMS improved soil quality by having a direct influence on soil aggregation and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus.

Table 30: Effect on phosphate content of soil, root and leaf after 15days of seedling transfer

Treatment	Total Phosphate Content (µg/gm)		
	Soil	Root	Leaf
Control	53.47±4.73	11.42±1.73	7.67±0.83
Fresh SMS of oyster	43.50±3.45	17.45±1.44	10.33±1.76
Oyster mushroom SMS leachate	47.51±3.33	13.3±1.12	10.65±1.32
Weathered SMS of oyster	39.50±3.21	11.42±1.39	9.05±1.11
SMC leachate of Button	41.1±3.02	12.51±1.12	9.65±0.91
SMC weathered of Button	48.39±4.12	13.70±1.83	10.25±0.93
Fresh SMC of Button	44.56±3.31	12.15±1.19	10.70±1.12
SMS + SMC	41.35±4.17	11.95±1.90	10.25±0.93

'±' standard error; ($P<0.5$);

Effect of spent substrates on chlorophyll content of *C. annuum*

Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates enhanced the total chlorophyll content of the leaves of treated plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both oyster mushroom and button mushroom leachate, and in fresh oyster mushroom substrate. The dual treatment of both the substrate also showed significant amount of chlorophyll content (Table 29).

Table 31: Effect of Spent mushroom substrate on chlorophyll content of *C. annuum*

Treatments	Chlotophyll a (µg/gm tissue)	Chlorophyll b (µg/gm tissue)	Total Chlorophyll (µg/gm tissue)
Control	8.38	6.38	14.76
Fresh SMS of oyster	12.92	4.72	17.64
Oyster mushroom SMS leachate	13.51	5.30	18.81
Weathered SMS of oyster	11.40	3.5	14.90
SMC leachate of Button	12.76	4.81	17.57
SMC weathered of Button	11.24	4.35	15.59
Fresh SMC of Button	10.32	3.37	13.69
SMS + SMC	12.40	3.41	15.81

Effect of spent mushroom substrate on total protein and carotenoid activity of *C. annuum*

Total protein content was also evaluated in leaves and fruits. The results revealed that the total protein content was maximum in the fruits of fresh oyster mushroom substrate and fresh button mushroom compost treated plants ranging between 200-250 µg/gm tissue while it was lower in case of treatment with oyster mushroom weathered substrate and the dual treatment of button mushroom and oyster mushroom substrate. Higher leaf protein was also observed in oyster mushroom leachate, button mushroom weathered compost treatment as well as dual application of both the substrates (Figure 68A). Among the pigments, carotenoid, being an antioxidant compound is also important and hence carotenoid content was estimated in the study. Carotenoid was estimated in the leaf as well as in fruits of *Capsicum annuum* L. and button mushroom leachate treated plants showed a high range of carotenoid compound (0.25-0.30 µg/gm tissue) followed by the oyster mushroom fresh substrate and button mushroom weathered compost treatment (0.15-0.20 µg/gm tissue). Mature pepper fruits are also rich in carotenoids, compounds with antioxidant and anti-carcinogenic capacity; furthermore, either immature or mature fruits contain a high concentration of antioxidant phenolic compounds (Figure 68B).

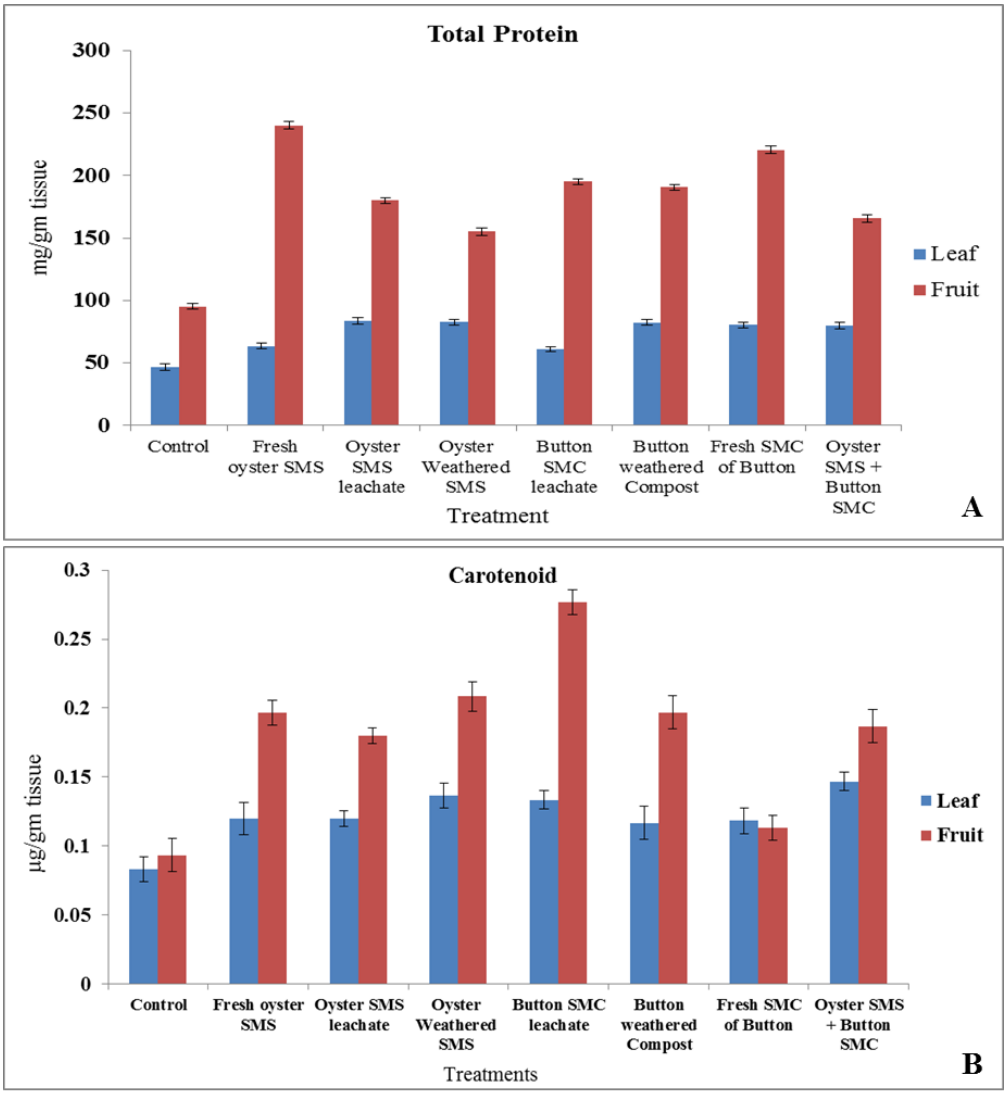


Figure 68: Effect of spent mushroom substrate on soluble protein (A) and carotenoid contents (B) of *Capsicum annuum*

4.18.3. Effect of spent substrates on chlorophyll content of *Solanum lycopersicum* and *Amaranthus* sp.

Photosynthesis is the most important physiological phenomenon then required for all plant kingdom and chlorophyll is the most important photosynthetic pigment responsible for photosynthesis. Results revealed that the use of spent mushroom substrates as potential biofertilizer helps in increasing the photosynthetic pigment in leaf in compare to the control plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both plant ie tomato and *Amaranthus* when treated with the spent mushroom substrates (Table 32).

Table 32: Effect of Spent mushroom substrate on chlorophyll content of tomato and *Amaranthus* sp.

Treatments	Chlorophyll a (µg/gm tissue)	Chlorophyll b (µg/gm tissue)	Total Chlorophyll (µg/gm tissue)
Untreated control	5.3067	2.188	7.4947
Treated *S. lycopersicum*	9.1791	6.5455	15.710
Untreated *Amaranthus*	4.329	1.874	6.203
Treated *Amaranthus*	6.743	2.397	9.14

4.19. Post-harvest processing of oyster mushroom

Processing of oyster mushroom is one of the major steps to utilize the cultivated mushroom. Shelf-life of oyster mushroom is very less in compare to milky mushroom and button mushroom. But it was very difficult to keep the cultivated mushroom fresh for 3-4 days. Thus processing is the most important step for the management of oyster mushroom. Processing technology of oyster mushroom was practiced to avoid the loss of cultivated mushroom (Figure 69). Fresh mushroom was harvested and was processed using different techniques. Following are the some of the processing techniques.

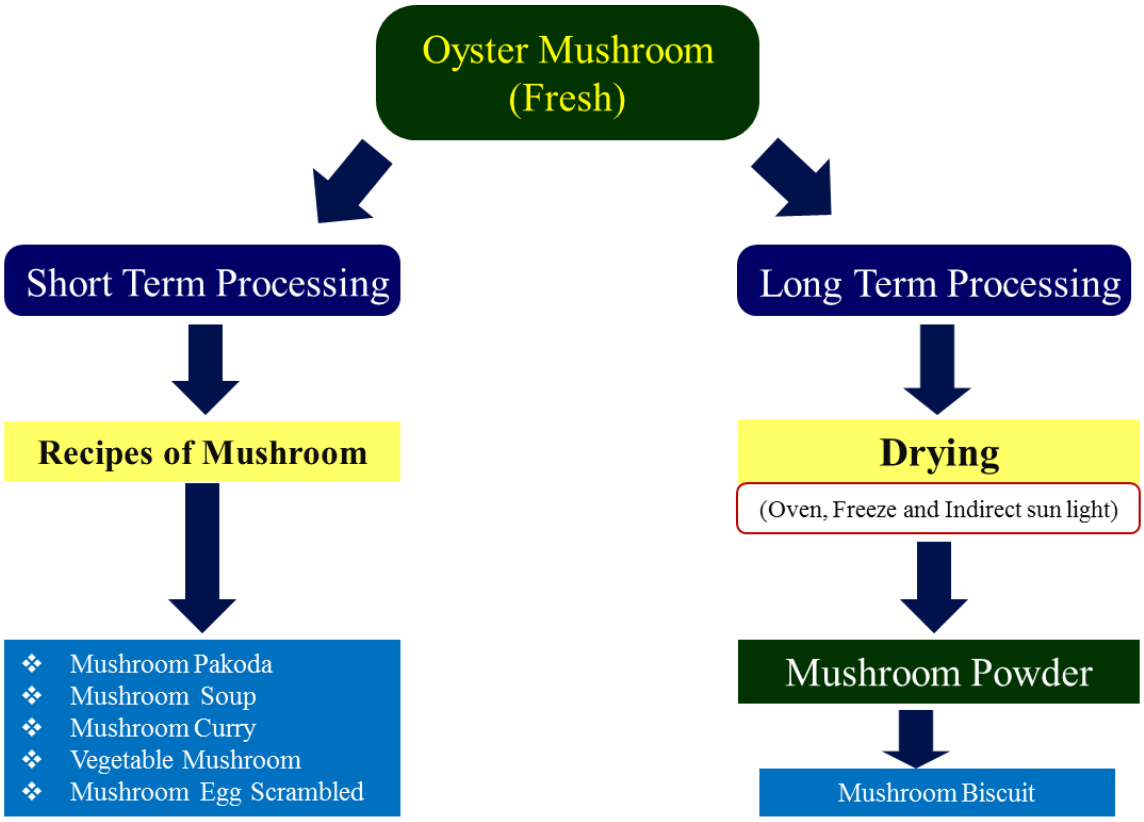


Figure 69: Schematic diagram of post-harvest processing of *Pleurotus* species

4.19.1. Short term processing

Shelf life is one of the major factors which affected the processing of oyster mushroom. Short term processing leads to packaging of mushroom for better preservation followed by making different recipes. Selection of fruiting body is one of the important steps for good quality packaging. Freshly harvested fruiting body was selected for packaging and sampling was done according to their size (Figure 70). The selected fruiting body was then cleaned and the basal part of the stipe was removed. Then the mushroom fruiting body was packaged in polypropylene bags according to their size, colour and species. Then the bags were sealed and weighted. The packaged mushrooms were then kept at 4^0 C for 3-4 days preservation.

Figure 70: Harvesting and packaging of oyster mushroom

Short term processing of oyster mushroom also includes the preparation of different recipes. A variety of delicious food items were prepared with oyster mushroom depending on personal choice and tested in the laboratory among the lab members for the evaluation of their taste and flavour. Mushroom curry, Vegetable mushroom with tomato, mushroom Pakoda and mushroom soup was prepared (Figure 71). These recipes were prepared using the cultivated different species of oyster mushroom. The mushroom soup was prepared following two different procedures like crushed mushroom soup and chopped mushroom soup and both the soups were delicious and tastes were completely different.

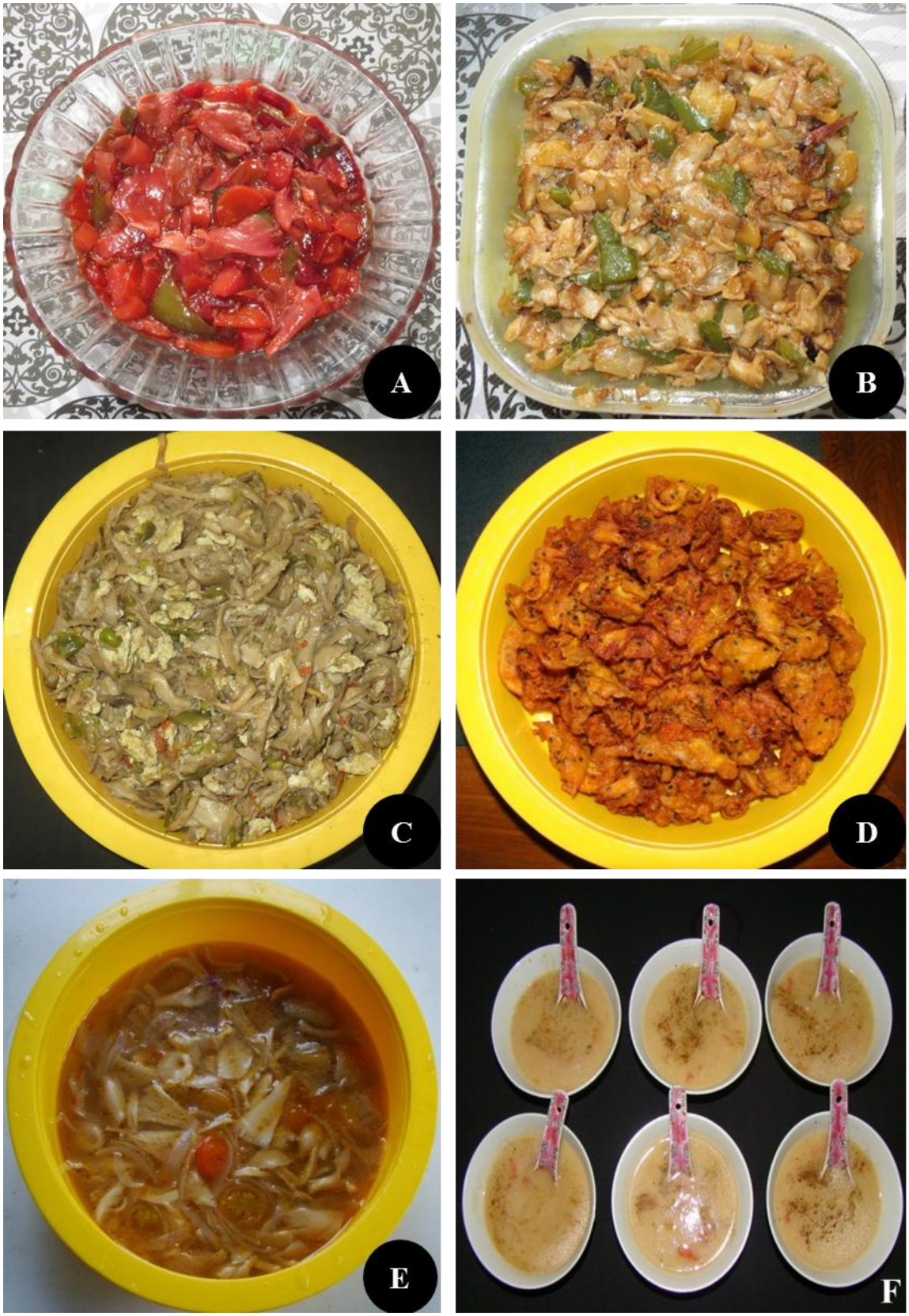


Figure 71: Different recipes of oyster mushroom (A) mushroom tomato curry, (B) vegetable mushroom, (C) egg mushroom scramble (D) onion mushroom pakoda, and (E-F) mushroom soup.

4.19.2. Long term Processing

Most of the mushrooms, being high in moisture and delicate in texture, it cannot be stored for more than 48 hours at the ambient conditions prevailing in the tropics. The spoilage of mushroom might be caused by the action of bacteria on the mushroom tissue and browning of mushrooms was due to a combination of auto-enzymatic and microbial action on the tissue. Postharvest practices have since been developed to extend the shelf life of fresh mushrooms. As far as processing technologies are concerned, sun drying of mushrooms is one of the simplest and oldest methods followed by the growers from the time immemorial. During the recent years, there has been an increased emphasis on the quality of fresh vegetables including mushrooms, which is reflected in the price of the produce. Effective processing techniques will not only prevent the post-harvest losses but also result in greater remuneration to the growers as well as to the processors. Value can be added to the mushrooms at various levels, right from grading to the readymade snacks. Technologies for production of some other products like mushroom based biscuits have been developed but are yet to be popularized. Attractive packaging of the value-added products is yet another area, which may be called the secondary value-addition.

Drying is perhaps the oldest technique known to the mankind for preservation of food commodities for long duration. It is the process of removal of moisture from the product to such a low level that microbial and biochemical activities are checked due to reduced water activity, which makes the products suitable for safe storage and protection against the attack by microorganisms during the storage. Mushroom contains about 90% moisture at the time of harvesting dried to a moisture level down below 10-12%. At a drying temperature of 55-60^{0}C, the insects and microbes on the mushrooms will be killed in few hours, which give us the dehydrated final product of lower moisture content with longer shelf-life (Figure 72 and 73). The temperature, moisture of the mushroom and humidity of the air affect the colour of the dried product. Dried mushrooms can be easily powdered and used in soups, bakery products, etc. Mushroom dried at higher temperature loose texture, flavour, and colour.

Figure 72: Dry mushroom of different *Pleurotus* species

Figure 73: Mushroom powder prepared from dried *Pleurotus* species

Figure 74: Mushroom biscuit prepared using dried oyster mushroom powder

Delicious and crunchy mushroom biscuits (Figure 74) were prepared by using the button/ oyster mushroom powder and various ingredients viz., maida, sugar, ghee (bakery fats), mushroom powder, backing soda, cashew nut crushed and milk powder. Biscuits were prepared to evaluate the quality of mushroom powder for long term processing in bakery industry. The biscuits were tasted among the several people and it was observed that the taste was very good along with the flavour of mushroom powder. Preparation of mushroom biscuit was successfully done and it was proven that the preparation was a good was to management of mushroom powder into a delicious health food material.

4.19.3. Biochemical characterization of powdered fruiting body of *Pleurotus* species

Processing practice is one of the major steps for the preservation of mushroom fruiting body. Various methods were adopted for preservation of the mushroom fruiting body. Dehydration of the fruiting body helps in long term preservation up to 6 months for *Pleurotus* species. Mushroom fruit body were harvested and dried under indirect sunlight and then it was powdered. Mushroom powder is also very nutritious for human health. Various biochemical constituents were analysed of the four cultivated species of *Pleurotus*. In case of total sugar content, it was found that the amount was higher in case of *P. djamor* followed by *P sajor-caju*, *P ostreatus* and *P. florida*. While in case of reducing sugar, *P ostreatus* and *P djamor* showed higher activity than *P. sajor-caju* and *P. florida* (Figure 75). Total protein content of the *Pleurotus* species was also evaluated

and it was found that *P djamor* and *P ostreatus* powder contains high amount of protein in compare to *P sajor-caju* and *P florida*. Evaluation of total lipid contend was also done and the results suggested that all the four species of *Pleurotus* consists very low amount of lipid content and among them *P florida* showed highest lipid content than that of the other species. Dietary fibre of mushroom is also an important biochemical constituent good for human health (Figure 76). The results revealed that *P. djamor* showed highest amount of dietary fibre followed by *P ostreatus, P florida* and *P. sajor-caju*.

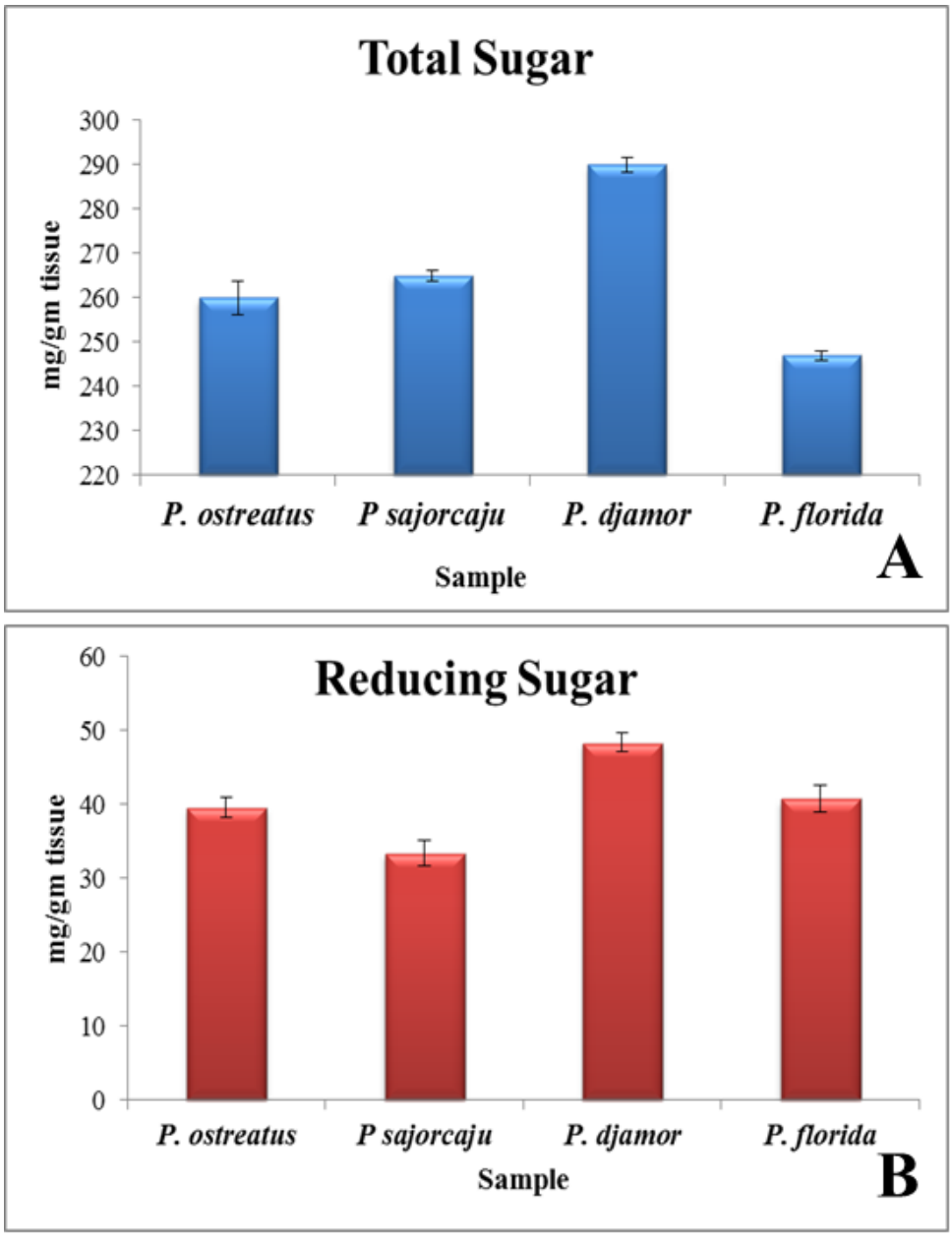


Figure 75: Evaluation of biochemical constituents of oyster mushroom powder (A) total sugar, (B) reducing sugar

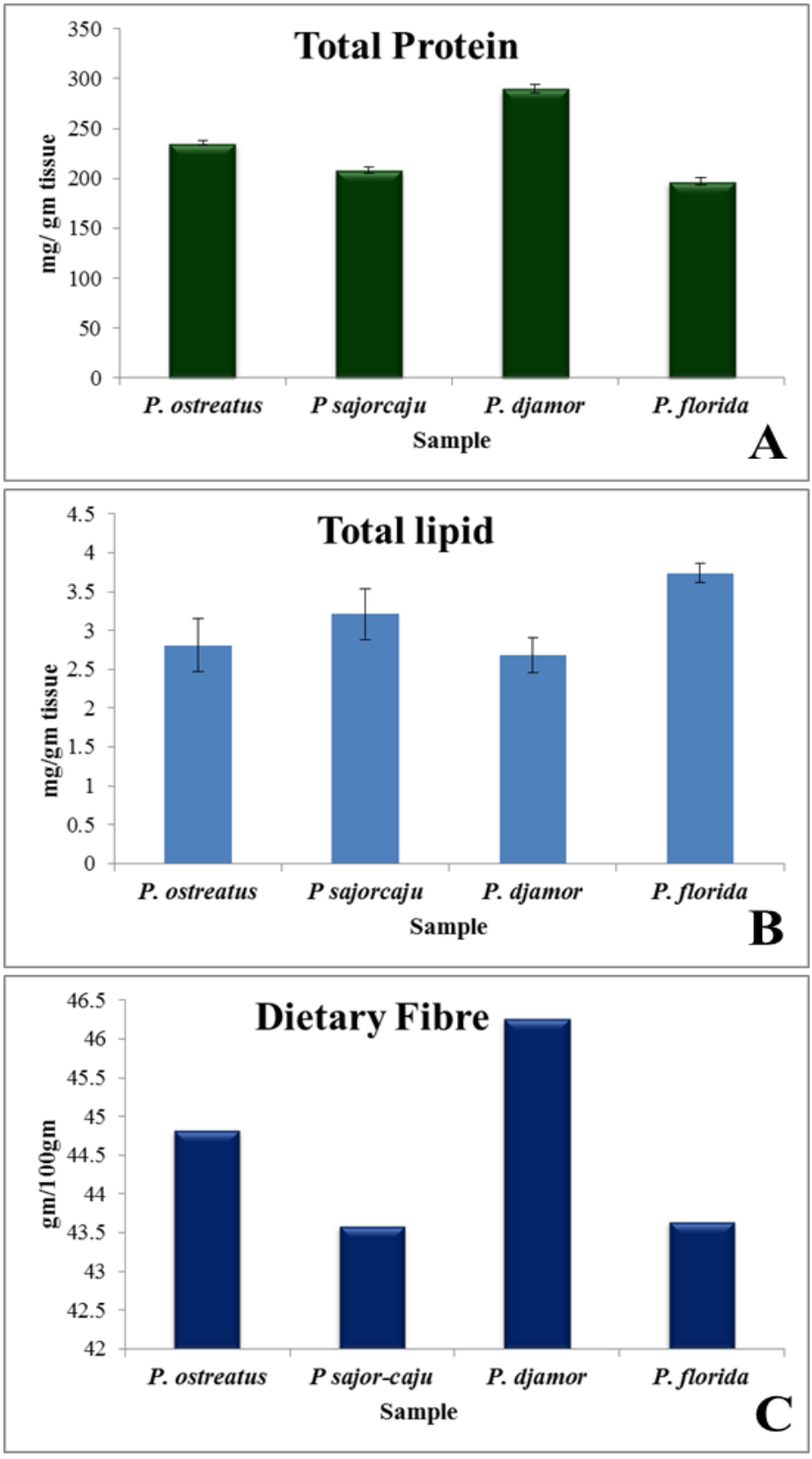


Figure 76: Evaluation of biochemical constituents of oyster mushroom powder (A) total protein, (B) total lipid and (C) dietary fibre

4.20. Management of Contaminants of oyster mushroom

Like other crops, mushrooms are also very much affected by different types of contaminants. Both biotic and abiotic agents are involved directly or indirectly which damages the mushroom results in reduction of production. Among biotic agents, it includes bacteria, fungi, viruses and even pests. Many of these organisms, acts as a competitor moulds which affects in the spawn run where as other attacks the fruiting body in different stages of development. The different types of mushroom contaminants found during the cultivation and their management are discussed as follows.

4.20.1. Ink Cap Disease

Ink cap disease is one of the major disease severely affected the cultivation of oyster mushroom caused by the attack of *Coprinus cometus, C. logopus.* Ink caps were appeared during the spawn run. They were slender, bell-shaped mushrooms. Initially they were cream coloured but later it turns into bluish black usually covered with scales (Figure 77). It was observed that the fungus grows in cluster on beds having a long stipe immerged deeply into the substrate.

Figure 77: Ink Cap disease caused by *Coprinus* sp.

Disease Management

Ink cap disease was one of the most severe diseases causing great loss in the production. Pretreatment of substrate was done by proper pasteurization; fresh substrate was used during cultivation; watering was limited during the colonization of the mycelia over the substrates; young pileus of ink caps were removed immediately; the contaminated bags were removed immediately from the room and was completely burnt to avoid further spread to another bags.

4.20.2. Cob Web Disease

Cob web is also commonly known as mildew or soft decay disease often caused by the fungus *Cladobotryum dendroides*. It was observed that the fungus extensively damages the substrate colonization as well as the fruiting body. A floccose white mycelium covers the substrate and gradually it affects the pileus and stipe and eventually results in decomposing the fruiting body (Figure 78). It was severely attacks with a white dense mould developed over the substrate first and finally results in degradation of the fruiting body. The white mycelia became turns into pinkish red. It was also observed that the higher humidity and temperature increase the chances of the disease.

Figure 78: Cobweb disease during cultivation of oyster mushroom

Disease Management

Cobweb disease was severely affects the production of oyster mushroom and it was observed that the disease caused by using the old substrates. Pasteurization was done properly so that the chance of contamination was reduced. Sanitation of the cultivation unit was done regularly using citronella oil and neem oil and other commercially available disinfectants to avoid the contaminants. Water spray to the mushroom bags was reduced at the time of summer and room temperature maintained by spraying water on the floor. The infected bag was removed immediately and burnt to avoid further contamination.

4.20.3. Green Mould Disease

Green mould disease is also a very common fungal disease caused by different species *Trichoderma*, *Penicillium* and *Aspergillus* and this disease is very well known Trichoderma blotch or green mould. Among the moulds, *Trichoderma* causes a great loss in the quality and quantity of mushroom production. Different species of *Trichoderma* affected the growth of mycelia of oyster mushroom and it was observed that the green mould grows faster than the mushroom mycelia and gradually it covers the whole substrate rapidly (Figure 79).

Figure 79: Green Mould disease caused by *Trichoderma* sp.

Disease Management

Green mould is a major competitor mould that grows very rapidly from one bag to another as the fungal spore spread through air. Very good hygiene was maintained in the production unit and proper pasteurization was done before spawning. Substrates were collected fresh and they were treated properly before spawning. 2% Formaldehyde was used to completely avoid the contamination. Moreover it was observed that sometimes the bag was contaminated by the green mould; so the contaminated bags were removed from the production unit to avoid further spread of the organisms.

4.20.4. Yellow Mould Disease

Yellow mould is also an important fungal disease commonly affects the growth of oyster mushroom often caused by the fungi *Myceliophthora lutea* and *Chrysosporium luteum*. It was observed that yellow mould developed on the substrate with dark yellow circular colonies and gradually it distributed throughout the substrate. Rapidly the substrate becomes dark brown or turns into black (Figure 80). Like the green mould, this organism is a competitor mould that grows before the mushroom mycelia covers the substrates and thus mushroom production was severely inhibited.

Figure 80: Yellow mould disease during cultivation of *Pleurotus* species

Disease Management

Unlike green mould disease, yellow mould is one common disease that affected rapidly over the substrate. Proper pasteurization was very much effective in controlling the disease. It was observed that the increased pasteurization time helps in reducing the disease severity of yellow moulds. Application of formalin (2%) was also found to be very effective in reducing the chances of yellow mould. Moreover yellow mould contaminated bags were discarded from the production unit and it was then burnt to stop further spreading of the fungal spores.

4.20.5. Bacterial Blotch of oyster mushroom

Oyster mushroom was cultivated throughout the year and during cultivation, several other organisms affected the production. Bacterial blotch was one of the major contaminants severely damages the mycelia and gradually reduces the production. *Pseudomonas* was one of the bacterial genus destructively affected the production. It was characterize by the brown spots or blotches over the substrates (Figure 81). Under favourable conditions, circular or irregular spots were observed on the surface of the substrate and the spot initially light and became dark within 2-3 days. The substrates become loose and very strong pungent smell was one major characteristic feature of the bacterial blotch. The enlargement of the spot was dependent on the environmental condition.

Figure 81: Bacterial blotch disease

Disease Management

It was observed that high relative humidity enhances the disease severity. Continuous persistence of water on the surface of the substrate and also under the mushroom bags helps in bacterial contamination and thus to avoid bacterial disease, proper ventilation along with the sanitation of the production unit was done regularly. Spraying of water was controlled during the summer and winter to avoid water logging on the bed.

4.20.6. Pest Disease of Oyster Mushroom

4.20.6.1. Sciarid fly

Sciarid fly, *Lycoriella auripila* causes severe damage to subtropical mushroom *Pleurotus* sp. tunnelling in the stalk and the cap by the maggot is the characteristic visible symptom. Growth may be often arrested when in the pin-head stage the maggot attack. Even when the infected pin-head develops into button, they become small brown

and leathery. Sciarids, the small fungal gnats, are mosquito type flies. Colour of flies varies from brown black to black. Body length varies from 1.5 to 3.5 mm depending upon the species. Antennae are long (14 annuli) which are held characteristically erect. Larvae feed on substrates, mycelium and mushrooms. Through on consumption of substrate and mycelia by the larvae, pH of the substrate changes and thus the growth of mushroom mycelium become slow down. As the infestation by the larvae is often in groups, bare patches without mushrooms can be seen on the beds (Figure 82A). When larval attack occurs at pin head stage, it was observed that further development of pinheads completely stops and pin heads eventually die. Adult Sciarids consume minute quantity of water and other liquids but do not feed on mushrooms. Flies also transport spores of the pathogenic fungi, virus infected fungi, nematodes and mites. Sciarid infestation can cause up to 50% reduction in crop. It was also observed that the larvae prefer to feed in moist areas and tend to move away from dry areas. Fully grown larvae are dirty white with visible longitudinal black streaks. Larvae are 5-8 mm in length. The larval period is of 16 days. They then go in to resting or developmental stage called pupation. In this stage larvae may appear dead but in fact they are undergoing changes within the larval skin. Just after pupation, colour of pupa changes to yellowish brown. Male pupa is comparatively smaller than the female pupa. These flies have been found to stay in the cropping rooms throughout the year. Temperature affects the duration of life cycle to great extent.

4.20.6.2. Phorid Fly

These are small hump backed black or light to dark brown flies measuring 1.9-2.0 mm in size. These flies are diminutive of house flies. Antennae are inconspicuous. Wing venation is reduced. The infested mushroom turns brown along the tunnel in the stipe. Attack at pinning stage restricts the further development of pin heads. Larvae also feed on mushroom mycelium in compost and casing soil. In case of button mushroom phorids can cause up to 46% loss in yield. Phorid flies are less harmful than sciarid flies if we compare damage per larva. In oyster mushrooms, particularly during rainy season 100% loss in yield has been reported. These are small hump backed black or light to dark brown flies measuring 1.9-2.0 mm in size (Figure 82B). They move rapidly with jerky movements. Adult phorids are most common in early summer and are attracted to light and swarm near windows and doors of the cropping rooms. Unspawned compost and fully grown mature compost are not so attractive to oviposition phorid flies. The

eggs are whitish, slightly curved, 0.3 mm long. The newly emerged larvae are nearly transparent. The mature larva is dirty white and measures 3.3-4 mm in length with pointed head and blunt rear end. Larvae feed on mushroom tissue and move upward into the cap forming tunnels in stipe. The infested mushroom turns brown along the tunnel in the stipe. Attack at pinning stage restricts the further development of pin heads. Larvae also feed on mushroom mycelium in compost and casing soil. In case of button mushroom phorids can cause up to 46% loss in yield. Phorid flies are less harmful.

Figure 82: Pest of mushroom Sciarid fly (A), phorid fly (B) and beetle fly (C&D)

4.20.6.3. Beetle fly

Beetle fly is one of the major pest devastatingly attacks the oyster mushroom. These were commonly found during the winter season which feed upon the fruiting body of oyster mushroom. It was observed that this pest affect mostly *P. florida* as it was cultivated during the winter. *Sphaerius* sp is commonly known as Beetle fly about 1-1.5cm in length, mostly black coloured with red spot on it (Figure 82C). Beetle fly

generally lay their egg on the substrates and they feed the mature fruiting body which results in decreasing the production rate of mushrooms.

4.20.6.4. Pest Disease Management

Oyster mushroom very severely affected by different pests during its colonization and fruiting body initiation. Several control measure was practiced to avoid the attack of mushroom pests. Hygiene is the primary method of pest control in mushroom farming. It is the foundation upon which success of all other control techniques depends. The objectives of any hygiene programme include exclusion of pests and diseases from production cycle, elimination of pest and pathogens and destruction of pest and disease present in a crop at its termination. Sanitation focuses on elimination or killing a pest. Routinely removing stumpage from the production unit, is a sound sanitary practice. Sanitary practices are designed not only to remove mushroom pests but to kill significant crop threats.

Screening of doors and ventilators mushroom flies can easily pass through ordinary wire screen and enter the mushroom house to breed on spawned compost and mushroom beds. Screening of doors and ventilators with nylon net of 35 meshes or more can effectively check the entry of flies in the cropping rooms (Figure 83).

Figure 83: Protective measures against insects and pests during mushroom cultivation practice inside the production unit

Polythene sheets coated with sticky material and attached to a fluorescent tube light in each cropping room help in controlling adult flies. Insects are attracted to white light

above 15ºC and to yellow light at lower temperature. Use of light trap (15 W yellow bulb and polythene sheet coated with mustard oil) is very effective for monitoring as well as for the management of the flies.

4.21. Promotion of mushroom cultivation to the rural people of North Bengal

Oyster mushroom is one of the most popular mushrooms cultivated throughout the world and India is one of the largest producers of oyster mushroom. Large number of people are now cultivating mushroom in commercial level throughout India. North Bengal is also popular for producing Oyster mushroom. Awareness of mushroom production is one of the major problems of North Bengal. Large number of unemployed youth are very much interest in mushroom cultivation. For the promotion of oyster mushroom cultivation, unemployed youth of Darjeeling district and Jalpaiguri district were selected and training was given to those persons who are interested in cultivation in large scale as well as in small scale. The hands-on training was based on the oyster mushroom which includes the cultivation of oyster mushroom with special reference to seasonal productivity of different species, promotion of pink oyster mushroom production and bottle and box cultivation for small scale production, preparation of mushroom spawn, post-harvest processing for long time preservation of oyster mushroom with special reference to different techniques drying, powder, biscuit preparation, management of spent mushroom substrates for crop improvement. A large number of unemployed youth are now cultivating different species of oyster mushroom and following are the list of some of the mushroom growers who are cultivating mushroom successfully and producing a great amount of mushroom every year.

Growers are now cultivating the oyster mushroom in a large scale production unit and they are very much known to the technical defaults. It was also observed that the growers are no longer limited their business into the mushroom cultivation but they are now trying to process the extra mushroom left after fresh selling. Growers are now drying the mushroom for long term preservation. In North Bengal, growers are very much interested in selling the fresh mushrooms. But now-a-days, growers are drying the mushroom and packaging quality was also modernized by them.

Table 33: List of trained mushroom growers successfully cultivating *P. ostreatus, P. sajor-caju* and *P. florida* in different part of Darjeeling and Jalpaiguri district.

Sl No	Name of the Growers	Area/ Location	GPS Location	Farm Size (sq.ft)	Farm Capacity (Bags)	Annual Production (kg)
District– Darjeeling						
1	Supok Singha	Naxalbari	26.6823^0E 88.1998^0N	400	1050	16,800
2	Sushanta Das			360	1000	16,000
3	Biswanath Barman			290	650	10,400
4	Jagannath Baroi	Bagdogra	26.7006^0E 88.3433^0N	540	800	12,800
5	Promod Thakur			750	1550	24,800
6	Iswar ch. Rajbanshi			200	350	6.000
7	Rebika Roy			180	300	5,500
8	Ujjal Biswas			350	550	8,500
9	Nugumanandu Barman			400	650	9,600
10	Hitendra Nath Roy			280	350	7,500
11	Nirmal singha	Batasi	26.7308^0E 88.1958^0N	240	450	7,500
12	Ashok Prashad Bhagat			320	600	10,000
District- Jalpaiguri						
13	Baburam Sarkar	Maynaguri	26.5738^0E 88.8214^0N	425	1000	16,000
14	Gopal Biswas			230	650	10,400
15	Shivshankar Roy	Balakoba,	26.5860^0E 83.5993^0N	400	900	14,400
16	Dinabandhu Barman			450	1200	19,200
17	Subhash Roy			320	750	12,000
18	Tapu Baramn			250	600	9,600
19	Chitramohan Biswas			300	550	8,000
20	Jay Kr. Mahanta			350	650	8,500
21	Utpal Biswas			300	750	9,000
22	Noresh Sarkar	Dhupguri,	26.5782^0E 89.0161^0N	360	850	13,600
23	Biashwjit Sarkar			440	1350	21,000
24	Amit kr. Sarkar	Ambari	26.6445^0E 88.5057^0N	360	950	15,200
25	Babun Sarkar			350	800	12,800
26	Sukallyan basu	Jalpaiguri	88.4853^0E 26.1627^0N	300	600	9,500
27	Bappa mandal			280	450	6,500
28	Ranjan Barman			350	850	10,500
29	Jay kumar Mahanta			500	1050	12,500
30	Narayan mandal			250	750	9,800
31	Subal mazumdar			280	600	8,500
32	Mani Bhusan Roy			320	725	11,300

Data collected from field survey of the trained mushroom growers

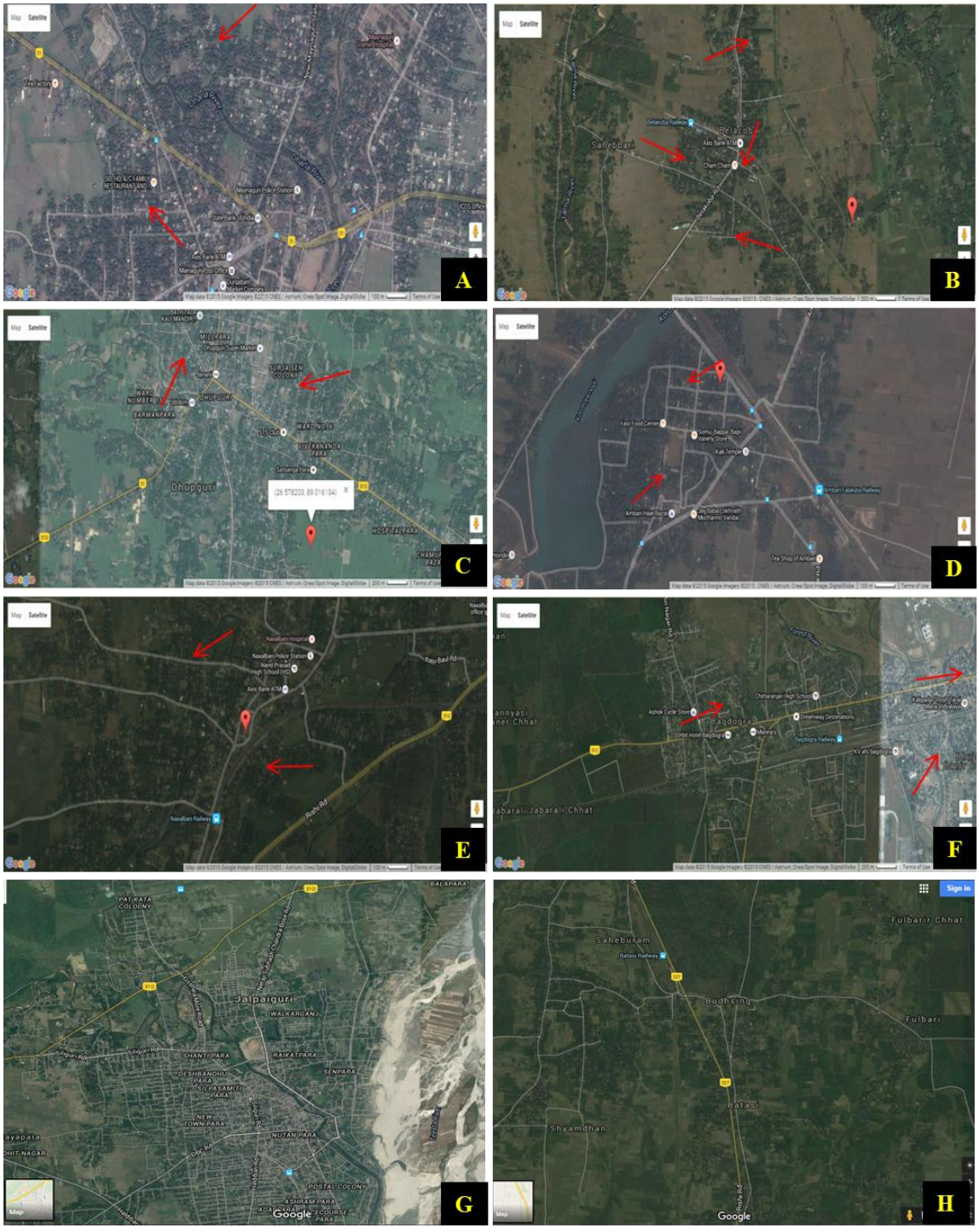


Figure 84: GPS location of mushroom farms established by the rural people after proper training in different districts of North Bengal (A) Mainaguri; (B) Belakoba; (C) Dhupguri; (D) Ambari; (E) Naxalbari, (F) Bagdogra, (G) Jalpaiguri and (H) Batasi

Figure 85: Promotion of oyster mushroom cultivation in different parts of North Bengal (A) Supak Singha Naxalbari, (B) Baburam Sarkar, Maynaguri, (C) Topu Barman, Belakoba, (D) Amit Kumar Sarkar, Aambari and (E) Jagonnath Baroi, Bagdogra

Figure 86: Demonstration of oyster mushroom cultivation practices during Krishi Mela organized by the Department of Agriculture, Govt. of West Bengal at Atharakhai Gram Panchayet, Shivmandir

Figure 87: Hands on training of cultivation process of oyster mushroom to the post-graduate students and unemployed youth (A) training for the unemployed youth, (B) students of Ananda Chandra College, Jalpaiguri, (C-D) Post-graduate students of NBU.

4.22. Cost benefit ratio of mushroom production in Darjeeling and Jalpaiguri district

Pleurotus is very common mushroom generally cultivated in North Bengal region. Different species of *Pleurotus* such as *Pleurotus ostreatus*, *Pleurotus sajor-caju P florida* and *P djamor* is being cultivated in this region. A large number of people is now involved in production and selling of *Pleurotus*. Unemployed youth, women self-help groups and retired persons are very active in production of oyster mushroom. Thus, a very good amount of mushroom is being produced every year in North Bengal. Darjeeling and Jalpaiguri district is very common in growing *Pleurotus* as the available raw material as well as the pleasant weather is very much favourable for the cultivation of *Pleurotus*. Depending upon the production capacity, growers are of three types, such as small, medium and large growers (Table 34). In this area, there are very good number of small mushroom growers in compare to large growers. On the other hand, depending upon the selling patterns, mushroom marketing is being done in four different channels which sequentially involves the growers, wholesalers, retailers and the consumers. In this region, most of the mushroom marketed through growers-wholesalers-retailer-consumer channel. On the other hand, small growers sell mushrooms directly to the market. To calculate the cost benefit ratio of mushroom marketing, market survey was done such as, champasari bazar, bidhan market, naxalbari hat, batasi hat, panitanki of Darjeeling district and fulbari, gatebazar, Jalpaiguri bazar and barivasa hat of Jalpaiguri district(Figure 88).

Table 34: Different types of mushroom selling pattern of mushroom in Darjeeling and Jalpaiguri district

Type of marketing	Small growers		Medium growers		Large growers	
	No of growers	Average qty. sold (kg)	No of growers	Average qty. sold (kg)	No of growers	Average qty. sold (kg)
Darjeeling District						
Grower-wholesaler-retailer-consumer	12	1,025	9	1885	3	1500
Grower- wholesaler-consumer	7	315	3	460	1	450
Grower-retailer-consumer	3	60	4	620	-	-
Grower-consumer	5	125	2	245	-	-
overall	27	1,525	18	3,210	4	1,950
Jalpaiguri District						
Grower-wholesaler-retailer-consumer	10	1450	7	1135	2	900
Grower- wholesaler-consumer	6	440	2	310	1	350
Grower-retailer-consumer	4	185	3	390	-	-
Grower-consumer	8	245	5	125	-	-
overall	28	2320	18	1960	4	1250

Data based on the market survey as well as the farm survey of different trained farmers in Darjeeling and Jalpaiguri districts

Table 35: Cost and returns of mushrooms in different categories of Darjeeling district

Particulars	Categories of farms			
	Small	Medium	Large	Average
District Darjeeling				
Mushroom production (kg)	1525	3210	1950	2228.5
Cost of production (Rs)	29737	60187	39975	43299.6
Average market rate (Rs/kg)	90	110	120	106.6
Gross returns (Rs)	137250	353100	234000	241450
Benefit cost ratio	**4.615**	**5.866**	**5.853**	**5.576**
District Jalpaiguri				
Mushroom production (kg)	2320	1960	1250	1843.3
Cost of production (Rs)	45240	38220	24375	28631.6
Average market rate (Rs/kg)	95	100	120	105
Gross returns (Rs)	220400	196000	150000	188800
Benefit cost ratio	**4.871**	**5.128**	**6.153**	**5.384**

Cost benefit ratio on the market survey data of selected markets of Darjeeling and Jalpaiguri district of North Bengal region

Figure 88: Mushroom sell at local markets; (A) fulbari haat, (B) Naxalbari, (C) Batasi haat, (D) Barivasa bazar and (E &F) departmental stores

It was observed that the cost of mushroom per kg ranges between Rs. 90-120. Market survey results also revealed that the cost benefit ratio much higher in case of large scale growers than that of the small growers. It was also observed that in all markets only fresh mushroom are being sold by the sellers. There are no such processed mushroom products such as canned of dried mushroom sold in the market. The use of compost has a positive relationship with the farm size. On the whole, in total variable cost, the average share of compost is maximum followed by labour charges and spawn. There is a positive relationship between mushroom production and farm-size. The income of mushroom growers goes up with the increase in farm size. The large growers adopt the better management practices, resulting into higher net income, that is followed by medium and small farmers. This demonstrates the applicability of "economies of scale" in mushroom cultivation. Of various channels, channel-I (Producer- Wholesaler-Retailer-Consumer) is the most common channel amongst different categories of mushroom growers, followed by the channel-II (Producer-Wholesaler-Consumer) in small and medium size farms, while channel-III only in case of large growers.