4. Results and Discussion
RESULTS AND DISCUSSION

4.1. Effect of Fungal Treatment on Substrate Composition

The corn cob substrate employed in this experiment contained (w/w): 91.66 % neutral detergent fibre (NDF), 69.75 % acid detergent fibre (ADF), 21.9 % hemicellulose, 35.93 % cellulose, 15.52 % lignin and 1.99 % crude protein.

The substrate for fermentation was prepared by mixing ground corn cob with distilled water in the ratio of 30:105 (w/v) and autoclaved for 1 h. It was then inoculated with mycelial culture of Pleurotus florida and incubated at 27 ±1 °C. Within 3 days, the white mycelial growth appeared which covered the whole surface of substrate within 10 days.

The change in composition of corn cob substrate during 40 days fermentation is presented in Fig. 1. The neutral detergent fibre (NDF) in the substrate decreased from 91.66 % to 86.32 % with a net loss of 5.83 % during 40 days fermentation. ADF also decreased from its initial 69.75 % to 68.14 % with a net loss of 2.30 %. Total 17.02 % hemicellulose was degraded during fermentation, while the degradation of cellulose was comparatively less and it was only at the level of 9.24 %. The lignin was degraded by 21.20 % during the fermentation. There was a constant increase in crude protein content of the substrate from 1.99 to 5.16 % during the course of corn cob fermentation.

In solid state fermentation of lignocellulosic substrate the fungal growth is completed in two phases. The primary phase, in which the substrate is colonized and the white mycelial mass covers the substrate and the secondary phase, in which the lignolytic enzymes are excreted and lignin degradation starts (Zadrazil, 1977). In the primary phase, there is no lignin degradation but hemicellulose and cellulose loss has been observed. The lignin degradation started at 10 days of treatment indicating that the secondary phase of Pleurotus florida growth started on 10th day onward during substrate fermentation. These results are in agreement with those reported by Kamra et al (1993). The increase in crude protein content of the substrate observed in this experiment might be at the cost of dry matter loss during fungal treatment. As the fungus (P. florida) employed in the present experiment is not known to fix atmospheric nitrogen, the increase in crude protein could be only at the cost of dry matter loss.
Fig. 1. Change in composition of unsupplemented corn cob substrate upon fermentation by *Pleurotus florida* during 40 days incubation
4.1.1. Effect of various additives on solid substrate fermentation

The substrate supplemented with various additives and inoculated with *P. florida* was incubated at 27 ±1 °C and analyzed at 5, 10, 15, 20, 25, 30 and 40 days interval for the determination of change in various constituents during the course of fermentation.

4.1.1.1. Effect of urea

In the 0.5 % (w/w) urea supplementation the NDF decreased continuously with a net loss up to 13.87 % on 40th day of fungal treatment. ADF also decreased gradually during the period of fermentation with a net loss of 4.58 %. The hemicellulose degradation started during primary phase of fermentation and the maximum degradation (10.52 %) was recorded on 25th day with a net loss of 42.88 % on 40th day. While, net loss in cellulose and lignin was recorded at 13.03 % and 27.09 %, respectively during 40 days of solid substrate fermentation. The crude protein increased constantly from 1.96 % at zero day to 6.88 % on the 40th day of incubation (Fig. 2a).

In the substrate amended with 1 % (w/w) urea, the NDF degradation started in primary phase of fermentation and continued in secondary phase with a net loss of 15.91 %. ADF degradation also started in primary phase but comparatively slower than NDF. The net loss in ADF content on 40 days fermentation was recorded as 6.72 %. Hemicellulose, cellulose and lignin degraded variably. The net loss in hemicellulose, cellulose and lignin was recorded as 31.54, 5.93 and 32.57 %, respectively during solid substrate fermentation. A continuous and gradual increase from 2.11 % to 7.92 % of crude protein content was observed during 40 days of *P. florida* treatment (Fig. 2b).

In corn cob substrate supplemented with 1.5 % (w/w) urea, the NDF degradation started well within 5 days of incubation and was degraded from 88.94 to 76.30 % with a net loss of 14.21 % on 40th day of fermentation. The ADF was also degraded in the same manner with a net loss of 11.99 % on 40th day of fungal treatment. The hemicellulose decreased by 21.84 %, while net loss in cellulose was recorded 7.51 % after fermentation up to 40 days. The net loss of lignin was reported 26.02 % within 40 days of incubation. The crude protein content increased continuously from 2.19 % and reached 8.02 % on 40th day of fermentation (Fig. 2c).
Fig. 2. Effect of urea supplementation at (a) 0.5 % (b) 1.0 % (c) 1.5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
The supplementation of urea to the substrate at 0.5 % (w/w) had influenced slightly more hemicellulose and cellulose degradation during the fermentation (Fig.2a). One % (w/w) urea addition led to the increased degradation of NDF and lignin with a marginally higher gain of crude protein. Urea at 1.5 % (w/w) supplementation resulted in higher degradation of ADF. The overall degradation of various constituents was slightly better upon supplementation with urea than unsupplemented control, but higher concentration of urea had adversely affected the degradation of almost all the constituents. Urea is a better source of nitrogen due to the presence of amino group. However, supplementation of substrate with excess urea leads to release of ammonia that is toxic for mycelial growth as it’s higher concentrations are inhibitory to fungal metabolism (Zakia, 1972; Tripathi and Yadav, 1992). In the present study, supplementation of corn cob substrate with higher concentration of urea resulted in comparatively less degradation of hemicellulose and cellulose, which may be due to the ammonification and release of excess ammonia, which is inhibitory to fungal metabolism (Zakia, 1972). Similar findings have been reported when various lignocellulosic substrates were supplemented with urea on solid substrate fermentation by Pleurotus florida (Fenn and Kirk, 1981; Kamra and Zadrazil, 1986; Kahlon and Dass, 1987; Venkatraman et al., 1989; Tripathi and Yadav, 1992).

4.1.1.2. Effect of ammonium sulphate

When the corn cob substrate was supplemented with 0.5 % (w/w) ammonium sulphate, the NDF degradation started during primary phase of fermentation leading to a decrease from 90.02 % at zero day to 72.85 % with a net loss of 19.07 % on 40th day of fungal inoculation. The net loss of ADF was recorded as 12.79 % during the course of corn cob fermentation. Degradation of hemicellulose, cellulose and lignin started during primary phase of fermentation and the net loss was 38.79, 13.55 and 20.84 %, respectively (Fig. 3a).

One per cent (w/w) supplementation of ammonium sulphate to the substrate led NDF, ADF, hemicellulose, cellulose and lignin degradation starting from primary phase of fermentation. NDF decreased from 89.94 to 74.35 %, with a net loss of 17.33 % and ADF decreased from 68.39 to 60.64 %, with a net loss of 11.33 %, on the 40th day of fermentation process. The hemicellulose and
Fig. 3. Effect of ammonium sulphate supplementation at (a) 0.5 % (b) 1.0 % (c) 1.5 % to corn cob substrate and change in composition upon fermentation by *P. floridea* during 40 days incubation.
cellulose in this case were degraded up to 36.68 and 19.94 %, respectively, while the net loss in lignin was recorded 26.20 %. The crude protein content gradually increased during the fermentation, which was raised from 2.11 % at zero day to 7.98 % on the 40th day (Fig. 3b).

In case of 1.5 % (w/w) ammonium sulphate supplementation to the substrate there was a variable degradation of the constituents leading to the net loss of 12.45 % and 5.75 % in NDF and ADF, respectively, during the period of incubation. The hemicellulose and cellulose were reduced up to 35.27 and 11.31 %, respectively after 40 days of inoculation with *P. florida*. The net lignin loss observed in this case was 24.53 %. The increase in crude protein content of corn cob during 40 days fermentation was almost equal to that of 1 % ammonium sulphate supplementation (Fig. 3c).

In the present experiment, the lower concentrations of ammonium sulphate had enhanced greatly the degradation of all the constituents of the substrate including NDF, ADF, hemicellulose, cellulose and lignin. The higher concentrations were found inhibitory to the degradation of lignocellulosic components of the substrate as compared to lower concentrations. Ammonium sulphate is a nitrogen rich supplement. The reduction in lignin degradation at high nitrogen concentration has been observed by many workers (Kirk *et al.*, 1976 and 1978). Suppression of lignin removal from lignocellulosics has also been observed by various researchers (Kirk *et al.*, 1976; Zadrazil and Brunnet, 1982; Munoj 1983; Srisodsuk and Flegel, 1984; Tripathi and Yadav, 1992). The adverse effect of higher nitrogen content on lignin degradation might be due to interference of nitrogen metabolism with lignin metabolism.

**4.1.1.3. Effect of gram flour**

On the addition of 2 % (w/w) gram flour to the substrate, NDF decreased continuously up to 25th day with a net loss of 17.65 % on 40 days of fermentation. ADF degradation followed the same pattern of NDF, with a net loss by 8.37 %. Hemicellulose and cellulose degraded in a variable pattern up to 40th day of fermentation with a net loss of 47.17 % and 17.10 %, respectively, while lignin was degraded continuously during the fermentation, with a net loss of 31.27 %. A continuous increase in crude protein content of corn cob substrate was from 1.74 % on day zero to 7.92 % on day 40 (Fig. 4a).
In the substrate amended with 3 % (w/w) gram flour, NDF and ADF degraded continuously up to 20th day following which the degradation was variable with a net loss of 15.54 and 8.43 %, respectively. The hemicellulose, cellulose and lignin degradation followed the same pattern as was observed with 2 % amendment, with a net loss of 38.33, 15.13 and 27.65 %, respectively. Contrary to the loss of other constituents of the substrate, crude protein content was increased from an initial 1.93 % at zero day to 8.17 % on 40th day (Fig. 4b).

In the substrate amended with 5 % (w/w) gram flour, NDF and ADF decrease followed the same pattern with a net loss of 12.83 and 5.70 %, respectively during 40 days fermentation. The hemicellulose degradation was continuous during the process, while, cellulose degradation was continuous up to 20th day. The net loss in hemicellulose and cellulose was 37.91 % and 9.18 %, respectively. Lignin was also reduced significantly by 25.89 % while, crude protein content was increased from 2.27 % on zero day to 8.29 % on 40th day of treatment (Fig. 4c).

The gram flour is a good source of organic nitrogen and carbon favouring growth of the fungal mycelium. The addition of gram flour to the substrate promotes the growth of fungus Pleurotus florida ( Gerrits, 1974 ). In the present experiment the gram flour supplementation, reduced the NDF, ADF, hemicellulose, cellulose and lignin degradation with increasing the levels of gram flour concentrations. At lower concentration ( 2 %) the gram flour enhanced the degradation of lignocellulosic constituents in the substrate as compared to unsupplemented control. The reduction in degradation at higher concentration might be due to the rise in temperature of substrate, resultant of higher concentrations of nitrogen. Gupta and Vijay ( 1990; 1991 ) reported that the supplementation of organic nitrogen sources above 2 % (w/w) on dry weight basis resulted in undue heating of substrate, which could inhibit the growth of mycelium and rate of degradation of substrate in vitro. Increased degradation of the constituents should correspond to better fungal growth in the substrate. Bano et al ( 1979 ) and Bano and Rajarathnam ( 1983 ) have reported increased fungal growth in substrate supplemented with gram flour. Supplementation of gram flour had resulted in increased crude protein content, which is indicative of the better growth of the fungus in the supplemented substrate.
Fig. 4. Effect of gram flour supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
4.1.1.4. Effect of soybean meal

The corn cob substrate supplemented with 2 % (w/w) soybean meal resulted in 17.23 % and 9.33 % net loss in NDF and ADF, respectively with a variable degradation over the period of fermentation. There was 41.95 % decrease in hemicellulose with a variable degradation during 40 days of fermentation. The cellulose and lignin were reduced variably with a net decrease by 15.63 % and 37.98 %, respectively. However, the crude protein content increased from 1.95 % to 7.59 % gradually during 40 days of fermentation (Fig. 5a).

Fig. 5b depicts that NDF and ADF were degraded gradually up to 20th day, following which degradation was variable with a net loss of 14.21 % and 6.75 %, respectively, during the fermentation process, after supplementation of substrate with 3 % (w/w) soybean meal. There was gradual decrease in hemicellulose and lignin, while cellulose degraded in a variable manner during the fermentation with a significant decrease. The hemicellulose, cellulose and lignin were degraded up to 38.05, 12.33 and 34.72 %, respectively on 40 days of incubation. Contrary to the decrease in other constituents, an increase in the crude protein content was noted from 1.94 at zero day to 7.87 % on 40th day of fermentation (Fig. 5b).

In the substrate amended with 5 % (w/w) soybean meal, both NDF and ADF degraded gradually up to 20th day of incubation with a respective net loss of 12.80 and 6.44 % on 40th day of incubation. Hemicellulose and lignin degraded gradually over the period of fermentation, while, cellulose degradation was variable throughout the process. The hemicellulose, cellulose and lignin decreased by 35.54, 7.17 and 26.21 %, respectively. The crude protein content was increased from 2.22 to 8.04 % (Fig. 5c).

The soybean meal rich in protein and fats was reported to promote the growth of mushroom mycelia by certain amino acids present in soybean protein (Randle, 1985). The growth has a direct correlation with the loss of dry matter and other cell wall constituents of the substrate. Similar results were reflected in the present observation as supplementation of lower level of soybean meal had greatly enhanced the degradation of various constituents of the substrate; at higher concentration the degradation was reduced, but was still higher than the unsupplemented control. At higher concentrations of supplement, reduced degradation may be the result of uncontrollable heatups and attack of weed moulds (Randle, 1985; Saxena and Rai, 1994). Crude protein content was greatly
Fig. 5. Effect of soybean meal supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
enhanced due to supplementation with soybean meal, which should be the result of better fungal growth (Gerrits, 1986; 1989).

4.1.1.5. Effect of mustard cake

Addition of 2 % (w/w) mustard cake to the substrate led to the continuous degradation of NDF and ADF up to 30th day of incubation with a net loss of 13.97 and 8.03 %, respectively during 40 days fermentation. Hemicellulose and lignin were gradually decreased during the complete duration of fermentation. The lignin degradation was variable throughout the incubation period. The net loss in hemicellulose, cellulose and lignin was recorded as 33.66, 17.16 and 40.33 %, respectively. The crude protein increased from 1.89 to 7.91 % on 40 days incubation (Fig. 6a).

There was a gradual and continuous decrease in the per cent composition of NDF, ADF, hemicellulose and lignin in the substrate supplemented with 3 % (w/w) mustard cake. Cellulose was degraded variably during the fermentation. The net loss in NDF, ADF, hemicellulose, cellulose and lignin was recorded as 15.74, 9.41, 35.65, 12.95 and 41.69 %, respectively. There was a gradual increase in crude protein content with incubation period from 1.96 % on zero day to 8.03 % on 40th day (Fig. 6b).

In the substrate supplemented with 5 % (w/w) mustard cake, the NDF, ADF, hemicellulose and lignin followed the same degradation pattern as observed in substrate supplemented with 3 % mustard cake. Net loss in NDF, ADF, hemicellulose, cellulose and lignin was observed as 16.94, 11.26, 34.89, 7.36 and 33.26 %, respectively on 40 days of fermentation. An increase in crude protein was observed from 2.29 to 8.35 % during 40 days fermentation (Fig. 6c).

In the present experiment, up to a limited level, the mustard cake supplementation provided better nutrients for fungal growth and increased degradation of lignocellulosic materials, but at higher levels it was inhibitory to the fungus resulting into decreased degradation of various constituents of the substrate. Crude protein content was also greatly enhanced following supplementation with mustard cake at lower level. Higher concentration of mustard cake contained more nitrogen which has proved inhibitory to fungal growth as same is inhibitory to fungal delignification (Kirk et al., 1978; Munoz et al., 1983; Tripathi and Yadav, 1992). The inhibitory effect of the mustard cake may also be due to
Fig. 6. Effect of mustard cake supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
and Yadav, 1992). The inhibitory effect of the mustard cake may also be due to presence of traces of mustard oil, which contains a biocidal substance named allylisothiocyanate (Singh and Singh, 1970; Bahl, 1991).

4.1.1.6. Effect of ground nut cake

In the substrate supplemented with 2 % (w/w) ground nut cake, the NDF and ADF were decreased gradually with a slight variation during the fermentation process with a net loss of 14.89 and 7.85 %, respectively. There was a continuous and gradual decrease in hemicellulose, cellulose and lignin with a slight variation in cellulose degradation. The net loss in hemicellulose, cellulose and lignin was recorded as 37.92, 13.52 and 39.12 %, respectively, during the 40 days fermentation. The crude protein content was increased gradually and continuously from 1.82 to 7.45 % during 40 days fermentation (Fig. 7a).

In the substrate amended with 3 % (w/w) ground nut cake, the NDF, ADF, hemicellulose, cellulose and lignin degraded gradually with a slight variance, during the phases of fermentation. The net loss in NDF, ADF, hemicellulose, cellulose and lignin was recorded as 16.22, 12.54, 28.35, 10.11 and 38.96 %, respectively. While, the crude protein content followed the same pattern of previous experiments and increased from 1.94 to 7.87 % on 40 days fermentation (Fig. 7b).

In case of 5 % (w/w) ground nut cake supplementation of substrate, the NDF, ADF, hemicellulose, cellulose and lignin were degraded variably with a net loss of 7.02, 1.27, 27.10, 7.24 and 33.44 %, respectively, over the period of incubation. Contrary to the other constituents, the crude protein content was increased from 2.22 to 8.04 % on 40 days fermentation (Fig. 7c).

In the present experiment the supplementation of the substrate with 2 % (w/w) ground nut cake induced degradation of almost all the cell wall constituents at lower concentration. There was highest protein content yield also at lower concentration. The higher concentration of supplement also led to better degradation of hemicellulose, cellulose and lignin, and better protein yield than unsupplemented control substrates. The above results are in accordance with the findings of other workers (Bano et al., 1979; Bano and Rajarathnam, 1983; Gupta and Vijay, 1994).
Fig. 7. Effect of ground nut cake supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
4.1.1.7. Effect of cotton seed cake

Supplementation of the substrate with 2 % (w/w) cotton seed cake resulted in gradual decrease of NDF and ADF with a net loss of 21.26 and 10.45 % respectively during 40 days fermentation. Hemicellulose, cellulose and lignin were also decreased following the pattern of NDF and ADF degradation. The loss in hemicellulose, cellulose and lignin was recorded as 58.34, 20.80 and 34.55 %, respectively. There was a considerable increase in crude protein from 1.85 to 7.86 % on 40 days incubation (Fig. 8a).

Three per cent (w/w) supplement of cotton seed cake to the substrate affected all the components studied (Fig. 8b). There was a gradual loss of NDF and ADF recorded throughout the fermentation process with a net loss of 15.76 and 9.17 %, respectively. The hemicellulose, cellulose and lignin degradation followed the same pattern and were decreased by 38.58, 18.50 and 35.30 %, respectively, during the fermentation. The crude protein was increased gradually from 1.98 to 7.97 % on 40th day of incubation.

Supplementation of the substrate with 5 % (w/w) cotton seed cake resulted in gradual decrease of NDF with a net loss of 16.78 % on 40th day of fermentation. The ADF was degraded maximum up to 30th day with a net loss of 7.09 % on completion of fermentation process. The hemicellulose was degraded variably leading to the net loss of 49.85 %, while, cellulose and lignin degraded gradually during the course of fermentation with a net loss of 12.78 and 27.63 %, respectively. The crude protein was increased from 2.12 to 7.35 % on 40 days fermentation (Fig. 8c).

The lower concentration of cotton seed cake supplementation induced the degradation of various lignocellulosic cell wall constituents. At higher concentration, the degradation recorded was lesser than lower concentration supplementation, but better in comparison to unsupplemented control substrate. Application of cotton seed cake supplements can cause uncontrollable heatups and are therefore, more prone to weed mould attack (Randle 1985; Saxena and Rai, 1994). These may be the reasons of decreased degradability of lignocellulosics at higher concentrations of this supplement. The results of the present study are in agreement with the studies carried out by earlier researchers. Bano et al (1979), Bisaria et al (1987) and Ertan (1988) reported better yield with the addition of cotton seed cake to the substrate, which is a result of better substrate degradation as per "better yield: better degradation of the substrate".
Fig. 8. Effect of cotton seed cake supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
4.1.1.8. Effect of molasses

Fungal treatment following the amendment of corn cob substrate with 2 % (w/w) molasses resulted in gradual degradation of NDF and ADF, that started during the primary phase of fermentation with a net loss of 15.20 and 8.06 %, respectively, after 40 days of treatment. The hemicellulose and lignin decreased gradually throughout, while cellulose degraded gradually up to 20\textsuperscript{th} day of fermentation. The net loss in hemicellulose, cellulose and lignin was recorded as 37.48, 8.83 and 36.05 %, respectively, on 40\textsuperscript{th} day of incubation. Crude protein was increased from 1.92 % on zero day to 7.55 % on 40\textsuperscript{th} day of fermentation (Fig. 9a).

With respect to the addition of 3 % (w/w) molasses to the substrate, the NDF, ADF, hemicellulose and lignin followed the same pattern of degradation and were decreased gradually during the period of fermentation. The lignin degradation was variable throughout the fermentation process. The net loss of NDF, ADF, hemicellulose, cellulose and lignin was recorded as 14.11, 8.57, 34.63, 6.94 and 32.59 %, respectively. The net gain in crude protein content was increased from 1.99 % on zero day to 7.86 % on 40\textsuperscript{th} day of solid state fermentation (Fig. 9b).

The substrate supplemented with 5 % (w/w) molasses resulted in the degradation of NDF, ADF, hemicellulose and lignin, which followed the same pattern of degradation during the period of fermentation. The pattern of degradation was same as observed in substrate supplemented with 3 % molasses. The lignin degradation was variable throughout the fermentation process. The net loss of NDF, ADF, hemicellulose, cellulose and lignin was recorded as 12.64, 6.99, 30.11, 3.99 and 26.31 %, respectively. The crude protein content was increased from 2.28 to 8.35 % on 40 days fermentation (Fig. 9c).

Molasses is a good source of nutrient and growth factors for microorganisms. The addition of molasses in the premix with substrate stimulated good fungal growth and the different levels of molasses affected the composition of substrate. There was a decrease in NDF, ADF, hemicellulose, cellulose and lignin but a corresponding increase in crude protein of the fermented substrate. The supplementation of substrate with free carbohydrates (molasses) led to the increased degradation of NDF, ADF, hemicellulose and lignin while reduced the cellulose degradation in comparison to the degradation of constituents in unsupplemented control substrate. The loss in substrate with respect to hemicellulose, cellulose and lignin was comparatively lesser in higher
Fig. 9. Effect of molasses supplementation at (a) 2 % (b) 3 % (c) 5 % to corn cob substrate and change in composition upon fermentation by *P. florida* during 40 days incubation.
concentrations of molasses. Therefore, loss in lignin and hemicellulose decreased with increase in the concentration of molasses. This observation is supported by the results of Yadav (1987).

4.2. Effect of Nitrogen Supplements on Enzyme Profiles

During the fermentation of the unsupplemented control and nitrogen supplemented substrate with *P. floruida*, various enzymes responsible for degradation of cellulose and hemicellulose were produced, including carboxymethyl cellulase, xylanase, β-glucosidase and β-xylosidase. The nitrogenous supplements *viz.* urea and ammonium sulphate were added at 0.5, 1.0 and 1.5 % (w/w), while gram flour, soybean meal, mustard cake, ground nut cake, cotton seed cake and molasses were supplemented at 2, 3 and 5 % (w/w) to corn cob substrate and their effect on various enzyme production by *P. floruida* during 40 days solid state fermentation was studied. The results are depicted in Figs. 10 to 25.

4.2.1 Effect of urea

In general, the production of various enzymes was comparatively less in urea supplemented substrates as compared to the unsupplemented corn cob during the course of fermentation (Figs. 10 & 11). Therefore, it can be stated that addition of urea had no effect or rather was slightly inhibitory for the production of various enzymes by *P. floruida*. Further, the production of CMCase, xylanase, β–glucosidase and β–xylosidase was maximum at 40, 10, 25 and 20 days, respectively during corn cob fermentation by *P. floruida*. In general, higher urea concentrations at 1.0 and 1.5 % (w/w) were found to be slightly inhibitory for enzyme production.

4.2.2. Effect of ammonium sulphate

In general, addition of ammonium sulphate was inhibitory for CMCase and xylanase production as the maximum enzymes were produced respectively during secondary (30 and 40 days) and primary phase of fermentation in unsupplemented corn cob substrate by *P. floruida* (Fig. 12). When substrate was supplemented with different levels of ammonium sulphate, the maximum CMCase activity was noted on day 30 with 1% (w/w) ammonium sulphate. The xylanase activity was
Fig 10. Carboxymethyl cellulase and xylanase production by *P. floridea* on solid substrate fermentation of corn cob supplemented with urea during 40 days fermentation. Error bar is showing standard deviation.
Fig 11. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with urea during 40 days fermentation. Error bar is showing standard deviation.
Fig 12. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with ammonium sulphate during 40 days fermentation. Error bar is showing standard deviation.
recorded maximum on 15th day of incubation in the substrate amended with 0.5 % (w/w) ammonium sulphate (Fig. 12).

Fig. 13 depicts that β-glucosidase production was more or less comparable in both ammonium sulphate supplemented and unsupplemented corn cob substrate throughout the period of fermentation and was maximum on 25th day. As such highest enzyme units (3.72) were produced with 1 % ammonium sulphate supplemented substrate, which was only slightly higher than the unsupplemented one. β-xylanidase profile was maximum on 20th day with higher units produced in unsupplemented substrate fermented by *P. florida*.

**4.2.3. Effect of gram flour**

The highest CMCase units (2.45) were obtained when corn cob substrate was supplemented with 5 % (w/w) gram flour and fermented by *P. florida* during 30 days incubation. However, the CMCase units produced in other levels of gram flour supplementation and control (unsupplemented) were only slightly less during 30-40 days fermentation (Fig. 14).

Regarding xylanase production, the higher enzyme units were produced in unsupplemented corn cob as compared to gram flour supplemented corn cob substrate. Therefore, it is apparent that supplementation of gram flour at all levels was inhibitory for xylanidase production (Fig. 14).

Fig 15 shows that β-glucosidase production was maximum in unsupplemented and 2 % (w/w) gram flour supplemented corn cob substrate during 25 and 30 days of solid substrate fermentation by *P. florida*. Furthermore, in general, enzyme units in gram flour supplemented substrate was maximum during 30-40 days fermentation and was more or less independent of the levels of supplementation.

Fig. 15 depicts that the production of β-xylanidase was maximum in unsupplemented substrate as compared to the gram flour added substrate during 20 days fermentation. Furthermore, the concentrations of gram flour supplementation had no effect as enzyme units produced at all levels were more or less the same.
Fig 13. β-Glucosidase and β-xylosidase production by P. florida on solid substrate fermentation of corn cob supplemented with ammonium sulphate during 40 days fermentation. Error bar is showing standard deviation.
Fig 14. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with gram flour during 40 days fermentation. Error bar is showing standard deviation.
Fig 15. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with gram flour during 40 days fermentation. Error bar is showing standard deviation.
4.2.4. Effect of Soybean

Figures 16 and 17 show that the pattern of all the four enzymes’ production upon supplementation of soybean meal was more or less similar to that of gram flour supplementation (Fig. 14 & 15). In general, higher units of various enzymes were produced in unsupplemented substrate than the soybean meal supplemented substrate except for the glucosidase enzyme which was produced maximally in 3% soybean meal supplemented corn cob substrate during 30 days solid substrate fermentation (Figs. 16 & 17). On the other hand, carboxymethyl cellulase, xylanase and β–xylosidase production was highest respectively on 40, 15 and 20 days fermentation of unsupplemented corn cob substrate by *P. florigida*.

4.2.5. Effect of mustard cake

In general, the production of CMCase, xylanase and β–xylosidase was maximum in unsupplemented than mustard cake supplemented corn cob substrate fermented by *P. florigida* during 40 days incubation. Further, the production of xylanase, β–xylosidase, CMCase and β–glucosidase was maximum on 15, 20, 40 and 25 days, respectively (Fig. 18 & 19). β–Glucosidase was produced maximum in the substrate amended with 3% (w/w) mustard cake. Mustard cake was found to be more inhibitory for xylanase production as compared to other three enzymes.

4.2.6. Effect of ground nut cake

The highest CMCase production was noted on 40 days fermentation in unsupplemented corn-cob substrate. However, the enzyme units produced in ground nut cake supplemented substrate were comparable during 30 days fermentation and only slightly less on 40th day (Fig. 20). Contrary to CMCase enzyme, the production of xylanase was maximum on 15th day in unsupplemented corn cob substrate. The higher level of ground nut cake was slightly inhibitory for these enzyme production during the same course of fermentation (Fig. 20).

With regard to β–glucosidase units, however, 2% (w/w) ground nut cake supplementation produced maximum enzyme on 25th day; 5% (w/w) ground nut cake being inhibitory (Fig 21). The production of β–xylosidase on the other hand was maximum on the 20th day in unsupplemented corn cob substrate fermented by *P. florigida* and was comparable with respect to ground nut cake supplemented substrate (Fig. 21).
Fig 16. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with soybean meal during 40 days fermentation. Error bar is showing standard deviation.
Fig 17. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with soybean meal during 40 days fermentation. Error bar is showing standard deviation.
Fig 18. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with mustard cake during 40 days fermentation. Error bar is showing standard deviation.
Fig 19. β-Glucosidase and β-xylosidase production by P. florida on solid substrate fermentation of corn cob supplemented with mustard cake during 40 days fermentation. Error bar is showing standard deviation.
Fig 20. Carboxymethyl cellulase and xylanase production by *P. florid*a on solid substrate fermentation of corn cob supplemented with ground nut cake during 40 days fermentation. Error bar is showing standard deviation.
Fig. 21. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with ground nut cake during 40 days fermentation. Error bar is showing standard deviation.
4.2.7. Effect of cotton seed cake

When corn cob substrate was supplemented with 2 % (w/w) cotton seed cake, the xylanase and β-xylosidase production was maximum during 20 days fermentation by P. florida (Fig. 22 & 23). However, the production of β-glucosidase was maximum in 3 % (w/w) cotton seed cake supplemented substrate on 25th day of fermentation. Contrary to that the production of CMCase was maximum in unsupplemented control substrate on 40th day of solid substrate fermentation.

4.2.8. Effect of Molasses

The production of CMCase, xylanase and β-xylosidase was found to be maximum in unsupplemented corn cob substrate during 40, 15 and 20 days fermentation. The molasses was found to be highly inhibitory for xylanase, CMCase and β-xylosidase, respectively in the decreasing order of significance (Fig. 24 & 25). Contrary to that, the production of β-xylosidase was maximum in corn cob substrate supplemented with 2 % (w/w) molasses on 25th day of solid substrate fermentation, which was comparable with the unsupplemented control substrate and other levels of molasses supplementation (Fig. 25).

The biological degradation of lignocellulosic agricultural byproducts is accomplished with the activity of various categories of enzymes such as (i) hydrolytic (cellulases and hemicellulases), (ii) oxidizing (lignin peroxidases) and (iii) esterases (for breaking ester bonds between hemicellulose and lignin). Among the hydrolytic enzymes, carboxymethyl cellulase, β-glucosidase, xylanase and β-xylosidase are the most important which act synergistically to hydrolyze cellulose and hemicellulose.

The levels of all enzymes responsible for degradation of cellulose and hemicellulose were very low in the initial stages of fermentation in all the cases of nitrogen supplementation as well as unsupplemented control. The slow appearance of these enzymes may be because of the fact that in the primary phase of growth the soluble carbohydrates are preferentially utilized (Platt et al., 1984; Singh et al., 1994).

In the unsupplemented substrate, with increasing the incubation period, carboxymethyl cellulase units were variable throughout the fermentation process. The same pattern of the enzyme production was also observed for each nitrogen supplementation. The enzyme production was not affected significantly, rather
Fig 22. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with cotton seed cake during 40 days fermentation. Error bar is showing standard deviation
Fig 23. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with cotton seed cake during 40 days fermentation. Error bar is showing standard deviation.
Fig 24. Carboxymethyl cellulase and xylanase production by *P. florida* on solid substrate fermentation of corn cob supplemented with molasses during 40 days fermentation. Error bar is showing standard deviation.
Fig 25. β-Glucosidase and β-xylosidase production by *P. florida* on solid substrate fermentation of corn cob supplemented with molasses during 40 days fermentation. Error bar is showing standard deviation.
reduced due to various amendments. Moreover, there was further slight decreased production of CMCase recorded at higher concentrations of nitrogen supplementation. The enzyme units increased initially up to 15\textsuperscript{th} day of fermentation then decreased up to 25\textsuperscript{th} day, followed again by an increase to maximum level on 30\textsuperscript{th} and 40\textsuperscript{th} day. Our findings are in agreement with the results of other workers who have also reported the maximum cellulase activity during the final days of fermentation (Gordon and Philips, 1989; Kerem \textit{et al.}, 1992; Xie \textit{et al.}, 2001).

The β-glucosidase production in the unsupplemented substrate was recorded variable. The enzyme activity was increased rapidly after 20\textsuperscript{th} day with a maximum increase on 25\textsuperscript{th} day. The β-glucosidase units with various supplements exhibited the similar pattern except for gram flour and soybean meal. It is evident from the Figs 15 and 17 that in the substrate supplemented with gram flour and soybean meal the enzyme activity was continuously increased up to 20\textsuperscript{th} day, followed by slight decrease on 25\textsuperscript{th} day and thereafter increased to maximum during 30\textsuperscript{th} – 40\textsuperscript{th} day of fermentation. The enzyme production was affected significantly due to the levels of various amendments. The β-glucosidase production was recorded same at lowest and highest concentrations of the supplements such as urea, ammonium sulphate, gram flour, soybean meal, mustard cake and cotton seed cake, while the production was increased in the substrate supplemented with 1 % (w/w) urea and ammonium sulphate and 3 % (w/w) gram flour, soybean meal, mustard cake and cotton seed cake. In the substrate supplemented with ground nut cake and molasses the enzyme production was decreased at higher concentrations.

The increase in CMCase production results in production of higher amount of cellulose by degradation of cellulose. The cellobiose acts as substrate for β-glucosidase resulting in the release of D-glucose. The cellulase and β-glucosidase enzymes are produced synergistically. β-Glucosidase hydrolyses cellobiose, which would otherwise accumulate during cellulose degradation, thereby repressing the cellulase synthesis (Horton and Keen, 1966; Loewenberg, 1984). The specific β-glucosidase activities are strongly induced by their natural substrates (Mallet and Debros, 2001). The same pattern has been proved in the present study when CMCase production was the lowest, while β-glucosidase production was recorded maximum on the 25\textsuperscript{th} day of corn cob substrate fermentation by
P. florida. This may be attributed to the accumulation of higher concentration of cellulobiose on 25th day that inhibited the CMCase production but β-glucosidase production was increased to hydrolyze cellulobiose.

The pattern for xylanase production in unsupplemented control substrate was variable with increasing the time of fermentation. The enzyme activity was increased to maximum during 10–15 days and then it was reduced to its lowest on 25th day, but again an increase on 30th and 40th day of incubation was observed. The similar pattern of enzyme production was recorded during each supplementation to the substrate. The xylanase activity was decreased with increasing concentration of various chemical and biological additives except for soybean meal addition, which led to slight increase in activity at higher concentrations. Sernanni et al. (1994) observed that the xylanase activity was recorded maximum after 11 days with P. ostreatus and L. edodes cultures. Singh et al. (1994) also reported the peak activity of xylanase after 10th day of fermentation.

The activity of β-xylosidase in the unsupplemented control substrate was increased continuously up to 20th day followed by slight decrease and becoming nearly constant thereafter until the final day of fermentation. The pattern of the enzyme production in nitrogen supplemented cases was nearly the same as it was recorded with control except for cotton seed cake. It is shown in Fig. 21 that the maximum enzyme units were produced in cotton seed cake supplementation on 15th day of fermentation. The enzyme production was affected by higher concentrations of various additives as the units were decreased at higher concentrations of all other additives except molasses and gram flour where an increased production was noted at higher levels.

The β-D-xylosidases exhibited hydrolytic activity on xylobiose and xylo-oligosaccharides, which was the result of xylanase activity on xylan (Garcia and Wood, 1993). The higher activity of xylanase and β-xylosidase indicate that xylanase attacked the main xylan chain together with β-xylosidase, which attacked on xylobiose or xylo-oligosaccharides and a variety of debranching enzymes (Biely, 1985; Gasparic et al., 1995).

It is evident from our results that CMCase and β-glucosidase activities are dependent on each other (Loewenberg, 1984). Similarly, xylanase and β-xylosidase activities are also dependent on each other (Garcia and Wood, 1993).
The decrease in the activity of various enzymes in the later stages of fermentation might be either due to proteolysis of these enzymes by the production and secretion of specific protease enzymes by white rot fungi (Dosoretz et al., 1990 a, b; Palmieri et al., 2000) or due to suppression of the genes coding for the enzymes (Wilson and Walker 1995).

The production of all the enzymes exhibited the typical pattern of enzyme induction and catabolite repression. The enzymes responsible for the degradation of hemicellulose and cellulose are inducible meaning thereby that their synthesis occurs only in the presence of specific inducers. However, all the inducible enzymes are supposed to be under the control of catabolite repression, i.e. the synthesis of the inducible enzymes does not occur even in the presence of specific inducer if easily metabolizable water soluble carbohydrates are also present. In the present study also the production of CMCase, xylanase, β-glucosidase and β-xylosidase distinctly appear to follow the same pattern in unsupplemented as well as nitrogen supplemented corn cob substrate fermentation during 40 days by *P. florida*. During the initial days of fermentation the presence of water soluble carbohydrates in the corn cob substrate does not allow the inducers to induce the synthesis of inducible enzymes. Once the water soluble carbohydrates are exhausted in the medium, the next most easily fermentable carbohydrate is hemicellulose, and therefore, xylanase followed by β-xylosidase are induced. The maximum units of CMCase are invariably produced in all the cases during 30-40 days fermentation and thereby indicating the utilization of cellulose in the end of the fermentation process. The alternate increase and decrease in the units of all the enzyme production could be attributed to the alternate availability of water soluble carbohydrates (monosaccharides, disaccharides and oligosaccharides), thereby exhibiting the effect of catabolite repression.

4.3. Effect of Nitrogen Supplements on Fruitbody Yield of *P. florida*

Amendments used in mushroom cultivation since the inception of supplementation in mushrooms are of both animal as well as plant origin, ranging from carbohydrate, protein and oil rich substances. Sinden and Schisler (1962) listed a range of products and compared the nutritional values of both carbohydrate rich and protein rich materials, alone and in combination. Protein rich materials gave better results. Supplementation from vegetable origin includes
vegetable meals such as, cotton seed meal, soybean meal, gram flour, ground nut cake, mustard cake, cotton seed cake and molasses. Lignocellulosic substrate supplementation with nitrogenous additives have been recommended for enhancing the mushroom yield of *Pleurotus* spp. (Pal and Paul, 1985; Goswamy *et al.*, 1987; Azizi *et al.*, 1990; Gupta *et al.*, 1991; Singh, 1998).

The cultivation of *P. florda* was carried out on corn cob substrate in polyethylene bags. Two kilograms of corn cob substrate with and without nitrogen supplements was filled in polyethylene bags after mixing with various nitrogen supplements at different levels (w/w). Fifty gram of fungal inocula was used as spawn for the spawning of each bag. No supplementation to the corn cob served as control. The mouths of spawned bags were tied and incubated in mushroom house at $27\pm{1}$ °C. The substrate was colonized by the fungal mycelia within 15 days in all sets of the experiments. Following substrate colonization, the polyethylene bags were tared to spray fresh water, after which the fructification was started and the fruitbodies were harvested during 30-40 days. The yield data were recorded in grams as fruit body yield of *P. florda*. The fructification of *P. florda* is shown in Plates I and II.

### 4.3.1. Effect of urea

Urea was added as a nitrogen supplement at 0.5, 1.0 and 1.5 % (w/w) to corn cob substrate for large scale oyster mushroom production by *P. florda* during 40 days of fermentation. There was 1430 g fruitbody yield from 2 kg unsupplemented control substrate, and 1675 g from the corn cob substrate added with 0.5 % (w/w) urea. In case of 1.0 % (w/w) urea supplementation the fruitbody yield was recorded as 1525 g, while, from the substrate supplemented with 1.5 % (w/w) urea, 1450 g of fruitbody yield was achieved (Fig.26). Accordingly, there was a considerable decrease in biological efficiency of *P. florda* with increasing the level of urea as nitrogen supplement, i.e., biological efficiency decreased from 83.75 to 76.25 and 72.50 % respectively in 0.5, 1.0 and 1.5 % levels of urea amended to the substrate. On the other hand, the biological efficiency of unsupplemented control set was only 71.50 %. These fruitbody yield results support our findings at flask level experiments that higher levels of urea led to decreased degradation of lignocelluloses, which in turn influenced fruitbody yield. However, the biological efficiency of the urea supplemented substrate was
Plate I. Fruitbody yield of *P. florida* on unsupplemented (a), urea (b), ammonium sulphate (c), gram flour (d) and soybean meal (e), supplemented corn cob substrate.
Plate II. Fruitbody yield of *P. florida* on mustard cake (a), ground nut cake (b), cotton seed cake (c) and molasses (d), supplemented corn cob substrate.
comparatively higher than unsupplemented control substrate. Our results are in agreement with the findings of Singh (1998), who also obtained exactly the same yield of *P. florida* sporophores when grown on unsupplemented mixture of sugarcane bagasse and wheat straw (1:1). In general, supplementation of urea increased the biological efficiency and yield response of *P. florida* sporophores, however, the increase was significant at low concentration (0.5 %), while it was marginal at 1.5 % (w/w) level of urea. Contrary to our findings, Singh (1998) reported decreased yield and biological efficiency with all the levels of urea supplemented, which could be attributed to the use of different lignocellulosic substrate (sugarcane bagasse) and higher concentrations (2-5 % w/w) of urea supplemented. The urea is known to have toxic effect at higher concentrations on the growth of all the microorganisms (Chang, 1972; Ralph and Kurtzman, 1994).

4.3.2. Effect of ammonium sulphate

The *P. florida* sporophores yield response on 0.5, 1.0 and 1.5 % (w/w) ammonium sulphate supplemented corn cob substrate was 1815, 1725 and 1600 g/2 kg substrate, respectively (Fig. 26). The results indicate that there was a significant decrease in fruitbody yield with increasing the concentration of ammonium sulphate. Accordingly, the respective biological efficiencies were 90.75, 86.25 and 80 %, respectively as compared to unsupplemented control (71.50 %). Ammonium sulphate supplementation exhibited the positive influence on fruitbody yield and biological efficiency, but the response was relatively better than that of urea supplementation. Similar to urea the higher concentrations of ammonium sulphate than 0.5 % (w/w) supplementation were found to be toxic for both yield response and biological efficiency. In other words, urea was comparatively more toxic than ammonium sulphate. Our results are in accordance with the findings of Singh (1998), who has reported better sporophore yield with ammonium sulphate supplementation as compared to urea addition. However, he reported decreased yield upon ammonium sulphate addition as compared to unsupplemented sugarcane bagasse substrate. We obtained 90.75 % yield response of *P. florida* sporophores on corn cob substrate supplemented with only 0.5 % (w/w) ammonium sulphate. This can well be attributed to the choice of substrate and level of supplementation for the growth and fruitbody yield of *P. florida*.
Fig. 26. Effect of chemical supplementation to corn cob substrate on fruitbody yield of *Pleurotus florida*. 
### 4.3.3. Effect of gram flour

Among various nitrogen supplements attempted in the present investigation, gram flour was found to be the poorest and yielded poor sporophore response than even urea at the lowest level of supplementation. However, at the higher concentration of gram flour and urea supplementation, the yield response with the former was comparatively much better than the later as a nitrogen source. However, both the sporophore yield response and biological efficiency in urea and gram flour supplemented corn cob substrate were better than the unsupplemented substrate. The fruitbody yield and biological efficiency resulted due to gram flour supplementation at 2, 3 and 5 % (w/w) were 1640, 1635, 1510 g/2 kg corn cob substrate and 82.00, 81.75 and 75.50 %, respectively (Fig. 27). The increased fruitbody yield and hence biological efficiency of various mushroom fungi due to gram flour supplementation to various substrates have also been reported by several researchers (Bano et al., 1978, 1979; Bano and Rajarathnam, 1983; Balakrishnan and Nair, 1995).

### 4.3.4. Effect of soybean meal

The supplementation of corn cob substrate with 2 % (w/w) soybean meal produced 1860 g fruitbody yield followed by 3 % and 5 % (w/w) levels, which gave an yield response of 1775 and 1580 g, respectively (Fig. 27). Thus, there was a marked decrease in sporophore yield with increasing the levels of soybean meal. The decreasing order of biological efficiency of *P. florida* was 93.00 > 88.75 > 79.00 % with increasing 2, 3, and 5 % (w/w) levels of soybean meal. However, both yield response and biological efficiency of soybean meal supplemented corn cob substrate was always higher than the unsupplemented substrate. The soybean meal is one of the most widely used supplement today, which generally gives the best results. This additive usually serves as a source of proteins and fat and is called first generation supplement (Abell, 1988). Our findings also indicate that soybean meal has been one of the best sources of nitrogen and other nutrients for growth and fructification of *P. florida*. The similar observation for the better yield response and biological efficiency of various mushroom fungi have also been reported by various researchers with soybean meal supplementation to various substrates (Bahram, 1989; Gerritis, 1986,1989; Balakrishnan and Nair, 1995).
4.3.5. Effect of mustard cake

The production of fruit bodies from the corn cob substrate supplemented with 2, 3 and 5 % (w/w) mustard cake were 1850, 1680 and 1550 g (Fig. 27), corresponding with the biological efficiency 92.5, 81.5 and 77.5 %, respectively, which was much higher, particularly at low levels of supplementation, as compared to the unsupplemented control corn cob substrate. Contrary to our findings, several researchers have found and reported negative response due to mustard cake supplementation (Vijay and Upadhyay, 1990; Bahl, 1991; Gupta and Vijay, 1994; Singh, 1998). The better yield at 2 % (w/w) level in our study also corresponded with better degradation of various constituents of corn cob substrate. However, higher level of nitrogen has been proved inhibitory to the fungal growth (Kirk et al 1978; Munoz et al 1983; Tripathi and Yadav, 1992), which in turn adversely affected the product yield and decreased the biological efficiency at higher levels of supplements.

4.3.6. Effect of ground nut cake

The lowest concentration at 2 % (w/w) of ground nut cake amendment to corn cob substrate employed in the present investigation resulted in relatively higher yield response of 1850 g/2 kg substrate, with biological efficiency of 92.50 %. However, when the level of ground nut cake was increased at 3 and 5 % (w/w), both the sporophore yield as well as biological efficiency tremendously decreased to 1420, 1230 g/2 kg substrate (Fig. 27) and 71, 61.5 %, respectively, which were even lower than unsupplemented control (71.5 %) corn cob substrate. The ground nut cake contains high content of protein, nitrogen and also oil, which at high level is believed to suppress the lignin degradation that may lead to reduced fruitbody yield. Further, the low lignin degradation may be the result of interference of nitrogen metabolism with that of lignin metabolism. Similar findings have also been reported by Bano and Rajarathnum (1983). In our study, the better sporophore yield at 2 % (w/w) ground nut cake supplementation corresponded with better degradation of hemicellulose and cellulose. These findings are also supported by the results of other researchers (Bano et al 1979; Bano and Rajarathnum, 1983; Gupta and Vijay, 1994). Bahl (1991) has reported that though, ground nut cake has higher nitrogen (7.7 %), the increase in yield was only 3.3 %. This shows that the increase is not proportional to the amount of

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nitrogen present in the material because it has been well established that higher nitrogen concentration is inhibitory for mushroom. The same reason is, therefore, attributed for very poor yield of mushroom in the present investigation at high levels of ground nut cake supplementation.

4.3.7. Effect of cotton seed cake

The cotton seed cake was found to be the best supplement yielding 1875, 1825 and 1750 g fruitbodies (Fig. 27), respectively at 2, 3 and 5 % (w/w) supplementation, with a corresponding biological efficiency of 93.75, 91.25 and 87.50 %, respectively as compared to unsupplemented control. Similar observations have been made by several other researchers (Bano et al., 1979; Bano and Rajrathnum, 1983; Balakrishnan and Nair, 1995). Bisaria et al (1987) have reported better yield with the addition of cotton seed cake to the paddy straw substrate. Bahl (1991) has also reported that the cotton seed was the best supplement to the compost for the production of button mushroom. Ertan (1988) also reported better yield with addition of cotton seed cake to the wheat straw substrate. In our study, the decrease in fruitbody yield at higher than 2 % (w/w) supplementation was due to lower degradation of various constituents of corn cob substrate by *P. florida*.

4.3.8. Effect of molasses

The molasses supplementation at 2, 3, and 5 % (w/w) to the corn cob substrate resulted in 1840, 1770 and 1630 g fruitbody yield/2 kg substrate (Fig. 27), with a concomitant biological efficiency of 92, 88.5 and 81.5 %, respectively as compared to unsupplemented control (71.5 %). High fruitbody yield from the molasses may be attributed to the availability of water soluble carbohydrates in addition to available nitrogen and other growth factors. The decrease in biological efficiency at higher levels of supplementation could be due to decrease in degradation of various constituents of corn cob substrate (Yadav 1987).

Supplementation with delayed release of nutrients has been found to be an important factor in production of *Pleurotus* species: *Pleurotus* species and their varieties are grown on a wide variety of substrates ranging from straws, cotton waste, pulp waste, saw dust, other cellulosic materials and agricultural byproducts. In several laboratory studies and commercial production trials, delayed release of
Fig. 27. Effect of biological supplementation to corn cob substrate on fruitbody yield of *Pleurotus florida*.
nutrients has proved effective for *P. ostreatus*, *P. sajor-caju* and *P. pulmonarius* by (a) decreasing the harvesting time, (b) decreasing in disease incidence, (c) increase in fruitbody yield of mushroom, and (d) increase in number of production cycles per room/year.

The growth requirements vary for different species of this mushroom. Under natural conditions, *Pleurotus* spp. mostly grows on dead parts of plants, which are generally poor in nutrients and vitamins. But, it has been established by various workers that both mycelial growth and fruitbody development depends on lignocellulosic materials or C:N ratio in the substrate, which should be at least 1:50 (Balakrishnan and Nair 1995).

The corn cob substrate does not have adequate amount of nitrogen and other nutrients required to start the fermentation process due to C/N ratio less than required. So, the compounding mixture is supplemented with other materials having nitrogen and carbohydrate source. The nitrogen contents in most of the substrates range between 0.5 to 0.8 %, hence addition of organic nitrogen in the substrate helps in getting higher mushroom yield (Pal and Paul, 1985; Goswamy *et al.*, 1987; Azizi *et al.*, 1990; Vijay and Upadhyay, 1990; Gupta *et al.*, 1991).

The oyster mushrooms give 80-90 % biological efficiency in 50-60 days of harvest. Betterley (1988) found that with supplementation, the biological efficiency up to 90 % could be obtained within 35 days of cropping. In the present study, the maximum biological efficiency up to 93.75 % has been achieved with 2 % (w/w) cotton seed cake supplementation to corn cob substrate.

The comparison of the results regarding supplementation of various supplements revealed that i) the fruitbody yield with all the supplements was higher as compared to the control, ii) the reduced yield response was noted in all the cases at the higher level of supplements. Gupta and Vijay (1991) also reported that supplementation above 2 % (w/w) on dry weight basis resulted in undue heating of compost, which increased the incidence of weed moulds. Further, Dhar and Kapoor (1990) have also observed decreased yields at dosage of supplements higher than 3 %. Our findings are in full agreement with the aforementioned observations, iii) Two supplements *viz.*, cotton seed cake and soybean meal, which are most widely used today gave the best results and therefore, they were so far considered as first generation supplements. However, our findings have revealed that other supplements such as ground nut cake, mustard cake and molasses can be safely added to this list at lower (2 % w/w) level of
supplementation, iv) the order of nitrogen supplements with regard to fruitbody yield could be presented as: cotton seed cake > soybean meal > mustard cake > ground nut cake > molasses > ammonium sulphate > urea> gram flour, as compared to the unsupplemented corn cob substrate (control), which yielded minimum fruitbody yield response. Schisler and Sinden (1966) found that ground soybean meal was a better supplement than defatted soybean meal. Soybean meal is rich in protein and fats which are supposed to increase mushroom yields when used as a supplement, by promoting growth of mycelium by certain amino acids present in soybean protein (Randle, 1985; Gupta and Vijay 1991).

4.4. Marketing and Economic Avenue of Mushroom Cultivation

Marketing and Economic avenue is the primary objective in any business enterprise, so too in mushroom cultivation. The growth and stability of the mushroom units are important to build up the production potential of the unit to ensure better income. Mushroom cultivation being one of the unique enterprises, where unlike other farm activities, competition would not be for land, but for labour and capital.

Species of *Pleurotus* are cheapest and easiest to grow among all the cultivated edible mushrooms. *Pleurotus* species do not require much exacting conditions, and can tolerate much higher concentration of carbon dioxide. Furthermore, since *Pleurotus* species can easily be dried either in sun or mechanically, there is no expenditure needed for establishment of canning unit. The ability of *Pleurotus* species to tolerate wider range of temperature, i.e. 15-28 °C (both during spawn run and primordial formation) makes it cheapest to grow without spending for sophisticated air conditioning unit.

Cultivation of *Pleurotus* spp. which is not more technical but skilled activity involves investment depending upon the size of the unit/production targets. Before starting this venture one should have sufficient knowledge of this activity and should survey the market properly for disposal of the produce and then one should frame his project minimum enough to be economically viable. For economic evaluation of this enterprise it would thus be important to understand various costs and return components that can be used for judging economic efficiency of this farm enterprise. The expenditures in the mushroom unit can be divided into:
a) Recurring and
b) non recurring expenditure

**a) Non-recurring expenditure**

i. Construction cost of thatched house 20' x 15' x 10'  
Rs. 10,000.00

ii. Sprayer pump- one  
Rs. 1,500.00

iii. Galvanized tubs of empty diesel drums painted inside (4)  
Rs. 1,000.00

iv. Thermometer (max and min, dry and wet)  
bulb thermometer and bed thermometer  
Rs. 200.00

v. Bamboo racks @ Rs. 500 each (9 nos.)  
Rs. 4,500.00

**TOTAL**  
Rs. 17,200.00

**b) Recurring expenditure (Cost of raw materials)**

i. Maize cob (corn cob) 5 qt. @ Rs. 50/qt  
Rs. 250.00

ii. Cost of 250 polyethylene bags  
(125-150 gauze thick and 18" x 22" cm) @ Rs. 0.84 per bag  
Rs. 210.00

iii. Cost of 85 spawn bottles @ Rs. 20 per bottle  
Rs. 1,700.00

iv. Labour wages for 40 days  
Rs. 2,320.00

(one labourer was hired from the market for six days during spawning and 34 days while cropping) @ Rs. 58/- per day

v. Chemicals  
a). Bavistin 7 g per treatment of 15 kg corn cob  
Rs. 100.00

Total bavistin requirement 200 g for 5 qt corn cob.

b) Formaldehyde 3.5 litre @ Rs. 30 per litre  
Rs. 105.00

vi. Miscellaneous charges (water and electricity; pesticide, small polyethylene bags for packing, etc)  
Rs. 300.00

**TOTAL**  
Rs. 4,985.00

Depreciation @ 10% + interest 10% on the construction of thatched house growing unit for 6 crops in a year (Rs. 1720 + 1720).  
Rs. 3,440.00

Depreciation and interest for one crop  
Rs. 573.00

Total expenditure for one crop (Rs. 4985 + 573)  
Rs. 5,558.00

Expected yield 80% biological efficiency (400 kg fresh mushroom)
<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production cost of 1 Kg oyster mushroom</td>
<td>Rs. 13.90</td>
</tr>
<tr>
<td>Income from sale @ Rs. 30 per kg</td>
<td>Rs. 12,000.00</td>
</tr>
<tr>
<td>Net profit from one crop</td>
<td>Rs. 6,442.00</td>
</tr>
<tr>
<td><strong>Annual profit (6×6442)</strong></td>
<td><strong>Rs. 38,652.00</strong></td>
</tr>
</tbody>
</table>