(3) RESULT AND DISCUSSION

3.1 QUALITATIVE ANALYSIS

Qualitative analysis was carried out to select the fungal copartner for the study. Analysis carried to identify and categorise the selected fungi on the basis of their lignocellulolytic and xylanolytic enzyme activity. These fungi were further subjected to identify their compatibility potential by performing paired interaction test and the best compatible fungal isolates were selected for further study.

3.1.1 Lignocellulolytic enzyme activity of fungi

(a) Screening of ligninolytic and cellulolytic enzyme activity of fungi

During the initial screening, a total of 12 fungal isolates were isolated on PDA medium. All the fungi were screened for extracellular ligninolytic, cellulolytic and xylanolytic activity on Malt extract agar medium containing 1% tannic acid, 1% CMC and 1% xylan as sole carbon source.

In identifying an unknown fungus for its potential use in paper industry one of the first question is whether this fungus is ligninolytic or cellulolytic. This is usually determined by the well known Bavendamn test. It is known since long that mycelia of certain higher fungi contain enzymes which catalyze the oxidation of phenols and related compounds. Bavendamn (1928) was the first to point out the difference between the white rot and brown rot fungi with respect to their oxidative enzymes. When white rot fungi cultivated on nutrient agar containing certain phenolic compounds as gallic acid or tannic acid, the white rot fungi produce a deeply coloured zone around the mycelium while the fungi brown rot do not. The results from the Bavendam test provided an evidence for the presence of ligninolytic enzyme activity.

Enzymatic activities of 12 fungal isolates have been screened by using solid media containing coloured indicator compound which enables visual detection of

enzyme production and darkbrown colour zone and its intensity indicating positive extracellular enzyme production and results obtained by Bavendamn's test has been represented in Table 1 and Plate 1 and Plate 2.

Out of 12 fungi all fungi except *Trichoderma reesei* showed positive reactions to tannic acid, indicating them to release lignin degrading enzymes and hence are potential lignin degraders. After seven days of incubation the medium which was substituted with tannic acid showed the culture with brown coloration zone when viewed from the lower side of the petridish indicating presence of ligninolytic enzymes. Petridish substituted with CMC (carboxy methyl cellulose) after one week incubation period when flooded with Congo red did not show clear zone except in TV and TH confirming the presence of ligninolytic enzymes and absence of cellulolytic enzymes.

(b) Screening of xylanolytic enzyme activity of fungi

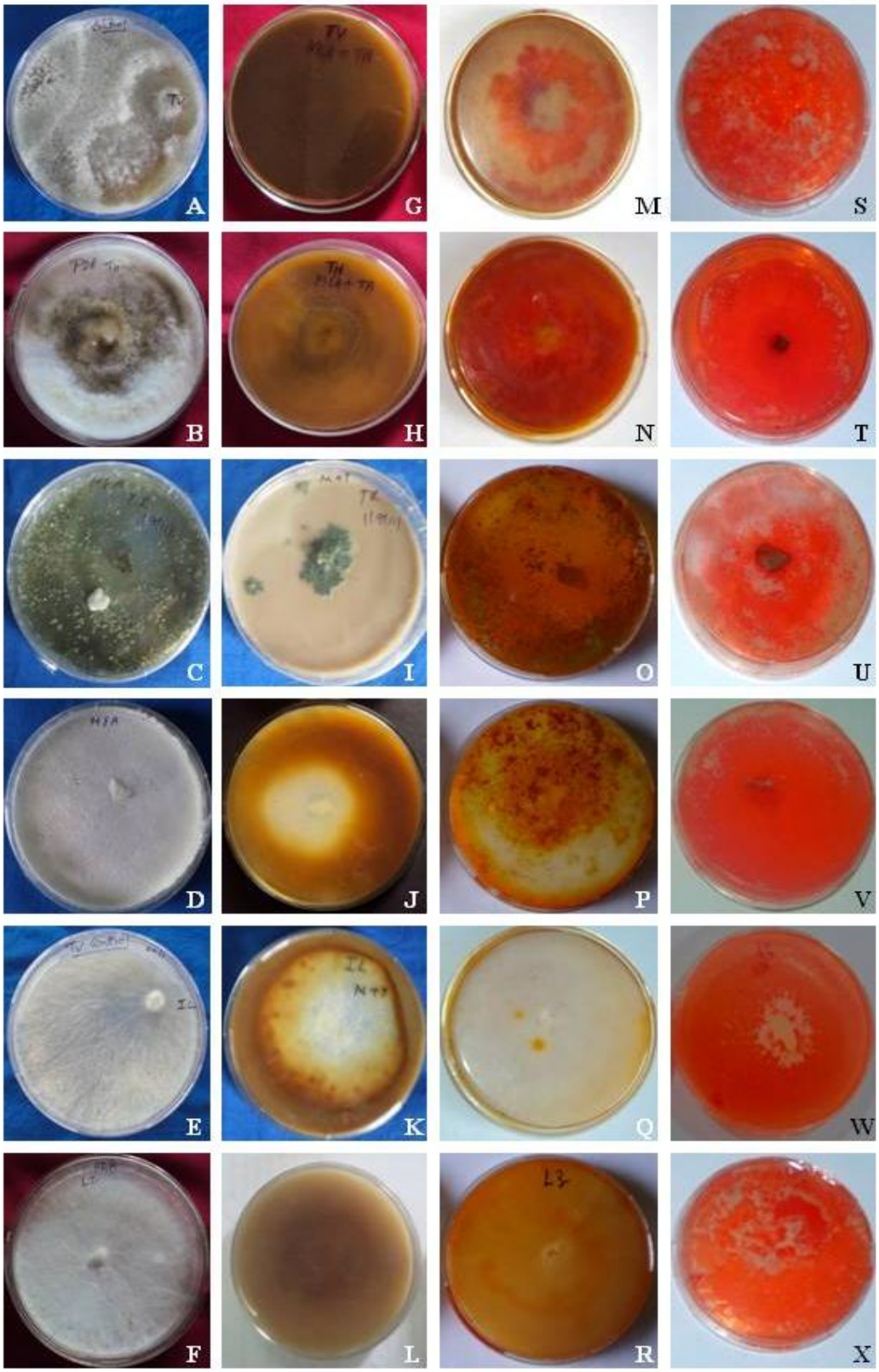
Xylanases in combination with cellulases have applications in food processing (Biely 1985) whereas xylanases without cellulases are important in paper industry as the quality of paper depends upon the amount of cellulose present in the paper. Therefore, to select potential cellulase-free xylanase producing fungi, rapid plate technique was carried out and enzyme activities (xylanase and cellulase) were measured. One of the major problems in screening large number of microbial strains for their xylanase producing ability, is the lack of single rapid reliable screening technique. Hence, solid agar screening method was used for screening. Initially, solid screening medium containing xylan as the sole carbon source developed for this purpose was employed by (Flannigan and Gilmour 1980).

Xylanase producing organisms were identified on the basis of the clearing zone formed around the colonies. Petriplates substituted with 1% xylan after one

week incubation period when flooded with congo red and washed by 1% NaCl solution, all the fungi except PO, DC and PC showed zone of clearance showing positive reaction for xylanase enzyme activity. The results of ligninolytic, cellulolytic and xylanolytic activity are represented in Table 1. All the screened fungi except TR produces ligninolytic enzymes. TV and TH showed positive reaction for ligninolytic, cellulolytic and xylanolytic activity. Except TV and TH all the test fungi shows negative reaction to celluloytic activity. TR shows an absence of ligninolytic and xylanolytic activity but positive xylanolytic activity.

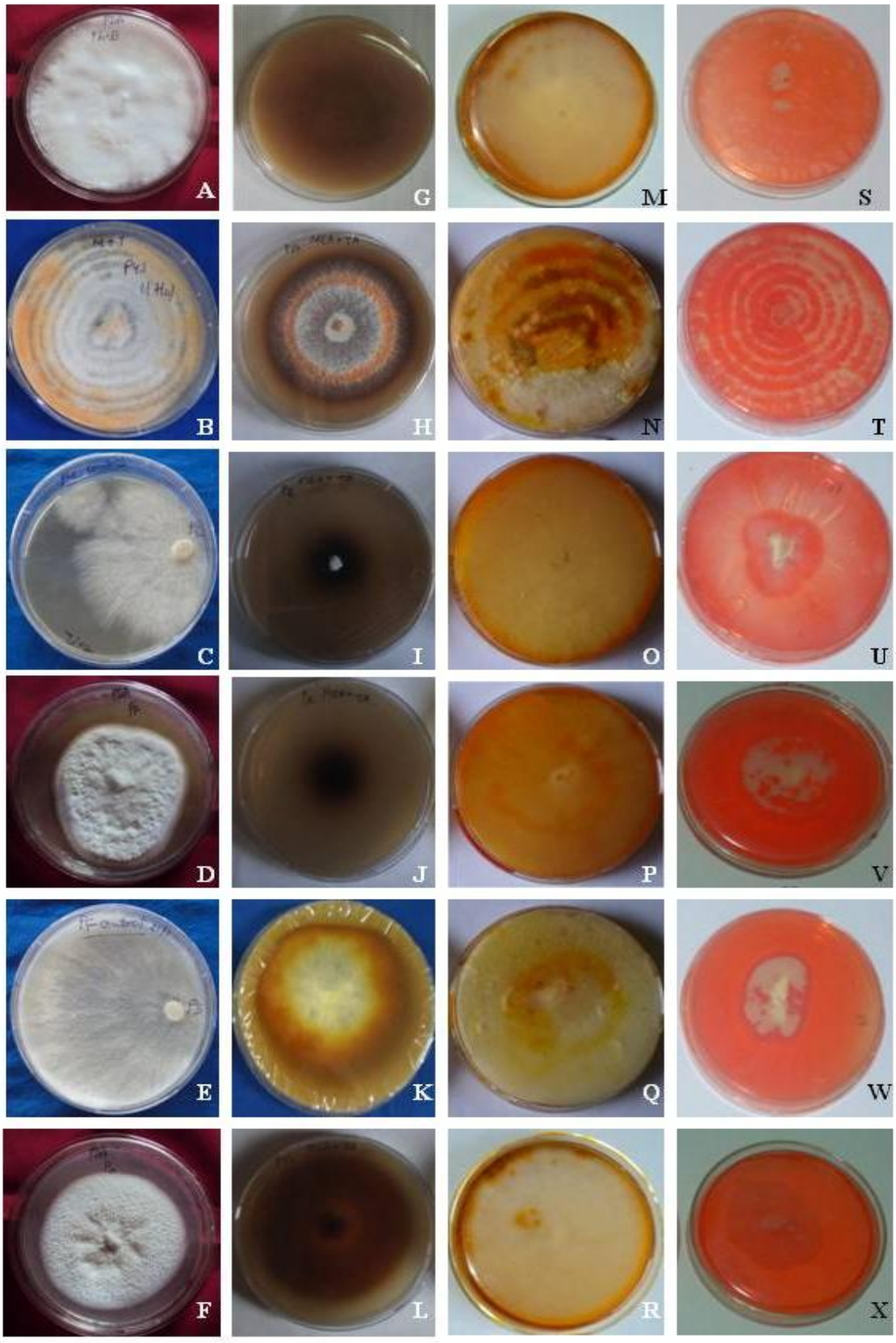
Fungal isolates	Ligninolytic	Cellulolytic	Xylanolytic
TV	Positive	Positive	Positive
TH	Positive	Positive	Positive
TR	Negative	Negative	Positive
PC	Positive	Negative	Positive
IL	Positive	Negative	Positive
DC	Positive	Negative	Positive
PHE	Positive	Negative	Positive
PYS	Positive	Negative	Positive
PE	Positive	Negative	Positive
PF	Positive	Negative	Negative
PS	Positive	Negative	Positive
PO	Positive	Negative	Negative

Table 1 Ligninolytic, Cellulolytic and Xylanolytic activity of different fungi



Fungal isolates showing ligninolytic, cellulolytic and xylanolytic activity

Plate 1



Fungal isolates showing ligninolytic, cellulolytic and xylanolytic activity

Plate 2

Plate 1

A-F: Growth of TV, TH, TR, PC, IL, DC on MEA medium

G-L: Growth of TV, TH, TR, PC, IL, DC on MEA medium + 1%Tannic acid

M-R: Growth of TV, TH, TR, PC, IL, DC on MEA medium + 1% CMC

S-X: Growth of TV, TH, TR, PC, IL, DC on MEA medium + 1% xylan

Plate 2

A-F: Growth of PHE, PYS, PE, PF, PS, PO on MEA medium

G-L: Growth of PHE, PYS, PE, PF, PS, PO on MEA medium + 1%Tannic acid

M-R: Growth of PHE, PYS, PE, PF, PS, PO on MEA medium + 1% CMC

S-X: Growth of PHE, PYS, PE, PF, PS, PO on MEA medium + 1% xylan

(3.1.2) Antagonistic activity and selection of fungi

(a) Paired interaction test

Various interactions of 12 different fungal isolates with each other were studied at three different incubation periods 3 days, 6 days and 9 days and even further days also. In these all combinations, growth of *Trichoderma* species was very fast compared to other fungal isolates and growth of *Pleurotus* species was found to be slower. The growth of other fungal isolates were slower then *Trichoderma* species but faster than *Pleurotus* species. Observations on the compatibility of the fungi in the co-culture / paired interaction tests could be distinguished into three categories.

1. Both fungi come in contact on the medium and growth of both fungal isolates are inhibited i.e. No further growth occurs once the two come in contact.

2. The two fungal isolates in the paired interaction test come in contact and growth of one is inhibited by the other but it is not killed. The fungal isolate grows on the counterpart.

3. The two fungal isolates in paired interaction test come in contact; one overgrows over the other and kills it.

A paired fungus was considered compatible once they come in contact and still each one grows over the other at its own pace with the formation of an overlapping zone which increases / advances towards both the sides. The growth of the fungal isolates with its counterpart has been represented individually in plate 3 to 26. Characteristic features of co cultures observed after the different incubation period have been represented in the text.

(1)*Trichoderma viride*

Trichoderma viride-Trichoderma harzianum (TV-TH) (Plate 3 A-C)

Both the fungi were grown equally on the 3rd day after inoculation. Sporulation of TV was started on the 6th day and at the same day both fungi came in to contact with each other and the growth of both fungi was stopped. The situation remains same on the 9th day and TV acts as an antagonist against Here both the fungi stopped growing further after they came in to contact with each other and showing dead lock type of interaction at the touching point.

Trichoderma viride-Trichoderma reesei (TV-TR) (Plate 3 D-F)

Both the fungi were grown equally on the 3rd day after inoculation. Sporulation of TR started on the 6th day and both fungi came in to contact with each other. TV did not grow further and growth of TR also restricted at the same time. On the 9th day situation remained same growth of both the fungi stopped. Here both the fungi stopped growing further after they came in to contact with each other and showing dead lock type of interaction at the touching point.

Trichoderma viride- Pleurotus eryngii (TV-PE) (Plate 3 G-I)

TV almost covered the whole plate on the 3rd day after inoculation and the growth of PE has just started here. Sporulation of TV was also started on the 3rd day. The growth of PE stopped on the 6th day and TV killed PE and acts as an antagonist. The situation remains same on the 9th day. Here the growth of PE has been started and TV over grew even on the inoculums disc of PE and killed it. This interaction is invasion/replacement.

Trichoderma viride- Pleurotus florida (TV-PF) (Plate 3 J-L)

It was seen that the growth of TV was very higher than PF and the growth of PS just started on 3rd day after inoculation and both fungi came in to contact with each other.

Plate 3 Interaction of *Trichoderma viridae* with other fungal isolates

A-C: TV-TH

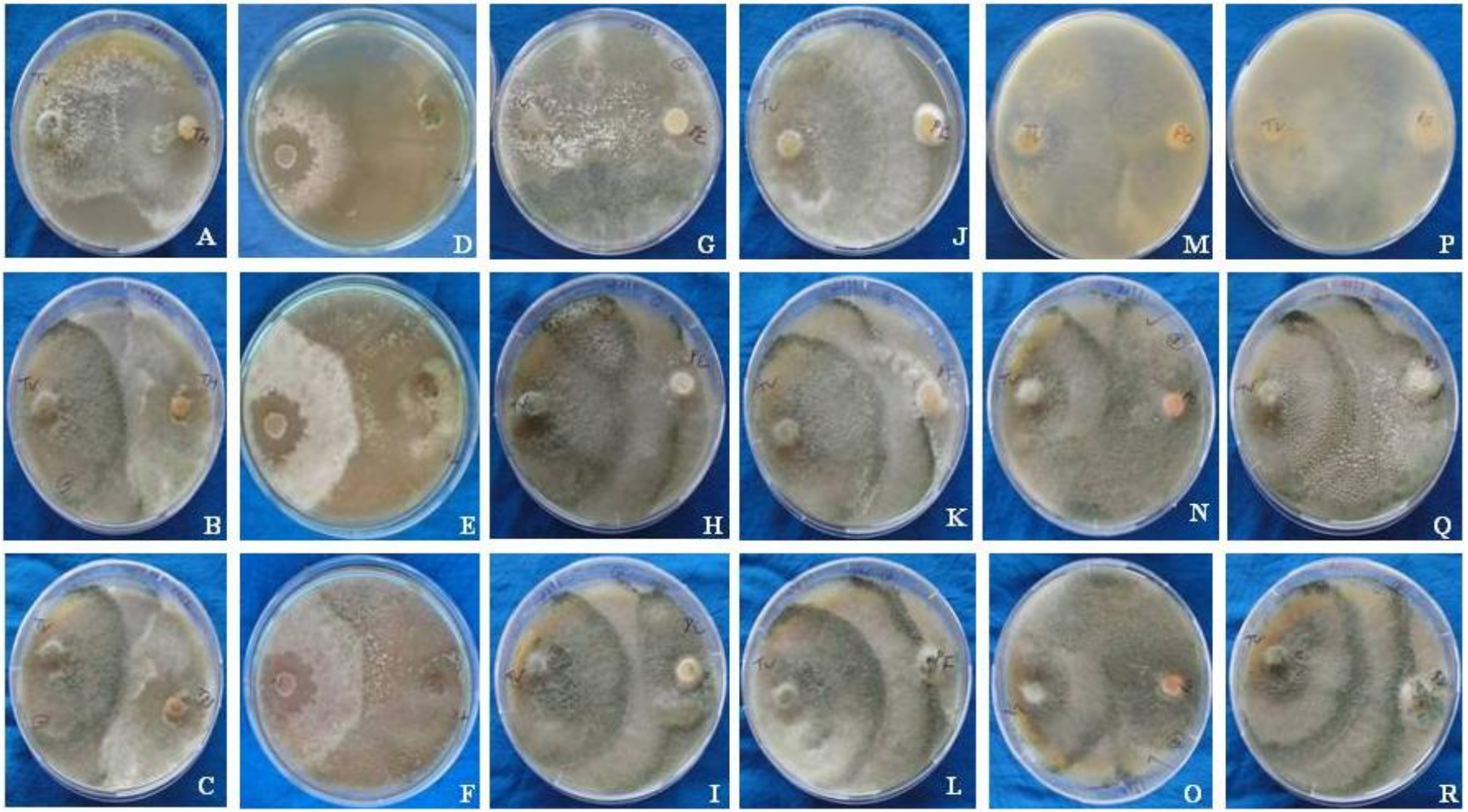
D-F: TV- TR

G-I: TV-PE

J-L: TV-PF

M-O: TV-PO

P-R: TV-PS



TV- TH TV-TR TV-PE TV-PF TV-PO TV-PS

Plate 3

Sporulation of TV was started on the 3rd day. The growth of PF inhibited by TV on the 6th day. TV over grew even on the inoculums disc of PF and killed PF on the 9th day and act as an antagonist against PF. This interaction is found to be invasion/replacement.

Trichoderma viride- Pleurotus ostreatus (TV-PO) (Plate 3 M-O)

TV almost covered the whole plate on the 3rd day. Sporulation has just started on the 3rd day after inoculation. The growth of PO started on the 6th day. TV over grew even on the inoculums disc of PO on the 9th day. The situation remains same even after one month also. TV act as an antagonist against PO and interaction is found to be invasion/replacement.

Trichoderma viride- Pleurotus sajorcaju (TV-PS) (Plate 3 P-R)

The growth of TV was more than PS on the 3rd day after inoculation. TV covered the whole plate on the 6th day and over grows on PS. The growth of PS stopped here. TV killed PS on the 9th day and act as antagonist against PS and interaction is found to be invasion/replacement.

Trichoderma viride-Irpex lacteus (TV-IL) (Plate 4 A-C)

The growth of TV was very higher than IL on the 3rd day after inoculation and sporulation of TV also occurred on the same day. Growth of IL also found on the 3rd day. On the 6th day growth of IL remains same but both the fungi came in to contact with each other. The growth of IL stopped on the 9th day but IL did not allow TV to grow further. Both fungi act as antagonist. This type of interaction is found to be deadlock at touching point.

Trichoderma viride- Daedaleopsis confragosa (TV-DC) (Plate 4 D-F)

The growth of TV was very high compared to DC on the 3rd day after inoculation and both the fungi came in to contact with each other on the same day. TV overgrows on

DC. DC grows further on the 9th day but after that the growth of DC becomes slow but it also over grew on TV. These two fungi are compatible with each other even the growth of TV was very rapid and this type of interaction is found to be partial mutual intermingling.

Trichoderma viride - *Phellinus pectinatus* (TV-PHE) (Plate 4 G-I)

The growth of TV was very higher than PHE on the 3rd day after inoculation. The both fungi came in contact with each other on the 6th day and PHE started to overgrow on TV. PHE overgrows on TV on the 9th day and show its compatibility with TV. This type of interaction was found to be partial mutual intermingling.

Trichoderma viride- Pycnoporus sanguineus (TV-PYS) (Plate 4 J-L)

The growth of TV was more than PYS on the 3rd day after inoculation as growth of PYS just started on this day. TV covers the whole plate and over grows on PYS. Sporulation of TV occurred on the 6th day and the growth of PYS stops here. TV killed PYS and the color of PYS inoculum change orange to black on the 9th day TV act as antagonist against PYS. Here TV overgrew on PYS till the inoculum disc and the interaction was found to be invasion/replacement.

Trichoderma viride- Phanerochaete chrysosporium (TV- PC) (Plate 4 M-O)

The growth of TV was more than PC and both the fungi came in to contact with each other on the 3rd day after inoculation. The growth of TV occurred and line of inhibition also clearly seen on the 5th day indicating the growth of PC was inhibited. On the 9th day TV covered the whole plate. Here the growth of PC restricted and TV overgrew on PC showing invasion/replacement type of interaction.

In the eleven interactions of different fungal isolates with *Trichoderma viride* it was very clearly seen that the growth of TV was very fast than other all fungal isolates.

Plate 4 Interaction of *Trichoderma viridae* with other fungal isolates

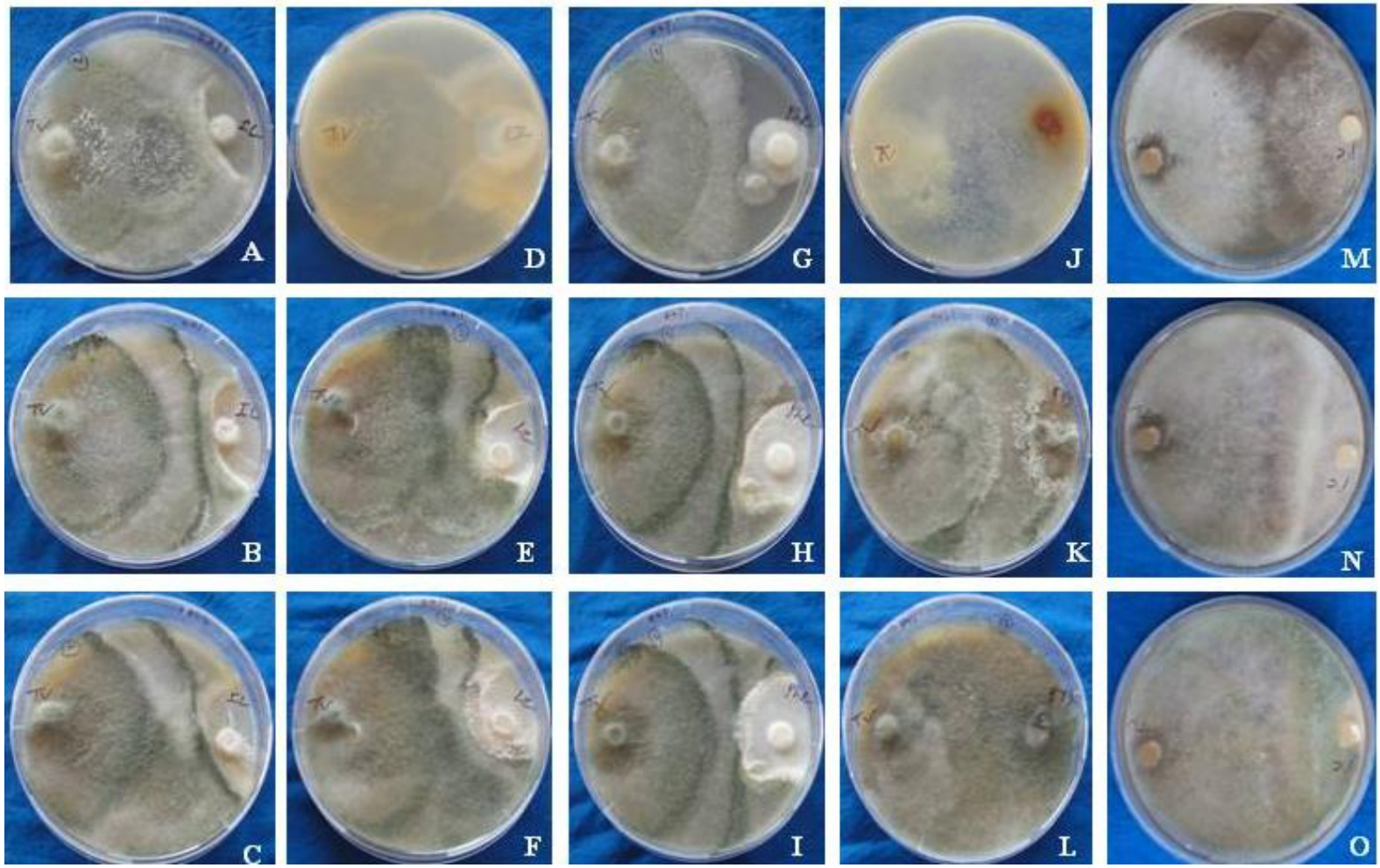
A-C: TV-IL

D-F: TV-DC

G-I: TV-PHE

J-L: TV-PYS

M-O: TV-PC



TV- IL TV-DC TV-PHE TV-PYS TV-PC

Plate 4

In all the interaction the sporulation of TV was started on the 3rd day except with TR, PC and PYS. The growth of *Pleurotus* species was very slow so it was replaced by TV and showed invasion/replacement while in the interaction with PYS and PC the growth of both the fungi slowed down due to antagonistic activity of TV and also showing invasion/replacement. The growth of other two *Trichoderma* species was faster and TV showed dead lock interaction at touching point with both TH and TR. In the interaction with DC and PHE though the growth of TV was fast it was partial mutual intermingling while with IL it showed deadlock at touching point. In all the eleven interactions of TV neutral intermingling and inhibition at distance was not observed.

(2)*Trichoderma harzianum*

Trichoderma harzianum-Trichoderma viride- (TH-TV) (Plate 5 A-C)

Both the fungi were grown equally on the 3rd day after inoculation. Sporulation of TV was started on the 6th day and at the same day both fungi came in to contact with each other and the growth of both fungi was stopped. The situation remains same on the 9th day and TV acts as an antagonist against TH. Here growth of both the fungi restricted after contact with each other so interaction was found to be dead lock at touching point.

Trichoderma harzianum- Trichoderma reesei (TH- TR) (Plate 5 D-F)

The growth of TR was more than TV on the 3rd day after inoculation and both fungi came in to contact with each other on the same day. Sporulation of TV was started on the 6th day and TH overgrows TR. The growth of both fungi stopped on the 9th day.

TR acts as an antagonist against TH. Here growth of both the fungi restricted after contact with each other so interaction was found to be dead lock at touching point.

Trichoderma harzianum- Pleurotus eryngii (TH-PE) (Plate 5 G-I)

The growth of TH was very higher than PE on the 3rd day after inoculation and the growth of PE has just started here. Sporulation of TH was started on the 6th day and the TH overgrew on PE and almost covered the whole plate and growth of PE stopped. The situation remains same on the 9th day. Here the growth of PE has been started and TH over grew PE. This interaction was found to be invasion/replacement.

Trichoderma harzianum- Pleurotus florida (TH-PF) (Plate 5 J-L)

The growth of TH was very higher than PF on the 3rd day after inoculation.TH overgrow on PF and almost covered the whole plate on the 6th day. The growth of PF still continued and PF also over grew on TH. In this interaction growth of TH was high but PF over grew on TH it was partial mutual intermingling type of interaction.

Trichoderma harzianum- Pleurotus ostreatus (TH-PO) (Plate 5 M-O)

The growth of TH was very higher than PO 3rd day after inoculation. Both the fungi came in to contact with each other on the 6th day. TH overgrows on PO and growth of PO was very less and stopped here. The situation remains same on the 9th day and TH act as an antagonist against PO and the interaction was found to be invasion/replacement as growth of PO was replaced by TH.

Trichoderma harzianum- Pleurotus sajorcaju (TH-PS) (Plate 5 P-R)

The growth of TH was more than PS on the 3rd day after inoculation. Both the fungi came in to contact with each other on the same day. TH covered the whole plate and over grows on PS on the 6th day and the growth of PS stopped here. Situation remained same on the 9th day. Here TH over grew on the growth of PS this interaction was found to be invasion/replacement.

Trichoderma harzianum-Irpex lacteus (TH-IL) (Plate 6 A-C)

The growth of TH was very higher than IL on the 3rd day after inoculation. Both the fungi came in to contact with each other on the same day. TH overgrow on IL.

Plate 5Interaction of *Trichoderma harzianum* with other fungal isolates

A-C: TH-TV

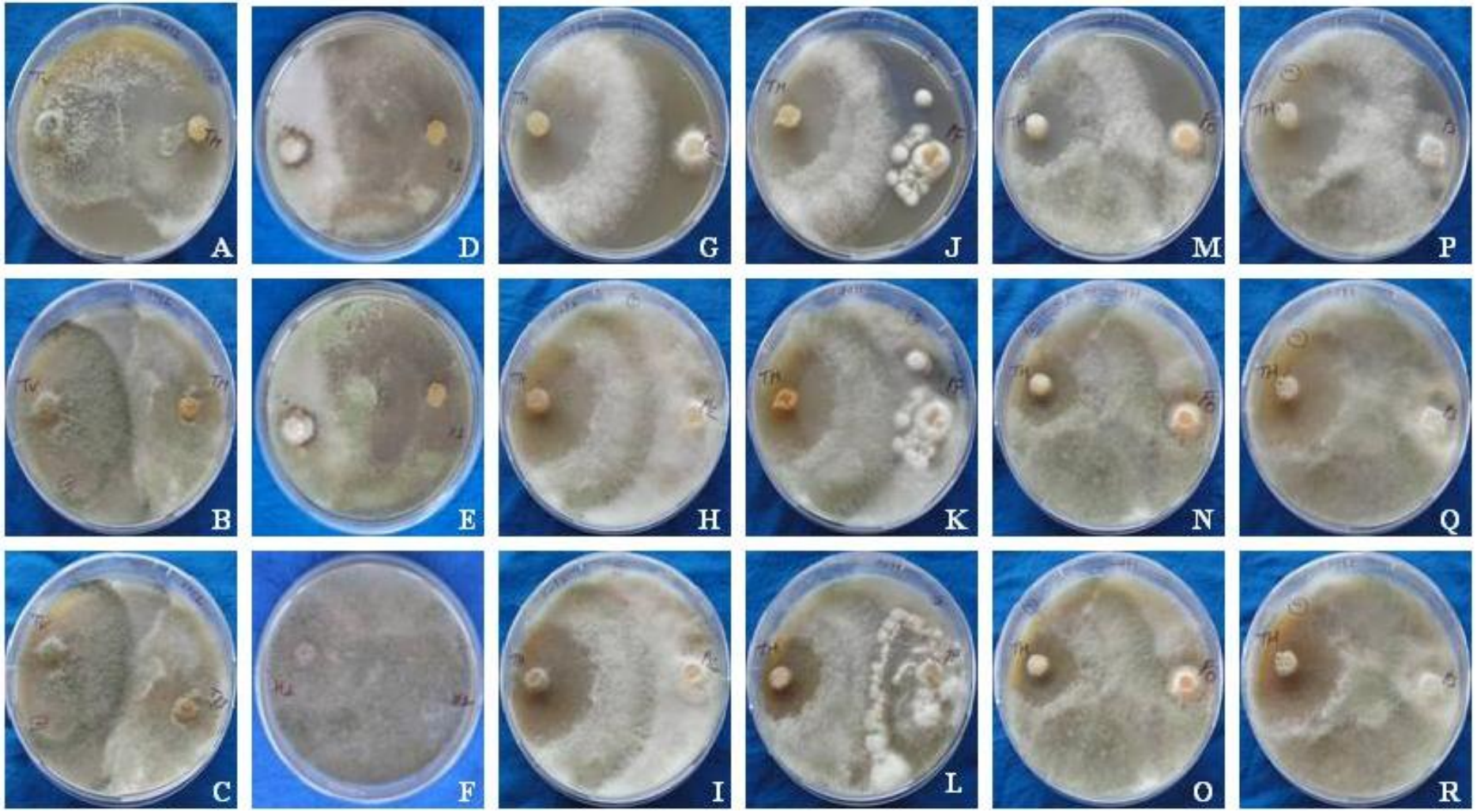
D-F: TH- TR

G-I: TH -PE

J-L: TH-PF

M-O: TH-PO

P-R: TH-PS



TH-TV TH-TR TH-PE TH-PF TH-PO TH-PS

Plate 5

TH almost covered the whole plate on the 6th day and the growth of IL was inhibited by TH on the 9th day and TH acts as an antagonist against IL. Here TH overgrew on IL and growth of IL was restricted so the interaction was found to be invasion/replacement.

Trichoderma harzianum- Daedaleopsis confragosa (TH-DC) (Plate 6 D-F)

The growth of TH was very high as compared to DC on the 3rd day after inoculation. Both the fungi came in to contact with each other and TH overgrows on DC on the 6th day. TH almost covered the whole plate on the 9th day but DC still overgrows on TH DC was found to be compatible with TH. The type of interaction was found to be partial mutual intermingling.

Trichoderma harzianum - Phellinus pectinatus (TH-PHE) (Plate 6 G-I)

The growth of TH was very higher than PHE on the 3rd day after inoculation. The both fungi came in contact with each other and TH almost covered the whole plate on the 6th day. PHE also overgrows on TH on the 9th day and further increase also showed its compatibility with TH. The type of interaction was found to be partial mutual intermingling.

Trichoderma harzianum- Pycnoporus sanguineus (TH-PYS) (Plate 6 J-L)

The growth of TH was more than PYS on the 3rd day after inoculation and both fungi came in contact with each other. TH over grew on PYS and growth of PYS was inhibited by TH on the 6th day. TH killed PYS and the color of PYS inoculum change orange to brown on the 9th day. The interaction was found to be invasion/replacement as the growth of PYS was restricted after overgrowth of TH.

Trichoderma harzianum - Phanerochaete chrysosporium (TH- PC) (Plate 6 M-O)

The growth of TH was more than PC and both the fungi came in to contact with each other on the 3rd day after inoculation. TH advanced up to ¾ of the petridish in 5 days.

Plate 6 Interaction of *Trichoderma harzianum* with other fungal isolates

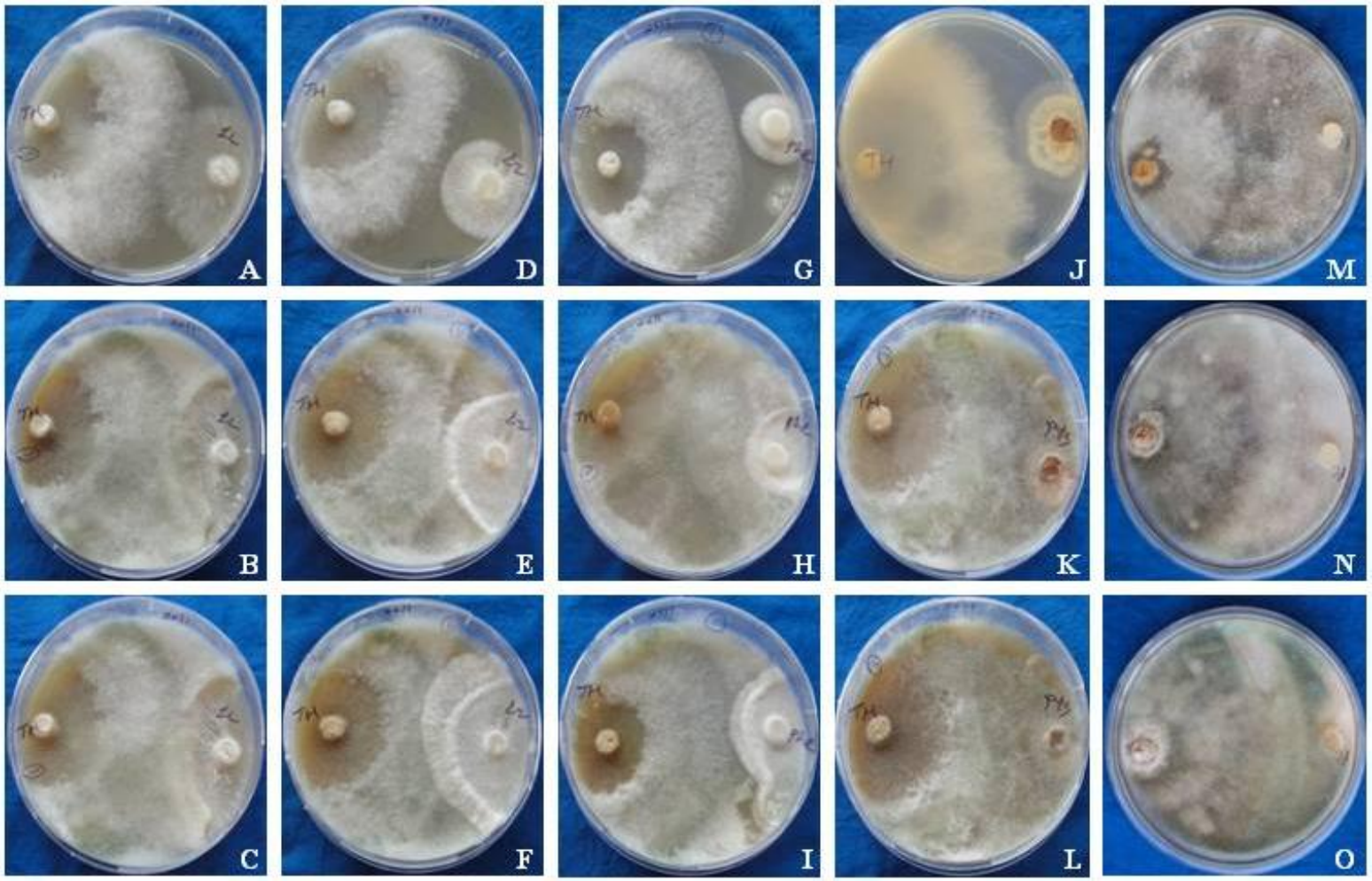
A-C: TH-IL

D-F: TH-DC

G-I: TH-PHE

J-L: TH-PYS

M-O: TH-PC



TH-IL TH-DC TH-PHE TH-PYS TH-PC

Plate 6

TH overgrows on PC and the growth of PC stop on the 10th day.TH cover the whole plate. Here TH overgrew on PC and it also restricted growth of PC and the interaction was found to be invasion/replacement.

In the eleven interactions of different fungal isolates with *Trichoderma harzianum* it was observed that the growth of TH was very fast than other all fungal isolates. The growth of *Pleurotus* species was very slow so it was replaced by TH and showed invasion/replacement except PF where the growth of PF continued on TH and showed partial mutual intermingling. The growth of other two *Trichoderma* species was faster and TH showed dead lock interaction at touching point with both TV and TR. In the interaction with DC and PHE though the growth of TH was very fast it was partial mutual intermingling while with IL, PYS and PC it showed invasion/replacement. In all the eleven interactions of TH neutral intermingling and inhibition at distance was not observed.

(3)*Trichoderma reesei*

Trichoderma reesei-Trichoderma viride (TR-TV) (Plate 7 A-C)

Both the fungi were grown equally on the 3rd day after inoculation. Sporulation of TR started on the 6th day and both fungi came in to contact with each other. TV did not grow further and growth of TR also restricted at the same time. On the 9th day situation remained same growth of both the fungi stopped. place. Here both the fungi stopped growing further after they came in to contact with each other and showing dead lock type of interaction at the touching point.

Trichoderma reesei- Trichoderma harzianum (TR-TH) (Plate 7 D-F)

The growth of TR was more than TV on the 3rd day after inoculation and both fungi came in to contact with each other on the same day. Sporulation of TV was started on the 6th day and TH overgrows TR. The growth of both fungi stopped on the 9th day

and TR acts as an antagonist against TH. Here growth of both the fungi restricted after contact with each other so interaction was found to be dead lock at touching point.

Trichoderma reesei - Pleurotus eryngii (TR-PE) (Plate 7 G-I)

TR almost covered the whole plate on the 3rd day after inoculation and the growth of PE was very slow and has just initiated. Sporulation of TR started on the 6th day and the growth of PE still continued with TR and grown till the inoculum disc of PE. Here the growth of PE was covered by TR so the type of interaction was found to be invasion/replacement.

Trichoderma reesei - Pleurotus florida (TR-PF) (Plate 7 J-L)

It was seen that the growth of TR was higher than PF and the growth of PF has started on the 3rd day after inoculation and both fungi came in to contact with each other. Sporulation of TR was started on the 6th day and the growth of PF was replaced by TR. TR overgrew PF and even surrounding disc of PF on the 9th day. The type of interaction was found to be invasion/replacement.

Trichoderma reesei - Pleurotus ostreatus (TR-PO) (Plate 7 M-O)

TR almost covered the whole plate and sporulation has also started on the 3rd day after inoculation. TR continued to grow and overgrows even on the inoculum disc of PO on 6th day. Growth of PO also started. PO continued to grow but after 15 days growth of PO was stopped and TR acts as an antagonist against PO. The type of interaction was found to be partial mutual intermingling. On the 10th day PS overgrows TR and continues till the inoculum disc of TR.

Trichoderma reesei -Irpex lacteus (TR-IL) (Plate 8 A-C)

Both the fungi grow equally and came into contact with each other on the 3rd day after inoculation. The sporulation of TR occurred and IL overgrows on TR and almost covered half of the plate on the 6th day.

Plate 7 Interaction of *Trichoderma Reesei* with other fungal isolates

A-C: TR-TV

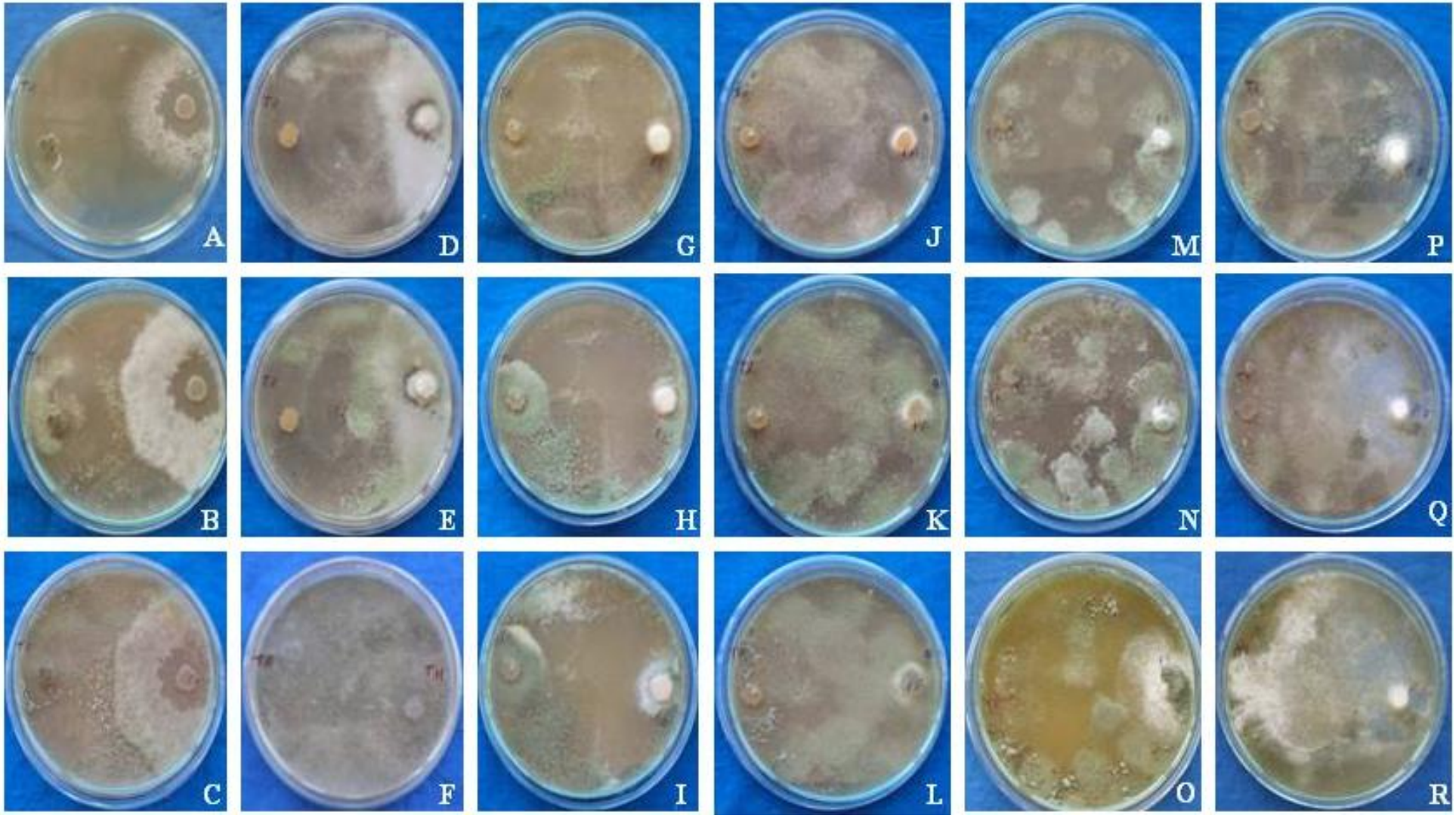
D-F: TR- TH

G-I: TR -PE

J-L: TR-PF

M-O: TR-PO

P-R: TR-PS



TR-TV TR-TH TR-PE TR-PF TR-PO TR-PS

Plate 7

The growth of IL continues even after 3 weeks of incubation period and found to be compatible with each other and the type of incubation was found to be mutual intermingling.

Trichoderma reesei - Daedaleopsis confragosa (TR-DC) (Plate 8 D-F)

The growth of TR was very high compared to DC and the sporulation of TR also occurred and both the fungi came into contact with each other on the 3rd day after inoculation. Growth of both the fungi seems to be restricted on the 6th day after coming in to contact with each other. DC slowly grows further after 10th day. DC covers more than half plate after 15th day of incubation and grows even after 4 weeks of incubation time. These two fungi are compatible with each other even the growth of TR was very rapid and type of interaction was found to be mutual intermingling.

Trichoderma reesei - Phellinus pectinatus (TR-PHE) (Plate 8 G-I)

The growth of TR was very higher than PHE on the 3rd day after inoculation. Both fungi came in contact with each other on the 6th day. PHE overgrows on TR on the 10th day. PHE continues to grow even after 20 days of incubation period. Both the fungi found to be compatible with each other and type of interaction was found to be mutual intermingling.

Trichoderma reesei - Pycnoporus sanguineus (TR-PYS) (Plate 8 J-L)

The growth of TR was more than PYS and TR almost covered the whole plate on the 3rd day after inoculation. Sporulation of TR occurred and PYS overgrows on TR on the 6th day. On the 10th day PYS continue to grow till the inoculum disc of TR and later covered the whole plate showing the compatibility with each other. The type of interaction was found to be mutual intermingling.

Trichoderma reesei- Phanerochaete chrysosporium (TR-PC) (Plate 8 M-O)

The growth of TR was more than PC. TR almost covers the whole plate and both the

fungi came into contact with each other on the 3rd day after inoculation. Sporulation of TR was started on the 6th day and the growth of PC also occurs and it overgrows on TR. The situation remains same on the 10th day PC completely overgrows on TR. Both the fungi found to be compatible with each other and showed mutual intermingling.

In the interaction of TR with other eleven fungal isolates the growth of TR was very fast but mutual intermingling was observed with PS, IL, DC, PHE, PYS, PC while partial mutual intermingling was seen with PO. Dead lock at touching point was observed with the interaction with TV and TH while with PE and PF invasion/replacement was noted.

(4) *Pleurotus eryngii*

Pleurotus eryngii- Trichoderma viride (PE-TV) (Plate 9 A-C)

TV almost covered the whole plate on the 3rd day after inoculation and the growth of PE has just started here. Sporulation of TV was also started on the 3rd day. The growth of PE stopped on the 6th day and TV killed PE and acts as an antagonist. The situation remains same on the 9th day. Here the growth of PE has been started and TV over grew even on the inoculums disc of PE and killed it. This interaction is invasion/replacement.

Pleurotus eryngii- Trichoderma harzianum (PE-TH) (Plate 9 D-F)

The growth of TH was very higher than PE on the 3rd day after inoculation and the growth of PE has just started here. Sporulation of TH was started on the 6th day and the TH overgrew on PE and almost covered the whole plate and growth of PE stopped. The situation remains same on the 9th day. Here the growth of PE has been started and TH over grew PE this interaction was found to be invasion/replacement.

Plate 8 Interaction of *Trichoderma reesei* with other fungal isolates

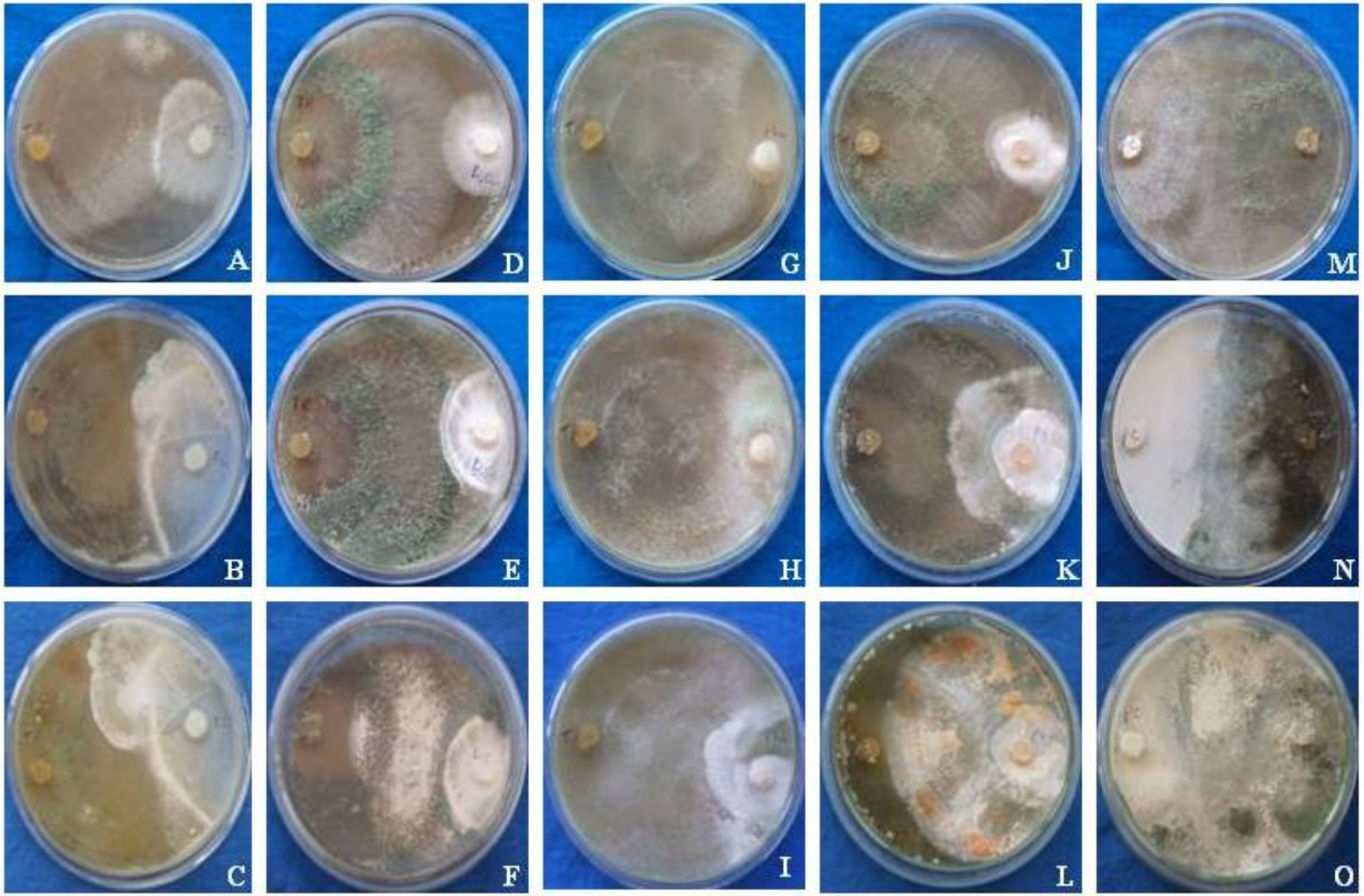
A-C: TR-IL

D-F: TR-DC

G-I: TR-PHE

J-L: TR-PYS

M-O: TR-PC



TR-IL TR-DC TR-PHE TR-PYS TR-PC

Plate 8

Pleurotus eryngii-Trichoderma reesei (TR-PE) (Plate 9 G-I)

TR almost covered the whole plate on the 3rd day after inoculation and the growth of PE was very slow and has just initiated. Sporulation of TR started on the 6th day and the growth of PE still continued with TR and grown till the inoculum disc of PE. Here the growth of PE was covered by TR so the type of interaction was found to be invasion/replacement.

Pleurotus eryngii- Pleurotus florida (PE-PF) (Plate 9 J-L)

It was seen that the growth of PE & PF both slower on the 4th day after the inoculation. The growth of PF is more than PE on the 7th day. Both fungi came in to contact with each other on the 10th day. A restriction zone is formed between the two fungi. Both the fungi stop at the line of contact and type of interaction was deadlock at touching point.

Pleurotus eryngii- Pleurotus ostreatus (PE-PO) (Plate 9 M-O)

The growth of PE & PO both was slower on the 4th day after the inoculation. The growth of PE is faster than PO on the 7th day. Both the fungi came in to contact with each other on the 10th day. After the contact with PE the growth of PO is inhibited. PE overgrows PO and an overlapping zone of 1.2 cm was present. PO is not killed. Here the type of interaction was found to be partial mutual intermingling as after formation of overlapping zone growth of PO was inhibited.

Pleurotus eryngii- Pleurotus sajorcaju (PE-PS) (Plate 9 P-R)

The growth of PE was slower than PS on the 4th day after inoculation. Both fungi came in to contact with each other & overlapping zone is clearly visible on the 7th day. After the contact with PS the growth of PE is inhibited. PS over grows PE and almost covers the whole petriplate on the 10th day and type of interaction was found to

be partial mutual intermingling as after over lapping occurred growth of PE was inhibited.

Pleurotus eryngii -Irpex lacteus (PE-IL) (Plate 10 A-C)

The growth of IL was more than PE and the contact between them occur. IL grows almost 2/3rd of the petridish on the 4th day after the inoculation. IL over grow on PE and the growth of PE is inhibited. The overlapping zone is clearly visible on the 7th day. IL growth increase & overlapping zone is also increased on the 10th day and type of interaction was found to be partial mutual intermingling as after over lapping occurred growth of PE was inhibited.

Pleurotus eryngii- Daedaleopsis confragosa (PE-DC) (Plate 10 D-F)

The growth of DC is more than PE on the 4th day after inoculation. Both the fungi came in to contact with each other on the 7th day, the growth of DC still more than the PE. DC over grow on PE & the growth of PE is inhibited on the 10th day and type of interaction was found to be partial mutual intermingling as after formation of over lapping zone growth of PE was inhibited.

Pleurotus eryngii- Phellinus pectinatus (PE-PHE) (Plate 10 G-I)

The growth of PE was slower than PHE on the 4th day after inoculation. The growth of PHE is still higher than the PE covering 80% of the petriplate on the 7th day. Both fungi came in to contact with each other on the 10th day. Growth of both fungi was further stopped. Here the growth of both the fungi was restricted after they came in to contact with each other so type of inhibition was deadlock at touching point.

Pleurotus eryngii- Pycnoporus sanguineus (PE-PYS) (Plate 10 J-L)

The growth of PYS is more than PE on the 4th day after inoculation. Both fungi came in to contact with each other on the 7th day and the growth of PE is inhibited. PYS over grow on PE and almost covered the whole plate on the 10th day.

Plate 9 Interaction of *Pleurotus eryngii* with other fungal isolates

A-C: PE-TV

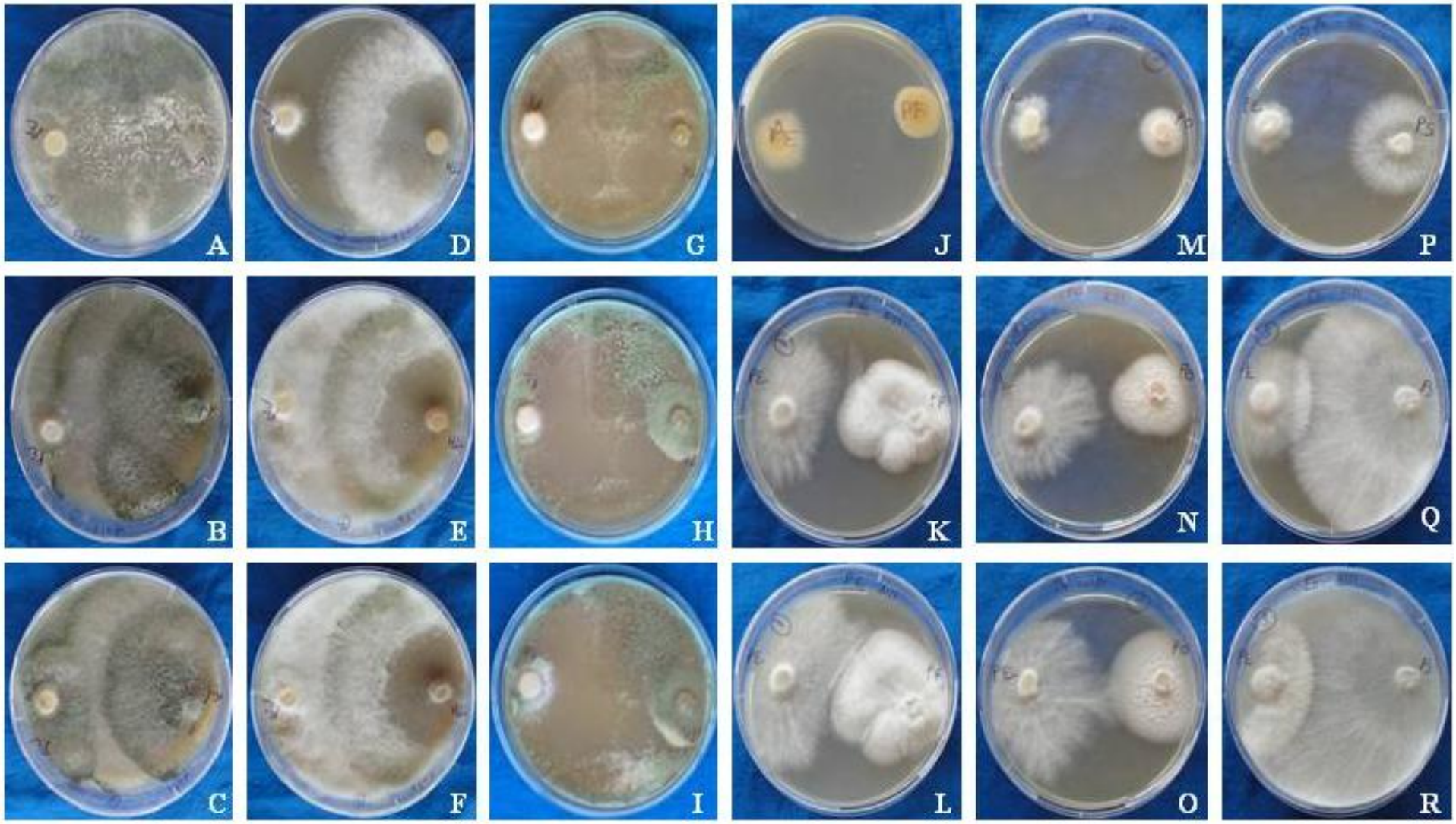
D-F: PE- TH

G-I: PE -TR

J-L: PF-PF

M-O: PF-PO

P-R: PF-PS



PE-TV PE-TH PE-TR PE-PF PE-PO PE-PS

Plate 9

The sporulation of PYS occurs reaching very close to the PE inoculum and type of interaction was found to be invasion/replacement as the growth of PE was replaced by PYS.

Pleurotus eryngii -Phanerochaete chrysosporium (PC-PE) (Plate 10 M-O)

The growth of PC was more than PE on the 3rd day after the inoculation. It almost advances up to more than half of the petriplate. Both fungi came in to contact with each other on the 5th day. PC grows completely fill the petriplate on the 8th day. PC covered the whole plate and the growth of PE was inhibited on the 11th day. Here the line of inhibition was clearly visible surround the growth of PE so type of interaction was deadlock at a distance.

In the eleven interactions of different fungal isolates with PE it was found that the growth of PE was slower compared to other. With *Trichoderma* species type of interaction found was invasion/replacement as the growth of *Trichoderma* species was very faster than PE. the same type of interaction was found with PYS also. The interaction of PE with IL, DC and other *Pleurotus* species was partial mutual intermingling except PF which showed deadlock at touching point. The interaction with PC was found to be deadlock at a distance which was not observed with the interaction in the *Trichoderma* species with other fungal isolates. Here neutral intermingling was not observed.

(5) *Pleurotus florida*

Pleurotus florida-Trichoderma viride (PF-TV) (Plate 11 A-C)

It was seen that the growth of TV was very higher than PF and the growth of PF has just started on the 3rd day after inoculation and both fungi came in to contact with each other. Sporulation of TV was started on the 3rd day. The growth of PF inhibited by TV on the 6th day. TV over grew even on the inoculums disc of PF.

Plate 10 Interaction of *Pleurotus eryngii* with other fungal isolates

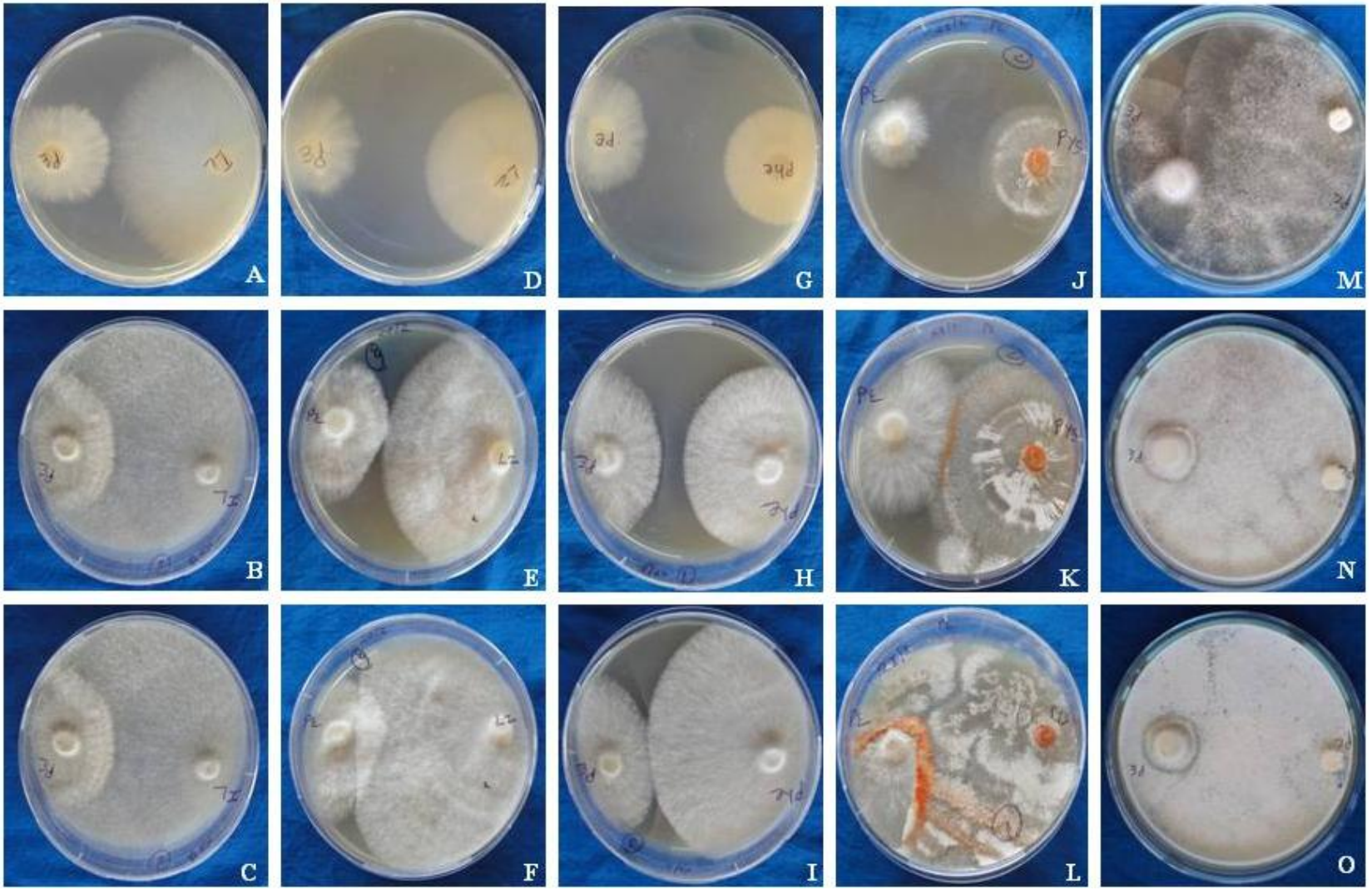
A-C: PE-IL

D-F: PE-DC

G-I: PE-PHE

J-L: PE-PYS

M-O: PE-PC



PE-IL PE-DC PE-PHE PE-PYS PE- PC

Plate 10

TV killed PF on the 9th day and act as an antagonist against PF. This interaction was found to be invasion/replacement.

Pleurotus florida-Trichoderma harzianum (PF-TH) (Plate 11 D-F)

The growth of TH was very higher than PF on the 3rd day after inoculation.TH overgrow on PF and almost covered the whole plate on the 6th day. The growth of PF still continued and PF also over grew on TH. In this interaction growth of TH was high but PF over grew on TH it was partial mutual intermingling type of interaction.

Pleurotus florida-Trichoderma reesei (PF-TR) (Plate 11 G-I)

It was seen that the growth of TR was higher than PF and the growth of PF has started on the 3rd day after inoculation and both fungi came in to contact with each other. Sporulation of TR was started on the 6th day and the growth of PF was replaced by TR. TR overgrew PF and even surrounding disc of PF on the 9th day. The type of interaction was found to be invasion/replacement.

Pleurotus florida-Pleurotus eryngii (PF-PE) (Plate 11 J-L)

It was seen that the growth of PE & PF both slower on the 4th day after the inoculation. The growth of PF is more than PE on the 7th day. Both fungi came in to contact with each other on the 10th day. A restriction zone is formed between the two fungi. Both the fungi stop at the line of contact and type of interaction was deadlock at touching point.

Pleurotus florida-Pleurotus ostreatus (PF-PO) (Plate 11 M-O)

The growth of PF is faster than the PO on the 4th day after inoculation. The growth of PF was still higher than PO on the 7th day. The growth of PO was also faster on the 10th day. Even after 30 days of inoculation the two fungi did not come in contact. The type of interaction was found to be deadlock at a distance as growth of both the fungi inhibited.

Pleurotus florida- Pleurotus sajorcaju (PF-PS) (Plate 11 P-R)

The growth of PF was slower than PS on the 4th day after the inoculation. Both the fungus came in to contact with each other on the 7th day and the growth of PF is inhibited. The growth of PS was higher and it overgrows on PF on the 10th and PS almost covered the plate. PS overgrows PF without killing it and advances towards the lid of the petriplate. Here growth of both the fungi continued and type of interaction was found to be mutual intermingling.

Pleurotus florida-Irpex lacteus (PF-IL) (Plate 12 A-C)

The growth of IL is more than PF on the 4th day after inoculation. Growth of PF is very slow. IL almost covered about 80% the whole plates on the 7th day & overgrows on PF and the growth of PF inhibited after coming in contact of IL. The IL covered the whole plate on the 10th day. IL completely overtakes the growth of PF but does not kill it. Here growth of PF was restricted after coming in to contact the type of interaction was found to be partial mutual intermingling.

Pleurotus florida-Daedaleopsis confragosa (PF-DC) (Plate 12 D-F)

The growth of DC is more than the PF reaching half of the petriplate on the 4th day after inoculation. The growth covering DC was also higher than PF on the 7th day. Both the fungi came in to contact with each other on the 10th day. After come in contact growth of both the fungi inhibited and the type of interaction was found to be deadlock at touching point.

Pleurotus florida - Phellinus pectinatus (PF-PHE) (Plate 12 G-I)

The growth of PHE is higher than the PF on the 4th day after inoculation. The growth of PHE is still higher than PF on the 7th day. Contact of both the fungi did not occur on the 10th day. Situation remained same for incubation period of 30 days. Here growth of both the fungi inhibited without coming in to contact with each other.

Plate 11 Interaction of *Pleurotus florida* with other fungal isolates

A-C: PF-TV

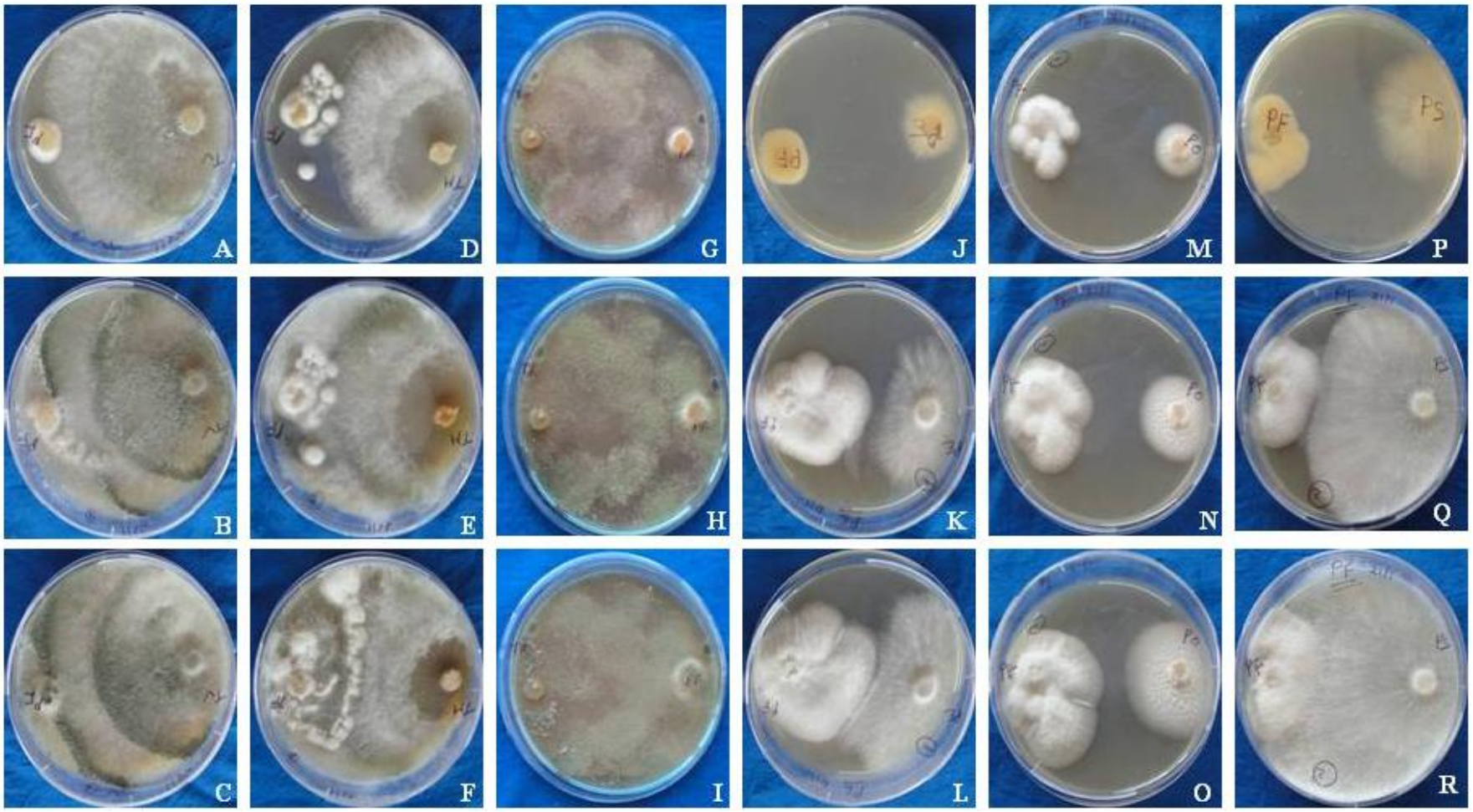
D-F: PF- TH

G-I: PF -TR

J-L: PF-PE

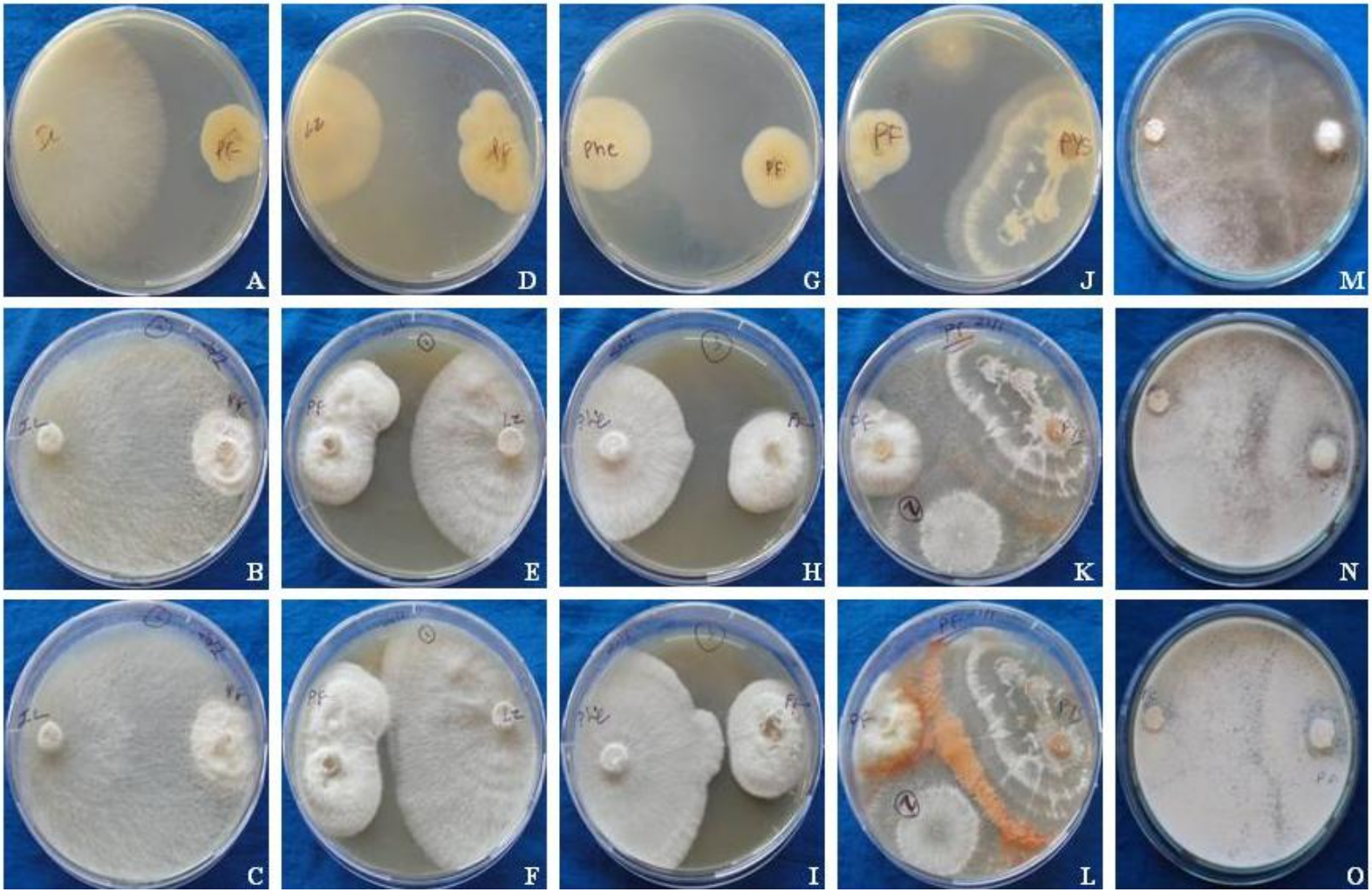
M-O: PF-PO

P-R: PF-PS



PF-TV PF-TH PF-TR PF-PE PF-PO PF-PS

Plate 11



PF-IL PF-DC PF-PHE PF-PYS PF-PC

Plate 12

Plate 12 Interaction of *Pleurotus florida* with other fungal isolates

A-C: PF-IL

D-F: PF- DC

G-I: PF -PHE

J-L: PF-PE

M-O: PF-PYS

P-R: PF-PC

The type of interaction was found to be dead lock at distance.

Pleurotus florida- Pycnoporus sanguineus (PF-PYS) (Plate 12 J-L)

The growth of PYS is higher than the PF on the 4th day after inoculation. Both the fungus came in to contact on the 7th day and PYS overgrows on PF and the growth of PF inhibited. The Sporulation of PYS appeared and it covered the whole plate on the 10th day. After coming in to contact with each other the growth of both fungi stopped and the type of interaction was found to be deadlock at touching point.

Pleurotus florida- Phanerochaete chrysosporium (PF-PC) (Plate 12 M-O)

The growth of PC was more than PF on the 3rd day after inoculation. PF appeared to grow very slow. The growth of PC was still denser indicated by the dense growth of mycelium within the entire petriplate and covering surrounding PF on 6th day and growth of PF was inhibited. Growth of PF did not occur on 9th day and situation remains same at later stage also. The type of interaction was found to be deadlock at touching point.

PF appears to be a very weak fungus which gets inhibited when comes in interaction with any of the other fungi except PS. PF is a very slow growing fungi but it grow with PS and found to be mutual intermingling while with IL it showed Partial mutual intermingling. In the interaction with TH the growth of PF was stopped and it showed partial mutual intermingling while with TV and TR it showed invasion/replacement. In the interaction with PE, DC, PYS and PC it showed deadlock at touching point while with PO and PHE it showed deadlock at a distance.

(6)*Pleurotus ostreatus*

Pleurotus ostreatus-Trichoderma viride (PO-TV) (Plate 13 A-C)

TV almost covered the whole plate on the 3rd day. Sporulation has just started on the 3rd day after inoculation. The growth of PO started on the 6th day. TV over grew even

on the inoculums disc of PO on the 9th day. The situation remains same even after one month also. TV act as an antagonist against PO and interaction is found to be invasion/replacement.

Pleurotus ostreatus-Trichoderma harzianum (PO-TH) (Plate 13 D-F)

The growth of TH was very higher than PO 3rd day after inoculation. Both the fungi came in to contact with each other on the 6th day. TH overgrows on PO and growth of PO was very less and stopped here. The situation remains same on the 9th day and TH act as an antagonist against PO and the interaction was found to be invasion/replacement as growth of PO was replaced by TH.

Pleurotus ostreatus -Trichoderma reesei (PO- TR) (Plate 13 G-I)

TR almost covered the whole plate and sporulation has also started on the 3rd day after inoculation. TR continued to grow and overgrows even on the inoculum disc of PO on 6th day. Growth of PO also started. PO continued to grow but after 15 days growth of PO was stopped and TR acts as an antagonist against PO. The type of interaction was found to be partial mutual intermingling.

Pleurotus ostreatus-Pleurotus eryngii (PO-PE) (Plate 13J-L)

The growth of PE & PO both was slower on the 4th day after the inoculation. The growth of PE is faster than PO on the 7th day. After the contact with PE the growth of PO is inhibited. PE overgrows PO and an overlapping zone of 1.2 cm was present. PO is not killed. Here the type of interaction was found to be partial mutual intermingling as after formation of overlapping zone growth of PO was inhibited.

Pleurotus ostreatus- Pleurotus florida (PO-PF) (Plate 13 M-O)

The growth of PF is faster than the PO on the 4th day after inoculation. The growth of PF was still higher than PO on the 7th day. The growth of PO was also faster on the 10th day. Even after 30 days of inoculation the two fungi did not come in contact.

Plate 13 Interaction of *Pleurotus ostreatus* with other fungal isolates

A-C: PO-TV

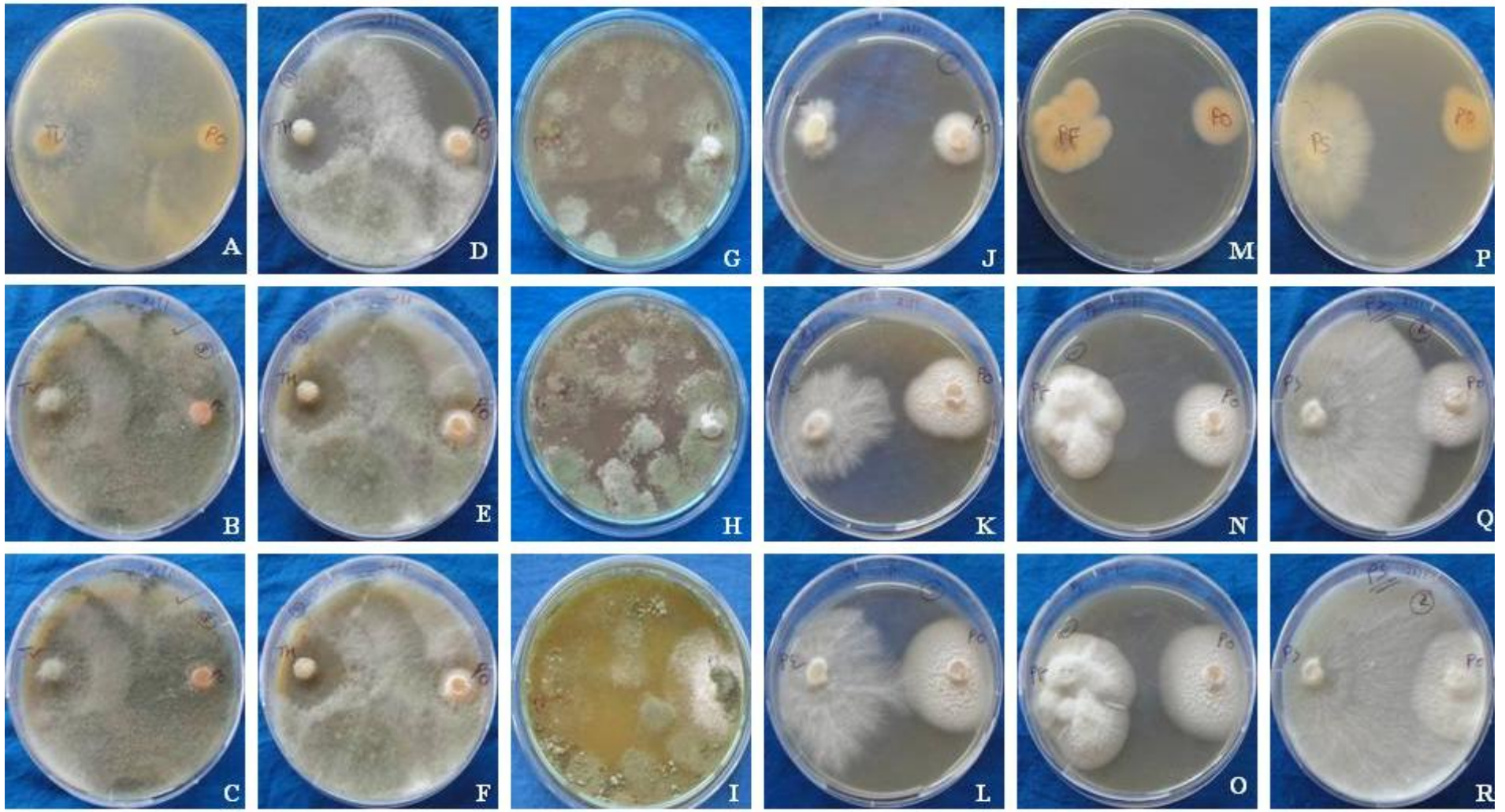
D-F: PO- TH

G-I: PO -TR

J-L: PO-PE

M-O: PO-PF

P-R: PO-PS



PO-TV PO-TH PO-TR PO-PE PO- PF PO-PS

Plate 13

The type of interaction was found to be deadlock at a distance as growth of both the fungi inhibited.

Pleurotus ostreatus-Pleurotus sajorcaju (PO-PS) (Plate 13P-R)

The growth of PS was more than PO on the 4th day after the inoculation. The both fungi came in to contact with each other on the 7th day. PS overgrow on PO and the overlapping zone of 1.2 cm visible. The PS almost covered the whole plate and growth of PO also continued but at a very slow rate on the 10th day. The growth of PO was not inhibited so PS and PO are compatible with each other and the interaction was found to be mutual intermingling.

Pleurotus ostreatus- Irpex lacteus (PO-IL) (Plate 14 A-C)

The growth of IL was more than PO reaching almost ½ of the petriplate on the 4th day after the inoculation. Both fungi came in to contact with each other on the 7th day and IL overgrow on PO and the growth of PO was further inhibited. IL almost covered the whole plate on the 10th day but growth of PO was not occurred further and the type of interaction was found to be invasion /replacement.

Pleurotus ostreatus- Daedaleopsis confragosa (PO-DC) (Plate 14 D-F)

The growth of DC was more than PO on the 4th day after the inoculation. Both fungi came in to contact with each other on the 7th day. DC overgrows on PO and the growth of PO stop on the 10th day. PO is not killed but growth is inhibited and DC overgrew till the inoculums disc of PO and showing invasion/replacement.

Pleurotus ostreatus-Phellinus pectinatus (PO-PHE) (Plate 14 G-I)

The growth of PHE was more than PO on the 4th day after the inoculation. Growth PHE is still higher than PO advanced into ½ of the petriplate on the 7th day. Both the fungi come in contact with each other on the 10th day and the growth of PO stopped at the region of contact. PHE has advanced up to 75% in the petri plate at this stage.

Plate 14 Interaction of *Pleurotus ostreatus* with other fungal isolates

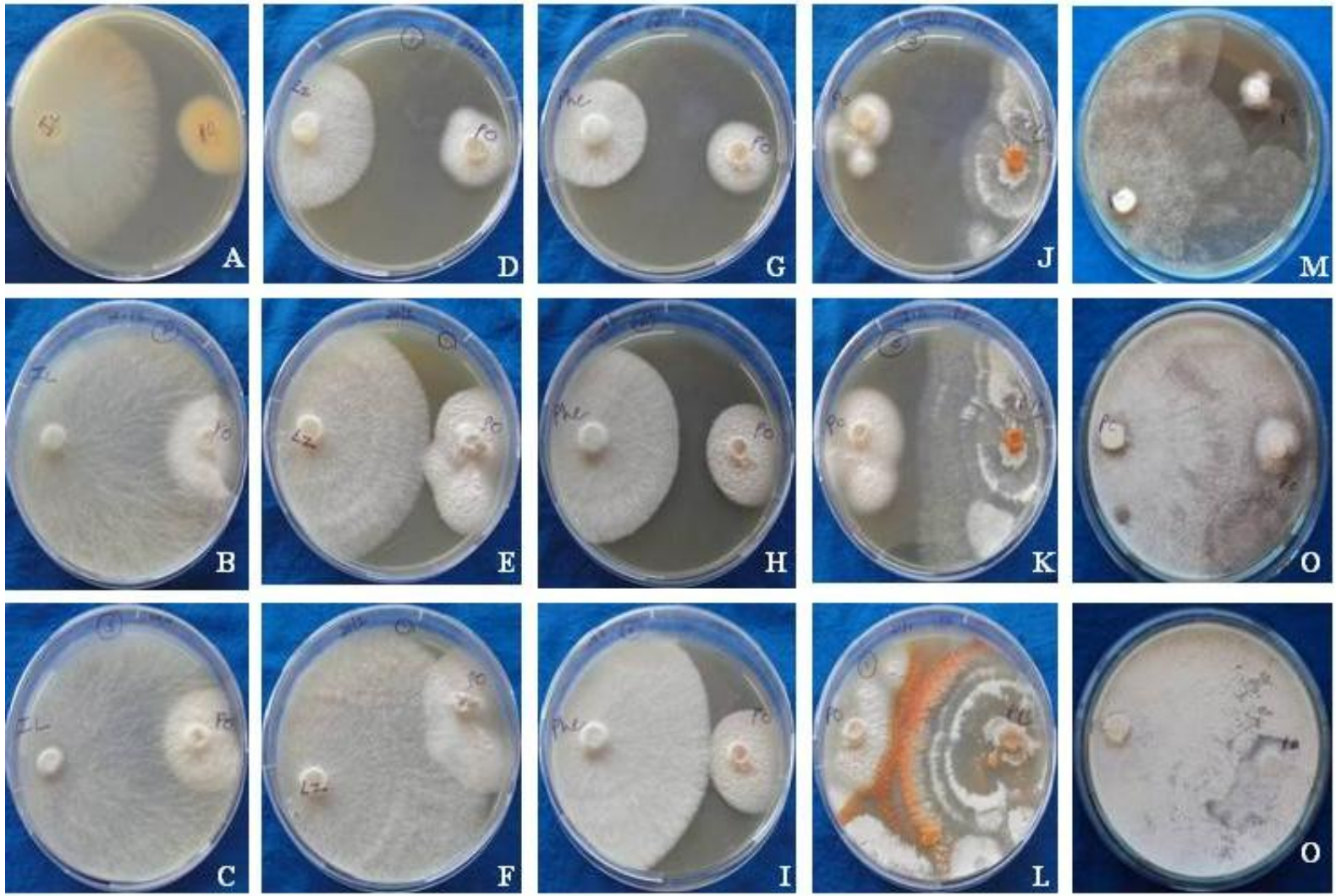
A-C: PO-IL

D-F: PO-DC

G-I: PO-PHE

J-L: PO-PYS

M-O: PO-PC



PO-IL PO-DC PO-PHE PO-PYS PO-PC

Plate 14

Here the type of interaction was found to be dead lock at touching point.

Pleurotus ostreatus- Pycnoporus sanguineus (PO-PYS) (Plate 14 J-L)

The growth of PYS was more than PO on the 4th day after the inoculation. The growth of PYS was 50% advanced petriplate on the 7th day. Both the fungi came in to contact with each other on the 10th day and further growth of PO and PYS stopped. The sporulation of PYS was clearly visible in concentric ring and even at the region of their contact showing deadlock at touching point.

Pleurotus ostreatus -Phanerochaete chrysosporium (PO-PC) (Plate 14 M-O)

The growth of PC was more than PO on the 3rd day after inoculation. The growth of PO was just started on the 3rd day. PC overgrows on PO and on the 6th day and both fungus come under contact with each other and the growth of PO also occurred. PC almost covered the plate on 10th day but growth of PO was stopped and showed partial mutual intermingling.

In the interaction of PO with other fungal isolates it was observed that with TV and TH it showed invasion/replacement while with TR it showed partial mutual intermingling. In the interaction of PO with other *Pleurotus* spp. three different types of interaction viz. partial mutual intermingling, deadlock at a distance and mutual intermingling with PE, PF and PS respectively. IL, DC, PHE, PYS and PC are fast growing fungi compared to PO and amongst them with IL and DC invasion/replacement could be seen while PYS and PHE showed deadlock at touching point and with Pc showed partial mutual intermingling. Here dead lock at a distance was not observed.

(7) *Pleurotus sajorcaju*

Pleurotus sajorcaju-Trichoderma viride (PS-TV) (Plate 15 A-C)

The growth of TV was more than PS on the 3rd day after incubation. TV covered the

whole plate on the 6th day and over grows on PS. The growth of PS stopped here. TV killed PS on the 9th day and act as antagonist against PS and interaction is found to be invasion/replacement.

Pleurotus sajorcaju-Trichoderma harzianum (PS-TH) (Plate 15 D-F)

The growth of TH was more than PS on the 3rd day after inoculation. Both the fungi came in to contact with each other on the same day. TH covered the whole plate and over grows on PS on the 6th day and the growth of PS stopped here. Situation remained same on the 9th day. Here TH over grew on the growth of PS this interaction was found to be invasion/replacement.

Pleurotus sajorcaju-Trichoderma reesei (PS-TR) (Plate 15 G-I)

The growth of TR was more than PS on the 3rd day after inoculation. TR covered the whole plate and growth of PS also continued on the 6th day. On the 10th day PS overgrows TR and continues till the inoculum disc of TR. Both fungi found to be compatible with each other and as growth of both fungi continues type of interaction was found to be mutual intermingling.

Pleurotus sajorcaju- Pleurotus eryngii (PS-PE) (Plate 13 J-L)

The growth of PE was slower than PS on the 4th day after inoculation. Both fungi came in to contact with each other & overlapping zone is clearly visible on the 7th day. After the contact with PS the growth of PE is inhibited. PS over grows PE and almost covers the whole petriplate on the 10th day and type of interaction was found to be partial mutual intermingling as after over lapping occurred growth of PE was inhibited.

Pleurotus sajorcaju- Pleurotus florida (PS-PF) (Plate 15 M-O)

The growth of PF was slower than PS on the 4th day after the inoculation. Both the fungi came in to contact with each other on the 7th day.

Plate 15 Interaction of *Pleurotus sajorkaju* with other fungal isolates

A-C: PS-TV

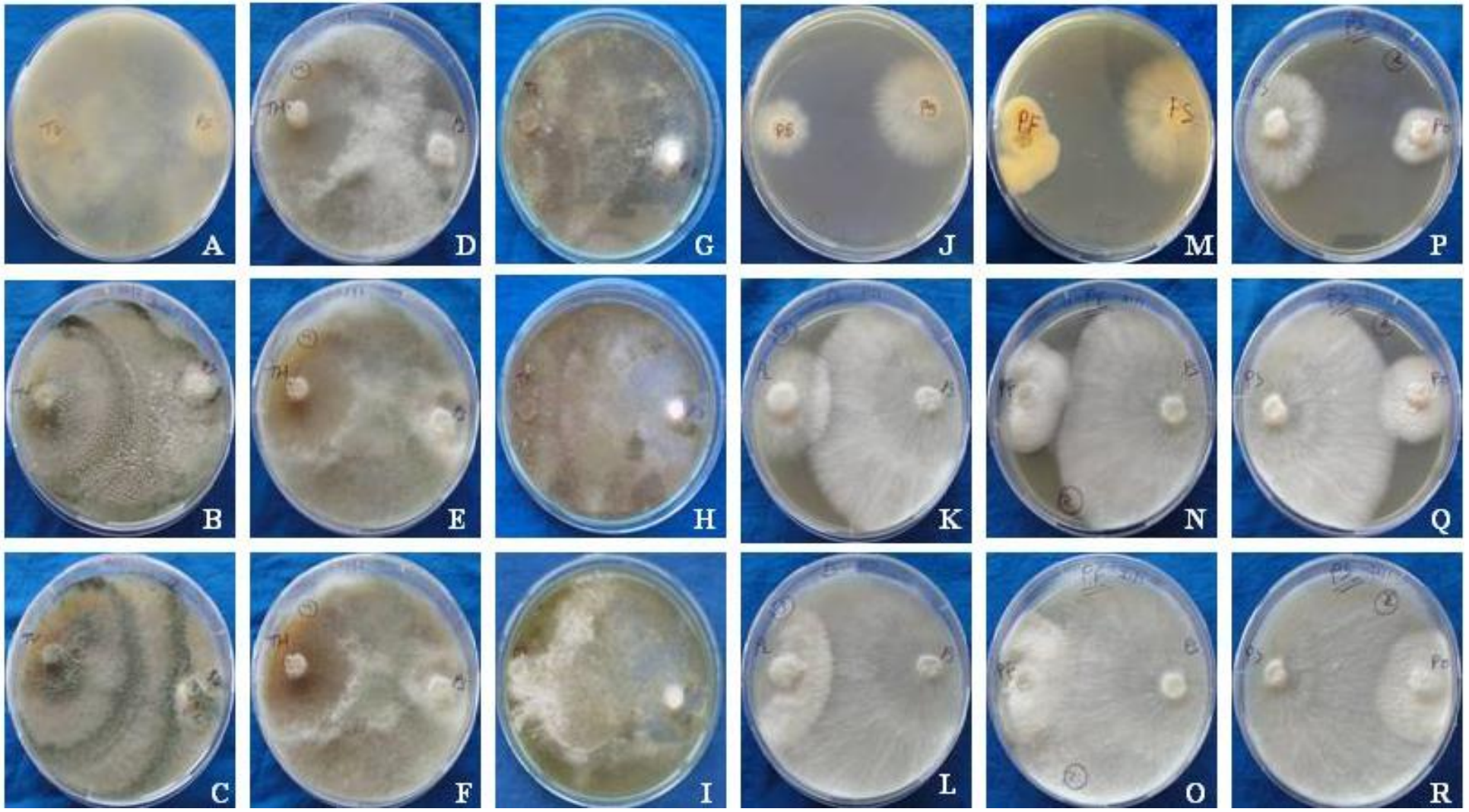
D-F: PS- TH

G-I: PS -TR

J-L: PS-PE

M-O: PS-PF

P-R: PS-PO



PS-TV PS-TH PS-TR PS-PE PS-PF PS-PO

Plate 15

The growth of PF is inhibited. The growth of PS was higher and it overgrows on PF on the 10th and PS almost covered the plate. PS overgrows PF without killing it and advances towards the lid of the petriplate. Here growth of both the fungi continued and type of interaction was found to be mutual intermingling.

Pleurotus sajorcaju- Pleurotus ostreatus (PS-PO) (Plate 15 P-R)

The growth of PS was more than PO on the 4th day after the inoculation. The both fungi came in to contact with each other on the 7th day. PS overgrow on PO and the overlapping zone of 1.2 cm visible. The PS almost covered the whole plate and growth of PO also continued but at a very slow rate on the 10th day. The growth of PO was not inhibited so PS and PO are compatible with each other and the interaction was found to be mutual intermingling.

Pleurotus sajorcaju – Irpex lacteus (PS-IL) (Plate 16 A-C)

The growth of IL was more than PS and both fungi come in contact with each other on the 4th day after the inoculation. IL and PS equally advance and come in contact. The IL overgrows on PS and overlapping zone was visible and the growth of PS stopped on the 7th day. IL growth increased and the overlapping zone was increased on 10th day. Overlapping zone of IL on PS is distinct. As growth of PS was inhibited the interaction was found to be partial intermingling.

Pleurotus sajorcaju- Daedaleopsis confragosa (PS-DC) (Plate 16 D-F)

The growth of PS was higher than DC on the 4th day after inoculation. The growth of DC was increased than the PS and contact between both fungi occur and the growth of PS was stops on the 7th day. DC overgrows on PS and further it covers the whole plate on the 10th day. DC goes on advancing over PS and does not kill it but growth is inhibited. Even after 3 weeks the growth of PS is slightly increased but still inhibited.

Plate 16 Interaction of *Pleurotus sajorkaju* with other fungal isolates

A-C: PS-IL

D-F: PS-DC

G-I: PS-PHE

J-L: PS-PYS

M-O: PS-PC

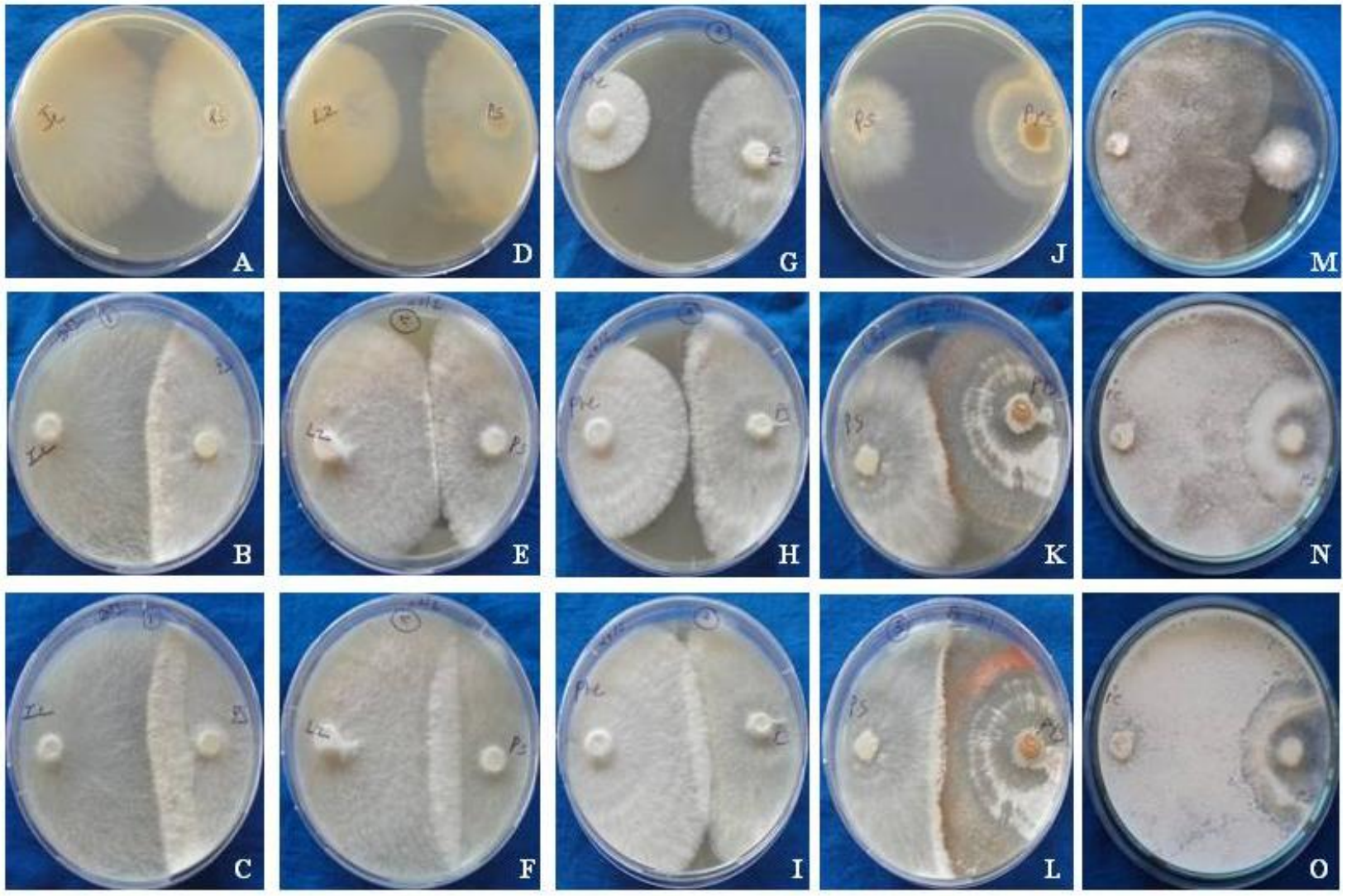


Plate 16

DC grows profusely covering the petriplate. Both are compatible but growth of PS is very slow and showed mutual intermingling type of interaction.

Pleurotus sajorcaju-Phellinus pectinatus (PS-PHE) (Plate 16 G-I)

The growth of PS was more than PHE on the 4th day after inoculation however the growth of PHE was slow. Both fungi came in to contact with each other on the 7th day. The growth of PHE was increase and PHE can overgrow on PS and the overlapping zone was form on the 10th day. The growth of PS does not stop here. PHE over grows PS further and the two continue to grow. The two are compatible and grow together and showed mutual intermingling type of interaction.

Pleurotus sajorcaju-Pycnoporus sanguineus (PS-PYS) (Plate 16 J-L)

The growth of PYS was more than PS on the 4th day after inoculation. Both fungi came in to contact with each other on the 7th day. At the zone of contact PYS sporulation gets covered by PS. Both fungi can overgrow on each other and overlapping zone is visible and at particular stage on the 10th day. Further the growth of PS first stops and then PYS stop. As growth of both the fungi stopped after coming in to contact the interaction was found to be dead lock at touching point.

Pleurotus sajorcaju - *Phanerochaete chrysosporium* (PS-PC) (Plate 16 M-O)

The growth of PC was more than PS and both fungi come under contact with each other on the 3rd day after inoculation. The growth of PS occurs and the growth of PC was further increase and the completely covered the petriplate on the 5th day. PS overgrows on PC on the 10th day but later the growth of PS was restricted indicating PC acts as an antagonist for PS and showed dead lock at touching point interaction type.

The Growth of PS in 5 days appears to be faster than the other three species of *Pleurotus.* PS advances fast and grew up to almost half of the petriplate in 5 days.

Although the growth of PS was fast but TH, TV replaced it and invasion/replacement interaction was observed while in interaction with TR it grew after the whole plate was covered by TR and showed mutual intermingling. The interaction of PS with other spp of *Pleurotus* mutual intermingling was observed with PF and PO and with PE partial mutual intermingling was observed. In the interaction with IL Partial mutual intermingling was seen and DC and PHE showed mutual intermingling with PS. PYS and PC showed dead lock interaction at touching point. in the interaction of eleven fungi with PS dead lock at distance was not observed.

(8)*Irpex lacteus*

Irpex lacteus- Trichoderma viride (IL-TV) (Plate 17 A-C)

The growth of TV was very higher than IL on the 3rd day after inoculation and sporulation of TV also occurred on the same day. Growth of IL also found on the 3rd day. On the 6th day growth of IL remains same but both the fungi came in to contact with each other. The growth of IL stopped on the 9th day but IL did not allow TV to grow further. Both fungi act as antagonist. This type of interaction is found to be deadlock at touching point.

Irpex lacteus- Trichoderma harzianum (IL-TH) (Plate 17 D-F)

The growth of TH was very higher than IL on the 3rd day after inoculation. Both the fungi came in to contact with each other on the same day. TH overgrew on IL and almost covered the whole plate on the 6th day and The growth of IL was inhibited by TH on the 9th day and TH acts as an antagonist against IL. Here TH overgrew on IL and growth of IL was restricted so the interaction was found to be invasion/replacement.

Irpex lacteus -Trichoderma reesei (IL-TR) (Plate 17 G-I)

Both the fungi grow equally and came into contact with each other on the 3rd day after

inoculation. The sporulation of TR occurred and IL overgrows on TR and almost covered half of the plate on the 6th day. The growth of IL continues even after 3 weeks of incubation period and found to be compatible with each other and the type of incubation was found to be mutual intermingling.

Irpex lacteus-Pleurotus eryngii (IL-PE) (Plate 17 J-L)

The growth of IL was more than PE and the contact between them occur. IL grows almost 2/3rd of the petridish on the 4th day after the inoculation. IL over grow on PE and the growth of PE is inhibited. The overlapping zone is clearly visible on the 7th day. IL growth increase & overlapping zone is also increased on the 10th day and type of interaction was found to be partial mutual intermingling as after over lapping occurred growth of PE was inhibited.

Irpex lacteus-Pleurotus florida (IL-PF) (Plate 17 M-O)

The growth of IL is more than PF on the 4th day after inoculation. Growth of PF is very slow. IL almost covered about 80% the whole plates on the 7th day & overgrows on PF and the growth of PF inhibited after coming in contact of IL. The IL covered the whole plate on the 10th day. Here growth of PF was restricted after coming in to contact the type of interaction was found to be partial mutual intermingling.

Irpex lacteus- Pleurotus ostreatus (IL-PO) (Plate 17 P-R)

The growth of IL was more than PO reaching almost ½ of the petriplate on the 4th day after the inoculation. Both fungi came in to contact with each other on the 7th day and IL overgrow on PO and the growth of PO was further inhibited. IL almost covered the whole plate on the 10th day but growth of PO was not occurred further and the type of interaction was found to be invasion /replacement.

Irpex lacteus- Pleurotus sajorcaju (IL-PS) (Plate 18 A-C)

The growth of IL was more than PS and both fungi came in to contact with each other

Plate 17 Interaction of *Irpex lacteus* with other fungal isolates

A-C: IL-TV

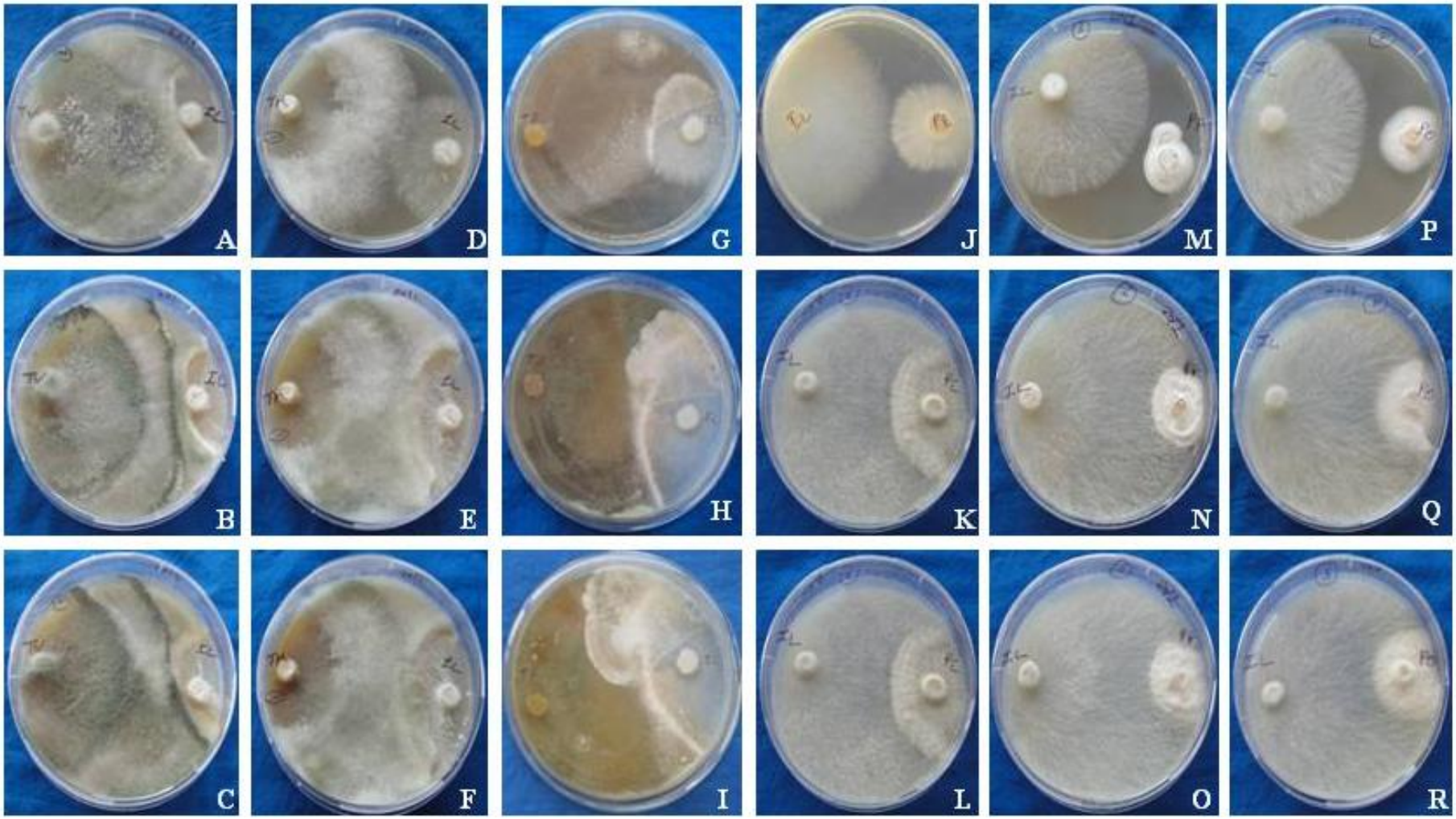
D-F: IL- TH

G-I: IL -TR

J-L: IL-PE

M-O: IL-PF

P-R: IL-PO



IL-TV IL-TH IL-TR IL-PE IL-PF IL-PO

Plate 17

on the 4th day after the inoculation. IL and PS equally advance and come in contact. The IL overgrows on PS and overlapping zone was visible and the growth of PS stopped on the 7th day. IL growth increased and the overlapping zone was also increased on 10th day. Overlapping zone of IL on PS is distinct. As growth of PS was inhibited the interaction was found to be partial intermingling.

*Irpex lacteus- Daedaleopsis confragosa (*IL-DC) (Plate 18D-F)

The growth of IL was more than DC on the 4th day after inoculation. Both fungus came in to contact with each other and DC overgrow on IL and the growth of IL was also continued on 6th day. Though the growth of DC continued over IL it did not inhibit by DC on the 9th day. Growth of both the fungi continued and overlapping zone of 2.7 cm was formed. Even after 3 weeks of incubation time both fungi grew to gather and interaction was found to be mutual intermingling.

Irpex lacteus-Phellinus pectinatus (IL-PHE) (Plate 18 G-I)

The growth of IL was more than PHE on the 3rd day after inoculation. Both fungi came under contact with each other on the 6th day and PHE started overgrow on IL. Both the fungi continued to overgrow and forming an overlapping zone of 1.5 cm and both the fungi were compatible with each other showed mutual intermingling.

Irpex lacteus- Pycnoporus sanguineus (IL-PYS) (Plate 18 J-L)

The growth of IL was more than PYS and both fungi came under contact with each other on the 5th day after inoculation. Both fungi overgrow on each other and overlapping zone appeared on the 8th day. The growth of both fungus inhibited by each other on the 11th day. At the region of contact orange red sporulation of PYS appears. The type of interaction was found to be deadlock at touching point.

Irpex lacteus- Phanerochaete chrysosporium (IL- PC) (Plate 18M-O)

The growth of PC was faster than IL. Both fungi came in to contact with each other

Plate 18 Interaction of *Irpex lacteus* with other fungal isolates

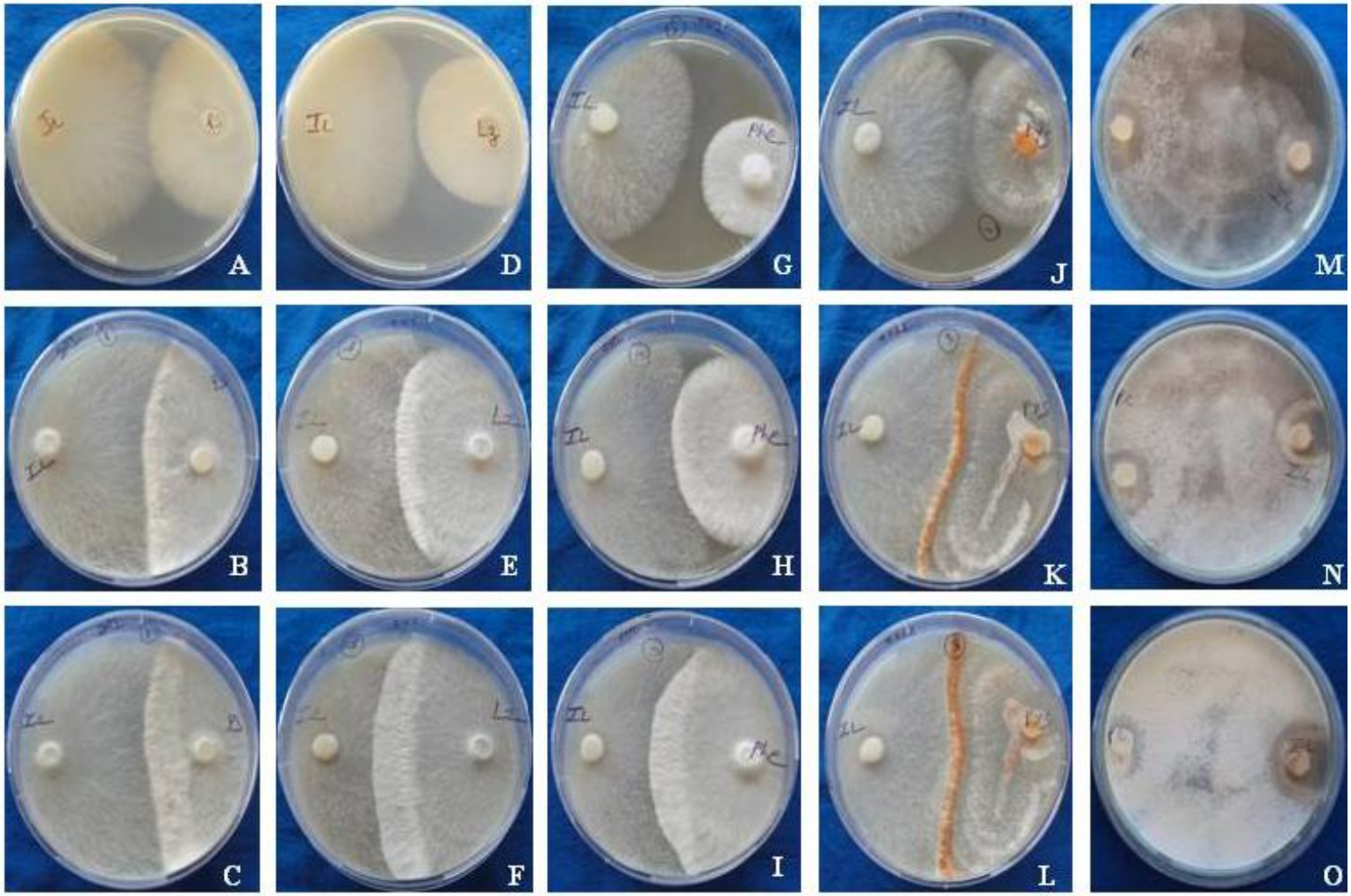
A-C: IL-PS

D-F: IL-DC

G-I: IL-PHE

J-L: IL-PYS

M-O: IL-PC



IL-PS IL-DC IL-PHE IL-PYS IL-PC

Plate 18

on the 3rd day after inoculation. The growth of PC was fast and the growth of PC continues and the growth of IL restricted on the 5th day. Growth of IL stopped on 8th day and line of inhibition was clearly seen. Situation remains same on the 10th day. PC acts as an antagonist against IL and type of interaction was found to be deadlock at touching point.

Irpex lacteus was found to be fast growing almost ½ the petriplate in 4-5 days. IL showed three different type of interaction with *Trichoderma* species viz dead lock at touching point, replacement and partial mutual intermingling with TV, TH and TR respectively. Partial mutual intermingling was observed with three *Pleurotus* species except PO which showed replacement. In the interaction with DC and PHE the growth of both the fungi were not inhibited and showed mutual intermingling while with PYS and PC dead lock interaction at touching point was observed.

(9) *Daedaleopsis confragosa*

Daedaleopsis confragosa -Trichoderma viride (DC-TV) (Plate 19 A-C)

The growth of TV was very high compared to DC on the 3rd day after inoculation and both the fungi came in to contact with each other on the same day. TV overgrows on DC. DC grows further on the 9th day but after that the growth of DC becomes slow but it also over grew on TV. These two fungi are compatible with each other even the growth of TV was very rapid and this type of interaction is found to be partial mutual intermingling.

Daedaleopsis confragosa- Trichoderma harzianum (DC-TH) (Plate 19 D-F)

The growth of TH was very high as compared to DC on the 3rd day after inoculation. Both the fungi came in to contact with each other. TH overgrows on DC on the 6th day. TH almost covered the whole plate on the 9th day but DC still overgrows on TH. DC was found to be compatible with TH and showed Partial mutual intermingling.

Trichoderma reesei - Daedaleopsis confragosa (Plate 19 G-I)

The growth of TR was very high compared to DC and the sporulation of TR also occurred and both the fungi came into contact with each other on the 3rd day after inoculation. Growth of both the fungi seems to be restricted on the 6th day after coming in to contact with each other. DC slowly grows further after 10th day. DC covers more than half plate after 15th day of incubation and grows even after 4 weeks of incubation time. These two fungi are compatible with each other even the growth of TR was very rapid and type of interaction was found to be mutual intermingling.

Daedaleopsis confragosa - Pleurotus eryngii (DC-PE) (Plate 19 J-L)

The growth of DC is more than PE on the 4th day after inoculation. Both the fungi came in to contact with each other on the 7th day, the growth of DC still more than the PE. DC over grow on PE & the growth of PE is inhibited on the 10th day and type of interaction was found to be partial mutual intermingling as after formation of over lapping zone growth of PE was inhibited.

Daedaleopsis confragosa - Pleurotus florida (DC-PF) (Plate 19 M-O)

The growth of DC is more than the PF reaching half of the petriplate on the 4th day after inoculation. The growth covering DC also higher than PF on the 7th day. Both the fungi came in to contact with each other on the 10th day. After come in contact growth of both the fungi inhibited and the type of interaction was found to be deadlock at touching point.

Daedaleopsis confragosa - Pleurotus ostreatus (DC-PO) (Plate 19 P-R)

The growth of DC was more than PO on the 4th day after the inoculation. Both fungi came in to contact with each other on the 7th day. DC overgrows on PO and the growth of PO stop on the 10th day. PO is not killed but growth is inhibited and DC overgrew till the inoculums disc of PO and showing invasion/replacement.

Plate 19 Interaction of *Daedaleopsis confragosa* with other fungal isolates

A-C: DC-TV

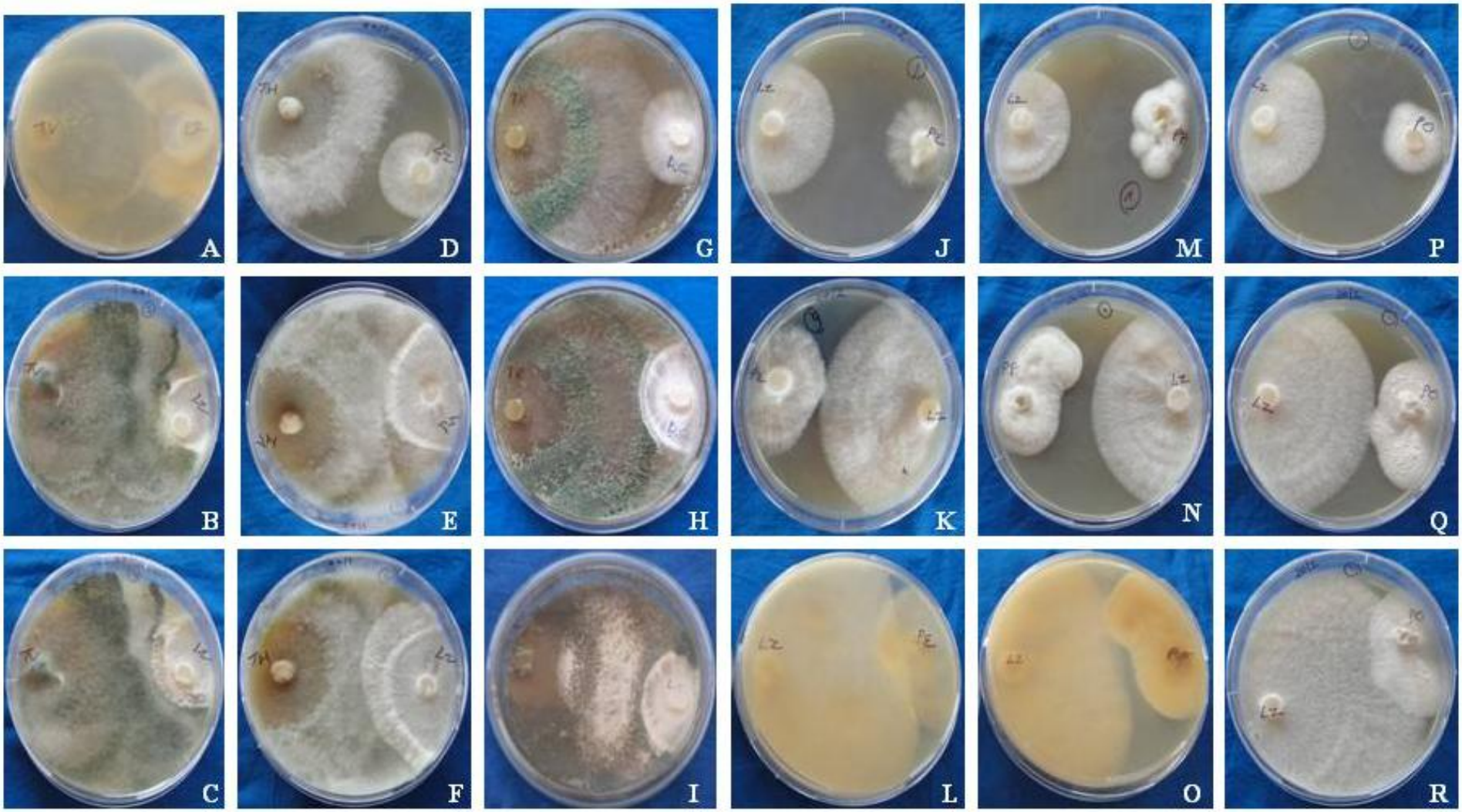
D-F: DC- TH

G-I: DC-TR

J-L: DC-PE

M-O: DC-PF

P-R: DC-PO



DC-TV DC-TH DC-TR DC-PE DC-PF DC-PO

Plate 19

Daedaleopsis confragosa - Pleurotus sajorcaju (DC-PS) (Plate 19 A-C)

The growth of PS was higher than DC on the 4th day after inoculation. The growth of DC was increased than the PS and contact between both fungi occur and the growth of PS was stops on the 7th day. DC overgrows on PS and further it covers the whole plate on the 10th day. DC goes on advancing over PS and does not kill it but growth is inhibited. Even after 3 weeks the growth of PS is slightly increased but still inhibited. DC grows profusely covering the petriplate. Both are compatible but growth of PS is very slow and showed mutual intermingling type of interaction.

Daedaleopsis confragosa - Irpex lacteus (DC-IL) (Plate 20 D-F)

The growth of IL was more than DC on the 4th day after inoculation. Both fungi came in to contact with each other and DC overgrow on IL and the growth of IL was also continued on 6th day. Though the growth of DC continued over IL it did not inhibit by DC on the 9th day. Growth of both the fungi continued and overlapping zone of 2.7 cm was formed. Even after 3 weeks of incubation time both fungi grew to gather and interaction was found to be mutual intermingling.

Daedaleopsis confragosa -Phellinus pectinatus (DC-PHE) (Plate 20 G-I)

The growth of DC was more than PHE on the 5th day after inoculation. The both fungus came in to contact with each other on the 8th day. PHE growth was inhibited on the 8th day and the growth of DC increase further but at particular stage when it comes in complete contact the growth of both fungi stopped. Both fungi survive but growth is inhibited. The type of interaction was found to be deadlock at touching point.

Daedaleopsis confragosa - Pycnoporus sanguineus (DC-PYS) (Plate 20 J-L)

The growth of DC and PYS was same reaching almost the middle of the petriplate on the 5th day after inoculation. The growth of DC increase on the 8th day than PYS.

Plate 20 Interaction of *Daedaleopsis confragosa* with other fungal isolates

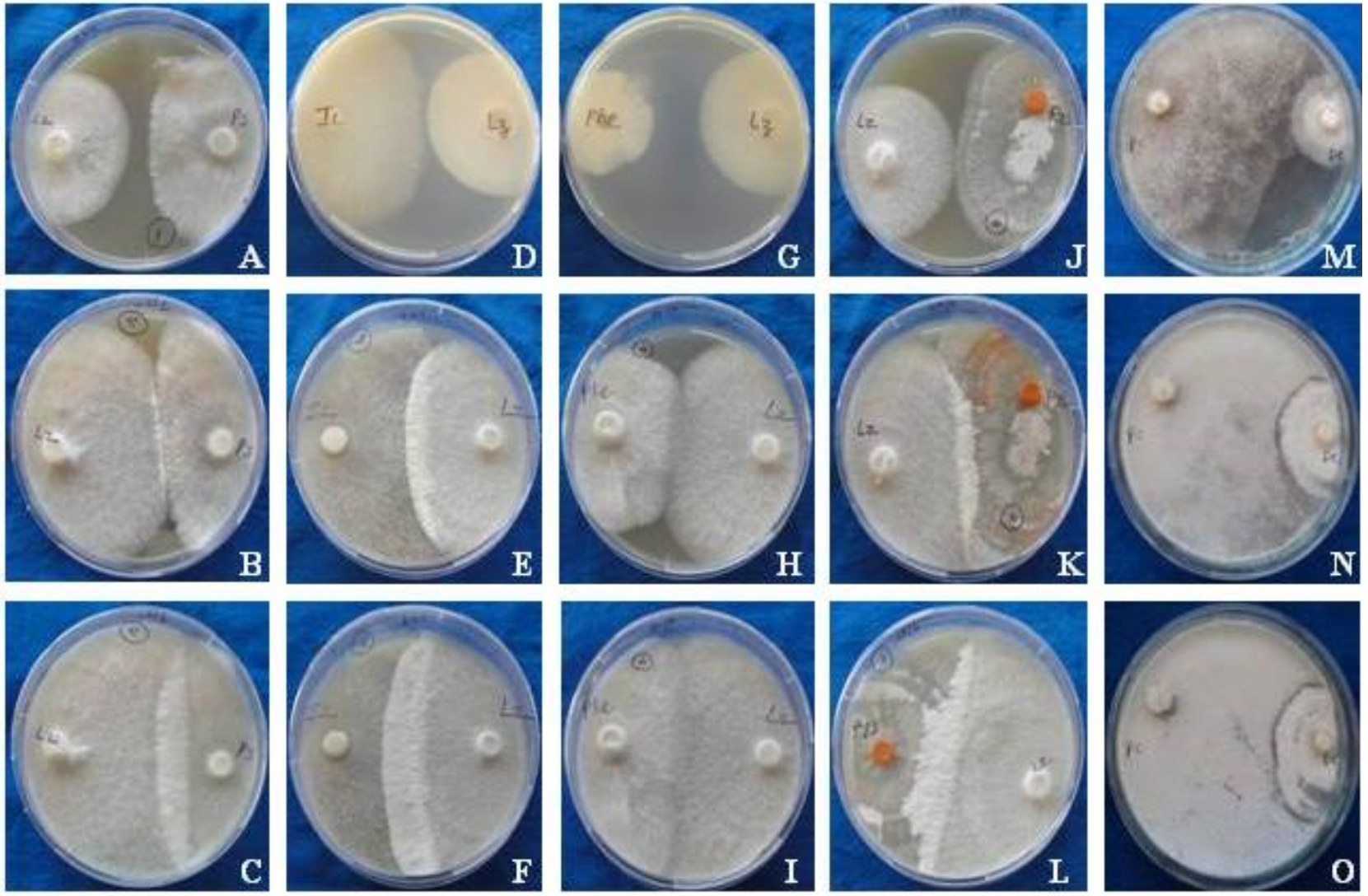
A-C: DC-PS

D-F: DC-IL

G-I: DC-PHE

J-L: DC-PYS

M-O: DC-PC



DC-PS DC-IL DC-PHE DC-PYS DC-PC

Plate 20

Both fungi comes in contact with each other. The growth of DC increased but PYS also continued to grow. Both fungi over grow on each other and overlapping zone was clearly visible. PYS growth was reduced and the growth of DC continued advancing along with PYS on the 11th day. Both the fungi grew on each other and the type of interaction was found to be mutual intermingling.

Daedaleopsis confragosa- Phanerochaete chrysosporium (DC-PC) (Plate 20 M-O)

The growth of PC was more than DC on the 3rd day after inoculation. The growth of DC seems to get restricted on the 5th day and thin line of inhibition appeared. Both fungi did not come in to contact with each other. The growth of both fungi occurred and inhibition line became visible clearly on the 10th day and both fungi are not compatible with each other. The type of inhibition was deadlock at a distance.

DC is a fast growing fungus on MEA and all the five types of interaction was observed. DC showed mutual intermingling with TR, PS, IL, PYS while with TV, TH, PE it showed partial mutual intermingling. In the interaction with PO invasion/replacement was observed. In the interaction with PF and PHE it showed deadlock at touching point and with PC dead lock at a distance was observed.

(10) *Phellinus pectinatus*

Phellinus pectinatus -Trichoderma viride (PHE-TV) (Plate 21 A-C)

The growth of TV was very higher than PHE on the 3rd day after inoculation. The both fungi came in contact with each other on the 6th day and PHE started to overgrow on TV. PHE overgrows on TV on the 9th day and show its compatibility with TV. This type of interaction was found to be partial mutual intermingling.

Phellinus pectinatus -Trichoderma harzianum (PHE-TH) (Plate 21 D-F)

The growth of TH was very higher than PHE on the 3rd day after inoculation. The both fungi came in contact with each other and TH almost covered the whole plate on

the 6th day. PHE also overgrows on TH on the 9th day and further increase also showed its compatibility with TH. The type of interaction was found to be partial mutual intermingling.

Phellinus pectinatus -Trichoderma reesei (PHE- TR) (Plate 21 G-I)

The growth of TR was very higher than PHE on the 3rd day after inoculation. Both fungi came in contact with each other on the 6th day. PHE overgrows on TR on the 10th day. PHE continues to grow even after 20 days of incubation period. Both the fungi found to be compatible with each other and type of interaction was found to be mutual intermingling.

Phellinus pectinatus -Pleurotus eryngii (PHE-PE) (Plate 21 J-L)

The growth of PE was slower than PHE on the 4th day after inoculation. The growth of PHE is still higher than the PE covering 80% of the petriplate on the 7th day. Both fungi came in to contact with each other on the 10th day. Growth of both fungi was further stopped. Here the growth of both the fungi was restricted after they came in to contact with each other so type of inhibition was deadlock at touching point.

Phellinus pectinatus -Pleurotus florida (PHE-PF) (Plate 21 M-O)

The growth of PHE is higher than the PF on the 4th day after inoculation. The growth of PHE is still higher than PF on the 7th day. Contact of both the fungi did not occur on the 10th day. Situation remained same for incubation period of 30 days also. Here both the growth of both fungi inhibited without coming in to contact with each other the type of interaction was found to be dead lock at distance.

.*Phellinus pectinatus -Pleurotus ostreatus* (PHE-PO) (Plate 21 P-R)

The growth of PHE was more than PO on the 4th day after the inoculation. Growth PHE is still higher than PO advanced into ½ of the petriplate on the 7th day. Both the fungi come in contact with each other on the 10th day and the growth of PO stopped at

Plate 21 Interaction of *Phellinus pectinatus* with other fungal isolates

A-C: PHE-TV

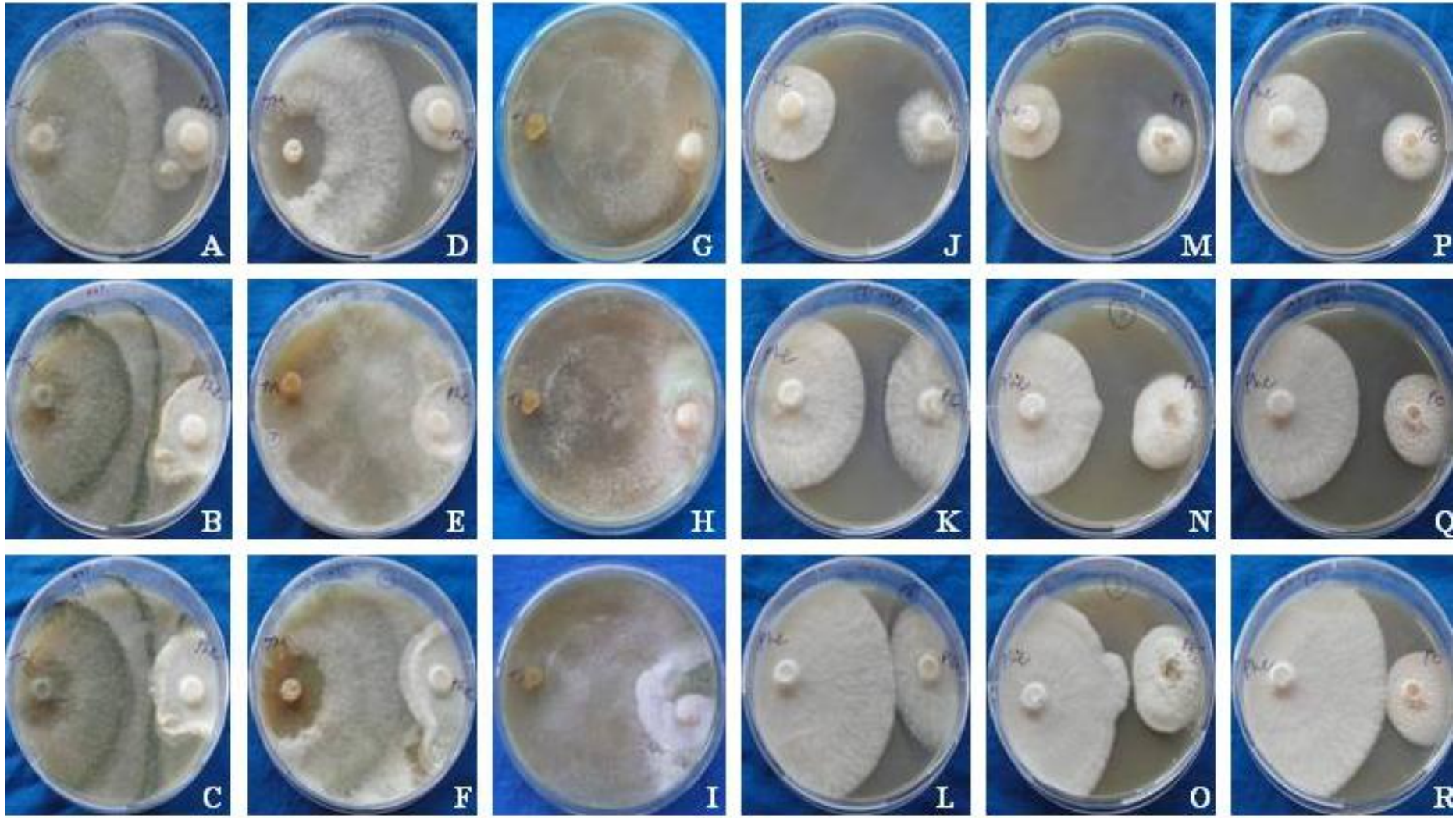
D-F: PHE- TH

G-I: PHE-TR

J-L: PHE-PE

M-O: PHE-PF

P-R: PHE-PO



PHE-TV PHE-TH PHE-TR PHE-PE PHE-PF PHE-PO

Plate 21

the region of contact. PHE at this stage has advance 75% in the petriplate and showing dead lock at touching point.

Phellinus pectinatus -Pleurotus sajorcaju (PHE-PS) (Plate 22 A-C)

The growth of PS was more than PHE on the 4th day after inoculation however the growth of PHE was slow. Both fungi came in to contact with each other on the 7th day. The growth of PHE was increase and PHE can overgrow on PS and the overlapping zone was form on the 10th day. The growth of PS does not stop here. PHE over grows PS further and the two continue to grow. The two are compatible and grow together and showed mutual intermingling type of interaction.

Phellinus pectinatus -Irpex lacteus (PHE-IL) (Plate 22 D-F)

The growth of IL was more than PHE on the 3rd day after inoculation. Both fungi came under contact with each other on the 6th day and PHE started to overgrow on the growth of IL. Both the fungi continued to overgrow and forming an overlapping zone of 1.5 cm and both the fungi were compatible with each other showed mutual intermingling.

Phellinus pectinatus -Daedaleopsis confragosa (PHE-DC) (Plate 22 G-I)

The growth of DC was more than PHE on the 5th day after inoculation. The both fungus came in to contact with each other on the 8th day. PHE growth was inhibited on the 8th day and the growth of DC increase further but at particular stage when it comes in complete contact the growth of both fungi stopped. Both fungi survive but growth is inhibited. The type of interaction was found to be deadlock at touching point.

Phellinus pectinatus -Pycnoporus sanguineus (PHE-PYS) (Plate 22 J-L)

The growth of PYS was more than PHE on the 5th day after inoculation. Both fungi came in to contact with each other on the 7th day. The growth of PHE was increase

Plate 22 Interaction of *Phellinus pectinatus* with other fungal isolates

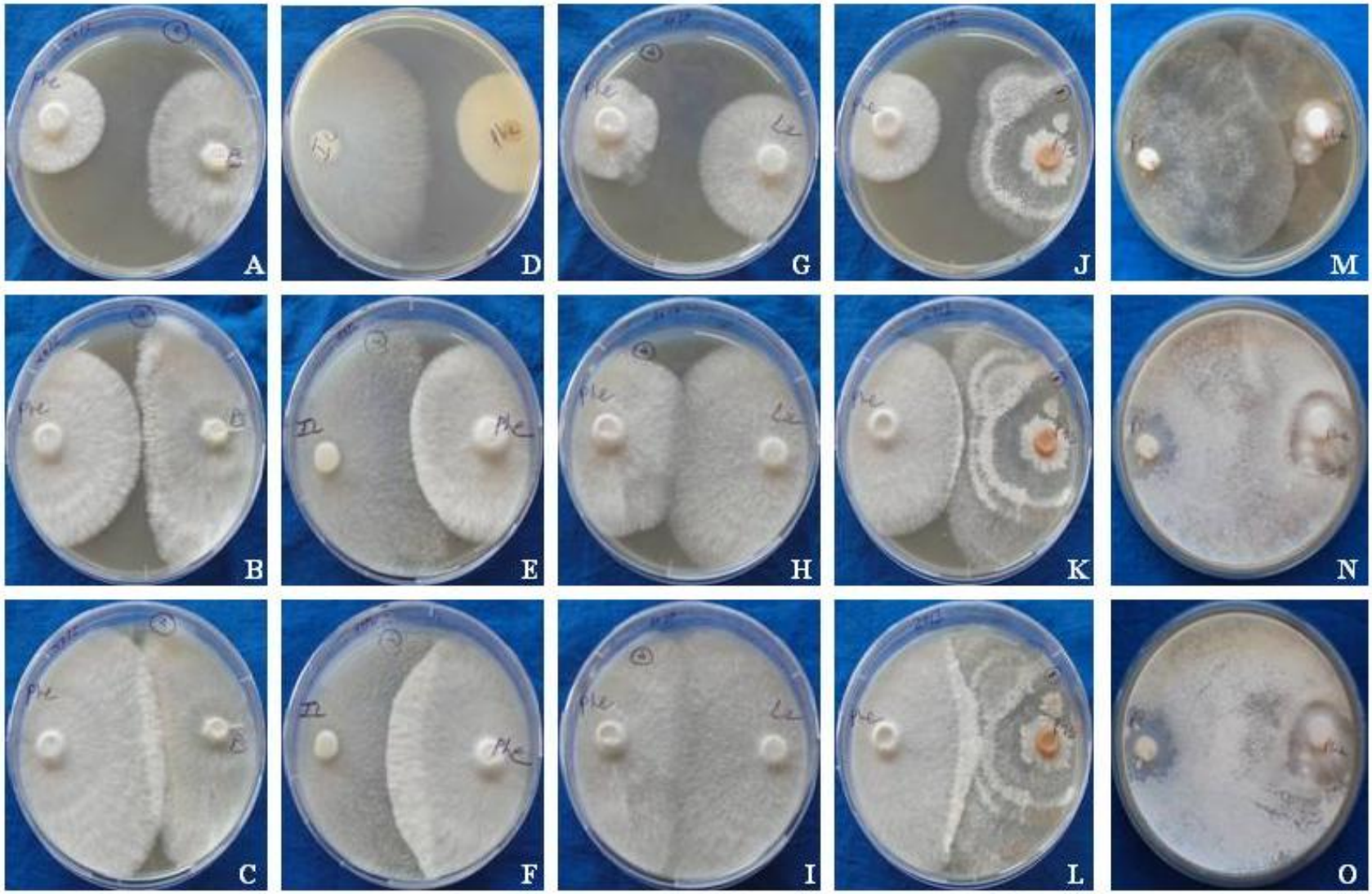
A-C: PHE-PS

D-F: PHE-IL

G-I: PHE-DC

J-L: PHE-PYS

M-O: PHE-PC



PHE-PS PHE-IL PHE-DC PHE-PYS PHE-PC

Plate 22

than PYS and it further increase and overlapping zone was visible and then further PHE overgrow on PYS and the growth of PYS inhibited on the 10th day. The overlapping zone does not expands indicating no inhibition in growth of both fungi and type of interaction was mutual intermingling.

Phellinus pectinatus- Phanerochaete chrysosporium (PHE- PC) (Plate 22 M-O)

The growth of PC was more than PHE on the 3rd day after inoculation. Both fungi come under contact with each other on the 5th day. The growth of PC was more than PHE but PHE further continue to grow on PC and forming an overlapping zone of 0.7 cm on the 10th day. PHE does not kill by PC but after 15th day growth of PHE was restricted. The interaction was found to be partial mutual intermingling.

In the interaction with PHE with other fungal isolates all the five types of interaction was noted Interaction of PHE with *Trichoderma* species was found partial intermingling with TV and TH while with TR it showed mutual intermingling. Partial mutual intermingling was also noted in the interaction with PYS and PC. In the interaction of PHE with *Pleurotus* spp. Deadlock interaction at a distance was observed with PF and deadlock at touching point with PE and PO while with PS mutual intermingling was seen. Mutual intermingling was also observed in the interaction with IL. Dead lock at touching point was noted in the interaction with PHE with DC.

(11)*Pycnoporus sanguineus*

Pycnoporus sanguineus-Trichoderma viride (PYS-TV) (Plate 23 A-C)

The growth of TV was more than PYS on the 3rd day after inoculation as growth of PYS just started on this day. TV covers the whole plate and over grows on PYS. Sporulation of TV occurred on the 6th day and the growth of PYS stops here. TV killed PYS and the color of PYS inoculum change orange to black on the 9th day TV

act as antagonist against PYS. Here TV overgrew on PYS till the inoculum disc and the interaction was found to be invasion/replacement.

Pycnoporus sanguineus-Trichoderma harzianum (PYS-TH) (Plate 23D-F)

The growth of TH was more than PYS on the 3rd day after inoculation and both fungi came in contact with each other. TH over grew on PYS and growth of PYS was inhibited by TH on the 6th day. TH killed PYS and the color of PYS inoculum change orange to brown on the 9th day. The interaction was found to be invasion/replacement as the growth of PYS was restricted after overgrowth of TH.

Pycnoporus sanguineus- Trichoderma reesei (PYS-TR) (Plate 23 G-I)

The growth of TR was more than PYS and TR almost covered the whole plate on the 3rd day after inoculation. Sporulation of TR occurred and PYS overgrows on TR on the 6th day. On the 10th day PYS continue to grow till the inoculum disc of TR and later covered the whole plate showing the compatibility with each other. The type of interaction was found to be mutual intermingling.

Pycnoporus sanguineus-Pleurotus eryngii (PYS-PE) (Plate 23 J-L)

The growth of PYS is more than PE on the 4th day after inoculation. Both fungi came in to contact with each other on the 7th day and the growth of PE is inhibited. PYS over grow on PE and almost covered the whole plate on the 10th day & the sporulation of PYS occurs reaching very close to the PE inoculum and type of interaction was found to be invasion/replacement as the growth of PE was replaced by PYS.

Pycnoporus sanguineus-Pleurotus florida (PYS-PF) (Plate 23 M-O)

The growth of PYS is higher than the PF on the 4th day after inoculation. Both the fungus came in to contact on the 7th day and PYS overgrows on PF and the growth of PF inhibited. The Sporulation of PYS appeared and it covered the whole plate on the 10th day. After coming in to contact with each other the growth of both fungi stopped.

The type of interaction was found to be deadlock at touching point.

Pycnoporus sanguineus-Pleurotus ostreatus (PYS-PO) (Plate 23 P-R)

The growth of PYS was more than PO on the 4th day after the inoculation. The growth of PYS was 50% advanced petriplate on the 7th day. Both the fungi came in to contact with each other on the 10th day and further growth of PO and PYS stopped. The sporulation of PYS was clearly visible in concentric ring and even at the region of their contact showing deadlock at touching point.

Pycnoporus sanguineus-Pleurotus sajorcaju (PYS-PS) (Plate 24 A-C)

The growth of PYS was more than PS on the 4th day after inoculation. Both fungi came in to contact with each other on the 7th day. At the zone of contact PYS sporulation gets covered by PS. Both fungi can overgrow on each other and overlapping zone is visible and at particular stage on the 10th day. Further the growth of PS first stops and then PYS stop. As growth of both the fungi stopped after coming in to contact the interaction was found to be dead lock at touching point.

Pycnoporus sanguineus-Irpex lacteus (PYS-IL) (Plate 24 D-F)

The growth of IL was more than PYS and both fungi came under contact with each other on the 5th day after inoculation. Both fungi overgrow on each other and overlapping zone appear on the 8th day. The growth of both fungus inhibited by each other on the 11th day. At the region of contact orange red sporulation of PYS appears. The type of interaction was found to be deadlock at touching point.

Pycnoporus sanguineus-Daedaleopsis confragosa (PYS-DC) (Plate 24 G-I)

The growth of DC and PYS was same reaching almost the middle of the petriplate on the 5th day after inoculation. The growth of DC increase on the 8th day than PYS. Both the fungi came in to contact with eachother. The growth of DC was increased but PYS also continued to grow. Both fungi overgrew each other and formed overlapping zone.

Plate 23 Interaction of *Pycnoporus sanguineus* with other fungal isolates

A-C: PYS-TV

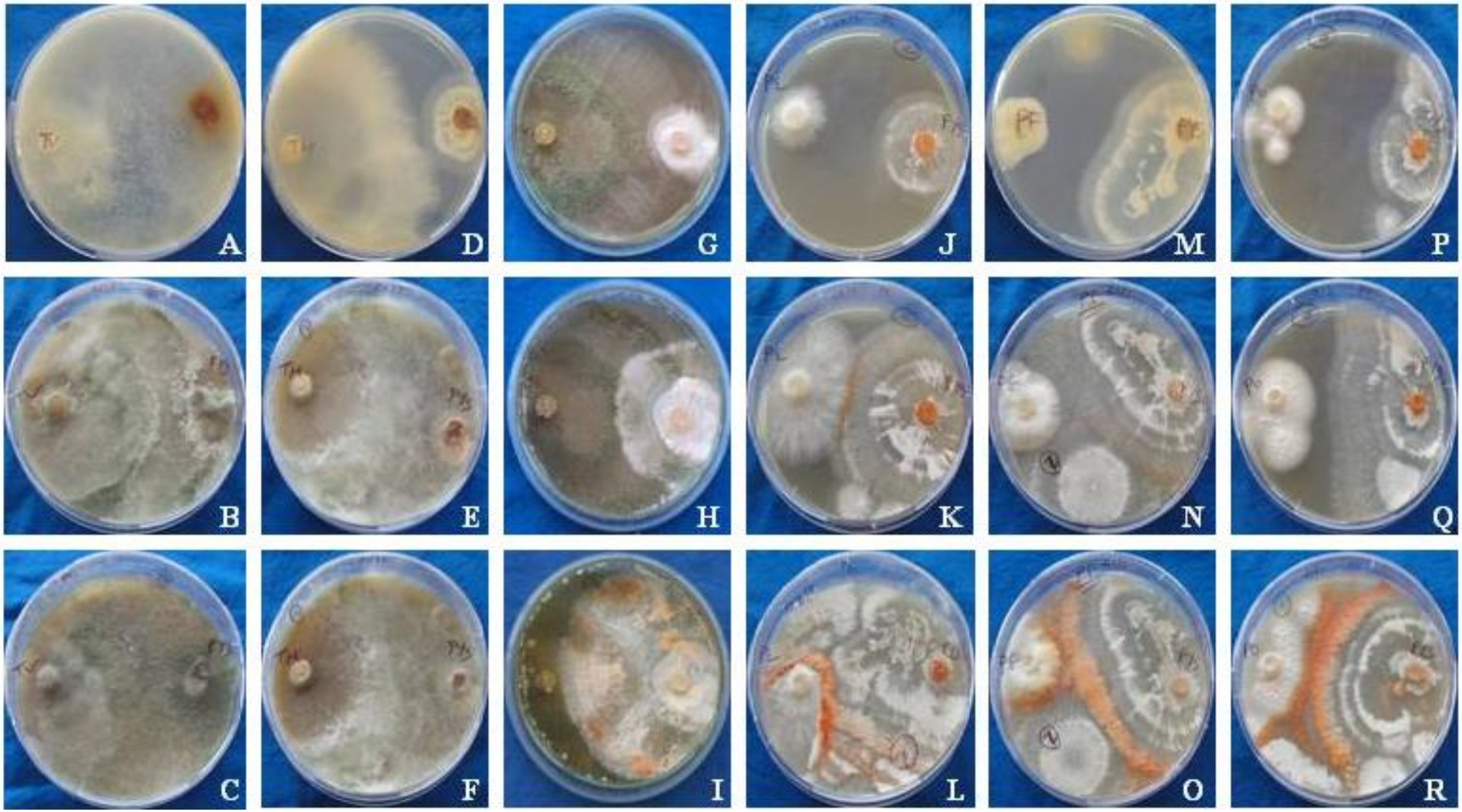
D-F: PYS- TH

G-I: PYS -TR

J-L: PYS-PE

M-O: PYS-PF

P-R: PYS-PO



PYS-TV PYS-TH PYS-TR PYS-PE PYS-PF PYS-PO

Plate 23

PYS growth was reduced and the growth of DC continued advancing along with PYS on the 11th day. Both the fungi grew on each other and the type of interaction was found to be mutual intermingling.

Pycnoporus sanguineus-Phellinus pectinatus (PYS-PHE) (Plate 24 J-L)

The growth of PYS was more than PHE on the 5th day after inoculation. Both fungi came in to contact with each other on the 7th day. The growth of PHE was increase than PYS and it further increase and overlapping zone was visible and then further PHE overgrow on PYS and the growth of PYS inhibited on the 10th day. The overlapping zone does not expands indicating no inhibition in growth of both fungi and type of interaction was mutual intermingling.

Pycnoporus sanguineus - Phanerochaete chrysosporium (PYS-PC) (Plate 24 M-O)

The growth of PC was more than PYS on the 3rd day after inoculation. Both fungi did not come under contact with each other but clear line of inhibition was seen on the 5th day after inoculation. Sporulation of PYS started on 8th day. The growth of both fungi inhibited by each other at the region of contact on the 11th day and type of interaction was found to be deadlock at touching point.

In the interaction of PYS with other eleven fungi mutual intermingling was observed with TR, DC and PHE while invasion/replacement was observed with TV, TH, PE. Deadlock at touching point was observed with PF, PO, PS, IL, PC. Partial mutual intermingling and deadlock at a distance was not observed in the interaction of PYS.

(12) *Phanerochaete chrysosporium*

Phanerochaete chrysosporium - Trichoderma viride (PC-TV) (Plate 25 A-C)

The growth of TV was more than PC and both the fungi came in to contact with each other on the 3rd day after inoculation. The growth of TV occurred and line of

inhibition also clearly seen on the 5th day indicating the growth of PC was inhibited. On the 9th day TV covered the whole plate. Here the growth of PC restricted and TV overgrew on PC showing invasion/replacement type of interaction. *Phanerochaete chrysosporium - Trichoderma harzianum* (PC-TH) (Plate 25 D-F)

The growth of TH was more than PC and both the fungi came in to contact with each other on the 3rd day after inoculation. TH advances up to almost ¾ of the petriplate in 5 days. TH overgrows on PC and the growth of PC stop on the 10th day.TH cover the whole plate. Here TH overgrew on PC and it also restricted growth of PC and the interaction was found to be invasion/replacement.

Phanerochaete chrysosporium-Trichoderma reesei (PC-TR) (Plate 25 G-I)

The growth of TR was more than PC and TR almost covers the whole plate and both the fungi came into contact with each other on the 3rd day after inoculation. Sporulation of TR was started on the 6th day and the growth of PC also occurs and it overgrows on TR. The situation remains same on the 10th day PC completely overgrows on TR. Both the fungi found to be compatible with each other and showed mutual intermingling.

Phanerochaete chrysosporium -Pleurotus eryngii (PC-PE) (Plate 25 J-L)

The growth of PC was more than PE on the 3rd day after the inoculation. It almost advances up to more than half of the petriplate. Both fungi came in to contact with each other on the 5th day. PC grows completely fill the petriplate on the 8th day. PC covered the whole plate and the growth of PE was inhibited on the 11th day. Here the line of inhibition was clearly visible surround the growth of PE so type of interaction was deadlock at a distance.

Phanerochaete chrysosporium -Pleurotus florida (PC-PF) (Plate 25 M-O)

The growth of PC was more than PF on the 3rd day after inoculation. PF appeared to

Plate 24 Interaction of *Pycnoporus sanguineus* with other fungal isolates

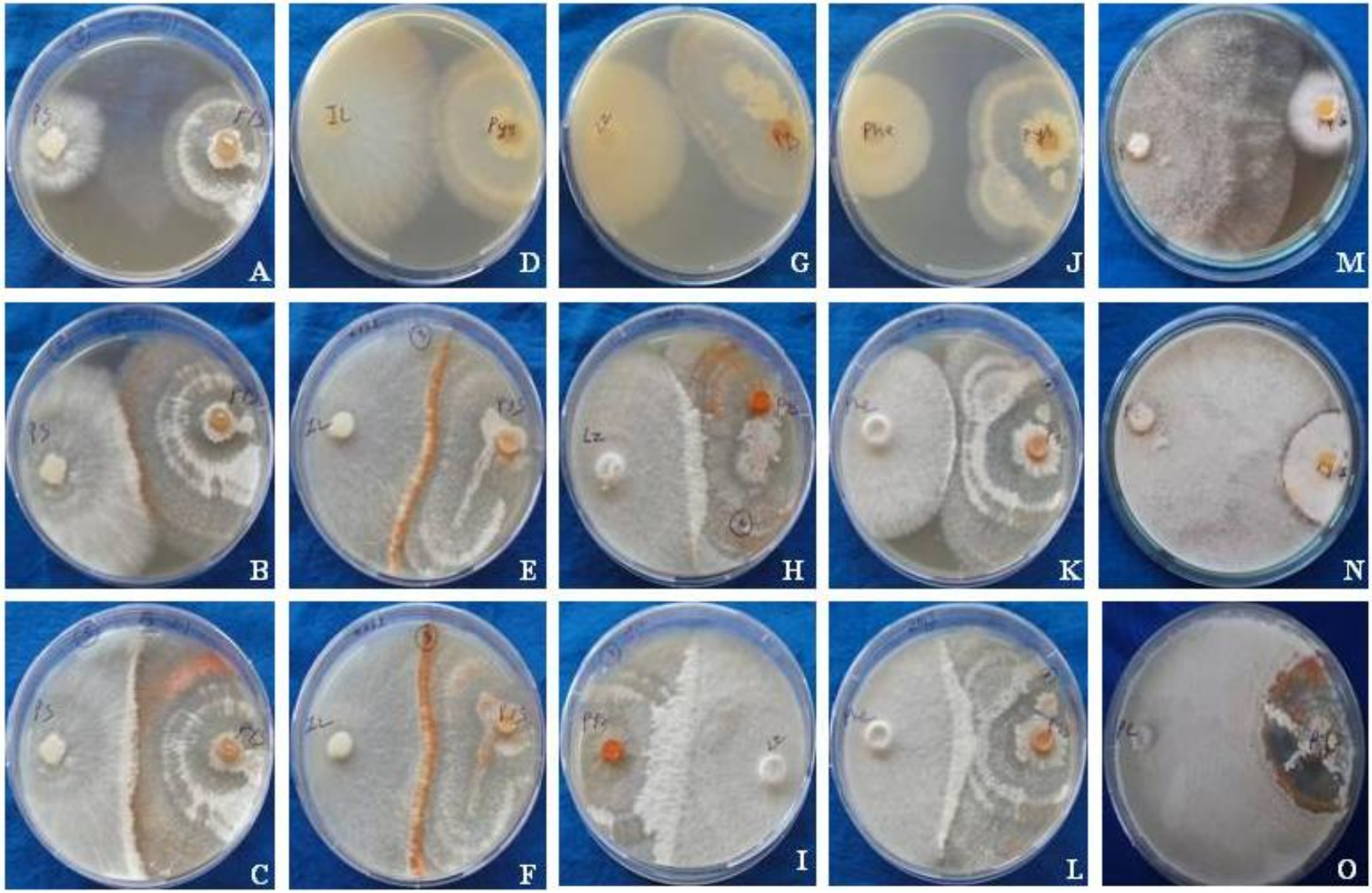
A-C: PYS-PS

D-F: PYS-IL

G-I: PYS-DC

J-L: PYS-PHE

M-O: PYS-PC



PYS-PS PYS-IL PYS-DC PYS-PHE PYS-PC

Plate 24

grow very slow. The growth of PC was still denser indicated by the dense growth of mycelium within the entire petriplate and covering surrounding PF on 6th day and growth of PF was inhibited. Growth of PF did not occur on 9th day and situation remains same at later stage also. The type of interaction was found to be deadlock at touching point.

Phanerochaete chrysosporium - *Pleurotus ostreatus* (PC-PO) (Plate 25 P-R)

The growth of PC was more than PO on the 3rd day after inoculation. The growth of PO was just started on the 3rd day. PC overgrows on PO and on the 6th day and both fungus come under contact with each other and the growth of PO also occurred.

PC almost covered the plate on 10th day but growth of PO was stopped and showed partial mutual intermingling.

Phanerochaete chrysosporium - *Pleurotus sajorcaju* (PC-PS) (Plate 26 A-C)

The growth of PC was more than PS and both fungi come under contact with each other on the 3rd day after inoculation. The growth of PS occurs and the growth of PC was further increase and the completely covered the petriplate on the 5th day. PS overgrows on PC on the 10th day but later the growth of PS was restricted indicating PC acts as an antagonist for PS and showed dead lock at touching point interaction type.

Phanerochaete chrysosporium -*Irpex lacteus* (PC-IL) (Plate 26D-F)

The growth of PC was faster than IL and both fungi come in contact with each other on the 3rd day after inoculation. The growth of PC was fast and the growth of PC continues and the growth of IL restricted on the 5th day. Growth of IL stopped on 8th day and line of inhibition was clearly seen. Situation remains same on the 10th day and PC acts as an antagonist against IL and type of interaction was found to be deadlock at touching point.

Plate 25Interaction of *Phanerochaete chrysosporium* with other fungal isolates

A-C: PC-TV

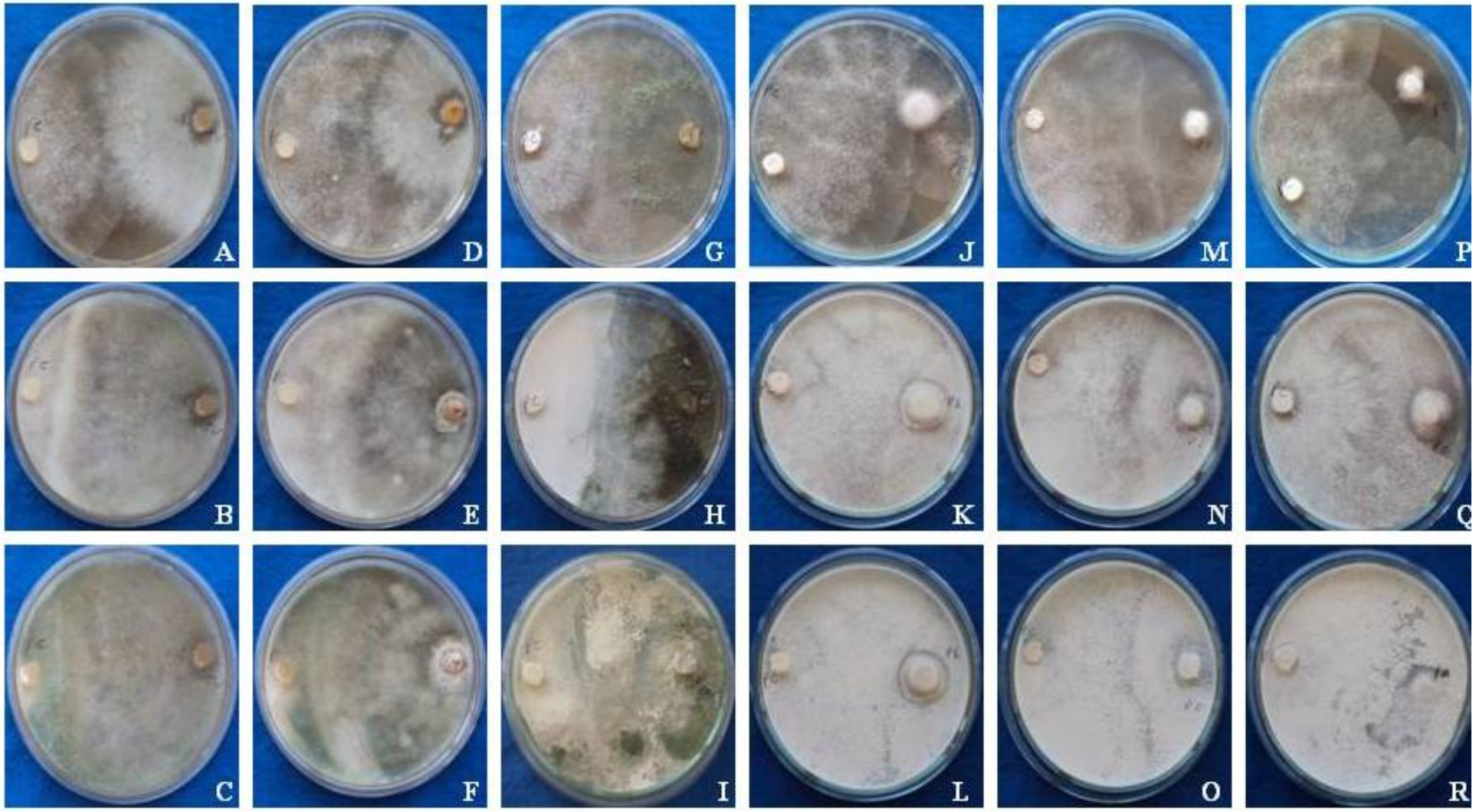
D-F: PC- TH

G-I: PC -TR

J-L: PC-PE

M-O: PC-PF

P-R: PC-PO



PC-TV PC-TH PC-TR PC-PE PC-PF PC-PO

Plate 25

Phanerochaete chrysosporium - *Daedaleopsis confragosa* (PC-DC) (Plate 26 G-I)

The growth of PC was more than DC on the 3rd day after inoculation. The growth of DC seems to get restricted on the 5th day and thin line of inhibition appeared. Both fungi did not come in to contact with each other. The growth of both fungi occurred and inhibition line became visible clearly on the 10th day and both fungi are not compatible with each other. The type of inhibition was deadlock at a distance.

Phanerochaete chrysosporium -*Phellinus pectinatus* (PC-PHE) (Plate 26 J-L)

The growth of PC was more than PHE on the 3rd day after inoculation. Both fungi come under contact with each other on the 5th day. The growth of PC was more than PHE but PHE further continue to grow on PC and forming an overlapping zone of 0.7 cm on the 10th day. PHE does not kill by PC but after 15th day growth of PHE was restricted. The interaction was found to be partial mutual intermingling.

Phanerochaete chrysosporium - *Pycnoporus sanguineus* (PC-PYS) (Plate 26 M-O)

The growth of PC was more than PYS on the 3rd day after inoculation. Both fungi did not come under contact with each other but clear line of inhibition was seen on the 5th day after inoculation. Sporulation of PYS started on 8th day. The growth of both fungi inhibited by each other at the region of contact on the 11th day and type of interaction was found to be deadlock at touching point.

In the interaction of PC with other eleven fungal isolates it was observed that PC is not compatible with most of the fungi it showed mutual intermingling with only TR and partial mutual intermingling with PO and PHE. It showed deadlock at touching point with PF, PS, IL, DC, PYS while dead lock at a distance was noticed with PE. in the interaction with TH and TV invasion/replacement was observed.

Plate 26 Interaction of *Phanerochaete chrysosporium* with other fungal isolates

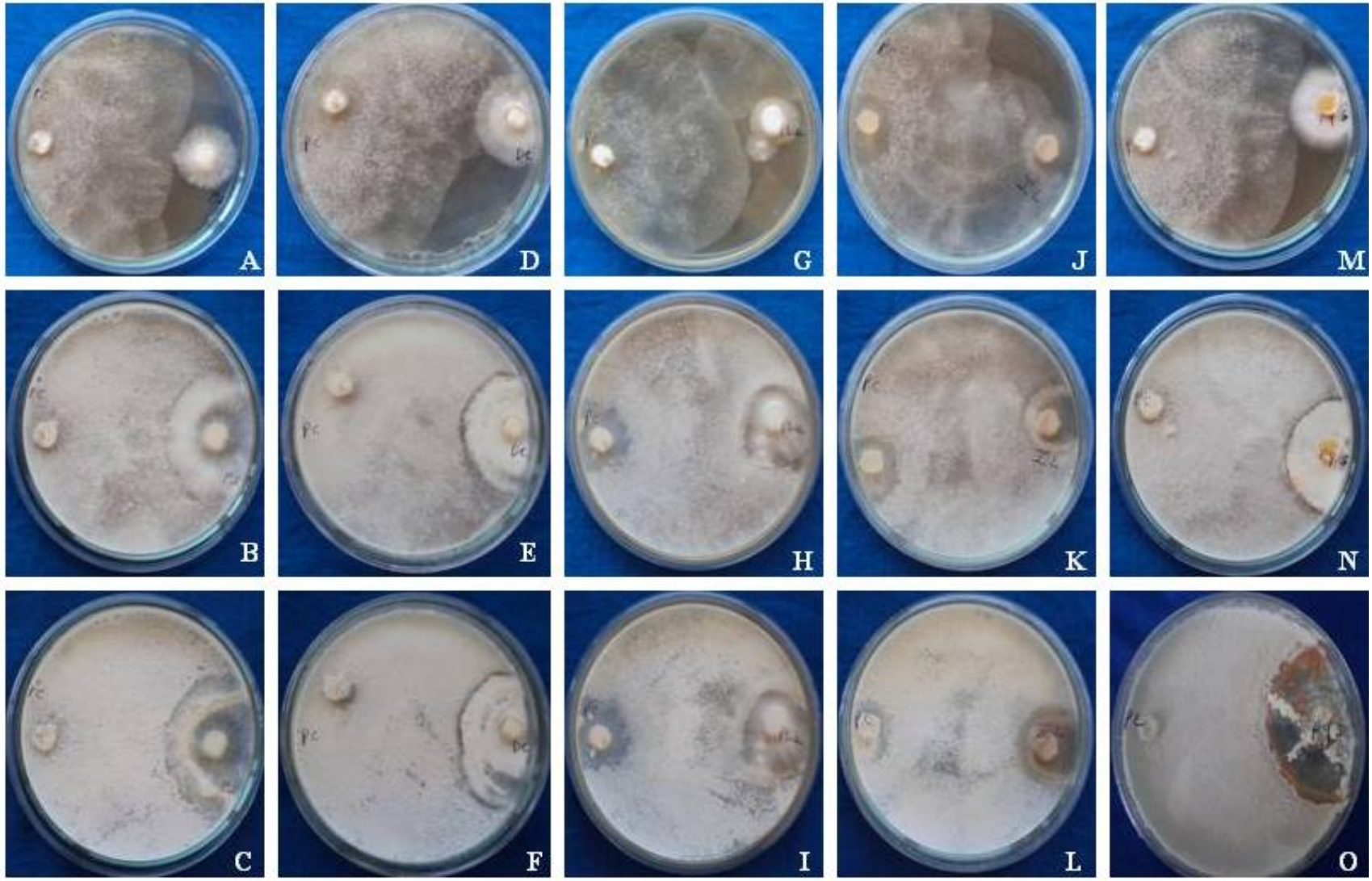
A-C: PC-PS

D-F: PC- DC

G-I: PC- IL

J-L: PC- PHE

M-O: PC- PYS



PC-PS PC-DC PC-PHE PC-IL PC-PYS

Plate 26

According to the classification proposed by Porter 1924 the data indicates the paired interaction test to show the following result.

Fungal isolates	TV	TH	TR	PE	PF	PO	PS	IL	DC	PHE	PYS	PC
TV	-	DAT	DAT	I/R	I/R	I/R	I/R	DAT	PMI	PMI	I/R	I/R
TH	DAT	-	DAT	I/R	PMI	I/R	I/R	I/R	PMI	PMI	I/R	I/R
TR	DAT	DAT	-	I/R	I/R	PMI	MI	MI	MI	MI	MI	MI
PE	I/R	I/R	I/R	-	DAT	PMI	PMI	PMI	PMI	DAT	I/R	DAD
PF	I/R	PMI	I/R	DAT	-	DAD	MI	PMI	DAT	DAD	DAT	DAT
PO	I/R	I/R	PMI	PMI	DAD	-	MI	I/R	I/R	DAT	DAT	PMI
PS	I/R	I/R	MI	PMI	MI	MI	-	PMI	MI	MI	DAT	DAT
IL	DAT	I/R	MI	PMI	PMI	I/R	PMI	-	MI	MI	DAT	DAT
DC	PMI	PMI	MI	PMI	DAT	I/R	MI	MI	-	DAT	MI	DAD
PHE	PMI	PMI	MI	DAT	DAD	DAT	MI	MI	DAT	-	MI	PMI
PYS	I/R	I/R	MI	I/R	DAT	DAT	DAT	DAT	MI	MI	-	DAT
PC	I/R	I/R	MI	DAD	DAT	PC	DAT	DAT	DAD	PMI	DAT	-

The short forms describing the nature of compatibility are as follows:

MI: Mutual intermingling

PMI: Partial mutual intermingling

I/R: Replacement

DAT: Deadlock at touching point

DAD: Dead lock at a distance

(b) Selection of fungi

Monocultures of fungal isolates for further quantitative analysis of enzymes were selected mainly on the basis screening experiment in which all the fungal isolates were tested for their ligninolytic, cellulolytic and xylanolytic enzyme activity using plate assay. Selection of coculture was done from the results obtained in the paired interaction test.

Depending up on the dark brown coloured zone in the plate containing MEA + Tannic acid and its intensity as positive indicator of Ligninolytic activity, absence of clear zone in media containing MEA + CMC as negative indicator of cellulolytic activity as well as presence of clear zone in the media containing MEA+ xylan as positive indicator of xylanolytic enzyme activity DC, PHE, IL, PS as monocultures were selected. The results of paired interaction test indicated cocultures of selected fungi IL+DC, IL+PHE, IL+PS, PS+DC, PS+PHE were found to be highly compatible with each other. The selected fungal isolates in mono and co cultures were subjected for further quantification of ligninolytic and hydrolytic activities in liquid culture media.

3.1.3 Discussion

In the present study twelve different fungal isolates, three belongs to *Trichoderma* i.e. *T. viride, T. harzianum, T. reesei*, four isolates of *Pleurotus* (*P. eryngii, P. florida, P. ostreatus, P. sajorkaju*) and other white rot fungi like *Irpex lacteus, Daedaleopsis confragosa, Phellinus pectinatus, Pycnoporus sanguineus, Phanerochete chrysosporium* were tested for their ligninolytic, cellulolytic and xylanolytic enzyme activity qualitatively by plate assay technique and by in vitro dual culture technique for the selection of compatible fungal co partner to be used efficiently in the pulp and paper industry for the process of biopulping and biobleaching. For the further study selection of fungi were done from the results of screening experiment and paired interaction test.

Bains *et al* (2006) evaluated ligninolytic and cellulolytic enzymes of wood degrading white rot fungi (*Ganoderma lucidum, Pleurotus florida, Polyporus volvatus, Polyporus* sp1, *Polyporus* sp2 and *Hymenochete* sp) and found that

Pleurotus florida secreting ligninolytic enzyme. Similar results were noticed in the present study also.

Atri and Sharma (2012) studied lignocellulosic enzyme activity of five wild species of *Pleurotus* namely *P. floridanus, P. pulmonaris, P. sapidus, P. cystidiosus* and *P. sajorkaju* using same substrate 1% tannic acid for ligninolytic enzymes and 1% CMC for cellulolytic enzymes. Results indicating that all the species of *Pleurotus* showing positive results for ligninolytic as well as cellulolytic enzyme activity. Here also *P. sajorkaju* showing presence of ligninolytic enzyme activity which agrees with their study but not showing cellulolytic enzyme activity may be due to difference in the species collected.

Boddireddy and Charya (2011) screened 30 fungal isolates for cellulase free xylanase producer amongst them 50% isolates were belonged to *Aspergillus* species and rest of were *Trichoderma* and *Penicillium* species. In the presented results also all three *Trichoderma* species were found to produce xylanase enzyme which agrees with their study.

In the interaction of the two different fungi the pattern of hyphal growth (one below the other) was observed by Skidmore and Dickinson (1976) and they explained the hyphal mode of growth by interacting fungi such as either above or below its colony, and its corollary. The partial mutual intermingling (represented diagrammatically in Figure given in the materials and methods by Porter 1924) was recognized by Skidmore and Dickinson as simply ‘intermingling’ and ‘neutral intermingling’ by Stahl and Christensen 1992.

A total of five different modes of outcomes of interacting fungi were observed as represented diagrammatically in the figure and can be compared with the citations of Porter 1924, Stahl and Christensen 1992. Porter described four outcomes, but did

not mention invasion/replacement. Stahl and Christensen combined mutual and partial intermingling into one 'neutral intermingling', which was described in separate events (basically in three steps) by Porter. They also combined the last two outcomes (i.e. inhibition at contact point as well as a distance) into deadlock with introduced the outcome of 'replacement'.

The dual culture test performed in the present study evaluated all the observed fungal interactions and noted that the hyphal contact between various fungal isolates occurred. As an interaction outcome of fungal isolates all the five types of possible interactions *viz* invasion/replacement, partial mutual intermingling and mutual intermingling, dead lock at touching point and dead lock at distance were observed.

Among these combinations the interactions of *Trichoderma* species with each other were found to be under absolutely fast growing where as with *Pleurotus* species they are fast-slow growing and with other white rot fungi found to be fast growing. The interactions between *Pleurotus* species were slow growing.

All the three species of *Trichoderma* showed deadlock at touching point with each other so that fungal mycelia of one community cannot enter the other territory. *T. viride* and *T. harzianum* showed invasion/replacement with all four species of *Pleurotus* except *P. florida* which was found to be compatible with *T. harzianum*. *T. reesei* showed invasion/replacement with *P. florida* and *P. eryngii* where as partial mutual intermingling and mutual intermingling with *P. ostreatus* and *P. sajorkaju* respectively. The other white rot fungi *I. lacteus*, *P. sanguineus*, *P. chrysosporium* showed invasion/ replacement with *T. viride* and *T. harzianum* except *I. lacteus* which showed dead lock at touching point with *T. viride*. *D. confragosa* and *P. pectinatus* showed partial mutual intermingling with *T. viride* and *T. harzianum* while all the five

white rot fungi were highly compatible with *T. reesei* as they showed mutual intermingling type of interaction.

Trichoderma species belonged to ascomycetes is the most studied genera for biocontrol agents for a wide range of plant pathogens because of their high reproductive capacity, ability to survive in harsh conditions and ability to utilize unfavorable nutrients (Cook and Baker 1983). The genus *Trichoderma* contains a large number of sub-strains which exert biocontrol against broad range of fungal species either directly or indirectly by modifying the environmental conditions like competing for nutrients and space, promoting plant growth and plant defensive mechanisms, or by mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the *Trichoderma* strain, the antagonized fungus and the environmental conditions, including nutrient availability, pH, temperature and iron concentration.

Highley and Recard (1988) found that on a malt agar medium an isolate of *Trichoderma viren, T.harzianum, T.polysporum* completely inhibited the growth of several white and brown rot fungi and killed them. In the present study also *T. viride* and *T. harzianum* were showing similar kinds of results except they were found to be compatible with *D. confragosa* and *P. pectinatus* with the overlapping zone which was very prominently observed even after 17 days of inoculation but *T. ressei* was compatible with most of the white rot fungi tested except *P. florida* and *P. eryngii* which was completely in contrast with their study. It was also noted that all three *Trichoderma* species were also not compatible with each other indicating interspecies antagonism amongst the species belonged to the same genera.

Trichoderma atroviridae was tested for its antagonistic potential against white rot wood decay fungi *Ganoderma adspersum, Ganoderma lipsiense, Inonotus*

liespidus, Polyporus squamosus and asomycete Kretzschmaria densta (Schubert *et al* 2008). *Trichoderma atroviridae* was consistently and highly competitive against most wood decay fungi with the exception of *Polporus squamosus* which showed resistance against antagonism in laboratory tests.

In the study conducted by Highley (1997) evaluation of *Trichoderma* (*Gliocadium*) *virens* against three white-rot fungi, *Trametes versicolor, Phlebia brevispora, Irpex lacteus,* and three brown-rot fungi, *Postia placenta, Neolentinus lepideus, and Gloeophyllum trabeum* was carried out. The results indicated that *T. virens* rapidly overgrow on the wood decaying fungi and kicked them. In the present study also the same observation could be seen. *T. viride* and *T. harzianum* rapidly overgrows *Irpex lacteus* and kills it once it has come in contact within 7 days of inoculation.

Tsujiyama and Minami (2005) reported that in the dual culture of *Trichoderma* with *Pleurotus ostreatus*, the hyphae of *P. ostreatus* did not grow around the boundary of *Trichoderma* due to secretion of antifungicide. In the present study also *P. ostreatus* showed invasion/ replacement with *T. viride* and *T. harzianum* whereas with *T. reesei* it showed partial mutual intermingling.

Paranthaman *et al* (2009) studied the effect of fungal co-culture for the biosynthesis of Tannic acid and Gallic acid from grape waste under solid stat fermentation. The co-culture *Penicillium chrysogenum* and *Trichoderma viride* produced highest activity of 84±U/g/min than other organisms.

Compatibility of *T. reesei* with the other white rot fungi (IL, DC, PHE, PYS, PC, PS, and PO) and *T. Viride* and *T. harzianum* compatibility with PHE and DC could be considered as a positive result for the present study as our aim was to select a co-culture which is potential for biopulping. *Trichoderma* is well known producer of

xylanase enzyme and white rot fungi are well known for production of lignin modifying enzymes, so white rot wood decay fungi together with *Trichoderma* species would increase the process of delignification as well as maintain brightness property of the fibres.

In the interaction between *Pleurotus* species amongst each other it was noted that all type of interactions were found except invasion/replacement. *P. florida* showed dad lock at touching point with *P. eryngii* and dead lock at distance with *P. ostreatus*. *P. eryngii* found to be compatible with *P. ostreatus* and *P. sajorkaju* as partial mutual intermingling was observed where as *P. sajorkaju* showed mutual intermingling with *P. florida* and *P. ostreatus*.

In the study of Naraian *et al* (2010) in vitro degradability of *Pleurotus* species, *P. florida, P. eryngii* and *P. ostreatus* and its mixed culture were evaluated. Results indicated that enzyme crude extracts of PE+PS, PE+PF and PF+PS showed more degradation of cell wall constituents because of their compatiblity with each other. The results of present study also reassembled with it as PE and PF were found to be compatible with PS but PE and PF were not compatible with each other. The result showing that PE, PF and PO were found to be compatible with PS but it is not necessary that PE, PF and PO also compatible with each other they showed antagonistic interaction.

Mata *et al* (2005) reported the changes in the lignocellulolytic enzyme activity when six different strains of *Pleurotus* were cultivated on coffee pulp in combination with three *Trichoderma* species. *P. djamor, P. ostreatus, P. pulmonarius* in confrontation with *T. viride* 637, 638 and *T. reesei* 639 showed significant increase in laccase and MnP enzyme production due to their compatibility. In the present study also similar compatibility was noted as *P. florida* and *P. ostreatus* showed partial

mutual intermingling with *T. harzianum* with *T. reesei* respectively whereas *P. sajorkaju* showed mutual intermingling with *T. reesei* indicating to be compatible with each other.

The result of the present study showed that although *Trichoderma* species is very well known for efficient biocontrol agents as they inhibit the growth of other fungal isolates but *T. reesei* was found to be compatible with most of the white rot fungi.

3.2 QUANTITATIVE ANALYSIS

An extensive research is being carried out to study ligninolytic enzymes of white rot basidiomycetes for efficient production of enzymes to be used in biotechnological applications. Different enzymes are utilized in the pulp and paper industry to dissolve lignin to obtain intact cellulose fibers. Our aim was to screen a fungal culture that produce high ligninolytic enzymes along with xylanase and absence or scanty production of cellulolytic enzymes, a priority in paper and pulp industry.

For screening of fungal isolates for their enzyme producing ability lack of single rapid reliable screening technique is one of the major problem. Hence, solid agar screening method was used for screening and confirmation was done for the enzyme production under submerged conditions by using the standard procedures. Lignin degrading enzymes like LiP, MnP, Laccase and AAO and hydrolytic enzymes viz xylanase and cellulase was determined after each incubation period (3-35 days).

3.2.1 Evaluation of Enzyme activities

Different enzymes are used in the paper and pulp industry for the purpose of biopulping and biobleaching. White rot fungi are very well known to produce ligninolytic as well as hydrolytic enzyme activities. Study of the ability of selected fungi to produce ligninolytic as well as hydrolytic enzyme activities in liquid culture media was carried out at various incubation periods (3-35 days) and for each incubation period fungal biomass produced by selected fungi in mono and co culture was also investigated.

In all the mono cultures and co cultures significant increase in bio mass was observed as the incubation period increased but a fall in biomass concentration was also noticed after reaching the peak which is due to death of fungal mycelium in the

culture medium due to limitations of nutrients in the media after certain incubation period. The concentration of biomass in monoculture and cocultures at various incubation periods has been represented in Table 1. In mono cultures the highest concentration of bio mass was obtained by PS on the 25th day (0.41±0.02) and lowest was obtained by IL on 25th day (0.22±0.01) while PHE and DC showed maximum bio mass on 20th day after inoculation 0.28±0.02 and 0.39±0.02 respectively. In co cultures all the combinations showed highest biomass production on 20th day of incubation period after inoculation (Figure 1).

IL with DC showed maximum fungal biomass and the lowest increase in the biomass was found in IL+PHE. In co cultures there was an increase in the fungal biomass compared to mono cultures and within a shorter incubation period.

According to Tlecuitl-Beristain *et al* 2008 the production of enzyme is usually associated with the growth phase In microbes so role of growth phase in enzyme production, the mycelia dry weight of all the fungal isolates in monocultures and co cultures were determined for confirmation and results showed that, there was no correlation between with the production of ligninolytic as well as hydrolytic enzymes and the dry weight of the fungal mycelium. Therefore we could say that enzyme production is not dependent on the fungal biomass.

In monocultures IL and PHE did not produce Lip while DC produced highest Lip activity on 25th day (2.47 U/L) and PS produced highest activity on 30th day (0.77 U/L). Gradual decline in Lip activity was observed on 30th day and 35th day respectively. The activity of LiP is as represented in Table 2.

In case of co culture all the combination showed maximum production of LiP on the 20th day except IL+PHE which did not produce Lip enzyme activity (Table 2). Amongst the cocultures studied the highest LiP activity was obtained by IL+DC (2.74 U/L) followed by PS+DC (2.55), PS+PHE (1.08U/L), IL+PS (0.92 U/L) (Figure 2).

Days	IL (gm)	DC (gm)	PHE (gm)	PS (gm)	IL+DC (gm)	IL+PHE (gm)	IL+PS (gm)	PS+DC (gm)	PS+PHE (gm)
3	0.05 ± 0.006	0.10 ± 0.01	0.10 ± 0.015	0.06 ± 0.005	0.28 ± 0.015	0.13 ± 0.015	0.16 ± 0.01	0.12 ± 0.02	0.12 ± 0.015
5	0.09 ± 0.01	0.12 ± 0.005	0.13 ± 0.006	0.06 ± 0.01	0.35 ± 0.01	0.17 ± 0.01	0.23 ± 0.02	0.28 ± 0.02	0.24 ± 0.01
10	0.11 ± 0.01	0.15 ± 0.005	0.14 ± 0.006	0.11 ± 0.01	0.49 ± 0.01	0.27 ± 0.02	0.33 ± 0.02	0.36 ± 0.005	0.32 ± 0.01
15	0.15 ± 0.01	0.28 ± 0.02	0.15 ± 0.006	0.18 ± 0.01	**0.60 ± 0.01**	**0.35 ± 0.01**	**0.42 ± 0.01**	**0.49 ± 0.02**	**0.45 ± 0.01**
20	0.17 ± 0.005	**0.39 ± 0.02**	**0.28 ± 0.02**	0.28 ± 0.01	0.48 ± 0.01	0.30 ± 0.02	0.38 ± 0.01	0.43 ± 0.01	0.41 ± 0.01
25	**0.22 ± 0.01**	0.35 ± 0.01	0.17 ± 0.01	**0.41 ± 0.02**	0.40 ± 0.01	0.26 ± 0.01	0.23 ± 0.02	0.36 ± 0.02	0.32 ± 0.01
30	0.18 ± 0.01	0.26 ± 0.02	0.15 ± 0.01	0.34 ± 0.02	0.31 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.23 ± 0.02	0.19 ± 0.02
35	0.16 ± 0.01	0.19 ± 0.02	0.12 ± 0.02	0.25 ± 0.005	0.21 ± 0.01	0.14 ± 0.02	0.12 ± 0.02	0.18 ± 0.01	0.15 ± 0.02

Table 1 Fungal Biomass produced in mono cultures and co cultures during different incubation periods

Similar to LiP activity, MnP activity was also not detected in PHE but DC showed initiation of MnP production on the 5th day and obtained maximum activity on the 25th day (1.75 U/L) further declining in the activity on 30th day (Figure 3). DC showed highest activity amongst all the monocultures studied followed by PS (1.00 U/L) which initiated MnP production after 5 days of incubation period. IL produced (0.75 U/L).

The MnP activity of mono cultures and co cultures are as represented in Table 3. The maximum activity of MnP in IL and PS was observed on 30th day and after that decline in the activity of MnP was noted.

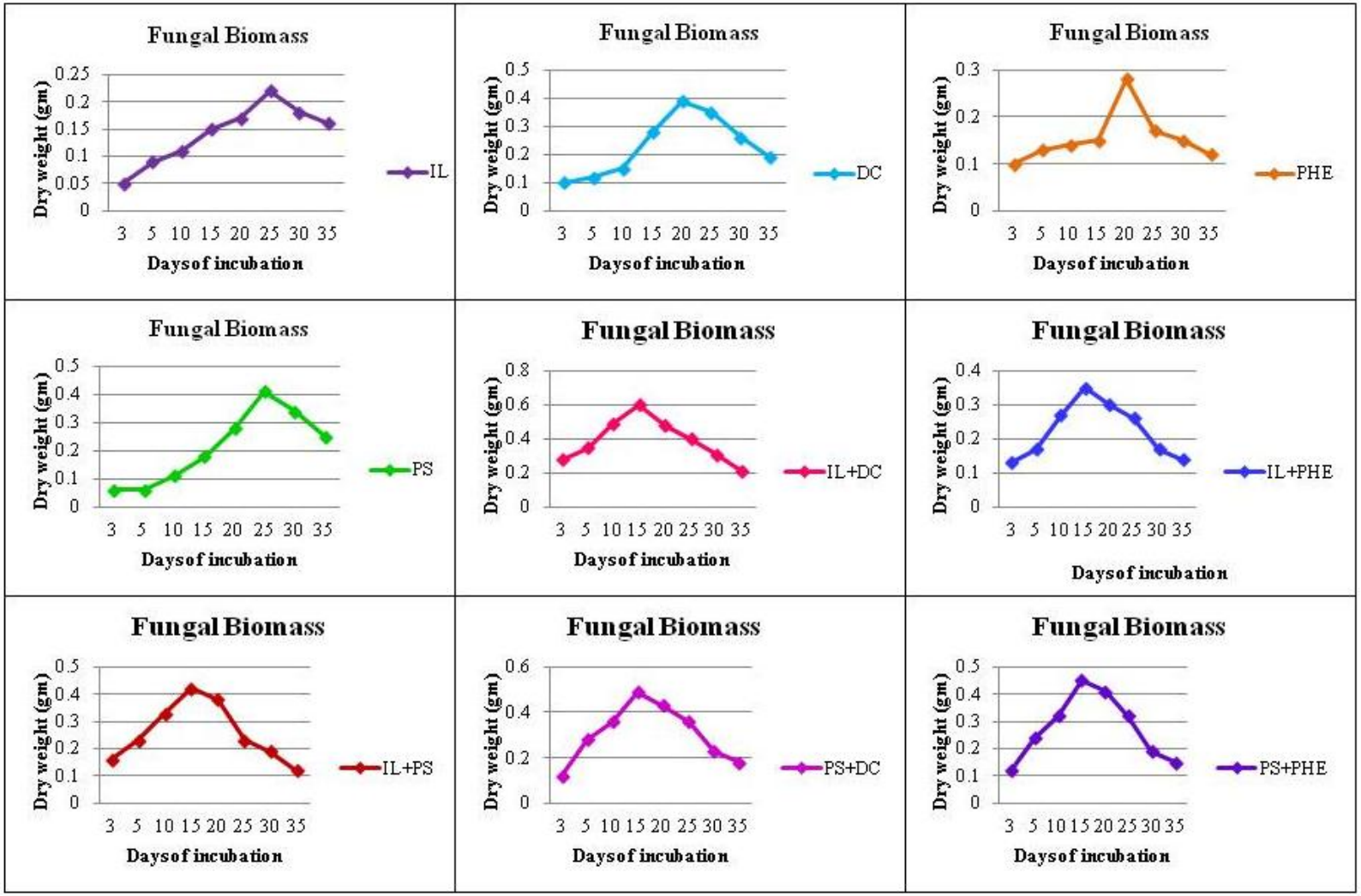


Fig. 1Bio mass produced by fungi in monocultures and cocultures at different incubation period

In the co culture maximum production was observed on 20th day and highest activity of MnP was obtained by IL+DC (1.97 U/L) followed by PS+DC (1.78 U/L), PS+PHE (1.25 U/L), IL+PS (0.94 U/L) and the lowest activity was observed in the combination of IL+PHE (0.79 U/L) Fig 3. After 20 days of incubation period decrease in the MnP activity gradually observed (Table 3).

In monocultures LiP activity was absent. LiP and MnP both the enzyme activity was absent in PHE but its co culture with IL showed lowest activity of MnP and its co culture with PS showed moderate MnP enzyme activity amongst co culture studied (Figure 3).

The Laccase activity was observed from the 3rd day and gradually increased and maximum activity of Laccase occurred in the culture filtrates of IL (1.94 U/L) followed by PS (1.79 U/L). DC showed the lowest Laccase activity (1.07 U/L) and PHE showed (1.22 U/L) as represented in Figure 4.

The highest activity of Laccase was observed in the culture filtrates of DC and PHE on the 25th day of incubation while IL and PS showed maximum activity on 30th day similar as observed in LiP and MnP production. After the peak of Laccase obtained there was a gradual decline in the production of Laccase enzyme was noticed (Table 4).

In case of co culture the highest activity was obtained on the 20th day after inoculation after that decline in the production of Laccase was observed. The maximum activity of Laccase was observed when IL co cultured with DC (5.45 U/L) as represented in the Figure 4 which is more than two fold increase if we compare the highest activity of mono culture in IL and five times more than in the mono culture of DC.

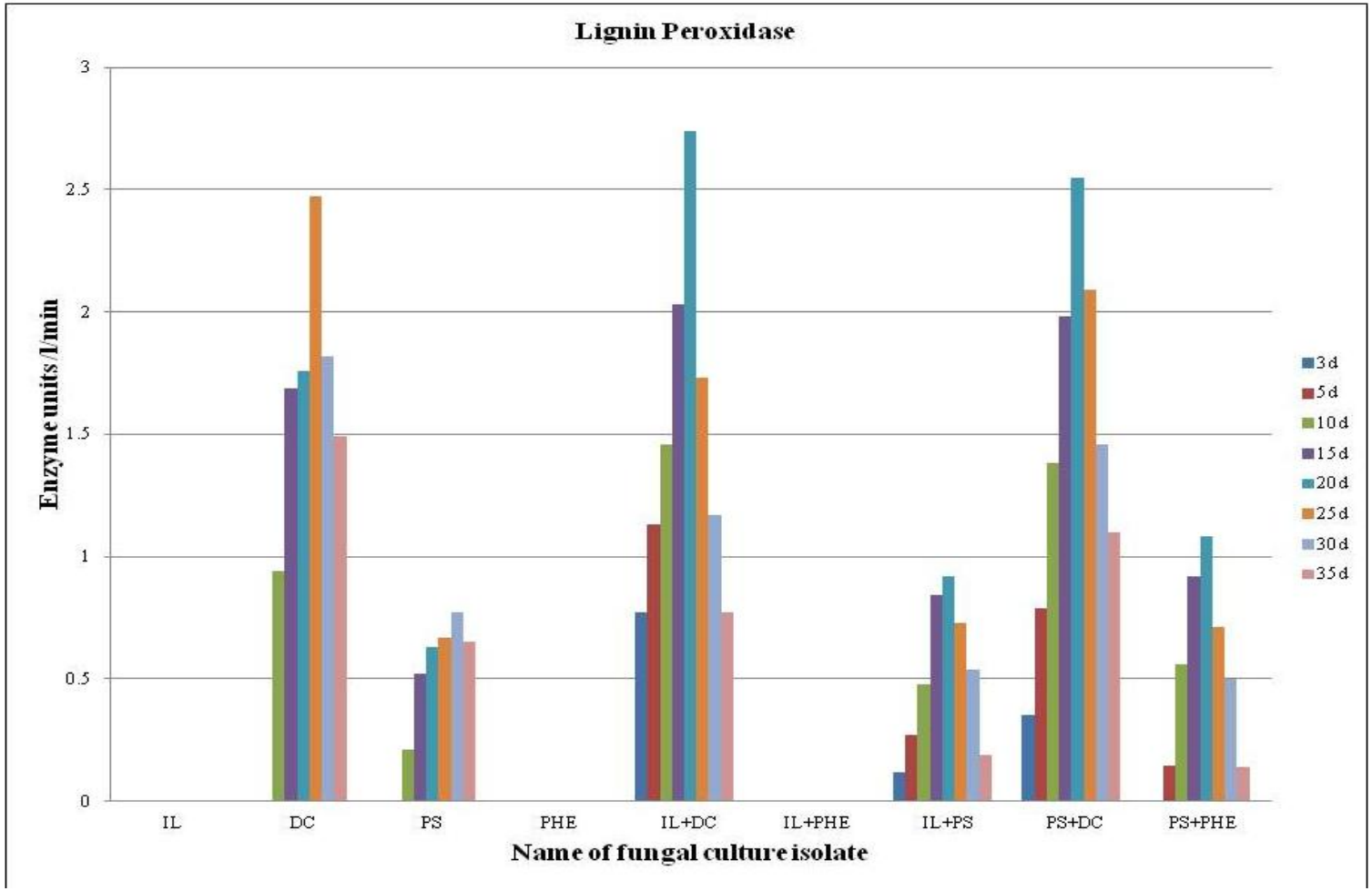


Fig. 2 LiP production in mono and co cultures at various incubation period

Days	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
3d	0	0	0	0	0.77±0.09	0	0.12±0.06	0.35±0.04	0
5d	0	0	0	0	1.13±0.062	0	0.27±0.037	0.79±0.07	0.15±0.09
10d	0	0.94 ±0.12	0	0.21±0.09	1.46±0.07	0	0.48±0.09	1.38±0.11	0.56±0.06
15d	0	1.69 ± 0.06	0	0.52±0.03	2.03±0.09	0	0.84±0.09	1.98±0.09	0.92±0.07
20d	0	1.76 ± 0.06	0	0.63±0.06	**2.74±0.096**	0	**0.92±0.09**	**2.55±0.09**	**1.08±0.13**
25d	0	**2.47 ± 0.03**	0	0.67±0.03	1.73±0.036	0	0.73±0.09	2.09±0.09	0.71±0.03
30d	0	1.82 ± 0.06	0	**0.77±0.09**	1.17±0.095	0	0.54±0.07	1.46±0.09	0.50±0.06
35d	0	1.49 ± 0.09	0	0.65±0.03	0.77±0.09	0	0.19±0.06	1.10±0.09	0.14±0.04

Table 2 Production of LiP by mono and co cultures of fungi at various incubation periods

Days	**IL**	**DC**	**PHE**	**PS**	**IL+DC**	**IL+PHE**	**IL+PS**	**PS+DC**	**PS+PHE**
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
3	0.15±0.03	0	0	0	0.39±0.09	0.21±0.03	0.19±0.06	0.73±0.07	0.15±0.09
5	0.27±0.03	0.08±0.04	0	0	0.92±0.09	0.40±0.09	0.50±0.06	0.90±0.13	0.56±0.06
10	0.33±0.02	0.40±0.03	0	0.08±0.03	1.30±0.13	0.48±0.09	0.79±0.07	1.28±0.03	0.94±0.06
15	0.39±0.03	0.77±0.03	0	0.33±0.03	1.53±0.09	0.52±0.09	0.84±0.09	1.36±0.09	1.07±0.06
20	0.50±0.03	1.00±0.06	0	0.43±0.06	**1.97±0.09**	**0.79±0.03**	**0.94±0.06**	**1.78±0.12**	**1.25±0.11**
25	0.54±0.03	**1.75±0.06**	0	0.50±0.06	1.74±0.03	0.50±0.06	0.56±0.06	1.46±0.09	0.81±0.06
30	**0.75±0.06**	0.58±0.09	0	**1.00±0.12**	1.00±0.06	0.19±0.06	0.10±0.03	1.44±0.11	0.56±0.06
35	0.20±0.09	0.23±0.09	0	0.18±0.12	0.66±0.09	0.08±0.03	0.08±0.03	1.11±0.09	0.25±0.06

Table 3 Production of MnP by mono and co cultures of fungi at various incubation periods

The maximum activity of Laccase in IL+DC was followed by IL+PS it showed (4.81 U/L) which is almost double than obtained in the mono cultures of both the individual fungi (Table 4).The other co cultures of IL+PHE, PS+DC, PS+PHE showed 2.11, 1.93 and 1.89 U/L respectively. The co culture of PS+PHE showed lowest Laccase activity (1.89 U/L) amongst all the co culture studied (Table 4).

AAO enzyme activity in mono cultures initiated from 3rd day after inoculation similar to laccase activity. Maximum AAO activity was observed on the 25th day in the culture filtrates of DC and PHE while in IL and PS cultures it was observed on the 30th day after inoculation. With further incubation a decline in activity gradually occurred (Table 5).

The highest activity of AAO was noticed in culture filtrates of IL culture (44.12 U/L) as represented in the Figure 5 followed by PS, PHE and DC which showed 39.83, 29.66 and 24.42 U/L. The results of AAO activity produced by mono cultures and co cultures are as represented in Table 5.

In the co culture the highest AAO activity was obtained on the 20th day after inoculation thereafter with a gradual decline in the AAO enzyme activity. The maximum activity of AAO was noted in the culture extracts of PS+PHE (61.59 U/L) and minimum activity was found in the culture extracts of PS+DC (39.97 U/L) however, it was higher than AAO produced in its mono cultures (Figure 5).

According to Biely 1985 Xylanase in combination without Cellulase is very useful for the paper and pulp industry. As the paper quality depends on the presence of undamaged cellulose fibers, screening of fungi which produced very less amount of cellulase / Cellulase free was conducted by submerged fermentation experiments and the results are represented in Table 6.

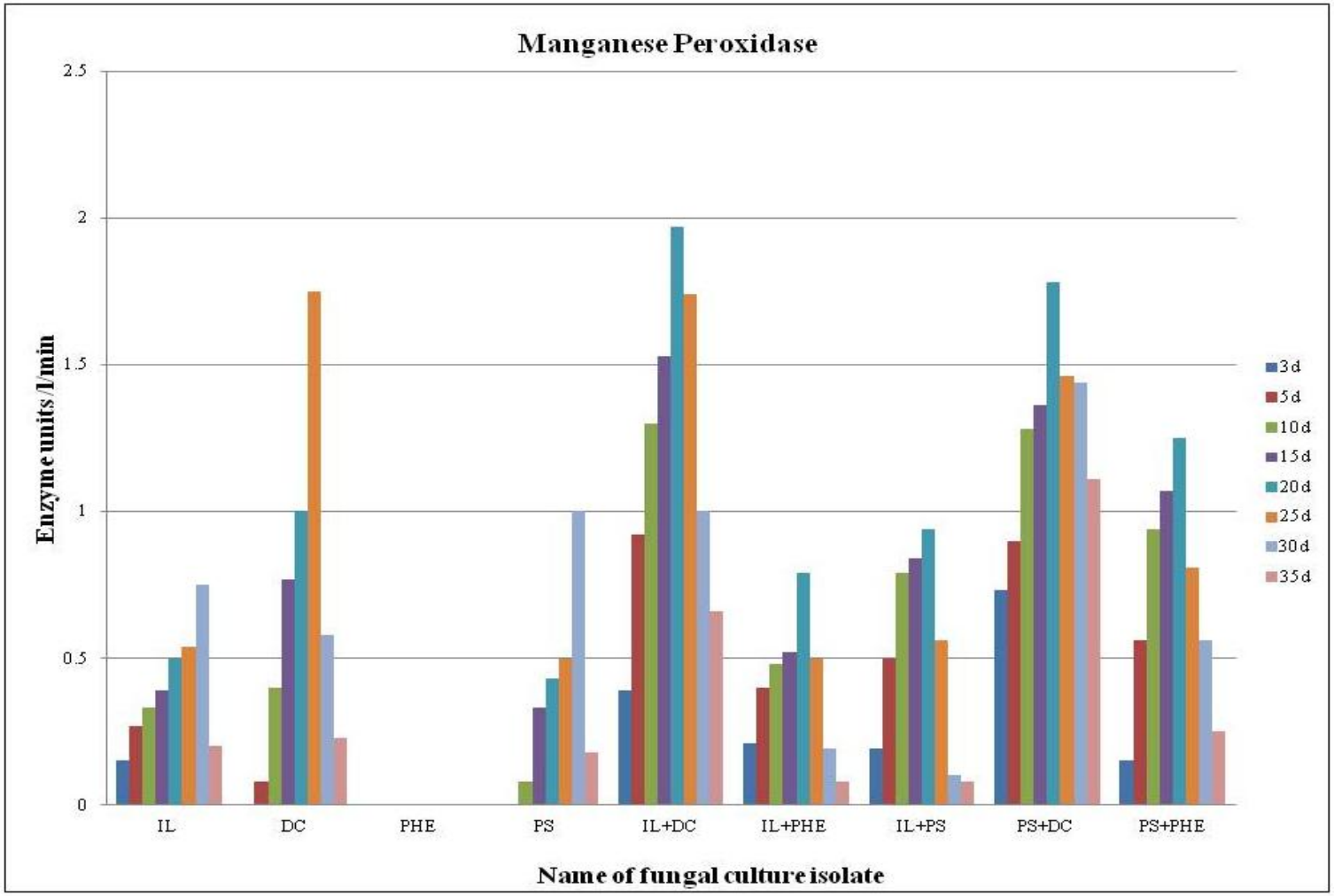


Fig. 3 MnP production in mono and co cultures at various incubation period

Days	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
3d	0.94±0.013	0.59±0.02	0.69±0.024	1.06±0.013	1.345±0.067	1.57±0.022	1.85±0.023	0.69±0.015	0.70±0.021
5d	0.99±0.015	0.76±0.017	0.90±0.064	1.17±0.010	1.48±0.018	1.69±0.011	1.98±0.018	0.76±0.018	0.77±0.013
10d	1.06±0.015	0.89±0.017	0.93±0.014	1.21±0.013	2.78±0.049	1.81±0.020	2.30±0.026	0.90±0.021	0.95±0.017
15d	1.15±0.026	0.93±0.014	0.95±0.012	1.28±0.015	3.12±0.042	2.06±0.021	3.15±0.026	0.97±0.018	1.18±0.020
20d	1.26±0.010	0.99±0.019	1.69±0.011	1.35±0.016	**5.45±0.030**	**2.11±0.019**	**4.81±0.061**	**1.93±0.015**	**1.89±0.021**
25d	1.57±0.02	**1.07±0.017**	**1.22±0.01**	1.39±0.66	1.51±0.019	1.22±0.010	2.21±0.019	1.00±0.016	1.11±0.017
30d	**1.94±0.012**	0.87±0.018	0.93±0.017	**1.79±0.019**	1.11±0.022	1.07±0.01	2.04±0.035	0.90±0.034	0.94±0.022
35d	1.26±0.012	0.52±0.03	0.78±0.04	1.49±0.016	0.73±0.03	0.72±0.04	1.26±0.03	0.63±0.04	0.58±0.02

Table 4 Production of Laccase by mono and co cultures of fungi at various incubation periods

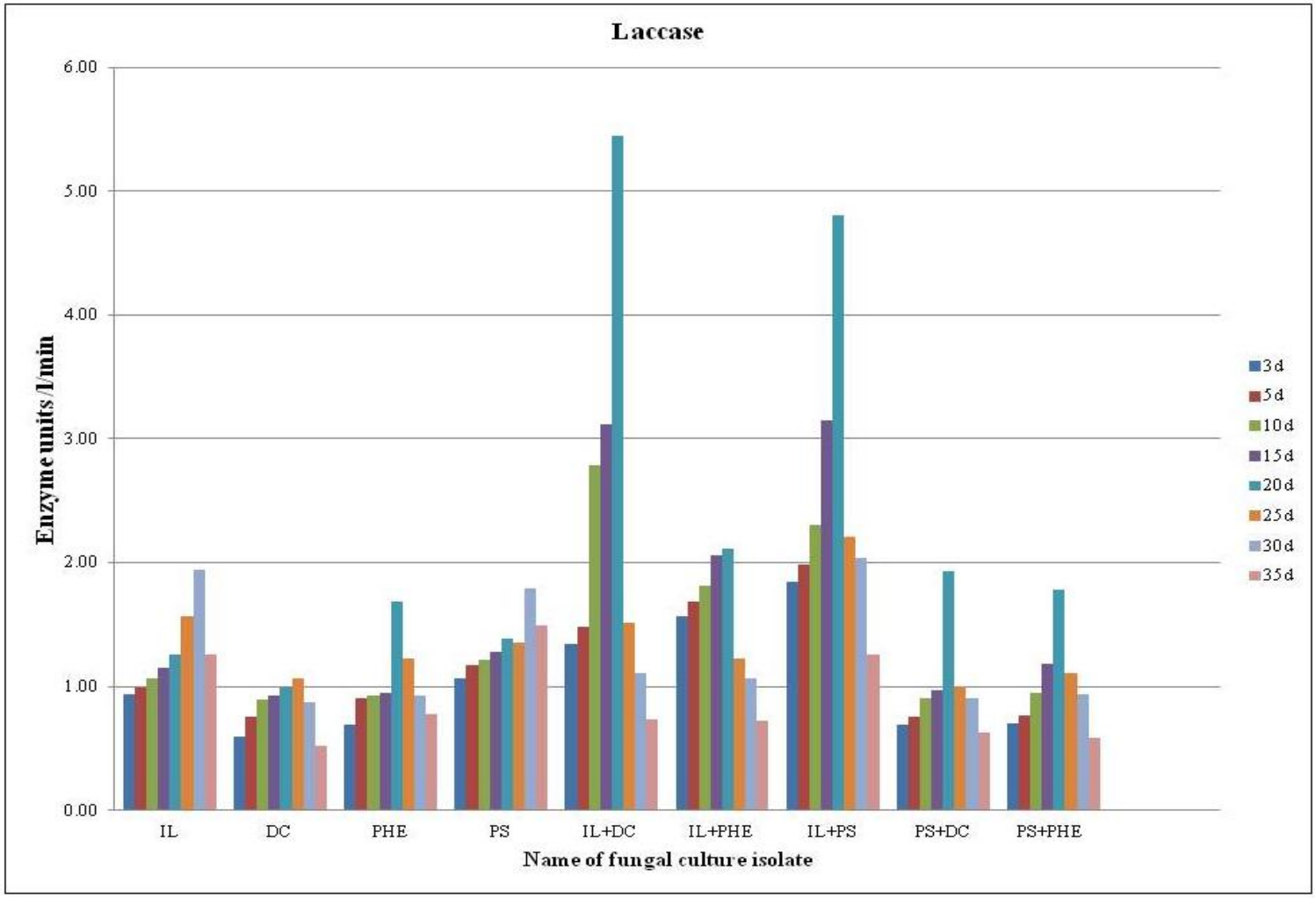


Fig. 4 Laccase production in mono and co cultures at various incubation period

It is very clear from the table 6 that in mono cultures of fungi the highest activity of Xylanase was obtained on 20th day in the culture filtrates of IL and DC while PHE and PS showed maximum xylanase activity on 25th day. After the peak was obtained gradual decline in the enzyme production was noticed similar to all the other enzymes.

The highest production of xylanase in mono cultures was observed in the IL (18.11 U/ml) as represented in Fig 6 which is followed by DC (13.86 U/ml), PHE (9.37 U/ml) and PS (3.21 U/ml). Amongst the monocultures studied lowest activity of xylanase was noticed in case of PS (Table 6). In co cultures the maximum activity of xylanase was obtained on 20th day after inoculation except for IL+DC which showed maximum activity on 20th day after which it has been declined gradually afterwards (Figure 6).

Maximum activity of xylanase was obtained in the combination of IL+DC (22.68 U/ml) as represented in the Figure 6 which is followed by IL+PHE, PS+DC, PS+PHE, IL+PS 19.49, 15.46, 10.86, 5.96 U/ml amongst which IL+PS showed the lowest activity of xylanase as represented in Table 6. Cellulase enzyme activity was not observed in monocultures as well as co cultures of the selected fungi.

Days	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
3d	20.70±0.21	13.27±0.14	19.62±0.17	18.69±0.13	24.99±0.16	24.18±0.10	18.16±0.12	19.54±0.11	20.94±0.10
5d	24.96±0.10	15.62±0.11	23.04±0.12	19.26±0.12	26.82±0.13	24.82±0.13	24.40±0.13	19.78±0.11	21.72±0.12
10d	26.41±0.14	19.64±0.10	26.89±0.20	22.13±0.20	28.40±0.10	26.45±0.13	24.82±0.10	21.33±0.12	24.77±0.10
15d	29.90±0.13	20.60±0.10	27.68±0.14	22.47±0.4	30.90±0.10	29.68±0.11	30.76±0.12	24.311±0.11	25.73±0.12
20d	32.42±0.15	22.53±0.10	28.44±0.12	28.41±0.15	**49.12±0.12**	**55.63±0.15**	**49.71±0.12**	**39.97±0.10**	**61.59±0.12**
25d	32.98±0.14	**24.42±0.11**	**29.66±0.12**	28.49±0.24	26.77±0.12	41.57±0.16	33.03±0.13	23.33±0.12	45.35±0.34
30d	**44.12±0.13**	23.50±0.10	28.75±0.12	**39.83±0.22**	24.31±0.16	24.75±0.16	23.14±0.10	22.32±0.12	26.43±0.11
35d	36.09±0.10	19.20±0.11	23.40±0.14	30.22±0.13	19.98±0.26	21.00±0.3	19.00±0.16	20.54±0.20	21.62±0.22

Table 5 Production of AAO by mono and co cultures of fungi at various incubation periods

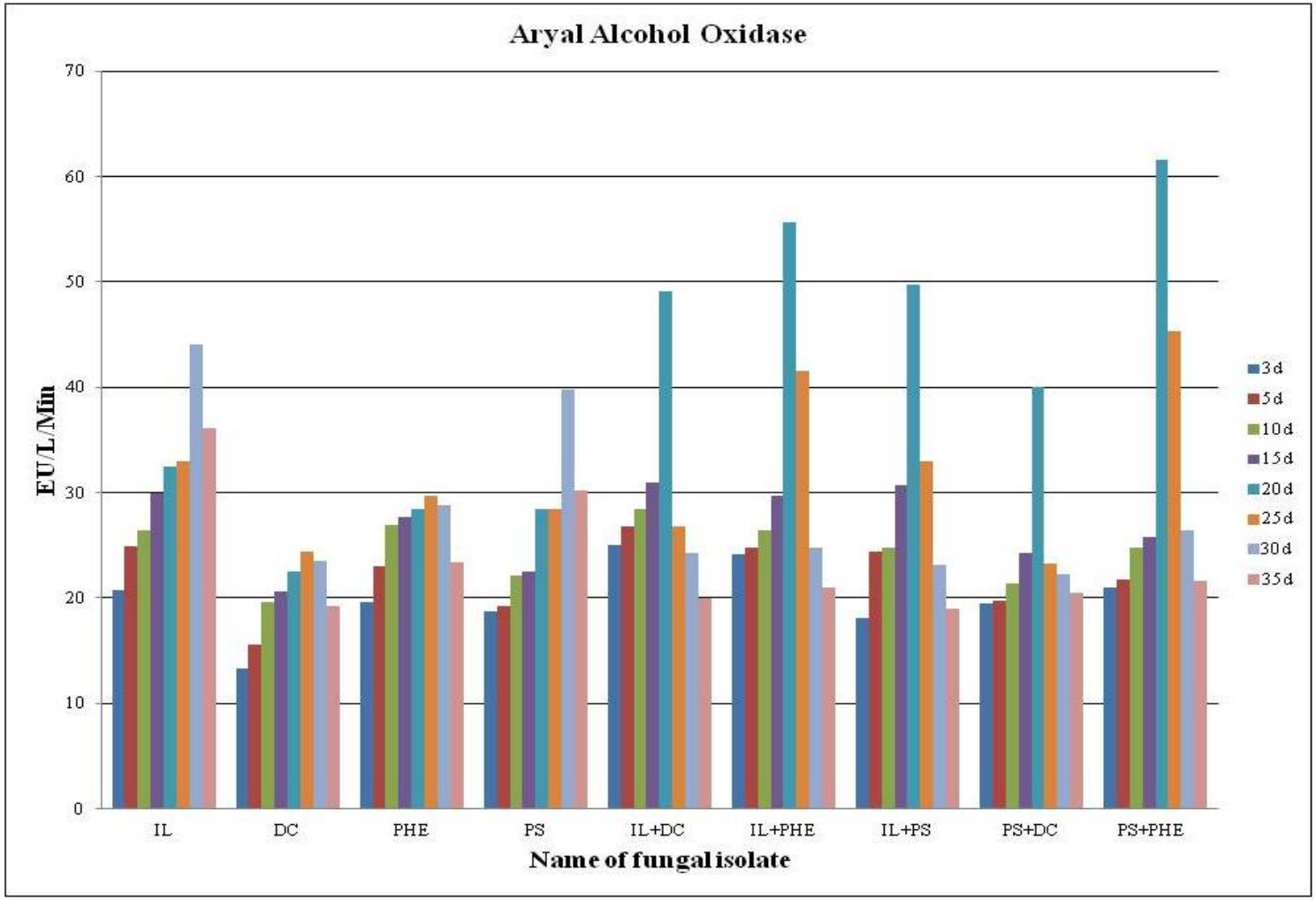


Fig.5 AAO production in mono and co cultures at various incubation periods

Days	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min
3	1.32±0.66	2.23±0.74	1.87±0.36	0.09±0.03	4.75±1.02	0.99±0.39	0.39±0.17	3.89±0.36	2.52±0.70
5	4.59±1.12	4.77±0.73	2.88±0.40	0.92±0.17	6.03±0.73	7.46±0.60	1.80±0.46	5.89±0.56	6.40±0.81
10	8.98±0.80	5.06±1.16	4.4±0.51	1.49±0.23	8.23±0.72	11.46±0.66	3.21±0.23	7.282±0.46	8.22±0.53
15	9.57±1.37	9.46±1.33	5.54±0.73	2.09±0.23	10.20±0.49	16.12±.77	4.99±0.42	13.97±0.42	9.37±0.46
20	**18.11±1.03**	**13.86±1.32**	6.86±0.80	2.57±0.19	**22.68±0.39**	**19.49±0.59**	**5.96±0.53**	**15.46±0.43**	**10.86±0.39**
25	6.05±0.46	12.83±0.93	**9.37±0.72**	**3.21±0.23**	3.72±0.87	12.25±0.66	4.26±0.36	10.16±0.49	8.11±0.54
30	1.96±0.49	1.65±0.72	5.61±0.63	2.2±0.26	3.37±0.52	8.82±0.57	1.98±0.30	6.89±0.59	5.01±0.49
35	0.79±0.21	1.19±0.37	3.65±0.70	1.36±0.23	2.27±0.46	5.61±0.54	0.89±0.26	3.43±0.39	2.68±0.51

Table 6 Production of Xylanase by mono and co cultures of fungi at various incubation periods

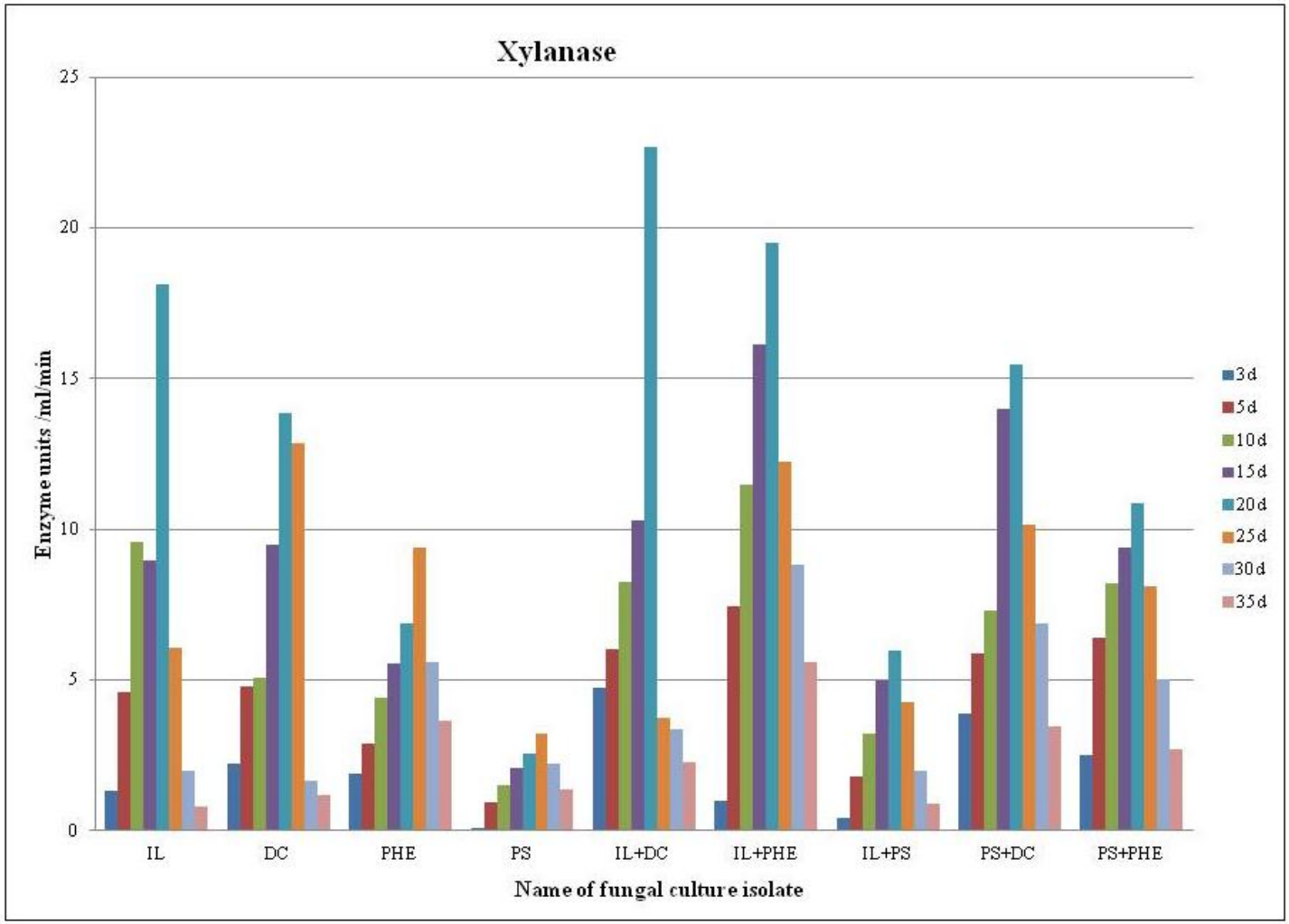


Fig. 6 Xylanase production in mono and co cultures at various incubation periods

3.2.2 Parameters affecting enzyme activity

Bio process parameters that can affect enzyme activities and productivities include incubation period, pH, temperature and agitation.

(a) Effect of Temperature

Temperature is an important parameter to detect the efficiency of enzyme production. So it is important to detect the temperature stability of the different lignocellulolytic enzymes (LiP, MnP, Laccase, AAO and Xylanase) produced by selected fungi in mono and co culture. The temperature stability of different ligninolytic enzymes secreted by both the fungi were detected by incubating the enzyme containing supernatants at 25, 35, 45, 55, 65, 75 ˚ C in water baths for four hours to allow ample time for denaturation and then carry out the enzyme assays.

The stability of different enzymes produced by the mono cultures and co cultures are represented individually in Table 7-11 and Figure 7-11.

In monocultures LiP activity of DC was stable in the temperature range of 5˚C to 45˚C while PS showed gradual increase in the activity from 5˚C to 25˚C and remains stable up to 55˚C after which a sudden decline is observed at 65˚. All the enzyme activities were becoming zero at 75˚C as represented in Table 7. IL and PHE showed no LiP activity.

In coculture maximum stability of LiP was observed in IL+DC which remains stable in the range of 5˚C to 55˚C after which slowly it declines at 65˚C and zero at 75˚C as represented in Fig. 7. In IL+PS, PS+DC, PS+PHE the activity of LiP was maximum at 25˚C and remains stable up to 55˚C and is zero at 75˚C.

In mono cultures of IL, DC and PHE, MnP activity was maximum at 25˚C and remains stable up to 55˚C with a decline at 65˚C and almost near to zero at 75˚C as shown in Table 8.

In co culture IL+DC, IL+PS, PS+DC showed maximum MnP activity at 25˚C and stability in the range of 5˚C. to 55˚C which reduced after 65˚C while co cultures of PS+PHE and IL+PHE showed gradually increase in MnP activity from 5˚C to 25˚C and after that it reduced slowly and reached to zero at 75˚C(Figure 8).

In all the mono and co cultures the enzyme Laccase remains stable from 15˚C to 55˚C obtaining its maximum activity at 25 ˚C and reduced to zero abruptly as represented in the Figure 9 and Table 9.

AAO activity at various temperature showed results same as observed in the Laccase production which is as represented in the Table 10. All the mono and co cultures showed maximum activity at 25˚C and remains stable in the range of 15˚C to 55˚C after that decline in the enzyme production was noted. In co culture only IL+PHE and IL+PS showed little less activity at 15˚C compared to 25˚C which was maximum (Figure 10).

In all the mono cultures and co cultures Xylanase remained stable in the range of 15˚C up to 55 ˚C (Table 11) after that slowly decrease in the production of xylanase was noticed at 65 ˚C which finally reached up to zero at 75˚C (Figure 11).

In mono cultures all the enzymes showed maximum activity at 25˚C and remained stable up to 55˚C thereafter a gradual decline at 65˚C was observed and at 75˚C the activity was almost nil. For the further experiments 25˚C considered to be optimum for the maximum production of enzymes.

Temp ^{0}C	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
5	0	1.67±0.09	0	0.10±0.04	1.95±0.06	0	0.5±0.06	1.59±0.09	0.62±0.06
15	0	2.28±0.09	0	0.56±0.06	2.68±0.09	0	0.75±0.03	2.39±0.06	1.00±0.06
25	0	**2.47±0.03**	0	**0.77±0.09**	**2.74±0.09**	0	**0.92±0.09**	**2.53±0.09**	**1.08±0.13**
35	0	2.23±0.03	0	0.63±0.03	2.47±0.09	0	0.87±0.06	2.38±0.06	0.90±0.04
45	0	2.19±0.03	0	0.52±0.03	2.38±0.09	0	0.75±0.06	2.13±0.06	0.83±0.09
55	0	1.78±0.09	0	0.48±0.03	2.36±0.06	0	0.62±0.06	2.00±0.06	0.77±0.09
65	0	0.82±0.06	0	0.04±0.03	1.92±0.06	0	0.25±0.06	1.07±0.06	0.54±0.03
75	0	0.17±0.03	0	0.03±0.04	0.10±0.09	0	0.04±0.03	0.31±0.06	0.020±0.03

Table 7 Effect of various Temperatures on LiP activity of monocultures and co cultures.

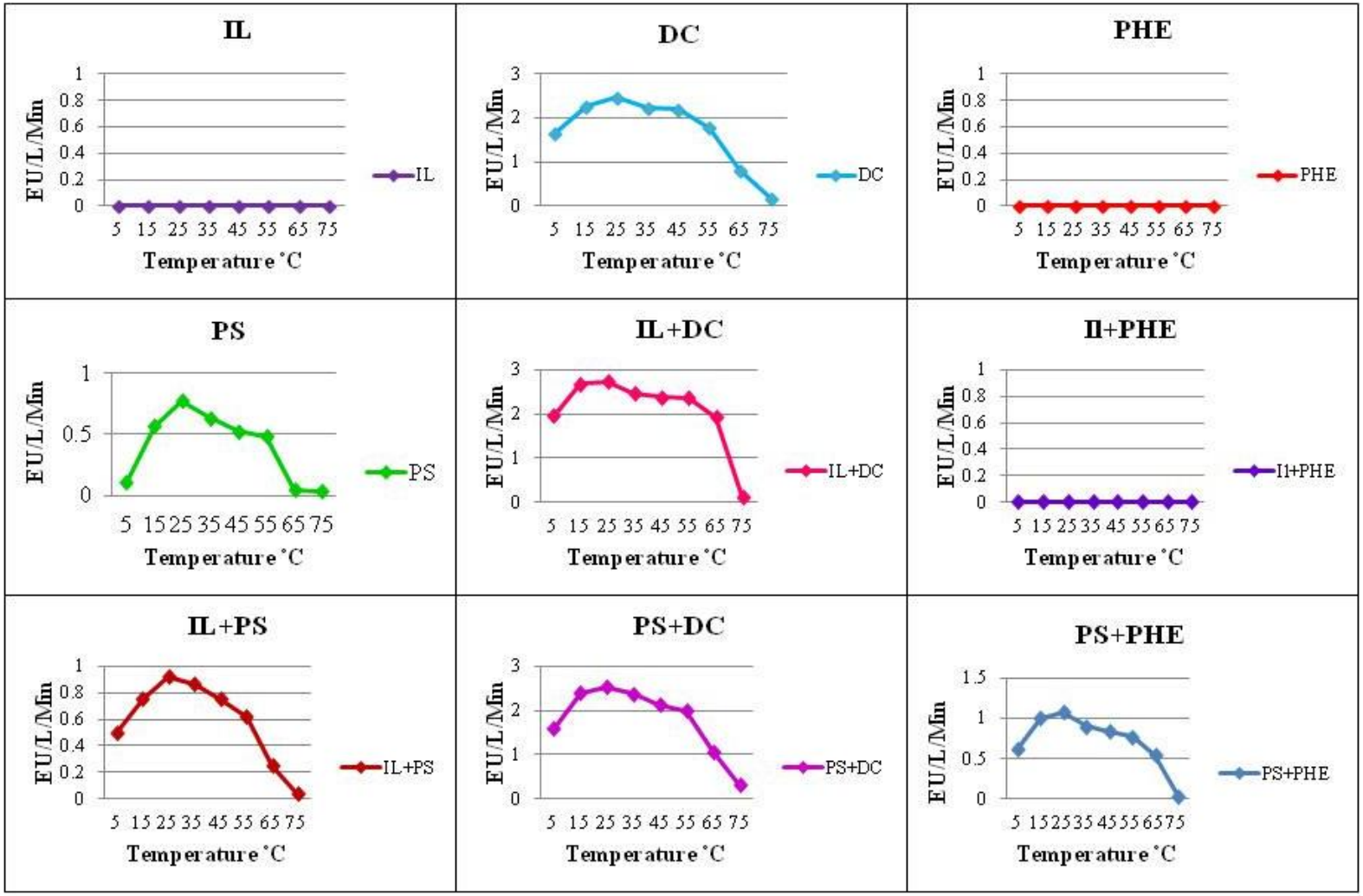


Fig. 7 Effect of various temperatures on LiP activity in monocultures and cocultures of fungi

Temp ^{0}C	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
5	0.17±0.04	0.79±0.09	0	0.25±0.06	1.15±0.09	0.18±0.06	0.25±0.06	0.94±0.12	0.33±0.09
15	0.63±0.06	1.50±0.06	0	0.88±0.06	1.75±0.12	0.62±0.06	0.75±0.06	1.69±0.06	1.06±0.06
25	**0.75±0.06**	**1.76±0.06**	0	**1.00±0.06**	**1.97±0.09**	**0.79±0.04**	**0.94±0.06**	**1.76±0.12**	**1.26±0.11**
35	0.62±0.06	1.63±0.06	0	0.87±0.06	1.82±0.06	0.69±0.06	0.88±0.06	1.61±0.09	1.13±0.06
45	0.50±0.06	1.50±0.06	0	0.79±0.09	1.61±0.09	0.50±0.06	0.82±0.06	1.44±0.06	0.94±0.06
55	0.43±0.06	1.38±0.12	0	0.56±0.06	1.44±0.06	0.38±0.06	0.66±0.06	1.21±0.09	0.71±0.09
65	0.19±0.06	0.84±0.09	0	0.31±0.06	0.77±0.09	0.25±0.06	0.37±0.06	0.88±0.12	0.35±0.09
75	0.08±0.03	0.10±0.03	0	0.08±0.04	0.15±0.09	0.04±0.03	0.06±0.06	0.52±0.09	0.06±0.06

Table 8 Effect of various Temperatures on MnP activity of monocultures and co cultures

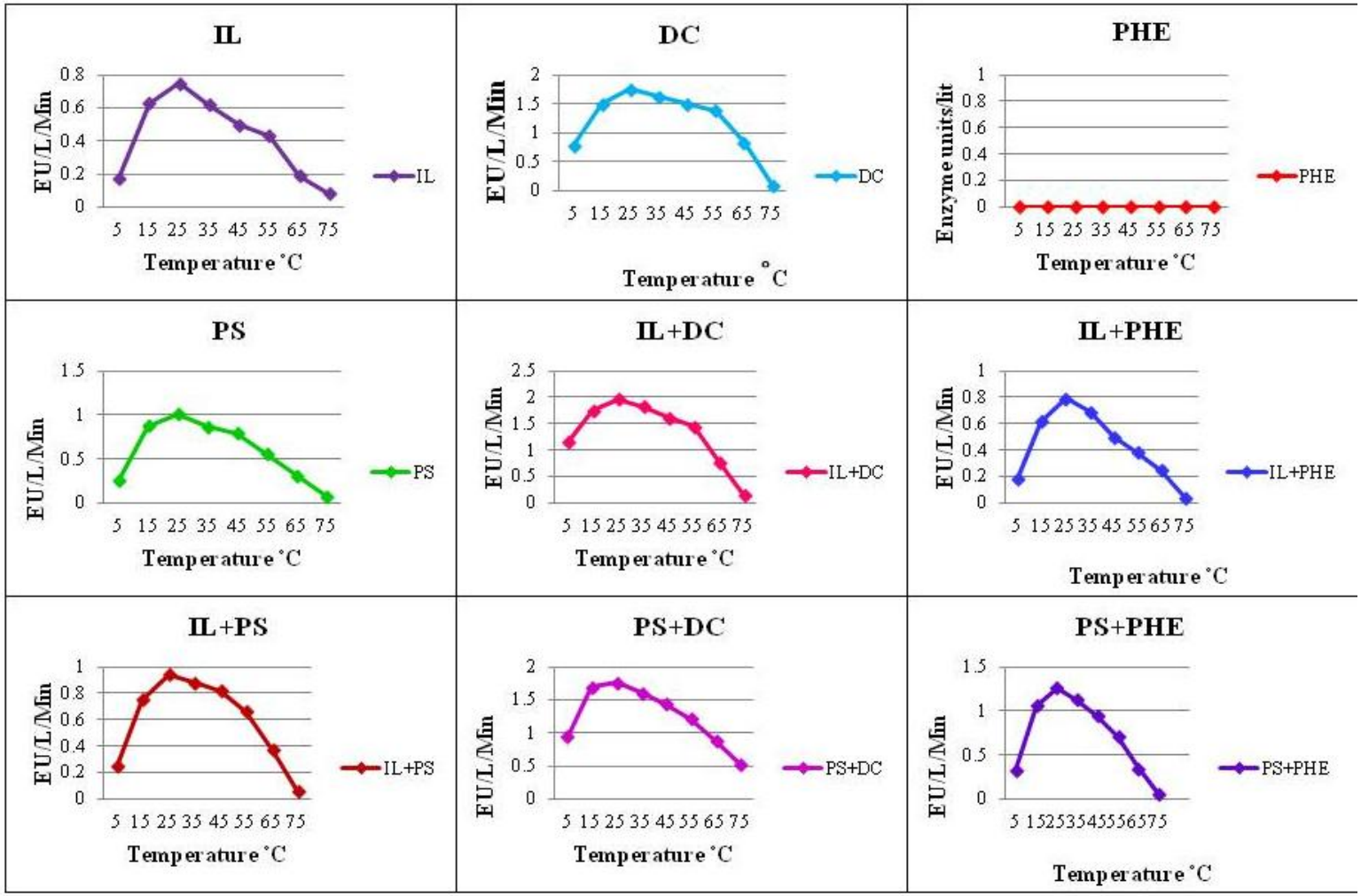


Fig. 8 Effect of various temperatures on Mn P activity in monocultures and cocultures of fungi

Temp ^{0}C	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
5	0.36±0.02	0.26±0.03	0.41±0.03	0.48±0.02	3.58±0.02	0.42±0.03	0.45±0.02	0.20±0.03	0.24±0.02
15	1.62±0.03	0.92±0.03	0.86±0.04	1.46±0.02	4.55±0.03	1.46±0.03	4.13±0.02	0.95±0.03	0.85±0.02
25	**1.94±0.01**	**1.07±0.02**	**1.22±0.01**	**1.79±0.02**	**5.45±0.03**	**2.10±0.02**	**4.81±0.06**	**1.12±0.01**	**1.18±0.02**
35	1.89±0.04	1.05±0.01	1.18±0.06	1.66±0.03	5.26±0.05	1.93±0.02	4.43±0.02	1.05±0.02	1.10±0.03
45	1.84±0.03	1.03±0.02	1.15±0.02	1.54±0.02	5.06±0.02	1.83±0.03	4.06±0.02	1.01±0.03	0.94±0.02
55	1.36±0.04	0.95±0.02	1.08±0.05	1.39±0.03	4.74±0.03	1.56±0.03	3.04±0.03	0.96±0.02	0.76±0.02
65	0.92±0.03	0.59±0.03	0.56±0.03	1.04±0.03	3.34±0.03	1.19±0.02	1.25±0.02	0.36±0.03	0.38±0.02
75	0	0	0	0	0	0	0	0	0

Table 9 Effect of various Temperatures on Laccase activity of monocultures and co cultures.

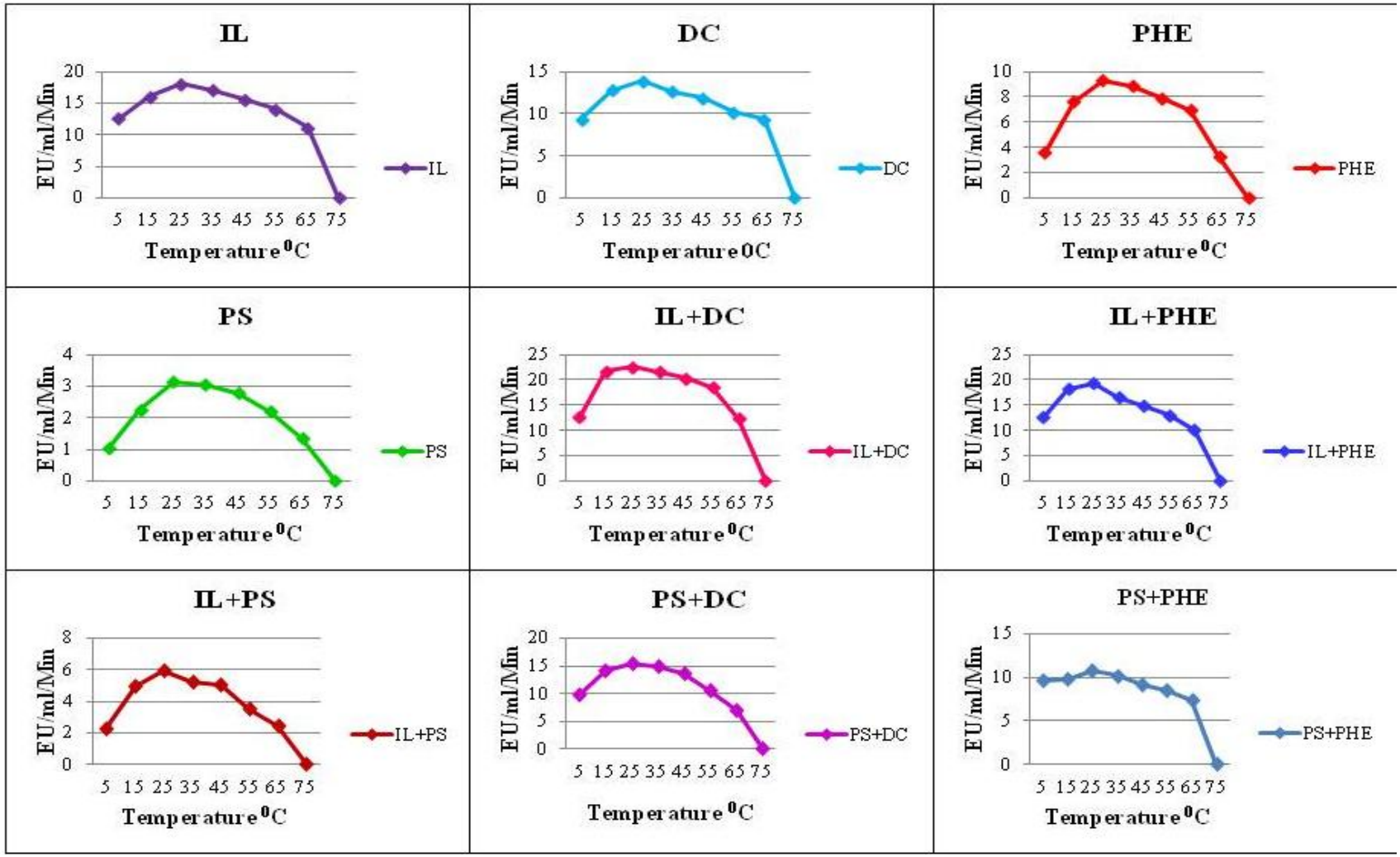


Fig. 9 Effect of various temperatures on Laccase activity in monocultures and cocultures of fungi

Temp	**IL**	**DC**	**PHE**	**PS**	**IL+DC**	**IL+PHE**	**IL+PS**	**PS+DC**	**PS+PHE**
^{0}C	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
5	9.84±0.17	10.24±0.25	12.08±0.11	11.86±0.22	10.43±0.15	8.45±0.17	12.72±0.15	12.82±0.15	9.84±0.17
15	41.98±0.20	21.10±0.13	29.19±0.18	36.90±0.17	44.79±0.15	36.82±0.18	41.33±0.15	37.01±0.15	41.98±0.20
25	**48.52±0.12**	**24.42±0.11**	**29.66±0.12**	**39.83±0.22**	**49.12±0.12**	**55.63±0.15**	**49.71±0.12**	**39.97±0.10**	**48.52±0.12**
35	46.82±0.16	24.03±0.16	29.05±0.15	37.65±0.18	46.70±0.15	54.20±0.18	44.20±0.13	38.86±0.16	46.82±0.16
45	45.71±0.11	22.11±0.15	28.29±0.13	36.05±015	42.26±0.13	49.71±.15	42.70±0.15	37.14±0.10	45.71±0.11
55	42.82±0.15	21.36±0.17	28.16±0.19	22.33±0.14	37.22±0.14	46.47±0.13	37.41±0.15	34.59±0.15	42.82±0.15
65	12.87±0.12	16.30±0.14	21.88±0.15	15.07±0.14	19.51±0.12	17.71±0.11	16.60±0.13	15.91±0.11	12.87±0.12
75	0	0	0	0	0	0	0	0	0

Table 10 Effect of various Temperatures on AAO activity of monocultures and co cultures.

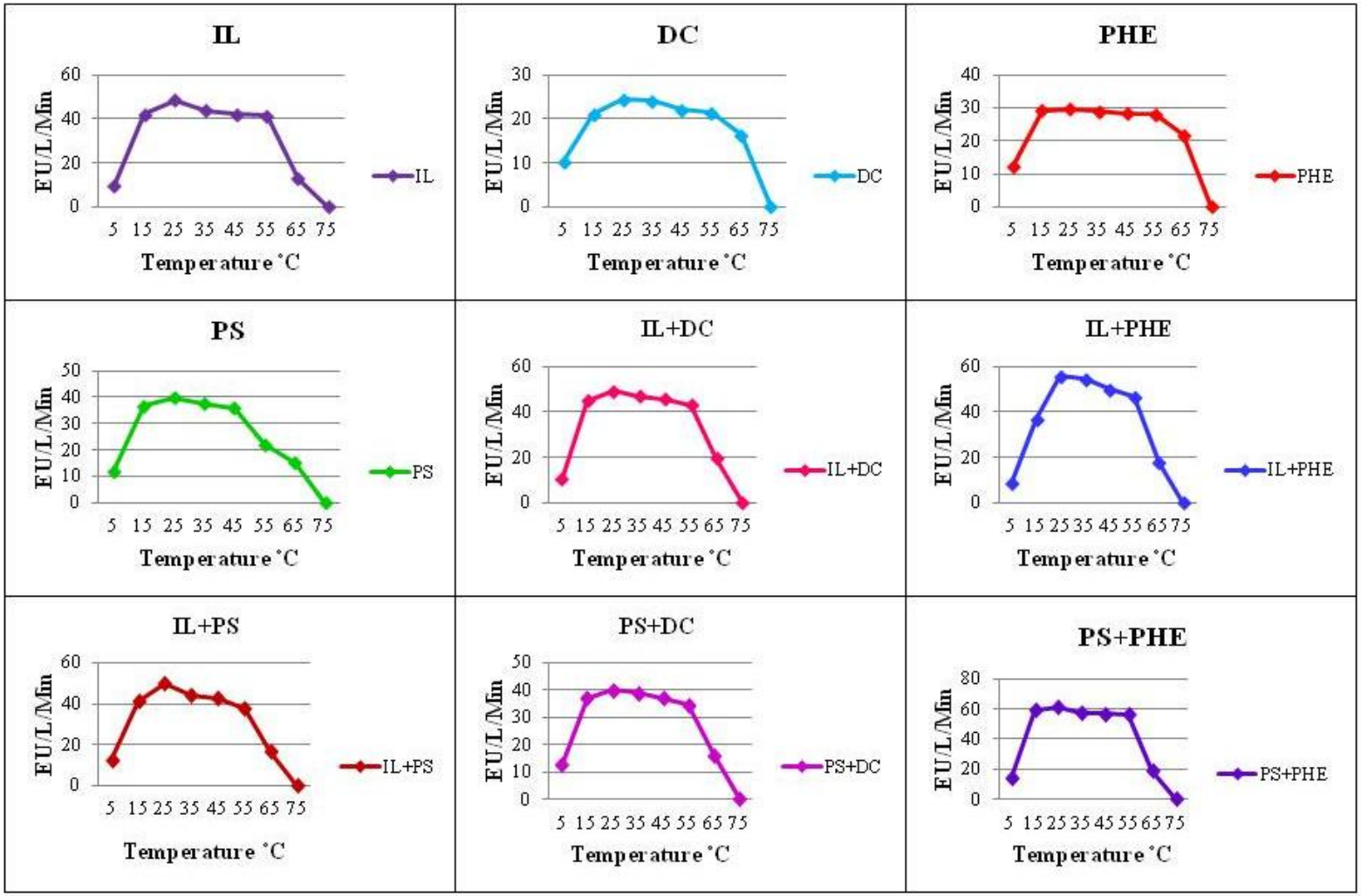


Fig. 10 Effect of various temperatures on AAO activity in monocultures and cocultures of fungi

(b) Effect of pH

It is important to study the pH stability of the different ligninolytic enzymes because in the paper industry the current pulping process takes place under extreme conditions (pH is highly basic and under high temperature). In the present study pH stability of the different ligninolytic enzymes (LiP, MnP, Laccase, AAO and Xylanase) have been evaluated. In order to study the effect of different pH on the enzyme production, the fungal enzyme extract samples were incubated at different pH and evaluated for the best stability. The maximum activity of the enzymes produced by monocultures and cocultures and its stability has been represented in Table 12-16 and Figure 12-16.

The maximum activity of LiP at various pH in mono culture and co culture of selected fungi indicates that LiP activity was not stable in the acidic range (1-3) but gradual increase in the LiP activity was occurred from pH 4 to pH 7. At pH 8 a decline in the LiP activity was appeared. Amongst the wide range of pH studied showed maximum LiP activity at pH 5 for both mono culture and co culture of the selected fungi (Figure 12and Table 12).

As represented in the Table 13 MnP enzyme showed lower production at acidic pH range (1-4) but remains stable in the range of pH (4-7) where as decline in the activity was observed at pH 8 indicating MnP was not much stable at acidic pH and alkaline pH (Figure 13).

In mono culture all the fungi showed laccase stability in the range of pH (4-7) where pH 5 was showing maximum activity and at basic pH enzyme activity was reduced (Figure 14). At acidic pH activity of Laccase enzyme was reduced and in case of co culture also maximum enzyme activity was observed at pH 5.

Temp ^{0}C	**IL**	**DC**	**PHE**	**PS**	**IL+DC**	**IL+PHE**	**IL+PS**	**PS+DC**	**PS+PHE**
	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min
5	12.63±0.43	9.35±0.83	3.59±0.53	1.034±0.16	12.69±0.43	12.60±0.30	2.31±0.19	9.78±0.26	9.63±0.35
15	16.01±0.27	12.76±1.15	7.65±0.23	2.28±0.16	21.73±0.36	18.28±0.36	4.98±0.27	14.19±0.24	9.83±0.30
25	**18.06±1.03**	**13.86±1.32**	**9.31±0.73**	**3.15±0.20**	**22.57±0.58**	**19.36±0.53**	**5.91±0.53**	**15.46±0.43**	**10.73±0.59**
35	17.11±1.32	12.65±1.01	8.84±0.83	3.05±0.16	21.56±1.33	16.61±0.57	5.23±0.43	14.82±0.20	10.21±0.26
45	15.62±1.49	11.88±0.66	7.94±0.52	2.79±0.10	20.35±0.83	14.91±0.19	5.02±0.20	13.55±0.20	9.13±0.29
55	14.08±1.32	10.23±0.66	6.95±0.23	2.2±0.20	18.48±1.19	13.00±0.26	3.53±0.26	10.53±0.26	8.49±0.29
65	11.08±0.37	9.35±0.36	3.27±0.40	1.34±0.20	12.32±1.37	10.07±0.28	2.44±0.30	6.95±0.30	7.38±0.16
75	0	0	0	0	0	0	0	0	0

Table 11Effect of various Temperatures on Xylanase activity of monocultures and co cultures.

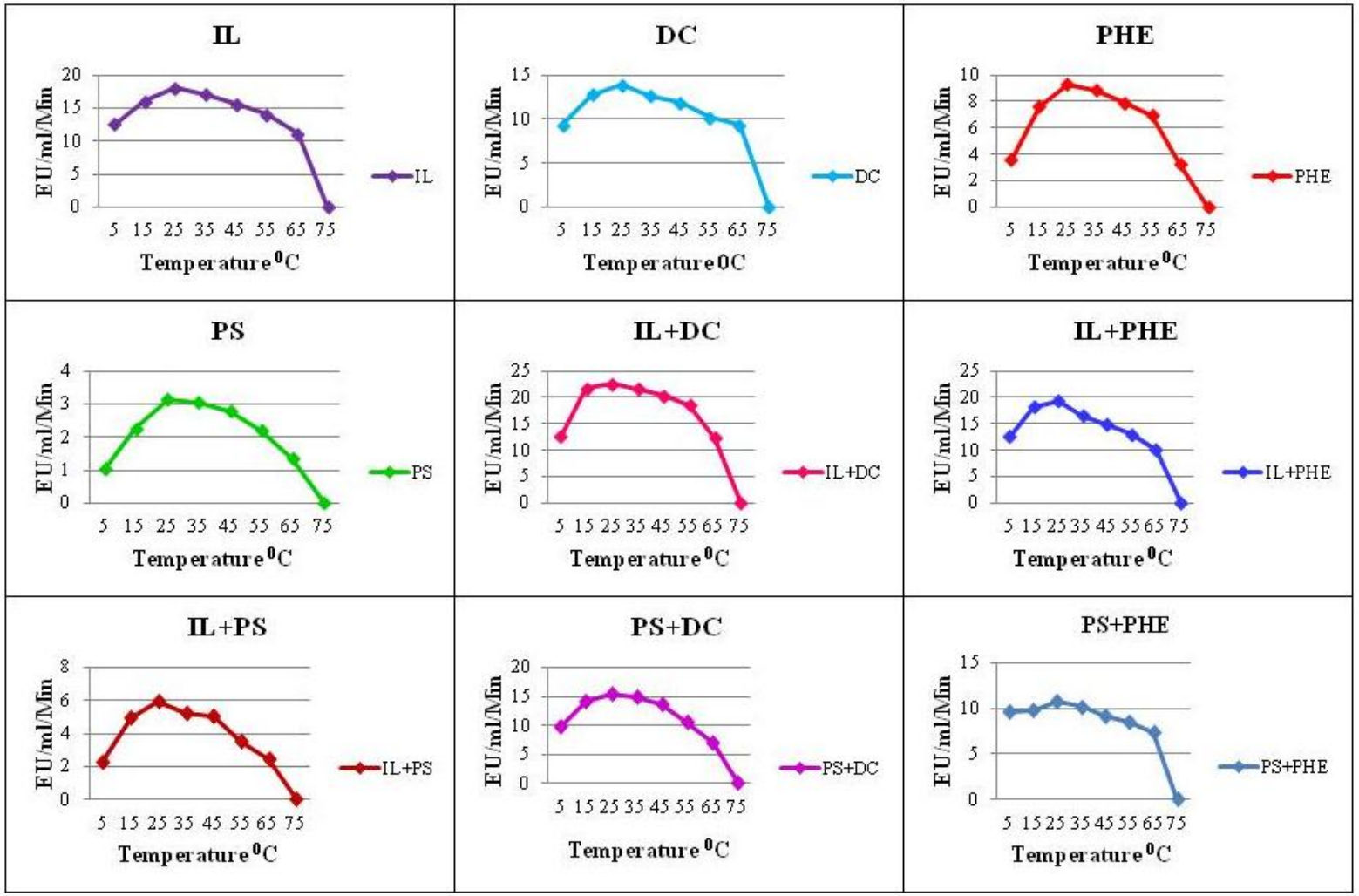


Fig. 11 Effect of various temperatures on Xylanase activity in monocultures and cocultures of fungi

. IL+DC, IL+PHE and IL+PS showed lower enzyme activity at pH 1-3 after that it increased up to 7 pH and decline was noted at pH 8 (Figure 14 and Table 14).

AAO activity in the mono culture and co culture of the fungi was found stable in the range of pH 4-6 and at neutral pH it starts to decrease as represented in the Table 15. At highly acidic pH and alkaline pH reduction in the AAO enzyme activity was observed. In all the co culture of fungi at pH 1 and 2 very less enzyme activity was observed and it is very clearly observed in the Figure 15.

Xylanase activity in mono cultures was stable in the range of pH 4 to 7 and showed maximum activity at pH 5 and at lower pH it decreased that indicates xylanase activity was not much stable at highly acidic pH as represented in Table 16. In co culture of fungi also same stability in the range of pH was observed and highest enzyme activity was noted at pH 5 (Figure 16).

All the enzymes showed maximum activity at pH 5 in mono culture and co culture both so pH 5 has been selected as optimum pH for the further experiment of optimization. Amongst the studied the mono cultures IL and DC and its co culture IL+DC was selected for the further experiments. Temperature 25˚C and pH 5 were selected as optimum parameters for the optimization experiments.

PH	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
1	0	1.51±0.06	0	0.25±0.04	0.69±0.06	0.18±0.06	1.13±0.04	0.13±0.06	0.25±0.04
2	0	1.92±0.09	0	0.43±0.06	1.06±0.06	0.37±0.06	1.46±0.06	0.29±0.09	0.43±0.06
3	0	2.26±0.06	0	0.56±0.06	1.25±0.06	0.56±0.06	1.84±0.09	0.54±0.09	0.56±0.06
4	0	2.36±0.03	0	0.69±0.06	1.38±0.06	0.67±0.09	2.11±0.09	0.77±0.09	0.69±0.06
5	0	**2.51±0.06**	0	**0.75±0.06**	**1.49±0.09**	**0.77±0.09**	**2.34±0.09**	**0.90±0.09**	**0.75±0.06**
6	0	2.47±0.09	0	0.71±0.09	1.40±0.06	0.75±0.06	2.26±0.06	0.86±0.09	0.71±0.09
7	0	2.45±0.06	0	0.62±0.06	1.21±0.09	0.62±0.06	2.07±0.09	0.69±0.06	0.62±0.06
8	0	1.69±0.08	0	0.42±0.09	0.67±0.09	0.19±0.06	0.98±0.09	0.41±0.09	0.42±0.09

Table 12 Effect of pH on LiP activity in Monocultures and co-cultures of the selected fungi

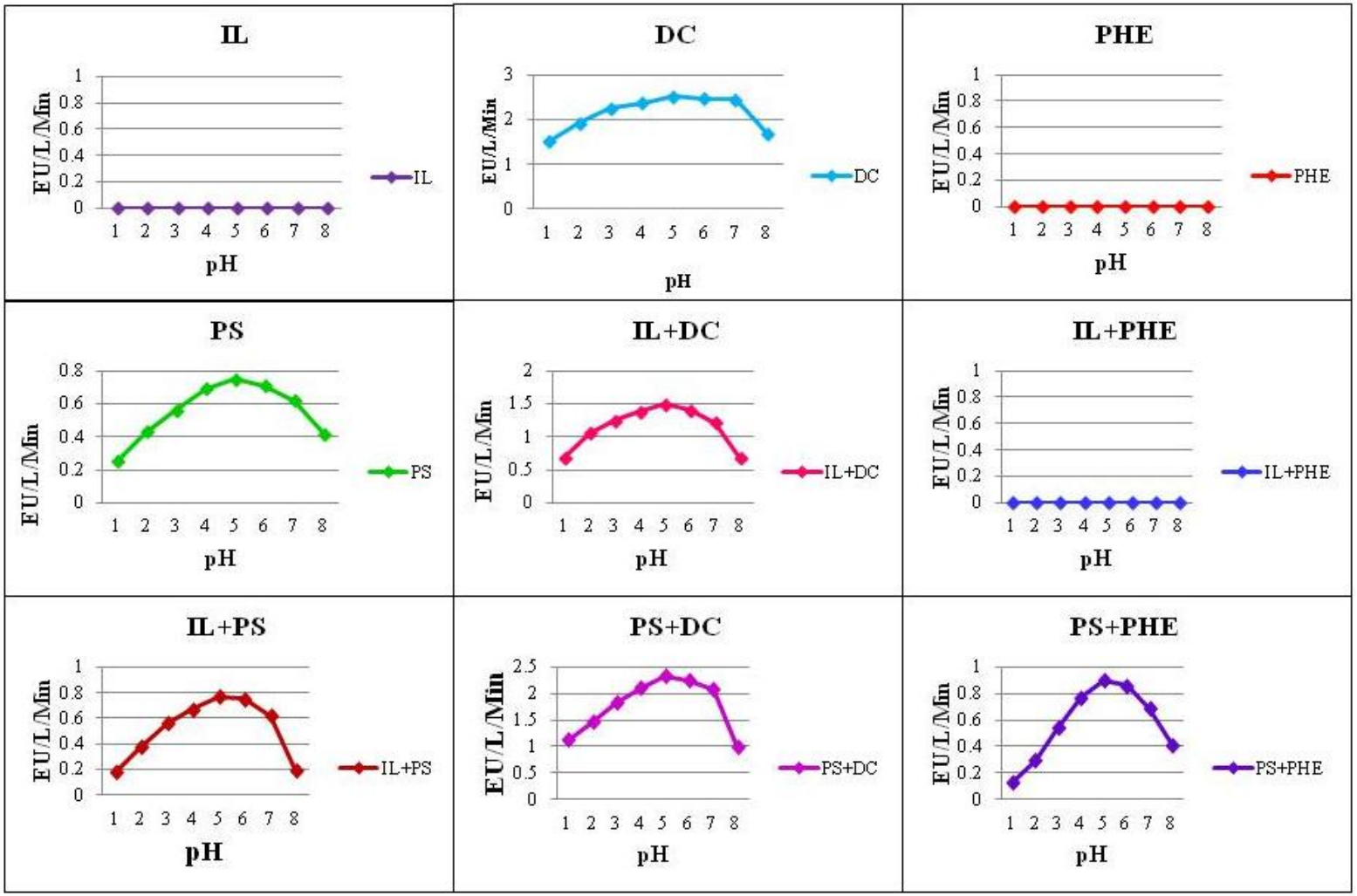


Fig. 12 Effect of pH on LiP activity in monocultures and cocultures of fungi

PH	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
1	0.10±0.04	0.35±0.04	0	0	0.31±0.06	0.15±0.07	0.15±0.07	0.40±0.09	0
2	0.23±0.09	0.56±0.06	0	0.10±0.04	0.69±0.06	0.33±0.09	0.19±0.06	0.75±0.06	0.19±0.06
3	0.35±0.09	0.84±0.09	0	0.19±0.06	1.17±0.09	0.38±0.06	0.42±0.09	1.11±0.09	0.67±0.09
4	0.57±0.09	0.98±0.09	0	0.56±0.06	1.55±0.09	0.54±0.09	0.75±0.06	1.38±0.06	0.84±0.09
5	**0.77±0.09**	**1.69±0.06**	0	**0.61±0.09**	**1.75±0.06**	**0.67±0.09**	**0.79±0.09**	**1.51±0.06**	**1.11±0.09**
6	0.75±0.06	1.57±0.06	0	0.50±0.06	1.30±0.09	0.54±0.09	0.75±0.06	1.44±0.06	1.00±0.06
7	0.69±0.06	1.48±0.09	0	0.25±0.06	1.11±0.09	0.44±0.06	0.50±0.06	1.05±0.09	0.86±0.09
8	0.21±0.09	1.19±0.06	0	0.17±0.07	1.05±0.09	0.31±0.06	0.38±0.06	0.90±0.09	0.44±0.06

Table 13 Effect of pH on MnP activity in Monocultures and co-cultures of the selected fungi

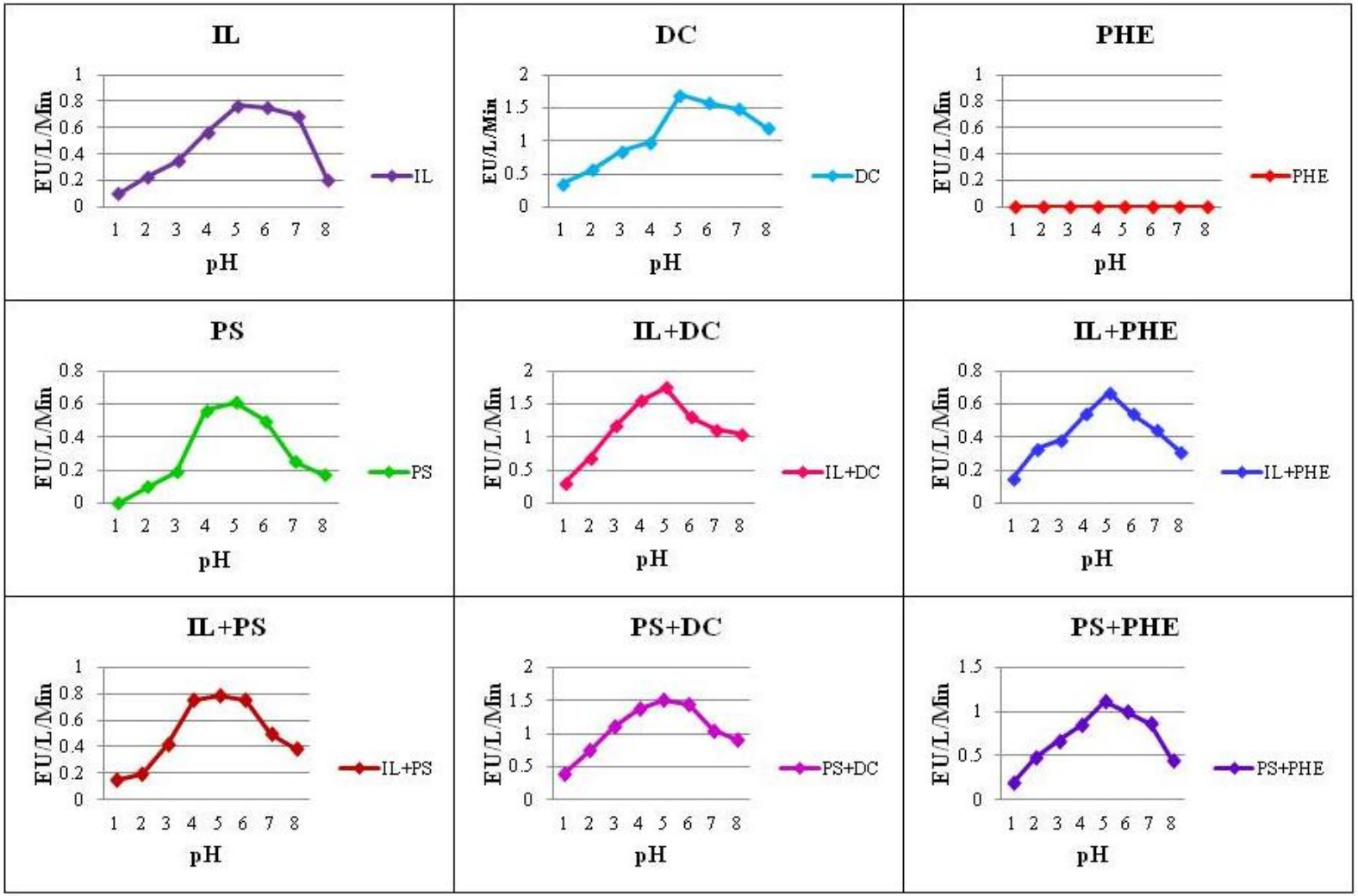


Fig. 13 Effect of pH on MnP activity in monocultures and cocultures of fungi

.pH	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
1	0.94±0.04	0.79±0.02	1.01±0.01	1.09±0.03	1.76±0.03	1.02±0.03	2.08±0.04	0.79±0.03	1.05±0.02
2	0.98±0.05	0.91±0.02	1.04±0.01	1.13±0.03	2.24±0.03	1.07±0.03	2.34±0.03	0.84±0.03	1.37±0.02
3	1.04±0.03	0.96±0.01	1.06±0.01	1.21±0.03	4.05±0.03	1.09±0.02	3.24±0.03	0.90±0.03	1.38±0.03
4	1.85±0.04	1.06±0.01	1.12±0.01	1.65±0.03	4.37±0.03	1.87±0.02	4.15±0.03	0.99±0.03	1.61±0.04
5	**1.90±0.03**	**1.08±0.02**	**1.20±0.01**	**1.76±0.03**	**4.82±0.03**	**1.99±0.03**	**4.37±0.03**	**1.12±0.04**	**1.68±0.03**
6	1.84±0.03	1.00±0.01	1.15±0.03	1.70±0.04	4.27±0.03	1.52±0.03	4.06±0.03	1.03±0.03	1.48±0.02
7	1.50±0.04	0.97±0.02	1.04±0.01	1.62±0.03	3.80±0.03	1.15±0.03	2.93±0.03	0.90±0.03	1.36±0.02
8	0.99±0.03	0.93±0.01	0.7±0.01	1.41±0.03	3.33±0.03	0.92±0.03	1.88±0.03	0.36±0.03	1.04±0.03

Table 14 Effect of pH on Laccase activity in Monocultures and co-cultures of the selected fungi

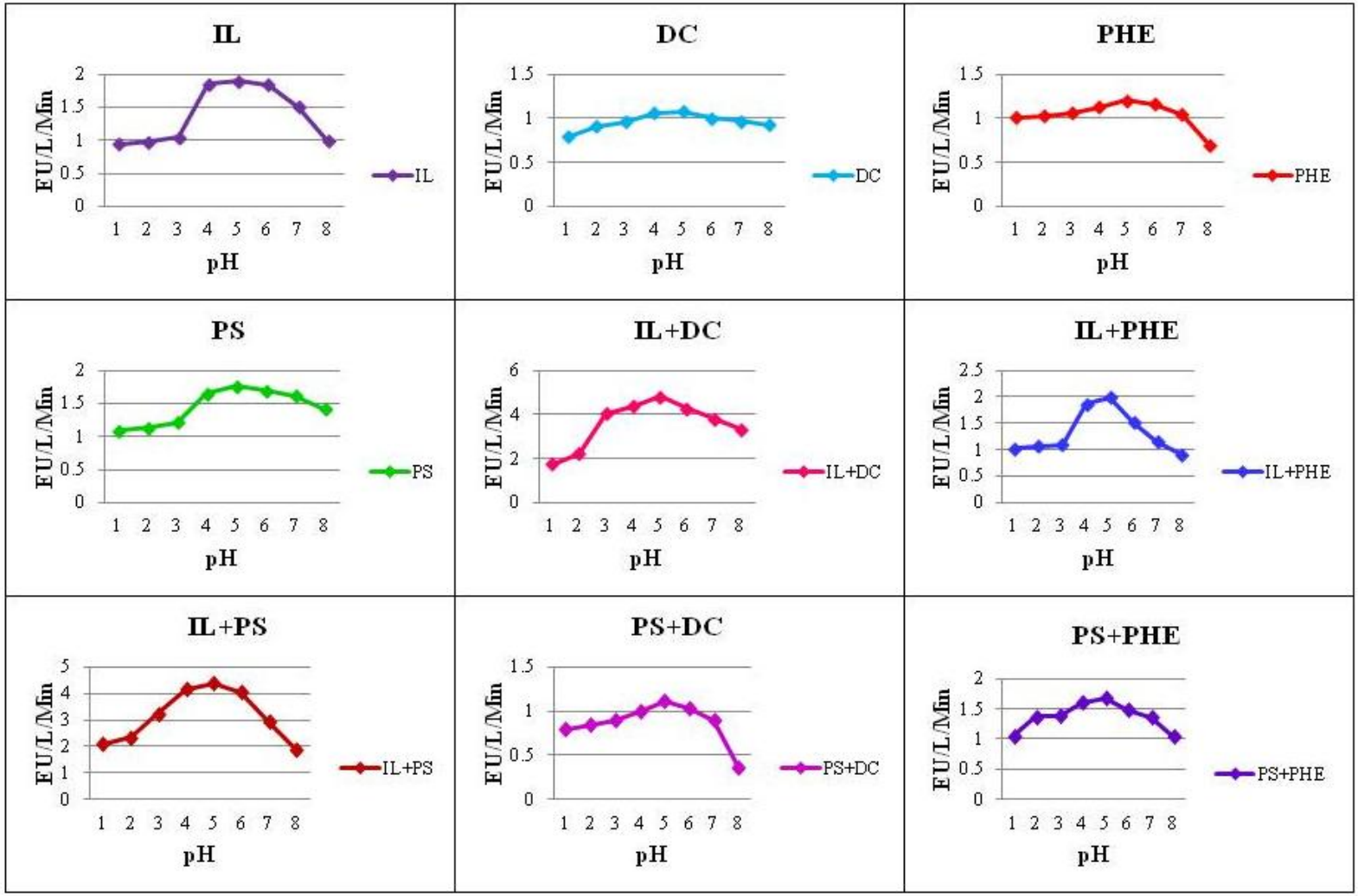


Fig. 14 Effect of pH on Laccase activity in monocultures and cocultures of fungi

pH	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min	EU/L/Min
1	25.92±0.15	21.75±0.14	15.92±0.24	27.16±0.10	28.41±0.10	36.76±0.11	28.56±0.14	26.40±0.12	31.62±0.13
2	27.58±0.10	22.50±0.10	16.61±0.16	27.93±0.10	28.62±0.10	38.44±0.11	29.2±0.12	27.75±0.13	38.08±0.10
3	28.27±0.11	23.13±0.36	22.87±0.10	28.34±0.10	41.22±0.10	42.50±0.12	38.15±0.1	32.43±0.11	45.82±0.11
4	46.76±0.10	23.34±0.10	26.57±0.16	35.56±0.10	45.79±0.14	52.71±0.12	46.18±0.11	38.63±0.11	59.68±013
5	**47.54±0.11**	**24.06±0.18**	**28.86±0.12**	**37.95±0.10**	**46.44±0.11**	**54.20±0.10**	**47.70±0.12**	**39.15±0.12**	**61.09±0.10**
6	46.70±0.10	20.34±0.31	27.99±0.16	36.58±0.12	45.83±0.12	53.05±0.13	46.17±0.10	38.57±0.12	60.67±0.11
7	38.76±0.11	10.75±0.14	16.47±0.10	36.09±0.11	38.86±0.10	51.05±0.10	45.82±0.11	37.93±0.11	57.15±0.10
8	31.51±0.10	10.54±0.12	15.70±0.11	34.08±0.11	29.05±0.11	50.08±0.10	37.72±0.10	32.99±0.11	51.04±0.10

Table 15 Effect of pH on AAO activity in Monocultures and co-cultures of the selected fungi

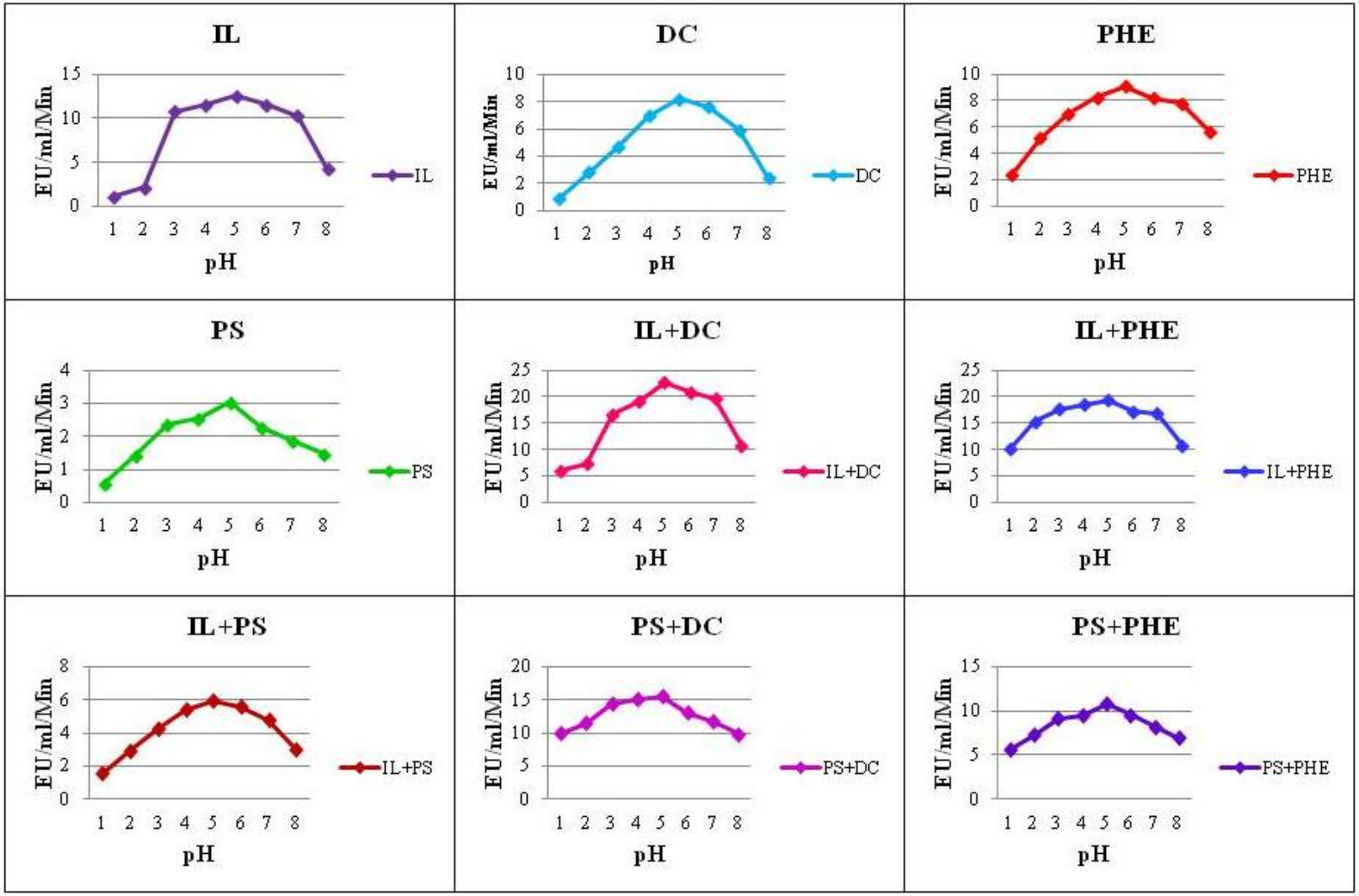


Fig. 15Effect of pH on AAO activity in monocultures and cocultures of fungi

pH	IL	DC	PHE	PS	IL+DC	IL+PHE	IL+PS	PS+DC	PS+PHE
	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min	EU/ml/Min
1	1.12±0.30	0.88±0.17	2.42±0.29	0.57±0.23	6.05±0.40	10.25±0.20	1.56±0.17	10.01±0.27	5.54±0.33
2	2.09±0.20	2.86±0.33	5.17±0.23	1.41±0.16	7.26±0.71	15.31±0.39	2.94±0.17	11.57±0.23	7.21±0.23
3	10.78±0.36	4.71±0.49	6.99±0.30	2.35±0.14	16.74±0.43	17.66±0.27	4.26±0.23	14.34±0.27	9.11±0.24
4	11.55±0.39	6.93±0.50	8.23±0.23	2.53±0.23	18.50±0.43	18.52±0.36	5.39±0.27	15.03±0.30	9.50±0.20
5	**12.54±0.50**	**8.18±0.46**	**9.10±0.36**	**3.04±0.13**	**19.03±0.33**	**19.31±0.33**	**5.96±0.53**	**15.46±0.43**	**10.86±0.39**
6	11.53±0.40	7.63±0.26	8.18±0.24	2.28±0.23	20.87±0.33	17.33±0.29	5.61±0.19	13.07±0.20	9.53±0.27
7	10.27±0.20	5.94±0.30	7.81±0.20	1.87±0.23	19.71±0.39	16.85±0.27	4.79±0.23	11.7±0.29	8.20±0.20
8	4.20±0.44	2.40±0.46	5.69±0.29	1.87±0.23	10.80±0.64	10.86±0.23	2.97±0.24	9.83±0.19	6.95±0.23

Table 16 Effect of pH on xylanase activity in Monocultures and co-cultures of the selected fungi

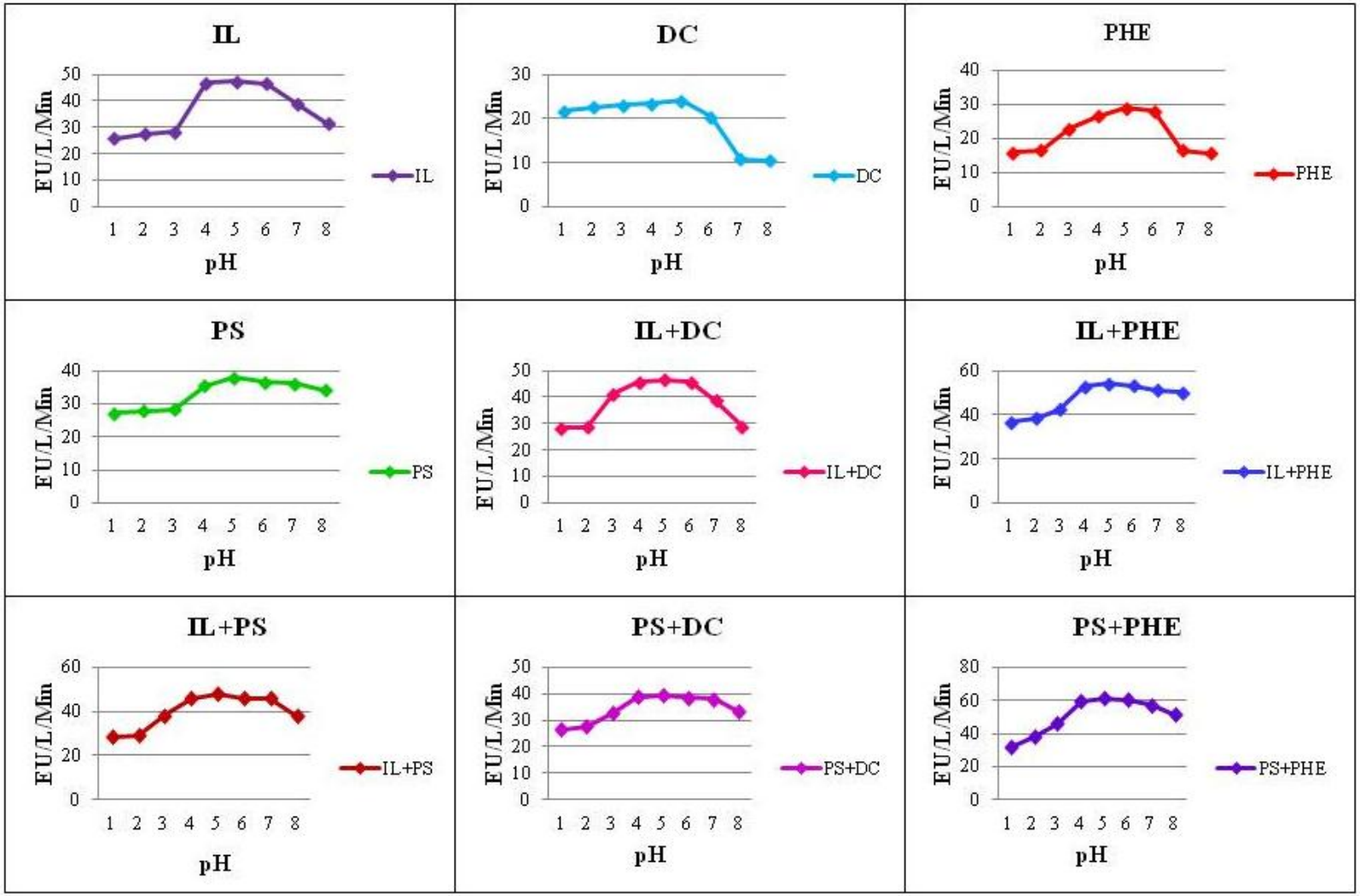


Fig. 16 Effect of pH on xylanase activity in monocultures and cocultures of fungi

3.2.3 Discussion

Recently, extensive research on basidiomycetes fungi has been carried out for isolating new organisms with tremendous secretion of ligninolytic enzymes as well as enzymes having important industrial application (Kiiskinen *et al* 2004, Mikiashvili *et al* 2004). The interest in the study of lignocellulolytic enzymes has developed due to their potential use in biotechnological processes. These enzymes can be employed to treat effluents in the textile, paper and pulp industries, to biobleaching cellulose pulp and during the *in vivo* biodelignification of wood chips, to clarify wines and juices, as well as in polymerization reactions.

However, not many comparative studies are available on ligninolytic enzymes production by these organisms, and especially little attention has been given to the evaluation of the hydrolytic system of these fungi (Baldrian and Gabriel 2003, Elisashvili *et al* 2006) so it may be beneficial to evaluate hydrolytic and oxidative enzymes activity of different fungi while cultivating either in solid state or submerged fermentation.

The objective of the present study was to investigate the ability of different fungal isolates and its co cultures to produce maximum main lignocellulose degrading enzymes (LiP, MnP, Laccase, AAO, xylanase and cellulase) by submerged fermentation in malt extract broth (MEB) medium. The culture filtrates of mono cultures and co cultures of selected fungi were evaluated at various incubation periods (3-35 days) as these enzymes play an important role in degradation of lignin, a major requisite of bio pulping.

The maximum fungal bio mass produced varied from 0.20 gm in IL to 0.60 gm in IL+DC. The results shown in Table 1 indicated co cultures of the fungi studied

viz IL+DC, IL+PHE, IL+PS, PS+DC and PS+PHE showed more biomass production as compared to their respective mono cultures.

In the co culture maximum activity of ligninolytic and xylanolytic enzymes were recorded on the 20^{th} day after incubation period which was obtained in monocultures on 25^{th} day by DC and PHE while taking 30 days in IL and PS. After the peak in the production of enzymes was noticed the gradually decrease of each enzyme activity was also recorded. Production of enzymes in the medium was decreased due to nutrient depletion which affects the metabolic activities of fungus (Patel *et al* 2009). In the studies of Cajthaml *et al* 2008 for PAH degradation maximum activity of lignocellulose degrading enzymes achieved by *Irpex lacteus* between 15-20 days of incubation period in Malt Extract Glucose medium while in our study it showed maximum activity between 25-30 days of incubation.

Mono cultures PS and DC produced all the ligninolytic as well as xylanolytic enzymes simultaneously, IL produced MnP and Laccase and PHE produced only laccase and AAO. Co cultures/mixed cultures produced all the enzymes except IL+PHE which showed an absence of LiP activity.

Amongst the mono cultures DC and IL were found to be potential enzyme producers. IL produced maximum activity of laccase, AAO and xylanase on 30^{th} day of incubation period while DC produced maximum amount of LiP and MnP on the 25^{th} day of incubation period. IL and PHE did not produce LiP in the maximum incubation period (25 days) and MnP was not produced by PHE. Overall PHE was found to be a poor producer of enzymes. IL significantly produces high amount of other important lignin degrading enzymes like laccase and AAO.

IL+DC is found to be the best co culture for production of enzymes within shorter incubation period. There was a significant 5 fold increase in laccase within

shorter incubation period of 20 days through LiP and MnP had no significant increase. Even the amount of xylanase showed a double fold increase within 20 days of incubation. Only AAO enzyme produced was maximum by IL+PHE. The low production can be compensated with the high production of the other enzymes.

In the studies of Hong *et al* (2012) Lip activity was found to be absent in four different white rot fungi (*Pleurotus ostreatus*, *Coriolus versicolor* , *Tyromyces albidus* and *Trametes gallica*) in static submerged fermentation using peat as a substrate indicating some white rot fungi may not be producing LiP activities. Kamitsuji *et al* (2004) also demonstrated that *Pleurotus* species have been recognized to produce no typical LiP activity. No LiP activity was detected in the *C.versicolor* cultured medium using Olive mill wastewater as the substrate (Ergul *et al* 2009). For lignocellulose degrading enzymes, some white rot fungi produce all of these enzymes while others produce only one or two of them. Results of the present study demonstrated that IL and PHE and its co culture showed absence of LiP activity.

In lignin degradation two major families of enzymes involved were Laccase and Peroxidases. All the major enzymes produced by some fungi, others produced only two of them, or even one also. In addition, reductive enzymes aryl alcohol oxidases, also plays role in lysis of lignin (Cullen 1997). Wood degrading fungi produce three groups of fungi but some produce only one or two groups was earlier described by De Jong *et al* (1994) and similar observations was noted in the present study. PHE did not produced LiP and MnP it only produce Laccase while IL produced MnP and Laccase only indicating that for the degradation of lignin all three enzymes are not required at a time.

Highest MnP and Laccase activity was observed in the co culture of IL+DC which showed Laccase activity 5.45 U/L while in mono culture IL and DC produced

1.94 and 1.07 U/L respectively. MnP activity noticed in co cultures of IL+DC was 1.97 and mono cultures of IL and DC showed 0.75 and 1.75 U/L respectively which is in accordance with the observations of Chi *et al* (2007) who has reported that laccase and MnP production was stimulated in co-cultures of *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* or *Physisporinus rivulosus*. Baldrian *et al* (2004) also noted stimulation of Laccase in the co culture of *Trametes versicolor* and *Pleurotus ostreatus.* In Malt extract medium higher ligninolytic enzymes like laccase and manganese peroxidase production is attributed as malt extract provides the complete pool of amino acids required for enzyme synthesis (Arora and Sandhu 1985).

All the four fungi in mono culture showed MnP production except PHE on 25^{th} to 30^{th} days. MnP activity ranged from 0.75 U/L in IL to 1.75 in DC while in co culture 1.97 U/L in IL+DC and 0.79 U/L in IL+PHE. In the study of four white rot fungi (*Pleurotus ostreatus, Coriolus versicolor* , *Tyromyces albidus* and *Trametes gallica*) by Hong *et al* (2012) all the fungi showed notable quantity of MnP except *Coriolus versicolor* which produce much activity of MnP 10 U/L on the 25^{th} day. The other three WRF in the same study produced MnP activities less than 2 U/L. *T. albidus* also showed some MnP activity with a peak of approximately 1.3 U/L.

Laccase activity gradually increased from 3^{rd} day of incubation till 25-30 days in mono cultures and after that a significant decline in the activity of laccase was observed. The highest activity was observed in the co culture on 20^{th} day of IL+DC (5.45 U/L) as compared to mono culture in which IL showed 1.94 U/L and DC showed 1.07 U/L. Laccase activity in the co culture increased fivefold and three fold than of DC and IL monocultures respectively. Ma *et al* (2011) described effect of co fungal treatment of *Irpex lacteus* and *Auricularia polytricha* on lignocellulosic

degradation which showed maximum activity on the 20th day which was more than that observed in the mono cultures of both fungi.

He *et al* (2010) showed laccase activity in the combination of *Trametes trogii* and *Trametes versicolor* to have increased 24.13 and 4.07 fold higher than *T versicolor* and *T trogii* respectively. Qi *et al* (2011) showed improved production of LiP and MnP simultaneously in the co culture of *Pleurotus ostreatus* and *Pleurotus radiata* which indicated potential of co culture in the lignocellulolytic biodegradation due to its synergistic action.

Kanmani *et al* (2009) discribed the laccase activity in co culture of *Rhizopus stolonifer* and *Phanerochete chrysosporium* increased gradually in fermentation and the maximum activity (5.1 IU/ml) was found to be on 28th days and Lip activity (8.1 IU/ml) was observed on the same day of fermentation by using coculture while *R. stolonifer* produced very low level of activity (3.5 IU/ml).

One of the main objectives of the present study was to screen the fungal isolate with higher xylanase activity with less or no cellulase producing ability. The results showed that all the four fungi selected showed absence of cellulase activity in mono culture as well as co culture. All the fungi secreted different levels of xylanase enzyme in the culture medium ranging from lowest 3.21 U/ml (PS) to highest 22.68U/ml (IL+DC).

Haq *et al* (2006) reported 30-50 per cent higher production of enzymes, CMCase (2.79 U/ml/min), FPase (1.75 U/ml/min) and xylanase (189.7 U/ml/min) in co-culture of *Aspergillus niger* and *Trichoderma viride.* Nair *et al* (2008) identified production of xylanase and cellulase activity in thirty four different fungi from which most of the fungi belonged to *Aspergillus* spp. All the strains produced xylanase along

with cellulase in solid state fermentation while in submerged fermentation 70% of the strains produced cellulase free xylanase enzyme.

Similar results were also obtained by Kamble *et al* 2012 in which six fungal isolates producing cellulase-free xylanase under submerged conditions were screened amongst which *Rhizopus oryzae* (205.0 U ml-1) and *Rhizopus microsporus* CB-10 (126.0 U ml-1) were the most prominent cellulase-free xylanase producers while *Hamigera insecticola* (66.0 U mL-1), *Rhizopus* sp. (75.10 U ml-1), *Aspergillus niger* (76.0 U ml-1), *Aspergillus flavus* (48.0 U ml-1), *Penicillium citrinum* (63.0 U ml-1) and *Penicillium sp.* (45.0 U ml-1) showed absence of cellulase in submerged fermentation.

High thermostability of enzymes is proved to be attractive and desirable characteristic of enzymes in the various industrial applications. To evaluate the effect of Temperature on the lignocellulolytic enzyme production in the range of 5 ˚C to 75˚C experiments were conducted and the stability of all the enzymes were investigated. The results showed all the enzymes were found to be stable between 15˚C to 55˚C with optimum temperature of 25˚C. Results indicated that at temperature higher than 55˚C all the enzymes lost their activity rapidly and at 75˚C the activity completely stops. All the enzymes were stable between pH 3-7 with optimum pH 5 for all the enzyme activities.

The maximum growth of white rot fungi and Lignin modifying enzymes production occurred at medium temperature (Toh *et al* 2003). Many authors (Zadrazil *et al* 1999, Arora and Gill 2001, Tekere *et al* 2001, Tripathi *et al* 2008) reported temperature optima of 25˚C to 37˚C for effective lignocellulolytic enzyme production. Aslam *et al* 2011 showed optimum temperature of MnP enzyme was found to be 30˚C.

Pure cultures and cocultures of *Thermomces lanuginosus* and *Aspergiluus flavus* produced LiP and MnP which were stable between 30˚C to 50˚C while laccase showed stability between 30˚Cto 60˚C (Ghani 2013).

Asgher *et al* (2006) reported optimum ligninolytic enzyme production in the solid state fermentation at 40˚C and pH 4. Due to three dimensional alteration in the structure of enzymes decrease in the activity at lower pH and temperature was observed (Kiran *et al* 2012).

Hossain and Anatharaman (2006) reported ligninolytic enzyme activity of *Trametes versicolor* was stable up to 45˚C. For many enzymes denaturation of proteins begins to occur at 45 to 50˚C temperatures. Sensitivity of a protein to denaturation at elevated temperatures can vary widely with medium pH and the influence of various temperatures – pH combinations may differ tremendously from enzyme to enzyme (Shuler and Kargi 1992, Pelczar *et al.* 1993).

Temperature in the range of 30˚C -35 ˚C pH in the range of 5-7 had a significant production of laccase reported by Mishra *et al* 2011. *Phanerochaete sordida* produced optimum Lignin degrading enzymes at pH 4-5 was described by Ruttimann *et al* 1993. A similar observation as present study was also described by Couto *et al* 2006 in which LiP and MnP produced maximum at 32˚C and pH 4.5.

Aslam and Asgher *et al* (2011) showed Laccase and MnP production in *Pleurotus ostreatus* was completely stable between pH 4-8. Highest activity of laccase 575U/L and 200 U/L for MnP was obtained at pH 4.

Laccase enzymes produced by most of the fungi were shown to be stable below 50˚C (Yaropolov *et al* 1994, Wood 1980, Goncovales 1996). IL and DC and its co culture produced thermostable enzymes so both are suitable strains for being used in paper pulp industry.

The result of the present study leads us to conclude that production of lignocellulolytic enzymes depends upon species. It has been observed that all the fungi used were capable of producing major lignocellulolytic enzymes and did not produce cellulase enzyme that is beneficial for the paper and pulp industry and mixed/co cultures of different species were most effective than monoculture extracts. High LiP, MnP, laccase, AAO and Xylanase activity as well as no cellulase activity proved to be good combination for lignocelluloses degradation and thus can be used for bio pulping of wood as well as due to xylanase helps in bio bleaching of the cellulose fibers and provides brightening to the fibers for the efficient production of paper in the industry.

3.3 IN VITRO DECAY EXPERIMENT

The wood of *Eucalyptus globulus* was selected for the present study as it is the most widely and traditionally used raw material in the pulp and paper industries for the production of paper. Section of *Eucalyptus globulus* wood blocks were observed to characterize the normal features of the wood elements and the alterations occurred in them due to fungal pretreatment by *Irpex lacteus* and *Daedaleopsis confragosa* experimented to facilitate bio pulping. Anatomical features pertaining to degradation has been observed after 45days, 3 months, 6 months and 12 months incubation period. Fungal pretreated wood blocks anatomically revealed that lignin layer was largely broken down and cellulose remained preserved. In later stages of decay wood elements break down and wood blocks showed fibrous consistency to be due to selective delignification preserving cellulose. The detailed features of the decay patterns are as shown in the Plate 2 and 3.

After in vitro decay experiment was conducted wood blocks were also analyzed biochemically to detect the percentage loss of the wood components.

3.3.1 Anatomical alterations

(a) Normal wood structure of *Eucalyptus globulus*

Secondary xylem of *Eucalyptus globulus* wood is diffuse porous with vessel elements having more or less same diameter throughout the wood hence with indistinct rings. Wood basically contained brown or shades of brown colour. Sapwood was light brown in colour while heartwood was dark brown color. The secondary xylem composed of vessel elements, parenchyma cells (Axial and ray) and fibers (Plate 1 A).

Vessels were exclusively solitary, circular to oval in shape (Plate 1 B) with simple perforation plate. Fibers are non septate and contained thin to thick wall. Axial

and ray parenchyma (Plate 1 B) possessed circular to oval bordered pits. Rays were mostly uniseriate (Plate 1 C) with oval to circular cluster of ray cells. Fiber cells contain bordered pits round to angular in shape (Plate D). Rays with procumbent, square and upright cells mixed throughout the ray (Plate 1 E).

(b) Features of decay in *Eucalyptus globulus* wood infected by *Irpex lacteus*

Wood blocks inoculated with *I. lacteus* showed appreciable alterations in the cell wall of the parenchyma cells after 45days of incubation. A selective delignification pattern was observed. Hyphae grow mainly in the vessel element along with the xylem rays. Fungal hyphae were abundantly found in vessel elements (Plate 2 A, B). Tangential view further confirms abundance of hyphae in vessel lumen. The fungal hyphae penetrate through pits and branch in the lumen of the vessel element with successive branchings (Plate 2 B). Radial view distinctly (Plate 2) C indicated occurrence of fungal hyphae in the fiber lumen adjacent to ray cells. At this stage initiation of the separation of middle lamella was also seen in the vessel element (Plate 2 D). Vessel element wall appears to have been separated out from the adjacent cells (Plate 2 D, arrow).

Initiation of the fungal decay is first observed in parenchyma cells surrounding vessel elements. Though the vessel element does not appear to be affected at this stage the initiation of fungal penetration is through vessel elements. Mycelia traversed through the vessel lumina via the pits and infected all the cell types of secondary xylem. Fungal hyphae invaded the neighboring cells through the pits present on the lateral walls (Plate 2 E) and further branches in the cell (Plate 2 F). Hyphae penetrating in to the adjacent cell through the pits present on their wall and when they traversed from one cell to other cell through pits, they tend to adjust their

diameter to the relatively narrow pit diameter and because of which enlargement of pits occurred (Plate 2 E arrow).

In tangential view interestingly complete degradation of xylem ray cell walls occurred and the fusion of ray cells takes place due to which pocket was formed in axial parenchyma and ray parenchyma respectively (Plate 2 G). Only outline of ray cells were observed (Plate 2 J). Plate 2 I arrow clearly depicts the entry of the fungal hyphae in to fiber cells through pits of the fibre cells adjacent to the ray cells. The hyphae further within the fiber lumen branches and enters the adjacent cell through pits (Plate 2 E) further traversing parallel within the lumen (Plate 2 I arrow). Since the ray cells were generally degraded rapidly, the fiber cells adjacent to them were often attacked first after 6 months of incubation. Plate 2 H showed the separation started from the ray cells due to which middle lamella gets dissolved and then proceeded in to secondary wall (S_2 layer) of adjacent fibers. Vessels are more resistant in contrast to fibers and axial parenchyma, while the rays were the most vulnerable cells to fungal enzymes. At later stage buckling of the fiber cells were noticed due to extensive degradation (Plate 2 K).

At the early stages of decay only areas of compound middle lamella are broken down (Plate 2 N) while the secondary wall layers show no detectable structural changes. This preferential degradation of compound middle lamella results in the cells becoming separated from their matrix and individual cells become separated from one another (Plate 2 N). Fine cell wall dissolutions appear aligned radially or perpendicular to the middle lamella. Fiber cells also showing numerous fine cracks, running perpendicular to the middle lamella and distinctly separating it from outer layer (Plate 2 O).

The stripy discoloration originated at first in the S_1 layer close to the middle lamella (Plate 2 L Arrowhead) and then extend in to the S_2 and S_3 layer. Subsequently, individual fiber clefts appear in these discolored areas extending from S1 to S3 layer of the secondary wall (Plate 2 M). At an early stage of decay in some of the fiber cells it is seen that individual lamellae of the secondary wall separate off towards the lumen (plate 2 L). In these cells the enzymatic activity of the hyphae appears to affect the inner S_2 layer of the secondary wall. The exuded enzymes spread out in all directions within the cell walls. Degradation of the walls appears to be with the diffusion of the enzymes from the lumen through the S_4 layer. The S_4 layer appears to be resistant to the enzymatic destruction and is present even with advanced degradation of S_2 layer. Enzyme advance preferentially between the lamellae following the direction of the fibrils, the cell wall collapsing into submicroscopic layers which leads to individual lamellae of the innermost secondary wall dissolving inwards to the lumen (Plate 2 M double arrowhead).

(b)Features of decay in *Eucalyptus globulus* wood infected by *Daedaleopsis confragosa*

Observations of wood decayed by *Daedaleopsis confragosa* indicates the fungal isolate appeared to be selective delignifying fungus. During the early stage of decay (45 days) wood blocks inoculated with *D. confragosa* showed the features of selective delignification. Middle lamella dissolution and separation of fibers occurred where as boreholes were also observed occasionally.

Transections showed fungal hyphae in the vessel lumen (Plate 3 A). Presence of fungal hyphae was also observed within the vessel lumen a Radial view. The fungal hyphae penetrates through pits and branch within the vessel lumen (Plate 3B). Magnified view of the ray cells confirms presence of fungal hyphae in the ray cells

traversing from one cell to the other cell (Plate 3C). At this stage there was no visual damage in the cell walls of the ray cells but fungal mycelium distinctly appeared to move from one cell to the next through the pits present on their walls. Longitudinal view further confirms the presence of fungal hyphae in fiber cells and ray cells (Plate 3 D)

After 6 months of incubation period considerable variation was observed. Due to degradation of ray cell walls the longitudinal cavity was formed. Degradation of S_2 layer became pronounced and resulting in the separation of the entire cell wall of the vessel element (Plate 3 E). As decay proceeds further vessel elements began to deform after 12 months and buckling of vessel wall was observed (Plate 3F). Vessel elements appeared deformed due to loss of rigidity and the vessel wall begins to crumble.

Similar to *I. lacteus* it is observed that degradation started with the parenchyma cells surrounding the vessel elements which showed presence of boreholes (Plate 3 G). At this stage thinning of ray cell walls were observed and sometimes lead to dissolution which results in the formation of cavity. Wood blocks exposed to 12 months of incubation period showed delignification the effect of which was more pronounced. At this stage presence of bore holes in the ray cells were more in number, became larger in size and irregular in shape due to fusion of two or more bore holes (Plate3 H). Presence of bore holes were also observed in the medullary ray (Plate 3 I, J).Tangential view showed clear degradation of middle lamella, stained blue with the astra blue which begins to lose integrity and individual ray cell were start separated from each other (Plate 3 K). Compared to the axial elements ray cells were more affected showing advanced thinning of the cell walls. Due to loss of rigidity parenchyma cells fused and formation of cavity or pocket was observed, the

small cavity gets fused to form relatively larger cavity (Plate 3 L). Xylem cells showed cell wall thinning.

As the incubation period proceeds further similar pattern of delignification was observed but with more pronounced effects. During this pronounced stage of degradation, long decay channels were formed in the fiber region due to degradation of middle lamella (Plate 3 M) and along with this S_2 layer of the fiber cells were also degraded. Degradation of middle lamella resulted in separation of xylem fibers from each other. Complete separation of fibers from each other was commonly noticed at this stage and due to pronounced degradation of S_2 layer buckling of fiber cells were noticed (Plate 3N). Plate 3 O shows enlarged view of fiber wall degradation. Middle lamella lost its integrity and the individual cells became separated from each other. Advanced erosion troughs were observed between the fiber cells.

In longitudinal section fiber cell contained more number of larger and irregular bore holes (Plate 3 P). Ray cells showed large erosion holes on the wall successively arranged one above the other. The ray cells appeared either partly or completely disintegrated. As the fungal hyphae traversed from one cell to other cell via pits present on their walls enlargement of pits were distinctly noted (Plate 3Q).

Plate 1: Normal wood structure of *Eucalyptus globules*

Magnification bar: A to G -10µM

A: Vessel elements and Parenchyma cells and fibers

B: Solitary vessel elements with circular to oval shape

C: Exclusively Uniseriate Rays

D: Fiber pits round to angular

E: Procumbent Ray cells in Radial view

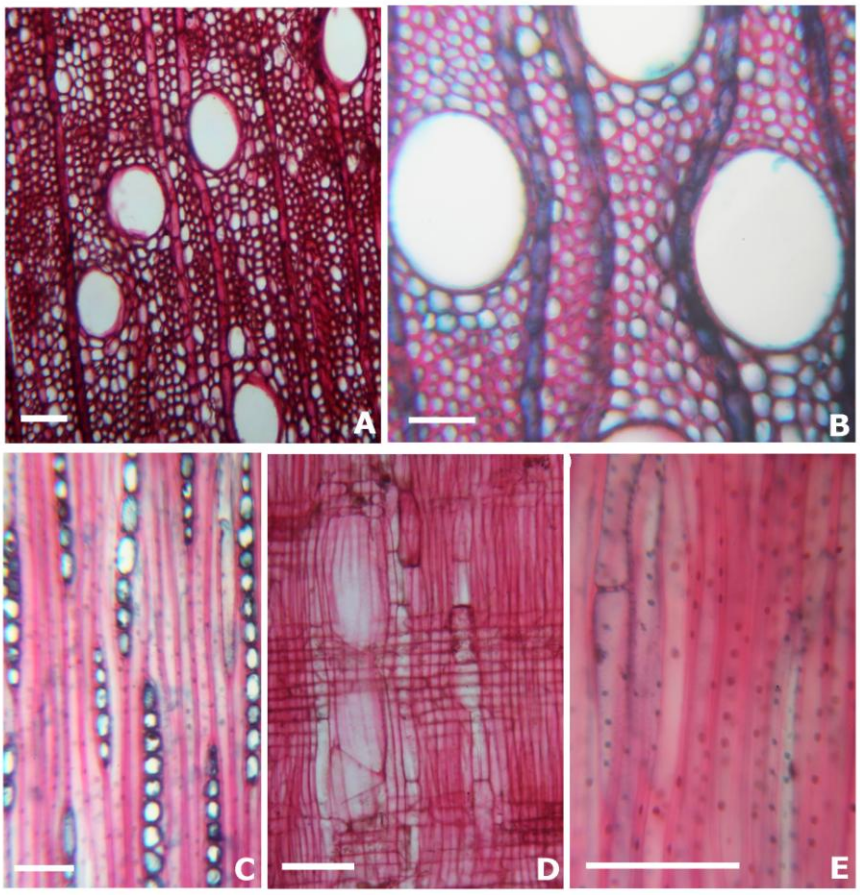


Plate 1

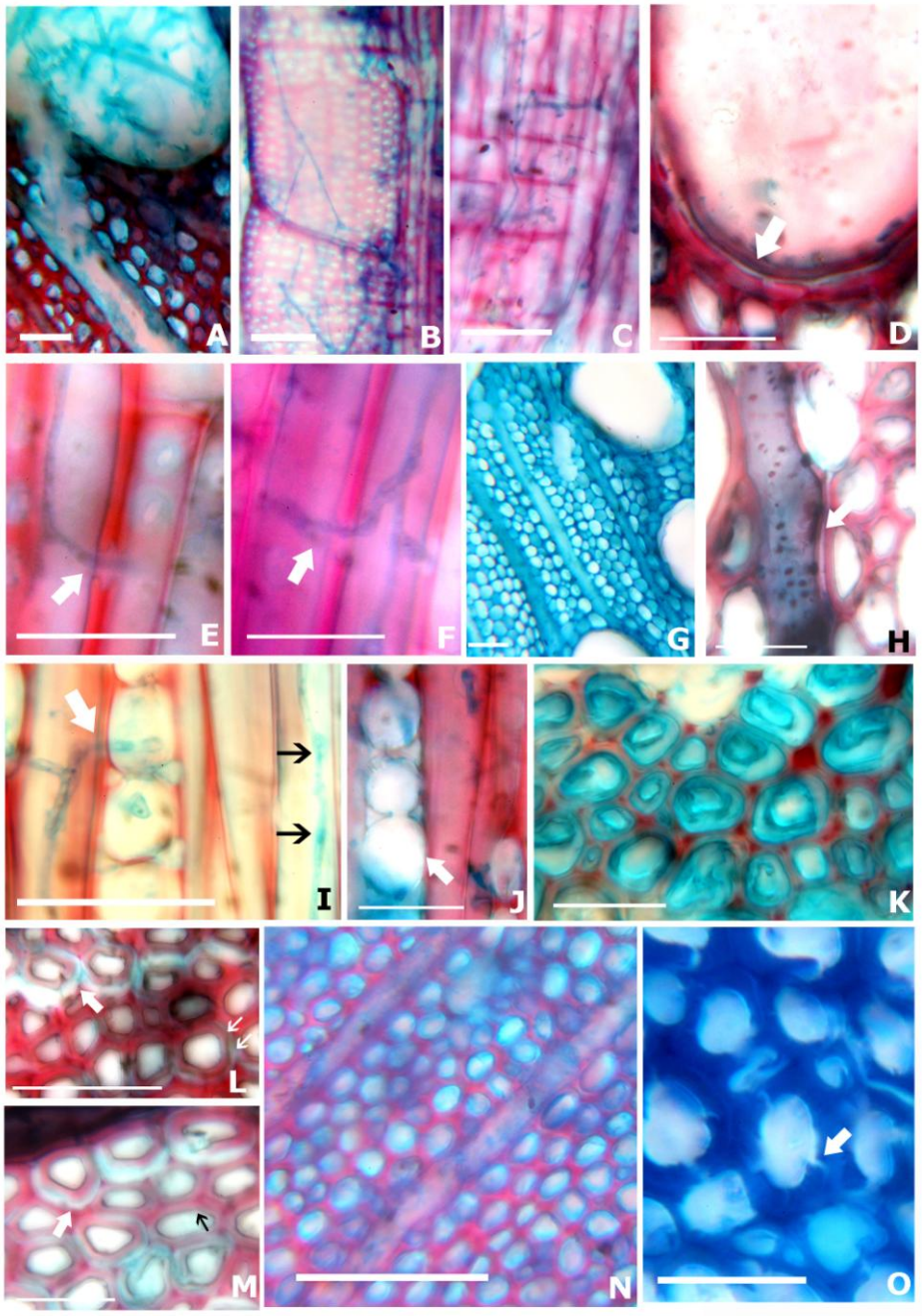


Plate 2

Plate 2: Pattern of decay in *Eucalyptus globules* wood infected with *I. lacteus*

Magnification bar: A to C, G -10µM, D to F, H to O - 5µM

A: Colonization of fungal hyphae in vessel elements

B: Penetration of fungal hyphae through vessel pits

C: Presence of fungal mycelia in ray and fiber cells

D: Vessel wall separation due to middle lamella degradation (Arrow)

E: Mycelia traversed through pits and enlargement of Pits (Arrow)

F: Branching of fungal mycelia in the fiber (Arrow)

G: Fusion of axial parenchyma cells

H: Separation of fibers from medullary ray (Arrow)

I: Presence of fungal hyphae in ray cell (Arrow)

J: Fusion of ray cells due to dissolution of ray cellwall

K: Buckling of fiber cells

L: Stripy degrdation of S_1 layer of the fiber

M: Formation of Zig-zag channel in the fiber region

N: Loosening of fiber cells

O: Cracks in S_2 layer of fiber and cleft formation

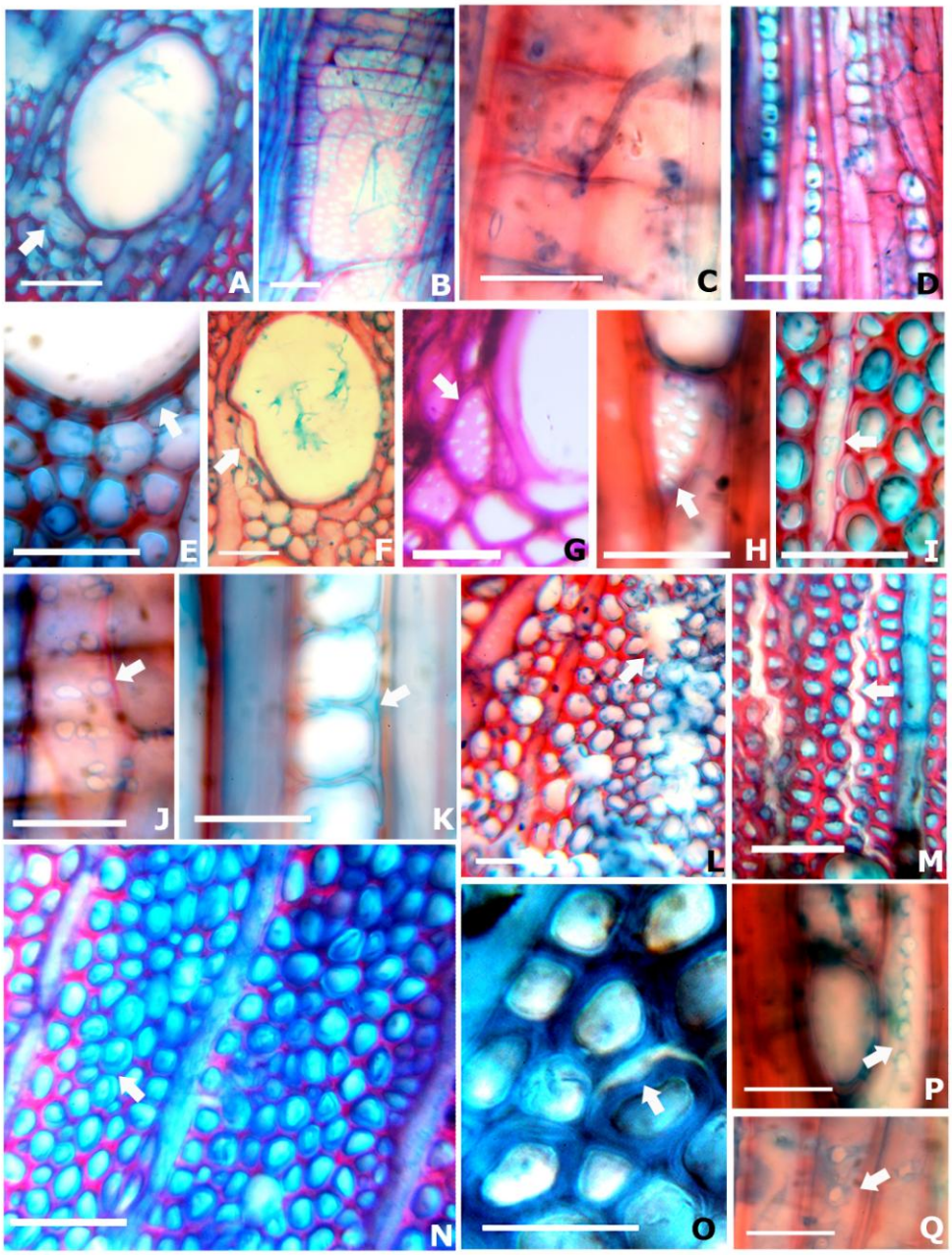


Plate 3

Plate: 3 Pattern of decay in *Eucalyptus globules* wood infected with *D. confragosa*

Magnification Bar: A to B, D to J, L to N -10 µM, C, G to K, O to Q - 5µM

A: Colonization of fungal hyphae in vessel elements and axial parenchyma cell fusion (arrow)

B: Penetration of fungal hyphae through vessel pits

C: Presence of fungal mycelia in ray cells

D: Presence of fungal mycelia in ray cells and fibers

E: Vessel wall separation and Fungal hyphae in axial parenchyma cells (arrow)

F: Buckling of vessel wall (arrow)

G: Boreholes in axial parenchyma cells (arrow)

H: Boreholes in ray parenchyma cells (arrow)

I: Bore holes in the medullary rays (arrow)

J: Bore holes in the medullary rays (arrow)

K: Middle lamella dissolution between ray cells (arrow)

L: Fusion of ray parenchyma cells (arrow)

M: Cleft formation between fiber cells (arrow)

N: S2 layer degradation in fiber cells (arrow)

O: enlarged view of S2 layer degradation in fiber cells (arrow)

P: Presence of bore holes in the fiber cells (arrow)

Q: Enlargement of fiber pits (arrow)

3.3.2 Biochemical analysis

For the wood decay test fungal inoculated culture bottles were incubated for a period of 12 months and loss of the lignin, cellulose and hemi cellulose content in the wood blocks were analyzed at four different incubation period i.e. 45 days, 3 months, 6 months and 12 months. In vitro decay tests in wood blocks of *Eucalyptus globulus* incubated with monoculture and co culture of *I. lacteus* and *D. confragosa* showed distinct response in colonization of hyphae and macroscopic appearance of wood after the treatment. Fungal mycelia completely ramified the wood blocks kept in mono and co culture within 8-10 days. In general, co culture showed quick colonization on wood blocks as compared to mono cultures. Both mono cultured and co cultured fungi began to degrade *Eucalyptus* wood within first week of incubation period. After desirable incubation period, the wood blocks when pressed they appeared to be soft especially in the longitudinal direction.

After 45 days of incubation period co cultured wood blocks displayed abundant mycelial mass on the wood blocks while *D. confragosa* in mono culture produced moderate fungal mycelia on the surface of the wood blocks and *I. lacteus* produce less fungal biomass during the whole period of decay but showed more mycelia in the internal surface when the wood blocks were cut for the further analysis of wood components. Several white coloured spots were observed on the wood blocks decayed by *I. lacteus*. These spots were present on the outer surface of the wood blocks which may be associated with the oxide deposits produced by some fungi (Eriksson *et al* 1990). The fungi in mono culture and co culture varied in its ability to degrade wood. The effect of fungal treatment on *Eucalyptus* wood in terms of percentage weight loss, lignin, cellulose, hemi cellulose loss and selectivity indices due to degradation are summarized in Table 1.

In both the pretreated mono cultured and co cultured wood blocks of *Eucalyptus*, fungi started to degrade *Eucalyptus* and gradual decreased in the components with increasing incubation period was observed. After 45 days of incubation period appreciable weight loss was observed in the wood blocks inoculated with both the fungi in its mono and co cultures and there after increasing with the succeeding days of incubation.

Incubation time	Name of fungi	Weight loss	Lignin loss	Cellulose loss	Hemicellulose loss	Selectivity value
45 days	IL	19.08±5.68	27.5±0.84	11.11±2.76	29.22±2.91	2.47
	DC	12.26±3.46	25.55±1.26	17.46±2.75	23.43±3.65	1.46
	IL+DC	27.93±6.33	30.83±0.83	23.80±4.75	24.08±5.78	1.29
3Months	IL	22.78±5.43	37.22±1.27	12.13±5.25	34.64±2.80	3.06
	DC	18.08±3.85	34.22±1.27	20.63±2.74	29.25±3.81	1.65
	IL+DC	36.18±12.26	40.86±0.84	31.74±2.74	30.99±4.62	1.29
6 Months	IL	67.36±6.38	40.83±1.66	28.57±4.76	28.85±5.34	1.43
	DC	23.62±3.92	37.5±0.83	23.8±4.76	30.25±3.70	1.57
	IL+DC	79.95±29.46	46.94±1.27	33.33±4.75	35.85±2.29	1.40
12 Months	IL	79.06±8.95	59.44±1.73	39.68±2.75	26.42±1.64	1.49
	DC	84.77±19.77	47.76±1.73	30.16±2.75	32.02±3.22	1.58
	IL+DC	87.39±20.49	68.34±2.20	41.26±2.75	39.21±4.18	1.65

Table 1 Percentage loss of wood components infected incubated with *Irpex lacteus* and *Daedaleopsis confragosa* in mono and co culture

The percentage weight loss in the wood blocks infected with *I. lacteus* was found to be 19.08% within one and half month of incubation period, increased up to 22.78% in three months, 67.36% within six months and 79.06% in 12 months of incubation period. *D. confragosa* showed less weight loss compared to *I. lacteus* till one and half month incubation period i.e 12.26% and it was enhanced up to 23.62%

after six months of time and after 12 months 84.77% weight loss was observed while in co culture weight loss ranged from 27.93% to 87.39% which was significantly more than the weight loss observed in the monoculture. The weight loss was almost double in co culture.

Percentage weight loss increased rapidly after one and half month and showed 67.36% by IL, 23.62% by DC and 79.95% by IL +DC after six months whereas after 12 months incubation period 79.06%, 84.77% and 87.39% weight loss was noticed in *I. lacteus, D. confragosa* and coculture of *I. lacteus*+ *D. confragosa* respectively which indicated very highly significant weight loss was observed in *D. confragosa* monoculture. Rapid increase in the weight loss observed may be due to the degradation potential of fungi and complete consumption of low molecular weight compounds present in the wood.

The lignin content of the wood was mainly affected by mono culture and co culture both. *I. lacteus* and *D. confragosa* both were efficient lignin degraders and the loss of lignin due to these fungi in mono and co culture is represented was shown in Table 1. Co culture of these fungi also showed significant weight loss as well as hemicelluloses loss. Percentage loss in the lignin content of wood *E globulus* showed gradual increase with increasing incubation period. After one and half month of incubation IL showed 27.5% lignin loss which increased 37.22% in 3 months, 40.83% after 6months of incubation time and 59.44% after 12 months and DC showed 25.55%, 34.22%, 37.5% and 47.76% lignin loss in the one and half month, 3 months, 6 months and 12 months of incubation period respectively while in co culture of IL and DC 30.83% loss of lignin was found within one and half month of incubation period which enhanced up to 40.86% in 3 months, 46.94% within six months and 68.34% in 12 months of incubation time. A significant increase in the degradation of

wood by co cultures noted and the result of percentage loss in the lignin indicated that the wood *E. globulus* was most susceptible to both the fungi in mono culture as well as in co culture.

The percentage cellulose loss in the wood blocks infected with *I. lacteus* was 11.11% within one and half month of incubation period, and increased up to 12.13%, 28.57% and 39.68% within three, six and twelve months respectively. Cellulose loss was more in wood blocks inoculated with *D. confragosa* in the initial stages. The loss of cellulose was 17.46% within one and half months, enhancing up to 20.63% in three months, 23.8% within 6 months and 30.16% in 12 months incubation period. In wood blocks inoculated with co culture, cellulose loss ranged from 23.80% to 41.26% which was significantly more than the cellulose loss observed in the monocultures.

The fungal interactions in wood showed stimulation in removal of easily attainable polymers such as hemicellulose. Both the fungi and its co culture hydrolyse hemicellulose significantly. The percentage hemicellulose loss in the wood blocks infected with *I. lacteus* was found to be 29.20% within one and half month of incubation period, and increased as the time of incubation increased up to three months after which it tend to decrease as higher loss of cellulose was noticed compared to initial incubation period.

Within three months hemicelluloses loss was found to be 34.64%, in six months decreased up to 28.85% and 26.42% was noticed after 12 months of incubation period. *D. confragosa* showed less hemicellulose loss as compared to *I. lacteus*, till one and half month of time i.e 23.43 % and it was enhanced up to 29.25% after three months of time. After 6 months and 12 months incubation period it was slightly higher compared to *I. lacteus* as 30.25% and 32.02% in six months and twelve months respectively while in co culture hemicellulose loss ranged from 24.08% to 39.21% which is clearly more than the loss of hemicellulose observed in the monoculture.

3.3.3 Discussion

Many microorganisms are likely to degrade wood. The most relevant of them are fungi because they produce important changes on the anatomical, physical and mechanical wood properties (Wilcox 1978, Highley *et al* 1994). Among fungi, Basidiomycetes are of interest as they are able to grow on lignocellulosic substrates and to exploit all wood components due to secretion of cellulases, xylanases and laccases. White rot fungi are of interest because they are one of the few groups of microorganisms that can selectively degrade lignin (Otjen and Blanchette 1985).

Macroscopically selective delignification was identified by light patches, as lignin degradation occurred preferentially and remains pure cellulose giving light appearance. Morphologically the wood decayed by the selective white rot fungi leaves fibers due to dissolution of middle lamella, rich region made up of lignin and the separation of individual cell elements from their matrix. Thus macroscopically we can assume selective delignification if the decayed wood has the fibrous consistency (Schwarze *et al* 2000).

Eucalyptus wood blocks decayed by *I .lacteus* and *D. confragosa* shows loosening of cells due to the degradation of middle lamella, *I. lacteus* and *D. confragosa* produced a selective delignification of the tissue with mainly the loosening and separation of cells and further concentric delignification of wall layers. Based on the degradation pattern fungal infection is categorized in to white rot, brown rot and soft rot (Blanchette 1991, Eaten and Hale 1993, Schwarze *et al* 1998). The white rot is further classified in to selective rot and simultaneous rot depending upon their degradation pattern. In selective delignification the most remarkable anatomical characteristic is the separation of cells by dissolution of middle lamella and in the simultaneous rot formation of erosion troughs, bore holes etc considered as major

characteristics. In the present study *I. lacteus* produced no boreholes, according to our findings in *Eucalyptus* blocks as clearly manifested by cell separation, a feature considered as the best indicator of the selective type of decay as agreed by (Anagnost 1998).

Degradation of middle lamella occurs in conjunction with extensive lignin degradation in the secondary wall. At the late stage, individual cells become separated from their matrix (Harting 1878, Blanchete 1984). Mechanism behind this kind of degradation is explained by Schwarze *et al* (2000). Hyphae penetrated in the lumen of the cell produce enzymes which in early stage of decay diffuse into secondary wall, after that diffusion extends to middle lamella and so preferential lignin degradation leads to the separation of individual cells. In present study the wood of *E. globulus,* infected with both (*I. lacteus* and *D. confragosa*) selected fungus showed the same results.

Buckling of the various cells is considered to be another important feature observed in the wood elements attacked by the selective white rot fungi. The buckling of the cell walls is based on the fact that after cell separation, the cellulose rich secondary walls can still be heavily stressed under tensile loading but easily fail under compression load (Schwarze 1995). In the present study buckling of vessel element in *Eucalyptus* wood infected by both the fungi (*I. lacteus* and *D. confragosa*) were noticed.

Pattern of degradation by *D. confragosa* distinctly differed from *I. lacteus* as in the later stage it produced features similar to simultaneous degraders. Formation of boreholes which is considered to be one of the main characteristic features of simultaneous rot (Liese 1970). However, in the initial stages loosening of cells due to middle lamella degradation prominently observed can be considered as the main

feature of selective rot. Presence of boreholes in the parenchyma tissues and medullary rays only not in the fiber cells which is actually beneficial for the process of pulping. In the later stage (in 12 months) only fiber cells showing presence of boreholes indicating *D. confragosa* to be selective rot fungi at initial stage and later on selectivity turns to simultaneous rot indicating *D. confragosa* showed dual pattern of degradation i.e. selective delignification in the initial stage (till the 6 months of incubation) followed by simultaneous rot during advanced stage (12 months of incubation) of decay.

It is important to note that when extensive selective delignification occurred in the wood, cellulose degradation take place in the later stages of the decay. As a result of this besides the initial change in the stiffness and compression strength, latter the tensile strength of the wood is severely modified (Pratt 1979, Schwarze 1995).

I. lacteus when co-cultured with *D. confragosa* produced anatomically no specific pattern of degradation both anatomically signs of lignin degraders and simultaneous degraders were observed. Chemical analysis indicated a significant increase in the degradation. Synergistic effect on wood degradation was noted when *I .lacteus* was co-cultured with *D. confragosa*.

During the maturation of woody cells, middle lamella with all the layers of wall impregnated with lignin. Lignification is especially pronounced in compound middle lamella i.e, between the rounded corners of fibers (Fergus and Goring 1970 a, b). In *D.confragrosa* degradation commenced in the corners of fibers along with middle lamella and in *I. lacteus* pretreated wood blocks middle lamella degrades and fiber cells get separated so loosening of parenchyma cells takes place and hyphae of both the fungi penetrates the cell wall and delignify the middle lamella. All of the these changes indicates clear degradation of lignin and cellulose was left relatively

during this kind of selective delignification (Schwarze *et al* 1995, 2004) which is most important for the paper making process. The lignin degradation occurred by these fungi in the fiber cells and middle lamella regions indicates the potential use of fungi in the bio pulping process.

Initially the separation of the cells due to dissolution of middle lamella, accentuation of secondary cell wall layer and buckling of the vessel walls were the characteristic feature of both the fungal isolates, but in the advanced stage of decay, bore holes in fiber cells were also observed in *D. confragosa*. At the advanced stage, formation of bore holes in *Eucalyptus* wood infected with *D. confragosa* is a character of the simultaneous rot (Anagnost 1998). This agrees with Anagnost (1998) who mentioned that selective white rot fungi can produce anatomical features similar to simultaneous white rot fungi in advanced stage of decay.

Koyani *et al* (2011) evaluated degradation pattern in *Azadirachta indica* wood infected with *Chrysosporium asperatum* and *Trichoderma harzianum*. In their study also dual pattern of degradation with both the fungal isolates were observed that is selective delignification in the initial stage and simultaneous rot during later stages of decay which resembled with the present study of decay with *D. confragosa*.

Koyani *et al* (2014) analysed wood blocks of *Tectona grandis* infected with *Irpex lacteus* and *Phanerochaete chrysosporium* and concluded that both the fungi showed selective delignification pattern identified by separation of xylem cells. Similar pattern was noticed in the present study where *I. lacteus* showed separation of xylem cells.

It was observed that in the early stage of decay fungal hyphae from vessel lumen traversed in the ray parenchyma cells associated with vessel elements for further distribution or invasion. Similar observation was also reported by other several

authors (Rayner and Boddy 1988, Schwarze 2007, Scharwaze *et al* 2004). Luna *et al* (2004) reported presence of fungal hyphae in various cells of populus wood infected with *Pycnoporus sanguineus* and *Ganoderma lucidum.* A similar observation was noticed in *Eucalyptus* wood infected with *I. lacteus* and *D. confragosa*. Both the fungal isolates having higher potential of lignin degradation with lesser amount of cellulose loss proved to be utilized for the process of Bio pulping in the manufacturing of paper.

Lignin is an important constituent of secondary wall (mainly xylem) in the plants found to be highly branched polymer made up of phenyl propanoid units. Lignin is considered as the second most abundant polymer next to cellulose (Boerjan *et al* 2003). Lignin is mainly associated in providing mechanical support to the plant, acts as a defense against microorganisms and even helps plants to stand in erect position (Wainhouse *et al* 1990, Boudet 2000, Lagaert *et al* 2009).

It was most commonly noticed that secondary xylem of plant is invaded by many microorganisms like fungi and bacteria. Now a day white rot fungi are of main interest as they can selectively degrade lignin without looses of cellulose (Otjen and Blanchette 1985). Thus white rot fungi are gaining more and importance in the process of biopulping and even in the process of bioremediation.

Hwang *et al* (2008) observed 38.0% weight loss in the *Pinus strobus* incubated with the same fungal isolate *I. lacteus* within 3 months incubation period, which was higher than that of the weight loss observed in the present study.

Koyani *et al* (2014) observed 15.06% weight loss in the *Tectona grandis* infected with *I. lacteus* within 3 months of incubation time which was lower than the weight loss observed in *E. globulus* infected with *I. lacteus* in present study within 3 months of time.

Ferraz *et al* (2003) reported 11.7% weight loss in the *Eucalyptus grandis* wood infected with the white rot fungi *Ceriporiopsis subvermispora* in the 3 months of incubation period which is less than what was observed in the wood blocks of *E. globulus* infected with *I. lacteus* in the present study within same incubation period of 3 months. Fernandes *et al* (2005) also showed 1.56-5.30% weight loss in the wood chips of *E. grandis* infected with *Phellinus flavomarginatus* within 2 months of incubation period.

Luna *et a*l (2004) reported about 50–60 % weight loss of poplar wood within 2–5 months by *Pycnoporus sanguineus* and *Gonoderma lucidum.* Therefore, it appears that weight loss differs from fungal species to species or within species and natural durability of the wood.

In the study of *E. globulus* wood degraded by *Grammothele subargentea* for 30 days 13.5% lignin loss was noted by Saparrat *et al* 2008 but in the present study within 45 days of incubation both the fungi in mono and co culture showed double amount of lignin loss, 27.5 % by IL 25.55% by DC and 30.83% by IL+DC in which it is clearly observed that the loss of lignin was also higher in the co culture of fungi.

Selectivity indices was considered as a common measurement of delignification efficiency and calculated as lignin loss/cellulose loss ratio (Hatakka 1983). Higher selectivity indices indicate better efficiency for preferential lignin degraders and lower selectivity value indicates higher cellulose loss during the pretreatment with fungi (Camarero *et al* 1994). As shown in Table 1 mono culture of IL showed 2.47, 3.06 and 1.49 selectivity value after 45 days, 3 months and 12 months of incubation period respectively where as mono culture of DC showed 1.46, 1.65 and 1.57 selectivity value after 45 days, 3 months and 12 months of incubation period respectively. Co culture of IL and DC indicated 1.29, 1.29, 1.40 and 1.79

selectivity values after four various incubation periods 45 days, 3 months, 6 months and 12 months respectively. IL showed better selectivity (3.06) than DC (1.65) and Mixed culture (1.79). As there was a significant increase in the selectivity value of wood pretreated with IL and DC up to 3 months, the results indicated both fungi to be high lignin degraders. The selectivity value decreased in mono cultures after 6 months of incubation period indicating both the fungi to degrade lignin preferentially in the early stages of incubation period and at a later stage being non selective lignin degraders as they also started to degraded cellulose fibers also. Selectivity value increased in co culture as the incubation time increased up to 12 months indicating that co culture had a great capacity to degrade lignin preferentially and proved it as efficient lignin degrader with lower indices as it was not converted to non selectivity even if the incubation time increased up to 12 months.

In the study of Nazarpour *et al* (2009) the effect of pretreatment of rubber wood with white rot fungi and its co culture namely *Ceriporiopsis subvermispora*, *Trametes versicolor*, and a mixed culture of *C. subvermispora* and *T. versicolor* was evaluated. The results indicated there was a gradual increase in the selectivity value observed from 30 days to 90 days in *C. subvermispora* and considered as an efficient lignin degrader with selectivity value 4.75 which was the highest value after 90 days of incubation compared to *T. versicolor* and co culture of *C. subvermispora* and *T. versicolor*. In *T. versicolor* and co culture of *C. subvermispora* and *T. versicolor* selectivity value decreased as the time of incubation increased which showed selective delignification in early stage only and their selectivity tends to turn in to non selectivity. In the present study co culture showed selective delignification up to 12months of incubation period where as in study of Nazarpour *et al* 2009 selective delignification was noted in the early stages only which then turned in to non

selectivity which was opposite to result of present study. The difference in the results of co culture might be due to the difference in the wood and the fungal isolates by which the pretreatment was carried out. It also indicates the lignin degradation was depended on the species of wood and the fungal species also.

The hemicellulose was found to be degraded more in the initial stages of decay in case of *I. lacteus* monoculture. Maximum hemicelluloses loss was obtained in IL within three months of incubation period and in the co culture it was obtained in the twelve months of incubation time. While cellulose loss was occurred at a slower rate indicating that the cellulose remained protected from the bio degradation during the whole wood decay experiment. hemicellulose was degraded most quickly, which may be due to its structural characteristic as hemicellulose consists of heterogeneous polymers of pentoses, hexoses and sugar acids which does not form tightly packed crystalline structures like cellulose and it is also more accessible to enzymatic hydrolysis.

The increase in the loss of hemicellulose content may be due to the reason that all the reducing sugars produced by the degradation was not converted to CO_2 and H_2O. Xu *et al* (2009) reported the similar observations as in the present study where maximum hemicelluloses loss was noted in the initial days in which corn stover degraded by *Irpex lacteus* CD2 showed 63.0% loss of hemi cellulose within 15 days of incubation only while in our study 29.22% was observed in IL within 45 days only which is quite less than the observed in the degradation of corn-stover while co culture showed 39.21% loss of hemicellulose after 12 months of incubation period.

Apparently, weight loss observed was lower as compared to total component loss in the initial stages which was due to differences in the methods of component determination because the component losses were measured as the true losses of

polymetric fractions of lignocellulose and the soluble fractions were removed during determination and it is not necessary that all the carbohydrates degraded must be converted to carbon dioxide and water (Xu *et al* 2009).

The chemical composition of decayed wood supports the view that energy for the fungus, irrespective whether paired or not, is mainly achieved through the degradation of hemicellulose. This structural softening of the wood due to decay, and grinding the wood before analyses, further exposed the cellulose fibres for removal of easily extractable glucose but the obtained results strongly indicate that the fungi leave cellulose intact and utilize hemicellulose as the primary energy and carbon source. These features in wood degradation are important in biopulping. To improve the efficiency of biodegradation the use of co cultures or mixed cultures of lignocellulolytic microorganisms could be considered as one of the current approaches.

Many of these cocultures or mixed cultures were reportedly more efficient in lignocellulolytic biodegradation because of producing high activity enzymes due to their synergistic action (Saxena and Vohra 2001, Bajpai *et al* 2001). Hence, the result of wood decay test directed towards identifying the best synergistic coculture which could successfully colonize and degrade the *E. globulus* wood without much loss of cellulose fibers and proved to be beneficial in the paper and pulp industry.

3.4 ENHANCEMENT OF ENZYME ACTIVITY

3.4.1 Enhancement experiment using chemical enhancers

Experiments were conducted to evaluate the enhancement in enzyme activities by supplementing the medium with well known chemical enhancers and effect of different conditions like pH, temperature and incubation period on the enzyme activity. From the results of earlier experiments to obtain maximum enzyme activities was 25 days, 30 days and 20 days for monocultures of *D. confragosa*, *I. lacteus* and coculture of *I. lacteus* +*D. confragosa* respectively. Maximum enzyme activity was noted at pH 5 and temperature 25˚C so these were conditions selected for the further experiments. A comparison of the enzyme activities in monocultures and cocultures were assessed after an incubation period of 20 days, as the coculture showed maximum enzyme activities on the 20^{th} day of incubation period. Also incubation period at which monocultures showed maximum enzyme activities (i.e. 25 days in *D. confragosa* and 30 days in *I. lacteus*) were compared with the activities obtained maximum in coculture incubation period (i.e. 20days). In order to select the best inducer concentration of different chemical enhancers and its effect on the lignocellulolytic enzyme production 3% MEB medium was supplemented with the different chemicals. Literature survey revealed that there is a wide range of chemicals which are known to enhance the enzyme activity. Five different known enhancers have been evaluated for its potential to enhance lignocellulolytic and xylanolytic activity in the studied mono and coculture. The enhancers we used are (Ethanol, Veratryl alcohol, Xylidine, Yeast extract, Peptone) in varying concentrations and media were autoclaved and inoculated with 9 mm disc of 10 days old culture. After the completion of desired incubation period crude enzyme culture filtrates were collected and assays were carried out.

(a) Influence of Ethanol (ETH) on enzyme production

To determine the effect of different concentration of ethanol on various lignocellulolytic enzyme production 3% MEB media was supplemented with (1%, 2%, 3%, 4% and 5%) concentrations of ethanol after autoclaving the media. Control was also kept in which ethanol was not added.

All the concentration of ethanol supported the growth of fungi in mono culture and co culture. The influence of ethanol on the enzyme production is represented in Table 1. In mono culture *I. lacteus* showed an absence of LiP activity and rest of lignocellulolytic enzymes were enhanced in 4% concentration of ethanol except laccase which showed optimum enhancement in 1% ethanol where as AAO activity was not enhanced. The growth of *D. confragosa* in media supplemented with 1% ethanol accompanied by significant increase in all the enzymes. In co culture all the enzymes were enhanced in 4% ethanol except laccase which enhanced in 1% ethanol and as similar to *I. lacteus* co culture showed no stimulation in the activity of AAO.

LiP activity was found to be absent in *I. lacteus* where as *D. confragosa* showed the maximum activity in 1% ethanol (4.35 EU/L/Min) after that LiP activity was hampered up to 5% concentration. In co culture of *I. lacteus* and *D. confragosa* highest activity of LiP was noticed in 4% ethanol concentration 4.52 U/L (Figure 1 A).

MnP activity obtained in the *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* showed gradual increase from 1% to 5% concentration of ethanol as compared to control. Maximum activity of MnP was obtained in the concentration of 4% 1.71 EU/L/Min in *I. lacteus* and 2.95 EU/L/Min in *I. lacteus* and *D. confragosa* co culture. In case of *D. confragosa* MnP activity enhanced from 1% up to 3% ethanol and the

highest was recorded in 1% ethanol 2.89 EU/L/Min after which clear decline was noted but up to 3% it is high compared to control (Figure 1 B).

The best result for enhanced laccase activity induction in *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* was obtained 2.61 EU/L/Min and 6.84 EU/L/Min respectively with 1% ethanol after which laccase activity seems to be declined. In *D. confragosa* no enhancement of laccase activity was observed indicating its inhibition with ethanol (Figure 1 C).

In AAO activity 1% ethanol provided maximum results in *D. confragosa* (28.27 EU/L/Min) where as *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* showed inhibition of AAO activity which is not enhanced in media supplemented with ethanol as inducer (Figure 1 D).

Enzymes	Fungal isolate	Control	Concentrations of Ethanol				
			1%	2%	3%	4%	5%
LiP EU/L/Min	IL	0	0	0	0	0	0
	DC	2.47±0.03	**4.35±0.09**	3.39±0.06	3.07±0.06	2.13±0.04	1.76±0.06
	IL+DC	2.74±0.096	2.78±0.04	2.82±0.04	3.01±0.06	**4.52±0.06**	2.89±0.06
MnP EU/L/Min	IL	0.75±0.06	0.94±0.06	1.25±0.06	1.51±0.06	**1.71±0.09**	1.19±0.06
	DC	1.75±0.06	**2.89±0.06**	2.00±0.06	1.88±0.06	1.06±0.06	0.96±0.04
	IL+DC	1.97±0.09	2.07±0.06	2.34±0.09	2.44±0.06	**2.95±0.06**	2.13±0.06
Laccase EU/L/Min	IL	1.94±0.012	**2.61±0.04**	2.08±0.02	1.92±0.04	1.50±0.03	1.22±0.03
	DC	1.07±0.017	1.00±0.04	0.88±0.02	0.87±0.02	0.79±0.03	0.74±0.02
	IL+DC	5.45±0.030	**6.84±0.03**	6.72±0.02	6.34±0.02	6.23±0.02	6.17±0.03
AAO EU/L/Min	IL	44.12±0.13	43.62±0.04	37.65±0.06	29.79±0.07	21.99±0.06	20.40±0.09
	DC	24.42±0.11	**28.27±0.06**	24.61±0.06	23.71±0.05	20.69±0.05	16.28±0.06
	IL+DC	49.12±0.12	48.95±0.05	41.37±0.04	40.43±0.08	39.12±0.07	38.35±0.06
Xylanase EU/ml/Min	IL	5.26±0.49	5.65±0.13	8.56±0.12	8.76±0.17	**10.62±0.12**	5.57±0.10
	DC	12.83±0.90	**14.76±0.11**	14.39±0.09	14.15±0.14	13.27±0.10	12.86±0.10
	IL+DC	22.68±0.39	23.26±0.12	24.19±0.13	24.61±0.10	**25.09±0.12**	19.65±0.11
Cellulase EU/ml/Min	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table: 1 Effect of Ethanol concentration on the lignocellulolytic enzyme activity

Xylanase activity was maximum enhanced when *D. confragosa* was inoculated in 1% ethanol containing MEB media (14.76 EU/ml/Min). Concentration of 4% ethanol leads to the highest xylanase activity in *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* 10.62 EU/ml/Min and 25.09 EU/ml/Min respectively (Figure 1 E)

Cellulase activity was absent in the control as well as in the media provided with the different concentration of ethanol (Figure 1 F).

Enzyme	Fungal isolate	Control	Concentration of Ethanol				
			1%	2%	3%	4%	5%
LiP	IL	0	0	0	0	0	0
	DC	1.76±0.06	**3.28±0.09**	2.11±0.09	1.88±0.06	1.57±0.06	1.06±0.06
	IL+DC	2.74±0.096	2.78±0.04	2.82±0.04	3.01±0.06	**4.52±0.06**	2.89±0.06
MnP	IL	0.50±0.03	0.71±0.04	0.94±0.06	1.19±0.06	**1.52±0.09**	0.94±0.06
	DC	1.00±0.06	**2.19±0.06**	1.76±0.06	1.13±0.06	0.94±0.06	0.81±0.04
	IL+DC	1.97±0.09	2.07±0.06	2.34±0.09	2.44±0.06	**2.95±0.06**	2.13±0.06
Lac	IL	1.26±0.010	**1.90±0.06**	1.51±0.05	1.18±0.08	0.97±0.05	0.75±0.08
	DC	0.99±0.019	0.89±0.04	0.81±0.05	0.77±0.05	0.66±0.07	0.55±0.05
	IL+DC	5.45±0.030	**6.84±0.03**	6.72±0.02	6.34±0.02	6.23±0.02	6.17±0.03
AAO	IL	32.42±0.15	30.48±0.04	24.72±0.06	20.35±0.05	18.94±0.06	16.83±0.07
	DC	22.53±0.10	**25.98±0.06**	22.88±0.06	20.84±0.05	17.96±0.05	13.92±0.06
	IL+DC	49.12±0.12	48.95±0.05	41.37±0.04	40.43±0.08	39.12±0.07	38.35±0.06
Xyl	IL	18.11±1.03	18.76±0.09	19.58±0.13	19.87±0.13	**20.10±0.11**	17.29±0.10
	DC	13.86±1.32	**15.45±0.09**	15.24±0.11	14.74±0.10	14.62±0.12	14.17±0.11
	IL+DC	22.68±0.39	23.26±0.12	24.19±0.13	24.61±0.10	**25.09±0.12**	19.65±0.11
Cell	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 2 Effect of Ethanol concentration on the lignocellulolytic enzyme activity after 20 days incubation period

D. confragosa showed double fold increase in LiP and MnP activity with 1% ethanol supplementation but enhancement of AAO and xylanase was not very significantly high. Laccase activity was inhibited with supplementation with ethanol. A double fold increase in LiP and MnP activity of coculture supplemented with 4% ethanol is observed. More than double fold increase in xylanase activity was also noted in monoculture of *I. lacteus* supplemented with 4% ethanol but in *D. confragosa* not very significant increase was noted.

Results in Table 2 showed effect of various ethanol concentrations on lignocellulolytic enzyme activities when both monocultures and coculture were incubated for 20 days. Up on comparison of results between monocultures and coculture it was noticed that in coculture LiP and MnP activity was enhanced nearly 1.5 fold, Laccase activity also enhanced very significantly (nearly threefold) and single fold increase in xylanase activity indicating very significant enhancement has occurred in coculture however AAO enzyme activity was not enhanced. LiP in *I. lacteus* was found to be absent and cellulase activity was also not stimulated even after addition of various concentrations of ethanol in the medium.

(b) Influence of Veratryl alcohol (VA) on enzyme production

To determine the effect of different concentration of veratryl alcohol on various lignocellulolytic enzyme production 3% MEB media was supplemented with (4mM, 8mM, 12mM, 16mM and 20mM) concentration of veratryl alcohol and autoclaved. Media with absence of veratryl alcohol was kept as control.

The results obtained here demonstrated a clear and significant enhancement in lignocellulolytic enzyme activity up to 16mM concentration of veratryl alcohol after which decline in the enzyme activity was noted from 20mM concentration (Table 3).

All the enzyme activity in monoculture and coculture supplemented with 4mM to 12mM veratryl alcohol showed maximum activity in 12mM and 16mM and with a further decline in enzyme activity with 20mM was observed. However it was also noticed that medium supplemented with 20mM veratryl alcohol showed LiP and xylanase activity more than the control which was not supplemented with veratryl alcohol in both monoculture and coculture. Cellulase activity was completely absent in the control as well as in monoculture and cocultures supplemented with veratryl alcohol. Results also indicated that coculture significantly showed pronounced enzyme activities compared to monocultures.

LiP activity was found to be absent in *I. lacteus* supplemented with different concentration of veratryl alcohol. Cultures of *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* showed 4.39 EU/L/Min and 4.65 EU/L/Min LiP activity respectively in 12mM concentration. A decline in the LiP enzyme activity was noticed from 20mM but the activity was higher than that observed in the control (Figure 2 A).

MnP activity gradually increased from 4mM to 12mM concentration. But concentration of veratryl alcohol higher than 12mM leads to a decline in the MnP activity. The maximum enhancement of MnP enzyme activity was noticed in 12 mM in which *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* produced 1.44, 2. 51, 2.70 EU/L/Min MnP activity respectively. MnP activity in 16 mM observed was still higher than the control. In 20mM MnP activity further declined appearing to be inhibited. (Figure 2 B).

Evaluation of laccase activity showed veratryl alcohol responsible to promote the activity from 4mM to 16mM and in 20 mM concentration it was suppressed. The highest activity of laccase in *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and

D. confragosa were 2.24, 1.23 and 6.76 EU/L/Min respectively in 16mM concentration while at higher concentration it declined. (Figure 2 C).

Activity of AAO enhanced from 4mM to 16 mM veratryl alcohol concentration in case of mono culture and after that activity of AAO seems to be declined in 20mM. In *I. lacteus* and *D. confragosa* highest AAO activity obtained were 61.50 U/L and 29.66 EU/L/Min in 12mM and 16mM respectively. In co culture of *I. lacteus* and *D. confragosa* showed 62.67 EU/L/Min in 12mM veratryl alcohol which declined in 16mM and 20mM concentration. (Figure 2 D).

Enzyme	Fungal isolate	Control	Concentration of Veratryl Alcohol				
			4mM	8mM	12mM	16mM	20mM
LiP EU/L/Min	IL	0	0	0	0	0	0
	DC	2.47±0.03	3.64±0.09	4.14±0.06	**4.39±0.06**	3.52±0.06	3.26±0.06
	IL+DC	2.74±0.096	3.99±0.06	4.20±0.07	**4.65±0.06**	4.08±0.06	3.80±0.06
MnP EU/L/Min	IL	0.75±0.06	1.04±0.06	1.13±0.06	**1.44±0.06**	1.25±0.06	1.00±0.05
	DC	1.75±0.06	1.95±0.06	2.13±0.06	**2.51±0.06**	1.84±0.06	1.51±0.06
	IL+DC	1.97±0.09	2.07±0.06	2.32±0.06	**2.70±0.06**	2.09±0.06	1.88±0.06
Laccase EU/L/Min	IL	1.94±0.012	1.98±0.02	2.03±0.02	2.13±0.02	**2.24±0.03**	1.82±0.03
	DC	1.07±0.017	1.09±0.04	1.07±0.03	1.13±0.07	**1.23±0.02**	1.03±0.04
	IL+DC	5.45±0.030	6.23±0.05	6.35±0.02	6.60±0.02	**6.76±0.05**	6.57±0.03
AAO EU/L/Min	IL	44.12±0.13	44.90±0.09	46.30±0.06	**61.50±0.08**	47.12±0.11	30.28±0.08
	DC	24.42±0.11	24.92±0.07	27.30±0.06	28.69±0.10	**29.66±0.07**	29.42±0.05
	IL+DC	49.12±0.12	45.55±0.08	47.05±0.07	**62.67±0.10**	46.27±0.10	39.71±0.07
Xylanase EU/ml/Min	IL	5.26±0.49	6.04±0.10	8.49±0.11	10.56±0.12	**10.82±0.10**	5.85±0.12
	DC	12.83±0.90	13.00±0.09	13.62±0.10	**14.94±0.13**	14.11±0.12	13.03±0.09
	IL+DC	22.68±0.39	29.97±0.10	31.50±0.13	42.26±0.10	**43.14±0.10**	29.54±0.12
Cellulase EU/ml/Min	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 3 Effect of veratryl alcohol concentration on the lignocellulolytic enzyme activity

Xylanase activity was enhanced in all the concentration of veratryl alcohol (4mM to 20mM) but the most suitable concentration for *I. Lacteus* and co culture of *I. Lacteus* and *D. confragosa* was 16mM in which the activity found to be 10.82 and 43.14

EU/ml/Min respectively which indicates almost four fold enhancement in the coculture. In *D. confragosa* the highest AAO activity obtained was 14.94 EU/ml/Min in 12mM concentration. After 12mM concentration xylanase activity seems to be declined up to 20 mM but still it was higher as compared to control (Figure 2 E).

Cellulase activity was found to be absent in control as well as in all the concentration of veratryl alcohol tested for enhancement (Figure 2 F).

Enzyme	Fungal isolate	Control	Concentrations of Veratryl Alcohol				
			4mM	8mM	12mM	16mM	20mM
LiP	IL	0	0	0	0	0	0
	DC	1.76±0.06	2.82±0.06	3.07±0.06	**3.32±0.06**	2.88±0.06	2.34±0.09
	IL+DC	2.74±0.096	3.99±0.06	4.20±0.07	**4.65±0.06**	4.08±0.06	3.80±0.06
MnP	IL	0.50±0.03	0.84±0.09	0.94±0.06	**1.21±0.09**	0.95±0.06	0.76±0.05
	DC	1.00±0.06	1.24±0.08	1.67±0.09	**2.07±0.06**	0.84±0.09	0.56±0.06
	IL+DC	1.97±0.09	2.07±0.06	2.32±0.06	**2.70±0.06**	2.09±0.06	1.88±0.06
Lac	IL	1.26±0.010	1.28±0.05	1.38±0.05	1.50±0.06	**1.54±0.07**	1.17±0.06
	DC	0.99±0.019	1.01±0.04	1.04±0.03	1.05±0.04	**1.08±0.04**	0.93±0.04
	IL+DC	5.45±0.030	6.23±0.05	6.35±0.02	6.60±0.02	**6.76±0.05**	6.57±0.03
AAO	IL	32.42±0.15	33.17±0.07	35.54±0.08	**49.16±0.05**	41.20±0.07	19.50±0.07
	DC	22.53±0.10	22.83±0.07	23.85±0.06	24.77±0.06	**27.32±0.05**	27.13±0.06
	IL+DC	49.12±0.12	45.55±0.08	47.05±0.07	**62.67±0.10**	46.27±0.10	39.71±0.07
Xyl	IL	18.11±1.03	19.20±0.09	19.49±0.11	20.29±0.12	**20.32±0.10**	19.07±0.10
	DC	13.86±1.32	14.12±0.12	15.1±0.11	**15.88±0.13**	15.62±0.14	13.89±0.11
	IL+DC	22.68±0.39	29.97±0.10	31.50±0.13	42.26±0.10	**43.14±0.10**	29.54±0.12
Cell	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 4 Effect of veratryl alcohol concentration on the lignocellulolytic enzyme activity after 20 days of incubation period

Coculture of *I. Lacteus* and *D. confragosa* showed maximum enhancement in all the enzyme activities except laccase and xylanase when supplemented with 12mM

veratryl alcohol compared to monocultures. A significant almost double fold increase in the activity has been noted compared to the control.

When monocultures and coculture both were incubated for 20 days it was noticed that in coculture significantly double fold increase in MnP activity, 4.5 fold increase in Laccase activity, 1.2 fold increase in AAO and 2.7 fold increase in xylanase enzyme activity compared to *I. lacteus* as indicated in Table 4. As compared to Monoculture of *D. confragosa* 1.5 fold enhancement in LiP and MnP activity, two fold in AAO and xylanase enzyme activity was observed in coculture where as laccase enzyme activity was found to be enhanced six fold which was very highly significant enhancement in coculture. Cellulase enzyme activity was found to be absent even after addition of various concentrations of veratryl alcohol to the medium.

(c) Influence of Xylidine (XYL) on enzyme production

The effect of different concentration of xylidine on various lignocellulolytic enzyme production was evaluated by supplementing 3% MEB media with (4µM, 7µM, 10µM, 20µM, and 30µM,) concentration of xylidine and after that the media was autoclaved. Medium with absence of veratryl alcohol was considered as control.

All lignocellulolytic enzyme activities were influenced positively when various concentrations of Xylidine was used as an inducer. Enzyme production gradually increased from 4µM to 30µM Xylidine concentration and the highest enzymes were produced in 30µM except laccase enzyme which was not enhanced in case of *D. confragosa* and AAO in *I. lacteus* was enhanced the most in 20µM after that it was declined as compared to control (Table 5).

LiP activity was not reported in *I. lacteus* when the media was supplemented with xylidine as an inducer. In *D. confragosa* and the co culture of *I. lacteus* and *D.*

confragosa LiP activity was gradually increased from 4µM to 30µM and the highest activity measured was 2.82 and 3.70 EU/L/Min respectively (Figure 3 A).

For enhancing MnP activity 20µM and 30µM were the best concentration and its production was maximum in 30µM concentration. In *I. lacteus, D. confragosa* and the co culture of *I. lacteus* and *D. confragosa* showed 1.51, 1.90 and 3.18 EU/L/Min MnP activity in 30 µM concentration respectively (Figure 3 B).

Laccase activity was detected maximum in the 30 µM concentration of xylidine. In *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* MnP activity was found to be 5.51 and 6.90 EU/L/Min respectively (Figure 3 C). In *D. confragosa* laccase activity was not enhanced from 4 µM to 30 µM which clearly indicated that the laccase activity was inhibited in *D. confragosa* when the media contain xylidine as an inducer.

The effect of different concentration of xylidine on AAO activity indicated that in *I. lacteus* the highest AAO activity was found in 20 µM after that the activity was declined in 30µM concentration as compared to control where as in *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* showed maximum AAO activity 47.10 and 51.29 EU/L/Min respectively in 30µM concentration of xylidine (Figure 3 D).

Xylanase enzyme activity significantly increased in *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* from 4µM up to 30µM and the highest activity was obtained 10.79 and 32.82 EU/ml/Min respectively in 30µM concentration showing threefold increase in the coculture where as in case of *D. confragosa* highest xylanase activity appeared in 20 µM xylidine after which a decline occurred in 30µM (Figure 3 E).

Cellulase activity was found to be absent when xylidine was provided as an inducer (Figure 3 F).

Coculture of *I. lacteus* and *D. confragosa* showed maximum enzyme activities with 30µM xylidine supplemented to the medium. Increase in the activity is about 1to 2 fold except AAO which does not show any significant increase. Monoculture of *D. confragosa* shows a double fold increase in AAO activity when supplemented with 30µM xylidine. However it was still lower than the coculture supplemented with the same concentration of xylidine.

Enzyme	Fungal isolate	Control	Concentrations of Xylidine				
			4µM	7µM	10µM	20µM	30µM
LiP EU/L/Min	IL	0	0	0	0	0	0
	DC	2.47±0.03	2.51±0.06	2.58±0.06	2.61±0.09	2.72±0.06	**2.82±0.06**
	IL+DC	2.74±0.096	2.86±0.04	2.99±0.09	3.18±0.06	3.37±0.06	**3.70±0.06**
MnP EU/L/Min	IL	0.75±0.06	1.07±0.06	1.10±0.09	1.26±0.05	1.39±0.07	**1.51±0.06**
	DC	1.75±0.06	1.26±0.06	1.31±0.06	1.40±0.04	1.69±0.06	**1.90±0.09**
	IL+DC	1.97±0.09	2.38±0.06	2.45±0.06	2.63±0.06	2.95±0.06	**3.18±0.09**
Laccase EU/L/Min	IL	1.94±0.012	1.81±0.02	1.88±0.02	2.01±0.02	2.65±0.04	**5.51±0.04**
	DC	1.07±0.017	0.78±0.02	0.75±0.02	0.75±0.03	0.74±0.02	0.74±0.02
	IL+DC	5.45±0.030	6.41±0.02	6.57±0.07	6.62±0.02	6.79±0.02	**6.90±0.02**
AAO EU/L/Min	IL	44.12±0.13	42.33±0.04	41.86±0.07	41.64±0.06	41.30±0.06	40.37±0.07
	DC	24.42±0.11	18.96±0.07	19.45±0.06	24.17±0.05	24.56±0.06	**47.10±0.05**
	IL+DC	49.12±0.12	45.11±0.08	45.75±0.08	46.20±0.08	50.51±0.04	**51.29±0.08**
Xylanase EU/ml/Min	IL	5.26±0.49	5.79±0.12	6.11±0.10	8.52±0.09	10.31±0.11	**10.79±0.10**
	DC	12.83±0.90	14.31±0.12	16.30±0.12	18.06±0.11	**19.16±0.11**	15.88±0.10
	IL+DC	22.68±0.39	26.40±0.10	27.10±0.10	27.97±0.09	29.54±0.13	**32.82±0.11**
Cellulase EU/ml/Min	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 5 Effect of Xylidine concentration on the lignocellulolytic enzyme activity

Lignocellulolytic enzyme activity of monoculture and coculture after 20 days of incubation period was represented in Table 6. The results indicated that LiP activity

was found to be absent in monoculture of *I. lacteus* but coculture showed 1.72 fold enhancement in LiP activity compared to monoculture of *D. confragosa*.

Similarly laccase activity in *D. confragosa* was not enhanced but in coculture nearly two fold higher laccase activity was noticed compared to *I. lacteus* monoculture which was very significant enhancement. In coculture 2.5 fold enhancement in MnP activity, 1.5 fold in AAO enzyme activity and 1.6 fold in xylanase activity noticed in coculture compared to both the monocultures showed enhancement was very significant.

Enzyme	Fungal isolate	Control	Concentrations of Xylidine				
			4µM	7µM	10µM	20µM	30µM
LiP	IL	0	0	0	0	0	0
	DC	1.76±0.06	1.82±0.06	1.94±0.06	2.00±0.06	2.07±0.06	**2.15±0.06**
	IL+DC	2.74±0.096	2.86±0.04	2.99±0.09	3.18±0.06	3.37±0.06	**3.70±0.06**
MnP	IL	0.50±0.03	0.79±0.06	1.04±0.07	1.14±0.06	1.22±0.08	**1.28±0.09**
	DC	1.00±0.06	0.74±0.06	0.83±0.09	0.90±0.09	0.94±0.06	**1.23±0.09**
	IL+DC	1.97±0.09	2.38±0.06	2.45±0.06	2.63±0.06	2.95±0.06	**3.18±0.09**
Lac	IL	1.26±0.010	1.15±0.06	1.46±0.07	1.81±0.06	1.99±0.04	**3.85±0.07**
	DC	0.99±0.019	0.73±0.04	0.73±0.03	0.70±0.03	0.68±0.04	0.67±0.04
	IL+DC	5.45±0.030	6.41±0.02	6.57±0.07	6.62±0.02	6.79±0.02	**6.90±0.02**
AAO	IL	32.42±0.15	29.45±0.05	28.93±0.07	28.74±0.05	**28.30±0.06**	27.47±0.05
	DC	22.53±0.10	16.74±0.07	18.29±0.06	19.10±0.05	22.97±0.06	**43.90±0.05**
	IL+DC	49.12±0.12	45.11±0.08	45.75±0.08	46.20±0.08	50.51±0.04	**51.29±0.08**
Xyl	IL	18.11±1.03	18.77±0.10	18.95±0.09	19.00±0.12	20.06±0.11	**20.29±0.11**
	DC	13.86±1.32	15.02±0.09	16.74±0.12	18.48±0.10	**20.11±0.13**	16.60±0.09
	IL+DC	22.68±0.39	26.40±0.10	27.10±0.10	27.97±0.09	29.54±0.13	**32.82±0.11**
Cell	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 6 Effect of Xylidine concentration on the lignocellulolytic enzyme activity after 20 days of incubation period

(d)Influence of Yeast extract (YE) on enzyme production

Effect of different concentration of yeast extract on lignocellulolytic enzymes were evaluated by providing various concentrations of yeast extract (0.1% g/L, 0.2% g/L, 0.4% g/L, 0.6% g/L and 0.8% g/L) in the 3% MEB medium. A control without yeast extract was also kept for each set of experiment. After completion of desired incubation time the culture filtrates were collected and enzyme assays were carried out.

Enzyme	Fungal isolate	Control	Concentrations of Yeast extract				
			0.1g/L	0.2g/L	0.4g/L	0.6g/L	0.8g/L
LiP EU/L/Min	IL	0	0	0	0	0	0
	DC	2.47±0.03	2.55±0.04	2.63±0.09	2.71±0.06	**3.25±0.06**	2.26±0.06
	IL+DC	2.74±0.096	2.95±0.06	3.07±0.06	3.26±0.06	**3.64±0.06**	2.95±0.06
MnP EU/L/Min	IL	0.75±0.06	0.94±0.06	0.99±0.04	1.25±0.06	**1.57±0.06**	1.38±0.06
	DC	1.75±0.06	1.34±0.04	1.39±0.06	1.46±0.06	**2.08±0.06**	1.32±0.06
	IL+DC	1.97±0.09	2.13±0.06	2.38±0.06	2.64±0.07	**2.91±0.09**	2.43±0.05
Laccase EU/L/Min	IL	1.94±0.012	1.46±0.02	1.65±0.02	1.69±0.02	**2.10±0.02**	2.02±0.02
	DC	1.07±0.017	0.59±0.07	0.62±0.05	0.64±0.07	0.70±0.07	0.64±0.02
	IL+DC	5.45±0.030	5.79±0.02	6.60±0.02	6.65±0.02	**6.73±0.02**	6.62±0.07
AAO EU/L/Min	IL	44.12±0.13	43.40±0.07	42.02±0.08	39.66±0.11	39.27±0.06	38.56±0.09
	DC	24.42±0.11	22.63±0.06	23.96±0.05	24.89±0.05	**27.69±0.05**	25.61±0.06
	IL+DC	49.12±0.12	48.39±0.09	47.97±0.06	46.80±0.04	46.67±0.05	45.98±0.08
Xylanase EU/ml/Min	IL	5.26±0.49	6.42±0.10	7.86±0.12	10.09±0.13	**10.87±0.11**	9.15±0.09
	DC	12.83±0.90	13.56±0.09	15.96±0.11	17.74±0.11	**18.98±0.10**	15.66±0.10
	IL+DC	22.68±0.39	26.40±0.11	26.85±0.10	27.98±0.10	**29.57±0.11**	27.70±0.09
Cellulase EU/ml/Min	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 7 Effect of Yeast extract concentration on the lignocellulolytic enzyme activity

Comparison of results in mono cultures and co culture clearly indicated that all the lignocellulolytic enzyme activity were significantly enhanced as the concentration of yeast extract increased and 0.6g/L proved to be the best concentration to obtain

highest enzyme production except AAO which was not enhanced in case of *I. lacteus* and co culture of *I. lacteus* and *D. confragosa.* The results are represented in Table 7.

Yeast extract did not have any effect on the LiP activity in *I. lacteus* as it was found to be absent even when the medium was supplemented with yeast extract as an inducer.

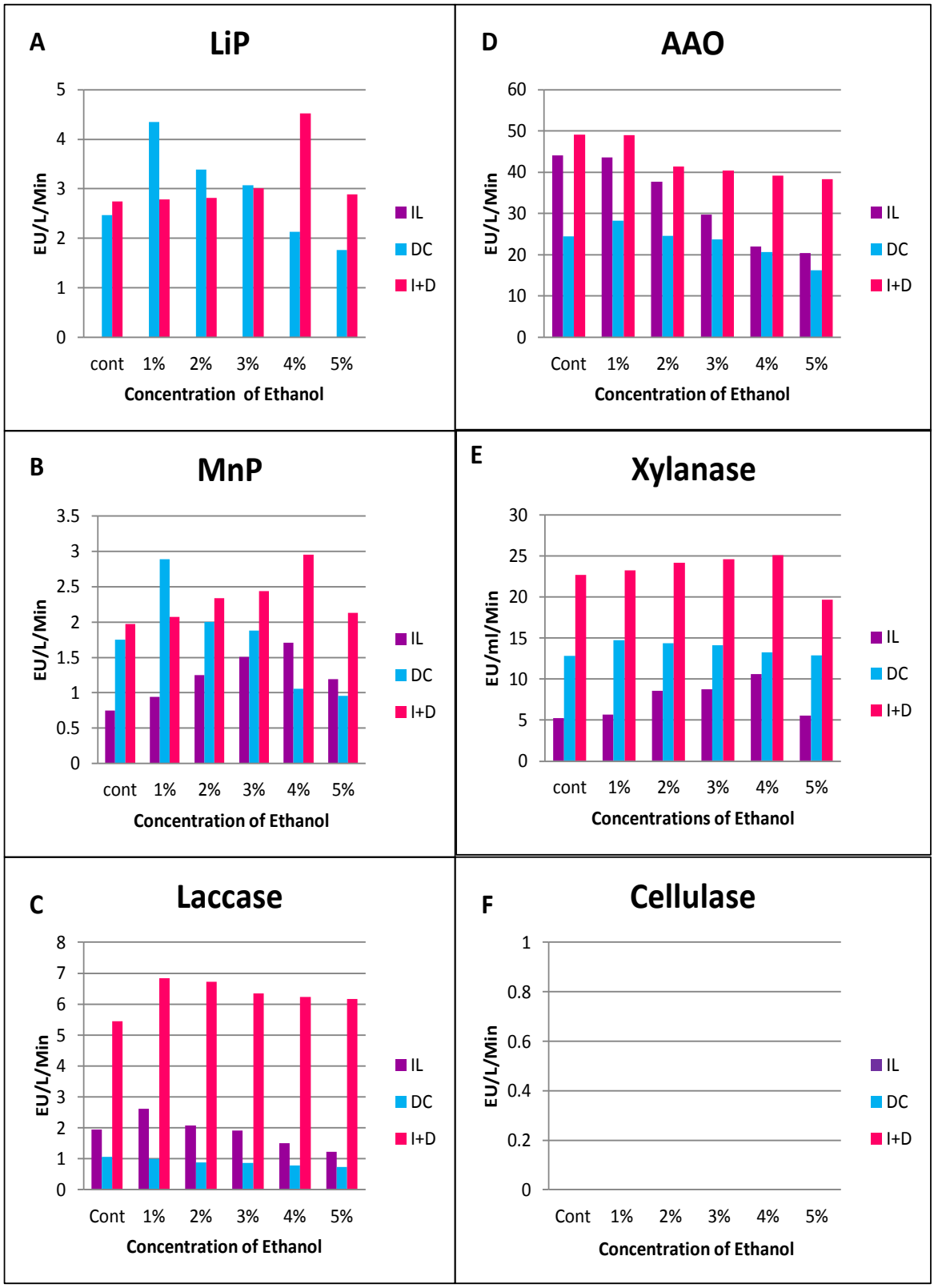


Fig. 1 Effect of Ethanol concentration on lignocellulolytic enzyme activity

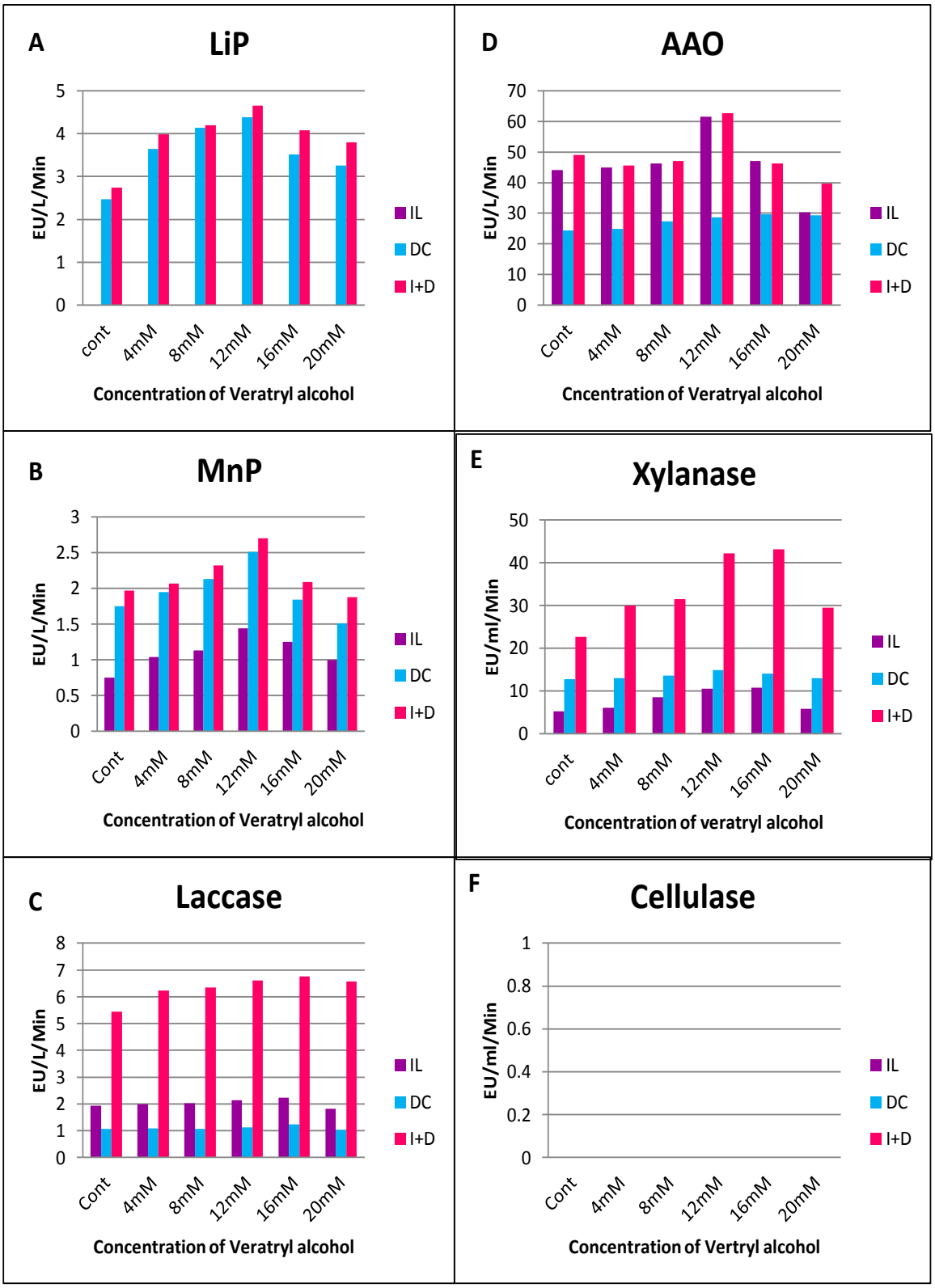


Fig. 2 Effect of Veratryl alcohol concentration on lignocellulolytic enzyme activity

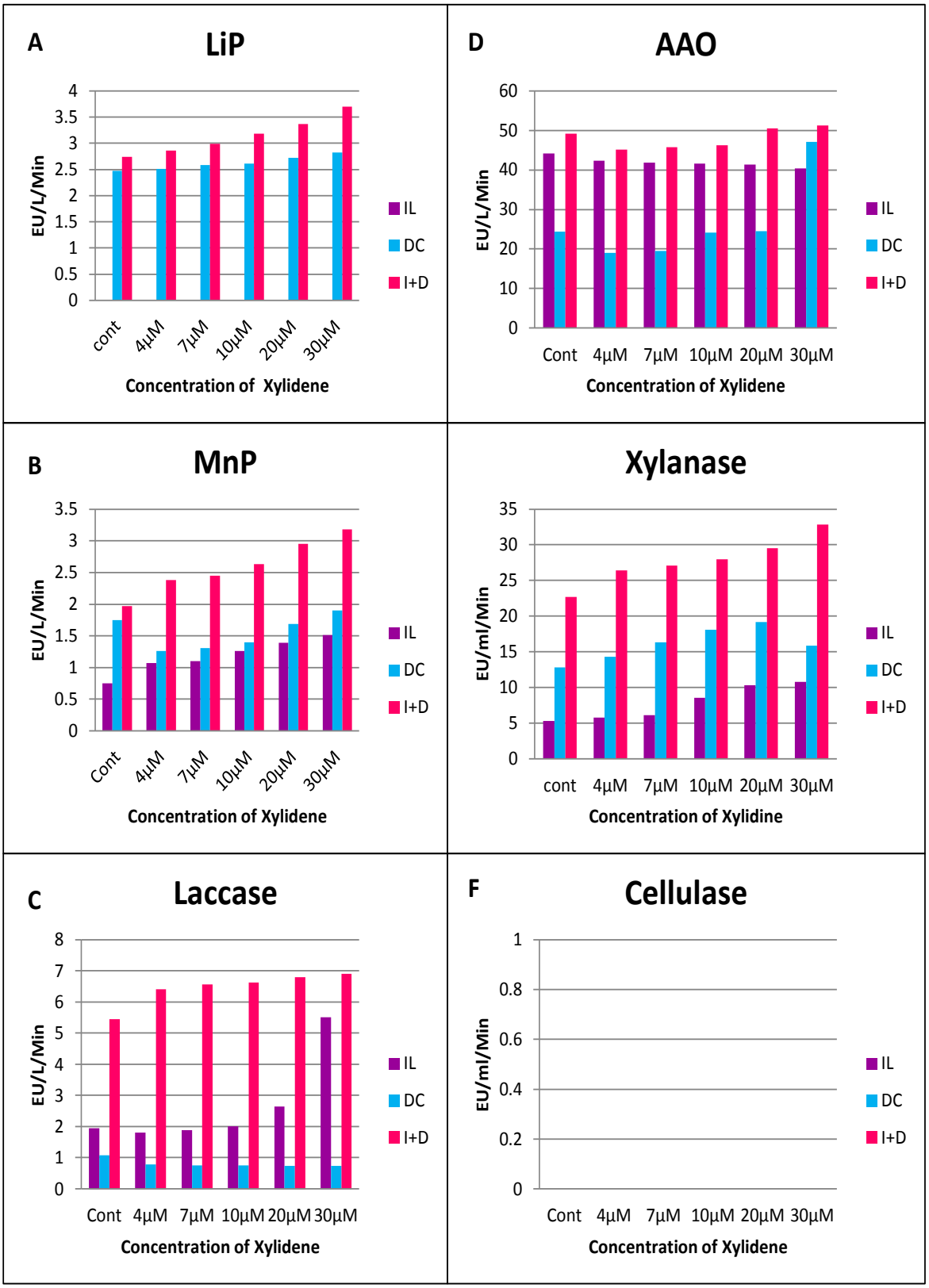


Fig. 3 Effect of Xylidene concentration on lignocellulolytic enzyme activity

LiP activity was enhanced in the range of 0.1g/L to 0.6g/L yeast extract concentration after that in higher concentration such as 0.8g/L activity of LiP was hindered and 0.6 g/L was found to the best for LiP activity. In *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* Li P activity obtained in 0.6 g/L were 3.25 and 3.64 EU/L/Min respectively after that it was inhibited (Figure 4 A).

Addition of yeast extract influenced MnP activity positively and it seems to be enhancing in the range of .1g/L to 0.6 g/L concentration and in 0.8g/L it was suppressed. *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* showed 1.57, 2.08 and 2.91 EU/L/Min respectively in 0.6% concentration of yeast extract where as in higher concentration 0.8g/L it was seems to be lowered (Figure 4 B).

Laccase activity obtained in 0.6% yeast extract was found to be the highest amongst all concentration studied, in 0.8% it was reducing but still higher than that of obtained in control in *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* where as in *D. confragosa* it was not stimulating. In *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* 2.10 and 6.73 EU/L/Min was found in 0.6 g/L concentration of yeast extract where as in higher concentration 0.8g/L it was seems to be reduced (Figure 4 C).

In the selection of best concentration of yeast extract, 0.6g/L resulted in highest activity (27.69 EU/L/Min) in *D. confragosa* which was lowered in 0.8g/L which was found to be higher compared to control. In case of *I. lacteus* and co culture of *I. lacteus* and *D. confragosa* the activity did not enhance (Figure 4 D).

Xylanase activity found to be maximum in 0.6 g/L concentration of yeast extract after which in 0.8 g/L it declined but still at a higher rate than the control. In *I.*

lacteus, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* 10.87, 18.98 and 29.57 EU/ml/Min respectively in 0.6% g/L and in 0.8g/L it was inhibited but higher than control (Figure 4 E).

Cellulase activity was not affected by addition of yeast extract to the media, it was found to be absent in all the concentrations of yeast extract (Figure 4 F).

Enzyme	Fungal isolate	Control	Concentrations of Yeast extract				
			0.1g/L	0.2g/L	0.4g/L	0.6g/L	0.8g/L
LiP	IL	0	0	0	0	0	0
	DC	1.76±0.06	1.92±0.09	2.03±0.09	2.09±0.05	**2.57±0.06**	1.42±0.07
	IL+DC	2.74±0.096	2.95±0.06	3.07±0.06	3.26±0.06	**3.64±0.06**	2.95±0.06
MnP	IL	0.50±0.03	0.71±0.04	0.84±0.10	0.96±0.08	**1.21±0.09**	1.03±0.09
	DC	1.00±0.06	0.69±0.06	0.81±0.06	1.10±0.09	**1.34±0.06**	1.05±0.10
	IL+DC	1.97±0.09	2.13±0.06	2.38±0.06	2.64±0.07	**2.91±0.09**	2.43±0.05
Lac	IL	1.26±0.010	1.01±0.04	1.09±0.05	1.20±0.03	**1.39±0.05**	1.33±0.03
	DC	0.99±0.019	0.48±0.04	0.52±0.04	0.59±0.03	0.64±0.04	0.61±0.03
	IL+DC	5.45±0.030	5.79±0.02	6.60±0.02	6.65±0.02	**6.73±0.02**	6.62±0.07
AAO	IL	32.42±0.15	31.84±0.06	30.96±0.06	30.23±0.06	29.95±0.05	28.83±0.06
	DC	22.53±0.10	20.46±0.08	21.83±0.06	22.70±0.07	**25.57±0.07**	24.85±0.07
	IL+DC	49.12±0.12	48.39±0.09	47.97±0.06	46.80±0.04	46.67±0.05	45.98±0.08
Xyl	IL	18.11±1.03	18.96±0.10	19.40±0.09	19.55±0.10	**20.35±0.09**	20.32±0.10
	DC	13.86±1.32	16.61±0.11	16.96±0.10	17.60±0.11	**19.94±0.11**	18.73±0.12
	IL+DC	22.68±0.39	26.40±0.11	26.85±0.10	27.98±0.10	**29.57±0.11**	27.70±0.09
Cell	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 8 Effect of Yeast extract concentration on the lignocellulolytic enzyme activity after 20 days of incubation period

There is a one fold increase in the enzyme activities when cultures (Mono and co) are supplemented with 0.6g/L yeast extract. It was also noted that AAO activity which showed a marginal increase in *D. confragosa* culture was found to be declining in *I. lacteus* and *I. lacteus* and *D. confragosa* coculture compared to the control which

had no enhancer supplemented in the media. Similarly laccase activity was found to be prominently declining on supplementation of enhancer yeast extract. A significant increase in the lignocellulolytic enzyme activities are not noticed when yeast extract was used as one of the chemical enhancer.

Table 8 represented lignocellulolytic enzyme activity when monocultures and coculture both were incubated for 20 days. It was noticed that AAO enzyme activity in coculture and LiP activity in monoculture of *I. lacteus* were not enhanced. LiP activity in coculture was enhanced nearly 1.5 fold significantly compared to *D. confragosa* monoculture. Similarly laccase enzyme activity was not enhanced in monoculture of *D. confragosa* but 5fold enhancement in laccase enzyme activity was observed compared to *I. lacteus* which was very highly significant. In coculture 1.5 fold enhanced xylanase activity and 2 to 2.5 fold enhanced MnP activity were noticed which was also very highly significant. Cellulase activity was not enhanced in both monocultures and cocultures even after addition of different concentrations of yeast extract as one of the enhancer.

(e)Influence of Peptone (PEP) on enzyme production

To evaluate the effect of peptone concentration on lignocellulolytic enzyme production 3% MEB medium was supplemented with various concentrations (0.1% g/L, 0.2% g/L, 0.4% g/L, 0.6% g/L and 0.8% g/L) and a control with no peptone was kept. After the completion of incubation period culture filtrates were collected and enzyme activity was determined.

Compared to the control all the lignocellulolytic enzyme activity were enhanced from 0.1g/L concentration of peptone. As the concentration of peptone increased the enzyme activity was seems to be increased up to 0.6g/L concentration after that even if the concentration of peptone was increased but the enzyme activity

was remained suppressed. 0.4g/L proved to be the best concentration amongst the various concentrations for all the enzymes except LiP which showed enhancement in 0.6g/L. The results were presented in Table 9.

LiP activity was not detected in *I. lacteus* even when the media was supplemented with peptone as an inducer. In *D. confragosa* and the co culture of *I. lacteus* and *D. confragosa* showed a linear increase in the LiP enzyme production from 0.1g/L to 0.6g/L: but the maximum enhancement was observed in 0.6g/L i.e., 2.82 EU/L/Min and 2.95 EU/L/Min respectively and when the fungi growth occurred in the media containing 0.8g/L peptone LiP enzyme production was declined (Figure 5 A).

Enzyme	Fungal isolate	Control	Concentration of Peptone				
			0.1g/L	0.2g/L	0.4g/L	0.6g/L	0.8g/L
LiP EU/L/Min	IL	0	0	0	0	0	0
	DC	2.47±0.03	2.76±0.06	2.82±0.09	**3.03±0.06**	2.82±0.06	2.51±0.06
	IL+DC	2.74±0.096	2.97±0.06	3.09±0.06	**3.25±0.06**	2.95±0.06	2.63±0.06
MnP\ EU/L/Min	IL	0.75±0.06	0.90±0.04	1.06±0.06	**1.19±0.06**	1.14±0.06	0.94±0.06
	DC	1.75±0.06	1.33±0.05	1.42±0.09	**2.08±0.06**	1.45±0.06	1.31±0.06
	IL+DC	1.97±0.09	1.95±0.06	2.08±0.06	**2.34±0.06**	2.00±0.06	1.89±0.06
Laccase EU/L/Min	IL	1.94±0.012	2.00±0.02	2.21±0.02	**2.37±0.02**	2.26±0.02	2.16±0.02
	DC	1.07±0.017	1.02±0.02	1.06±0.02	**1.13±0.02**	1.26±0.02	1.24±0.02
	IL+DC	5.45±0.030	5.62±0.02	5.67±0.02	**5.97±0.02**	5.76±0.04	5.70±0.06
AAO EU/L/Min	IL	44.12±0.13	44.89±0.08	46.39±0.06	**49.79±0.05**	46.90±0.05	44.94±0.04
	DC	24.42±0.11	23.29±0.06	23.38±0.045	**26.09±0.04**	24.60±0.07	23.36±0.07
	IL+DC	49.12±0.12	49.23±0.11	49.66±0.05	**50.42±0.04**	49.71±0.08	49.20±0.09
Xylanase EU/ml/Min	IL	5.26±0.49	6.71±0.09	8.42±0.10	9.88±0.14	**11.07±0.10**	10.75±0.12
	DC	12.83±0.90	13.76±0.10	14.22±0.10	**15.06±0.12**	14.40±0.10	13.80±0.11
	IL+DC	22.68±0.39	37.86±0.13	38.55±0.11	**39.43±0.12**	38.53±0.09	36.39±0.11
Cellulase EU/ml/Min	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 9Effect of peptone concentration on the lignocellulolytic enzyme activity

Results of MnP activity indicated that 0.4g/L could be considered as the best concentration in which the maximum activity was found after that in 0.6g/L and 0.8g/L concentration it was found to be reduced compared to control. The maximum activity of MnP found in *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were 1.19, 2.08 and 2.34 EU/L/Min respectively and at higher concentration it appeared to be decreased (Figure 5 B).

In presence of 0.4g/L concentration of peptone maximum laccase activity was obtained and at higher concentration it was inhibited. The highest laccase activity found in mono cultures of *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were 2.37, 1.13 and 5.97 EU/L/Min respectively which was showing hindrance at 0.6g/L and 0.8g/L concentration (Figure 5 C).

Mono cultures of *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* represented the highest activity of AAO, 49.79, 26.09, 50.42 EU/L/Min respectively in 0.4g/L peptone concentration. After that even if the media was provided with higher concentration of peptone up to 0.8g/L (Figure 5 D).

Xylanase activity enhanced in the range of 0.1g/L to 0.8g/L peptone concentration but the most effective concentration was 0.4g/L for *D. confragosa* and co culture of *I. lacteus* + *D. confragosa* which showed 15.06 and 39.43 EU/ml/Min respectively where as in case of *I. lacteus* it was found maximum 11.07 EU/ml/Min in 0.6 g/L after that the production of xylanase was declined (Figure 5 E).

Cellulase activity was not initiated even if media was supplied with peptone as one of the inducers, it showed a clear absence of cellulase activity (Figure 5 F). All enzyme activities were enhanced to the maximum when medium was supplemented with 0.4g/L peptone. However the increase was only about one fold except in xylanase by coculture of *I. lacteus* and *D. confragosa* which showed a double fold increase in its activity when supplemented with 0.4g/L peptone.

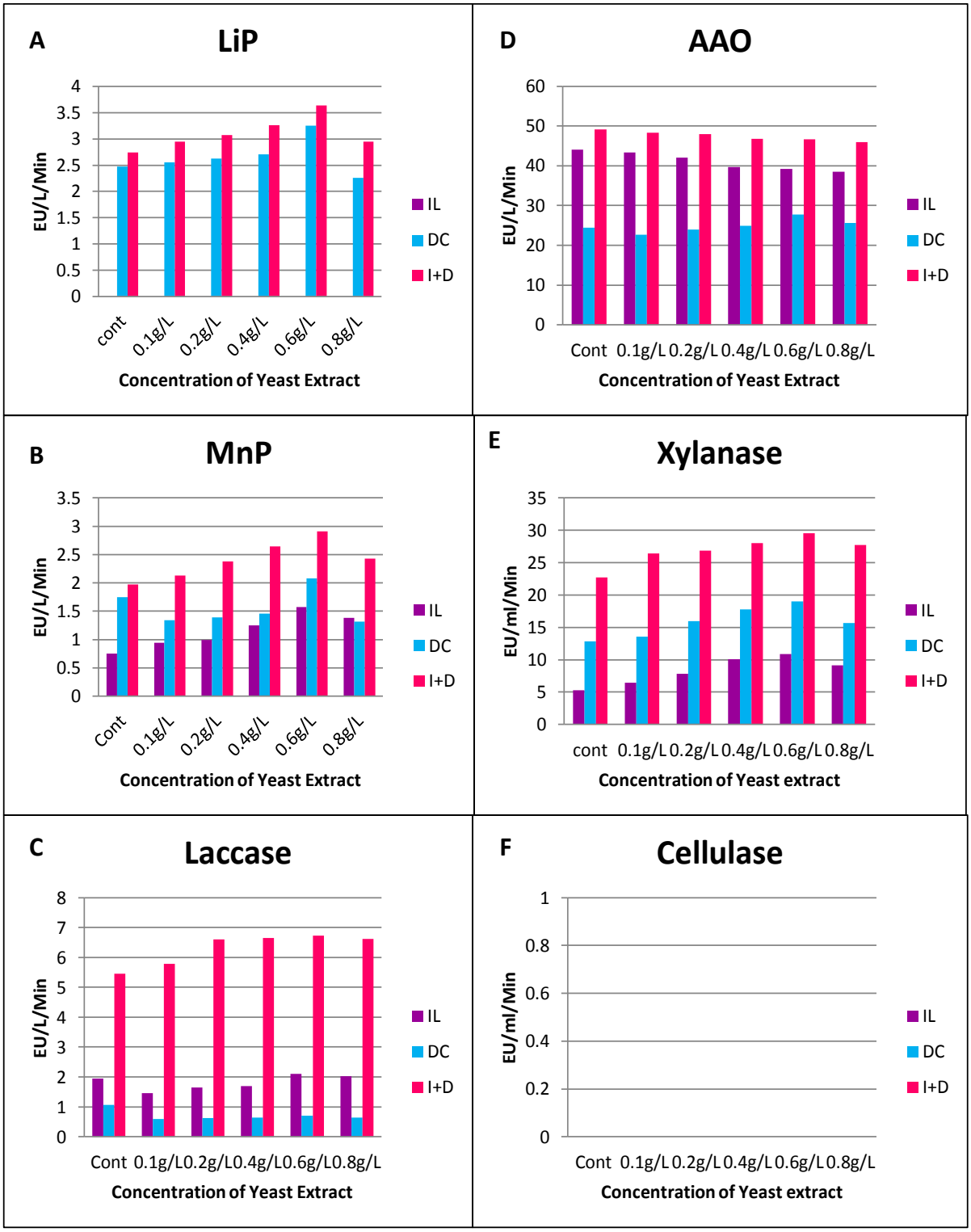


Fig. 4 Effect of Yeast extract concentration on lignocellulolytic enzyme activity

Monocultures and coculture when incubated for 20 days lignocellulolytic enzyme activity was enhanced significantly as shown in the Table 10. In coculture 2.6 fold enhanced MnP activity, 4.06 fold enhanced laccase enzyme activity, 1.39 fold AAO enzyme activity and 1.82 fold xylanase enzyme activity was enhanced significantly compared to *I. lacteus*. However LiP activity in *I. lacteus* was not stimulated even after addition of Peptone in various concentrations to the culture medium.

Enzyme	Fungal isolate	Control	Concentrations of Peptone				
			0.1g/L	0.2g/L	0.4g/L	0.6g/L	0.8g/L
LiP	IL	0	0	0	0	0	0
	DC	1.76±0.06	2.03±0.09	2.19±0.09	**2.32±0.06**	2.11±0.09	1.80±0.06
	IL+DC	2.74±0.096	2.97±0.06	3.09±0.06	**3.25±0.06**	2.95±0.06	2.63±0.06
MnP	IL	0.50±0.03	0.66±0.09	0.83±0.04	**0.90±0.07**	0.80±0.08	0.67±0.05
	DC	1.00±0.06	0.86±0.07	1.06±0.09	**1.36±0.05**	0.98±0.07	0.82±0.06
	IL+DC	1.97±0.09	1.95±0.06	2.08±0.06	**2.34±0.06**	2.00±0.06	1.89±0.06
Lac	IL	1.26±0.010	1.33±0.03	1.38±0.03	**1.47±0.04**	1.41±0.03	1.28±0.04
	DC	0.99±0.019	0.88±0.03	0.94±0.03	**1.01±0.03**	1.08±0.03	0.98±0.03
	IL+DC	5.45±0.030	5.62±0.02	5.67±0.02	**5.97±0.02**	5.76±0.04	5.70±0.06
AAO	IL	32.42±0.15	33.99±0.07	34.21±0.05	**36.18±0.05**	34.76±0.07	32.61±0.08
	DC	22.53±0.10	22.26±0.08	22.96±0.06	**24.81±0.07**	24.06±0.05	22.63±0.06
	IL+DC	49.12±0.12	49.23±0.11	49.66±0.05	**50.42±0.04**	49.71±0.08	49.20±0.09
Xyl	IL	18.11±1.03	18.81±0.12	18.98±0.10	20.00±0.10	**21.63±0.13**	19.62±0.11
	DC	13.86±1.32	14.16±0.10	14.77±0.10	**17.26±0.12**	17.06±0.12	15.89±0.09
	IL+DC	22.68±0.39	37.86±0.13	38.55±0.11	**39.43±0.12**	38.53±0.09	36.39±0.11
Cell	IL	0	0	0	0	0	0
	DC	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0

Table 10 Effect of Peptone concentration on the lignocellulolytic enzyme activity after 20 days of incubation period

As compared to monoculture of *D. confragosa* coculture showed significantly 1.5 fold enhanced LiP and MnP activity, 2fold AAO and xylanase enzyme activity whereas laccase activity was enhanced nearly fivefold which was found to be very highly significant. Cellulase enzyme activity was found to be absent in all medium supplemented with various concentrations of Peptone.

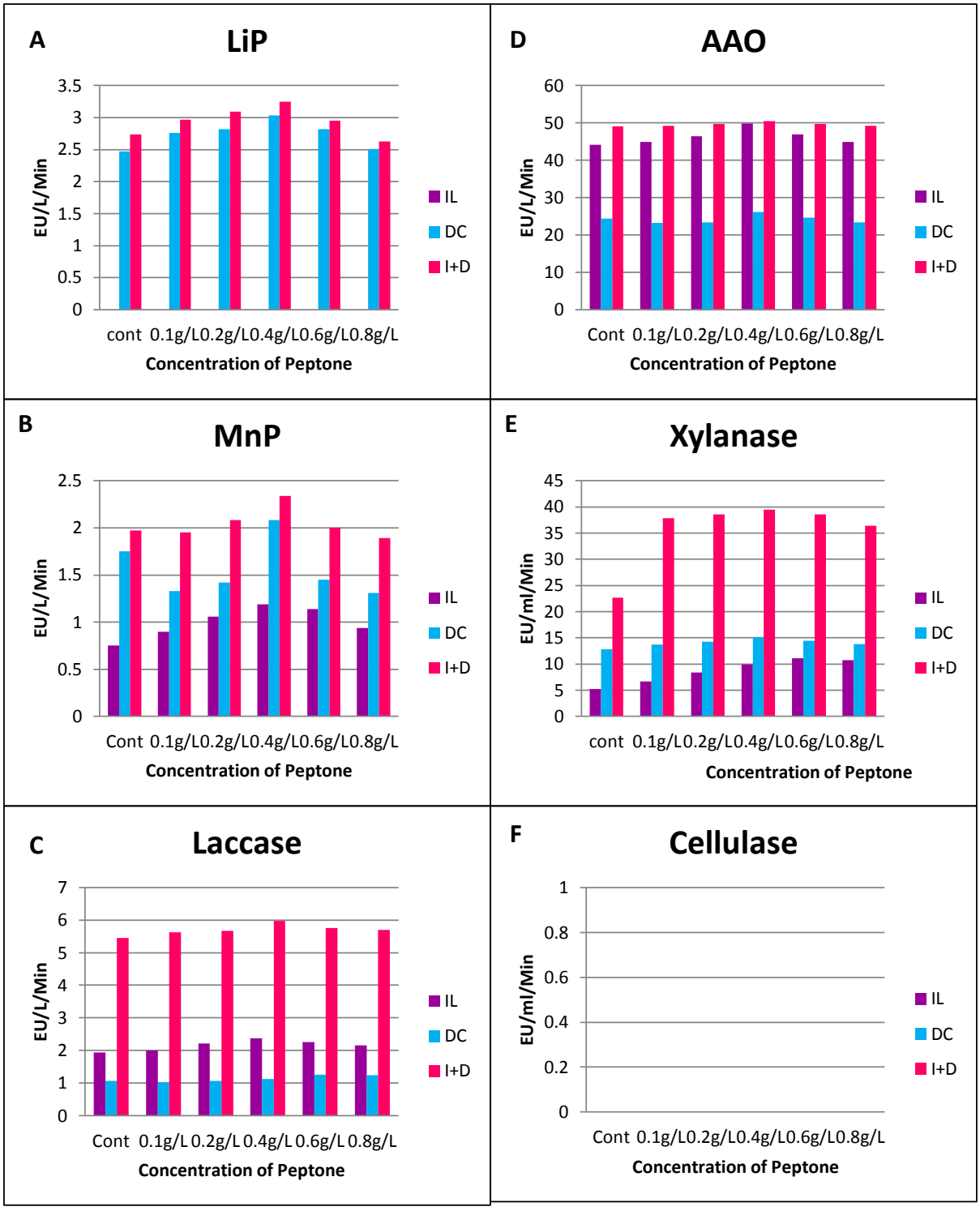


Fig. 5 Effect of Peptone concentration on lignocellulolytic enzyme activity

3.4.2 Effect of lignocellulosic substrates on the activity of enzymes

Experiments were conducted to study the effect of different conditions like pH, temperature and incubation period on the enzyme activity. From the results of earlier experiments the optimum incubation period for coculture 20 days, 25 days and 30 days for monocultures of *D. confragosa* and *I. lacteus* respectively. Similarly the maximum enzyme production was noted at pH 5 and temperature 25 ˚ C so these were conditions selected for the further experiments. A comparison of the enzyme activities in monocultures and cocultures were assessed after an incubation period of 20 days, as the coculture showed maximum enzyme activities on the 20^{th} day of incubation period. Also incubation period at which monocultures showed maximum enzyme activities (i.e. 25 days in *D. confragosa* and 30 days in *I. lacteus*) were compared with the activities obtained maximum in coculture incubation period (i.e. 20days). The white rot basidiomycetes have a capability to produce ligninolytic and hydrolytic enzymes simultaneously during the fermentation of lignocellulosic materials. The selection of appropriate lignocellulosic substrates suitable for the fungal growth and target enzymes is the key step in developing efficient technology of enzyme production.

The effect of lignocellulosic substrates significantly depends on its concentration in the medium. To select the most effective lignocellulosic material four different lignocellulosic substrates (Apple peels, Banana peels, Mandarin peels, Ash gourd pulp) in various concentration 1%, 2%, 3%, 4%, 5%, 6%, 8% and 10% were supplemented in 3% MEB (Malt extract broth) medium and autoclaved. All the culture bottles were inoculated with a 9 mm disc of 10 days old culture. All the lignocellulolytic substrates promoted the excellent growth of fungi. The visible signs of growth were seen 2-3 days after inoculation and colonization of substrates

increased with an increase in incubation period. After the completion of incubation period enzyme assays were carried out to evaluate the effect of substrates on the enzyme activity. The enzymatic analysis of crude enzyme extract showed that various substrates in different concentration have different enhancement effects on the individual enzyme production.

(a) Influence of Apple peels (AP) on enzyme production

Effect of different concentration of apple peels on the lignocellulolytic enzyme activities are represented in Table 1. Addition of 1%, 2%, 3%, 4% and 5% apple peels resulted in a significant increase in the production of all lignocellulolytic enzymes. All the enzymes were significantly enhanced in the concentration of 5% apple peels. Higher concentration of apple peels (6%, 8% and 10%) resulted in lowering the amount of enzyme activity compared to the maximum obtained in 5%concentration. All the monocultures and coculture showed an absence of cellulase activity.

I. lacteus showed an absence of LiP activity in the media supplemented with apple peels. *D. confragosa* showed enhanced LiP activity from 1% to 10% concentration of apple peels compared to control. Maximum enhancement was observed in 5% (2.93 EU/L/Min) after which the LiP production was declined up to (2.53 EU/L/Min) which was still higher than control (2.47 EU/L/Min). Co culture of *I. lacteus* and *D. confragosa* also showed maximum enhancement of LiP enzyme activity in the media supplemented with 5% apple peels (3.53 EU/L/Min) thereafter with a decline in the activity (2.80 EU/L/Min) was observed. However it was same as in *D. confragosa* ie, higher compared to control (Figure 1 A).

In mono culture and co culture both the MnP activity was enhanced in the range of 1% to 10% concentration of apple peels but the highest activity was noticed

in 5% apple peels after which decline in the MnP activity observed as compared to control.

I. lacteus showed MnP activity in the range of 0.79 to 0.89 EU/L/Min in 1% to 10% apple peels in which the maximum was observed in 5% 1.94 EU/L/Min after that decline in the enzyme activity was recorded which was still higher than observed in the control 0.75 EU/L/Min. MnP activity in *D. confragosa* increased from 1.75 EU/L/Min to 1.80 EU/L/Min in the range of 1% to 10% apple peels but the highest MnP activity was found in 5% apple peels (2.23 EU/L/Min) after that decline was clearly observed up to 10% concentration of apple peels which was still higher than the observed in control (1.75 EU/L/Min) (Figure 2 A). In case of co culture *I. lacteus* and *D. confragosa* attained the maximum MnP activity in 5% apple peels 2.87 EU/L/Min after which it declined till the 10% concentration of apple peels which was still higher than the MnP activity observed in control (Figure 2 A).

Laccase activity was enhanced from 1% to 10% concentration of apple peels amongst which 5% proved to be the best concentration which results in the highest enhancement of laccase activity. A decline in the laccase activity with an increase in the concentration of apple peel above 5% was noted in both the monocultures as well as co culture.

The maximum activity of laccase noted in *I. lacteus* was 3.30 EU/L/Min and in *D. confragosa* 4.74 EU/L/Min in 5% apple peels. The co culture of *I. lacteus* and *D. confragosa* showed 10.24 EU/L/Min laccase activity in 5% apple peels which was almost double fold as compared to control (Figure 3 A).

AAO activity was enhanced in the range of 1% to 10% apple peels in which 5% was the best concentration for enhancement after which decline in the AAO

activity was noted but in 10% also the activity of AAO observed was higher than the control.

Conc of Apple peels	Fungal isolate	Enzyme					
		LiP EU/L/Min	MnP EU/L/Min	Lac EU/L/Min	AAO EU/L/Min	Xylanase EU/ml/Min	Cellulase EU/ml/Min
Cont	IL	0	0.75±0.06	1.94±0.012	44.12±0.13	5.26±0.49	0
	DC	2.47±0.03	1.75±0.06	1.07±0.017	24.42±0.11	12.83±0.90	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.79±0.04	2.03±0.05	44.88±0.14	6.72±0.10	0
	DC	2.55±0.04	1.80±0.04	1.84±0.08	31.04±0.19	13.15±0.10	0
	IL+DC	3.05±0.09	2.07±0.07	5.51±0.09	52.73±0.12	23.23±0.22	0
2%	IL	0	0.82±0.06	2.09±0.09	47.00±0.18	8.71±0.10	0
	DC	2.57±0.06	1.88±0.06	2.50±0.04	31.87±0.16	14.05±0.11	0
	IL+DC	3.24±0.09	2.26±0.06	6.23±0.06	60.38±0.15	24.95±0.14	0
3%	IL	0	0.94±0.06	2.54±0.04	49.02±0.19	9.82±0.16	0
	DC	2.64±0.06	1.92±0.09	2.71±0.08	35.32±0.15	15.37±0.13	0
	IL+DC	3.34±0.06	2.51±0.06	6.79±0.06	62.15±0.15	24.43±0.21	0
4%	IL	0	1.17±0.04	2.62±0.08	54.45±0.16	10.64±0.11	0
	DC	2.78±0.04	2.00±0.06	4.23±0.06	37.57±0.11	17.24±0.10	0
	IL+DC	3.43±0.09	2.57±0.06	7.06±0.07	72.05±0.17	24.62±0.17	0
5%	IL	0	**1.94±0.06**	**3.30±0.10**	**55.40±0.19**	**11.22±0.12**	0
	DC	**2.93±0.09**	**2.23±0.04**	**4.74±0.08**	**40.93±0.13**	**19.93±0.10**	0
	IL+DC	**3.53±0.09**	**2.87±0.04**	**10.24±0.08**	**98.41±0.18**	**29.58±0.25**	0
6%	IL	0	1.25±0.07	3.24±0.10	53.43±0.13	9.83±0.10	0
	DC	2.83±0.06	2.08±0.06	3.69±0.08	39.13±0.14	17.35±0.10	0
	IL+DC	3.28±0.09	2.76±0.06	9.37±0.05	97.19±0.11	23.56±0.19	0
8%	IL	0	1.09±0.09	2.89±0.06	45.59±0.17	8.00±0.14	0
	DC	2.59±0.09	1.85±0.1	3.31±0.09	37.68±0.12	15.93±0.14	0
	IL+DC	3.01±0.06	2.26±0.06	8.38±0.07	90.68±0.15	23.44±0.21	0
10%	IL	0	0.89±0.06	2.58±0.05	44.82±0.10	6.18±0.16	0
	DC	2.53±0.09	1.82±0.06	2.30±0.08	36.84±0.15	14.58±0.11	0
	IL+DC	2.80±0.09	2.09±0.06	7.42±0.07	82.10±0.10	23.25±0.17	0

Table 1 Effect of different concentrations of Apple peels on ligninolytic enzyme activity

In *I. lacteus* maximum AAO activity (55.40 EU/L/Min) was noticed and in *D. confragosa* the highest activity noted was 40.93 EU/L/Min in 5% apple peels concentration. The co cultures of *I. lacteus* and *D. confragosa* showed 98.41 EU/L/Min AAO activity in 5% apple peels after which decline in the AAO activity was noticed which was still higher than the control (Figure 4 A).

In mono culture and co culture the maximum xylanase activity noted was in the 5% apple peels concentration after which clear decline in the enzyme production was observed but which was still higher compared to the enzyme production in control. The maximum activity of xylanase observed in *I. lacteus* was 11.22 EU/ml/Min and in *D. confragosa* 19.93 EU/ml/Min in 5% apple peels. The co culture of *I. lacteus* and *D. confragosa* showed 29.58 EU/ml/Min xylanase activity in 5% apple peels after that decline in the activity was noted which was higher than the control (Figure 5 A).

Amongst the studied enzymes AAO and Xylanase production was found to be significantly high with apple peels as the substrate. Compared to the other enzymes both monocultures and co culture showed double fold increase in laccase enzyme activity. Similarly xylanase enzyme also shows 113.30% and 55.34% increase in *I. lacteus* and *D. confragosa* respectively where as 30.42% increase in the enzyme activity was found in the coculture. AAO and xylanase showed more enhancements as compared to laccase and other lignin degrading enzymes, in both monocultures and coculture.

Overall fungal isolates *I. lacteus* and *D. confragosa* when mono cultured or cocultured showed an absence of cellulase enzyme activity when supplemented with apple peels. Except AAO enzyme activity the other ligninolytic enzymes (LiP, MnP, laccase and xylanase) were found to be higher in monoculture of *D. confragosa*

compared to *I .lacteus*. Coculture of *I .lacteus* and *D. confragosa* showed not very significant enhancement in all the enzyme activity compared to monocultures.

Concentration of Apple peels	Fungal isolate	Enzyme					
		LiP	MnP	Lac	AAO	Xylanase	Cellulase
Cont	IL	0	0.50±0.04	1.26±0.010	32.42±0.13	18.11±1.03	0
	DC	1.76±0.06	1.00±0.06	0.99±0.019	22.53±0.11	13.86±1.32	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.56±0.06	1.35±0.06	33.37±0.14	19.31±0.15	0
	DC	1.82±0.06	1.06±0.06	1.57±0.08	25.29±0.18	14.24±0.20	0
	IL+DC	3.05±0.09	2.07±0.07	5.51±0.09	52.73±0.12	23.23±0.22	0
2%	IL	0	0.60±0.07	1.51±0.09	34.94±0.12	19.66±0.17	0
	DC	1.88±0.06	1.10±0.06	1.93±0.06	26.28±0.14	14.67±0.13	0
	IL+DC	3.24±0.09	2.26±0.06	6.23±0.06	60.38±0.15	24.95±0.14	0
3%	IL	0	0.69±0.06	1.91±0.09	37.66±0.12	21.11±0.13	0
	DC	2.05±0.009	1.21±0.09	2.15±0.09	28.77±0.14	16.32±0.11	0
	IL+DC	3.34±0.06	2.51±0.06	6.79±0.06	62.15±0.15	24.43±0.21	0
4%	IL	0	0.87±0.06	2.10±0.08	40.45±0.13	23.31±0.15	0
	DC	2.09±0.09	1.38±0.06	2.51±0.06	31.21±0.14	18.05±0.15	0
	IL+DC	3.43±0.09	2.57±0.06	7.06±0.07	72.05±0.17	24.62±0.17	0
5%	IL	0	**1.13±0.06**	**2.71±0.08**	**43.35±0.16**	**25.53±0.20**	0
	DC	2.19±0.06	**1.62±0.06**	**3.03±0.07**	**34.71±0.16**	**20.76±0.17**	0
	IL+DC	**3.53±0.09**	**2.87±0.04**	**10.24±0.08**	**98.41±0.18**	**29.58±0.25**	0
6%	IL	0	1.06±0.06	2.47±0.10	39.15±0.19	24.47±0.18	0
	DC	2.07±0.06	1.44±0.06	2.83±0.06	32.59±0.14	18.07±0.19	0
	IL+DC	3.28±0.09	2.76±0.06	9.37±0.05	97.19±0.11	23.56±0.19	0
8%	IL	0	0.81±0.09	2.24±0.06	38.23±0.13	22.25±0.13	0
	DC	1.96±0.09	1.25±0.06	2.70±0.06	30.56±0.14	16.61±0.20	0
	IL+DC	3.01±0.06	2.26±0.06	8.38±0.07	90.68±0.15	23.44±0.21	0
10%	IL	0	0.69±0.06	1.63±0.06	35.65±0.15	19.38±0.16	0
	DC	1.90±0.09	1.19±0.06	2.18±0.09	26.86±0.16	16.06±0.18	0
	IL+DC	2.80±0.09	2.09±0.06	7.42±0.07	82.10±0.10	23.25±0.17	0

Table 2 Effect of different concentrations of Apple peels on ligninolytic enzyme activity after 20 days of incubation period

When monocultures and coculture both were incubated for 20 days enhancement in the lignocellulolytic enzyme activities were represented in the following Table 2. Results indicating that 5% apple peels were proved to be the best concentration for enhancement in all the lignocellulolytic enzyme activities. However LiP activity in *I. lacteus* monoculture was found to be absent even after addition of apple peels as substrate. Coculture showed 1.61 fold enhanced LiP activity compared to monoculture of *D. confragosa* where as 2.53v fold enhancement in MnP activity, 3.77 fold enhancement in Laccase activity, 2.21 fold in AAO activity and 1.13 fold enhancement in xylanase activity were noticed compared to monoculture of *I. lacteus*. When compared to D. confragosa monoculture 1.77 fold enhanced MnP activity, 3.37 fold enhanced Laccase activity, 2.83 fold enhanced AAO activity and 1.42 fold enhanced xylanase activity were observed. These results indicating very highly significant enhancement in all the enzyme activities were obtained when 20 days incubation period was considered as optimum for monocultures and cocultures.

(b) Influence of Banana peels (BP) on enzyme production

Monocultures *I. lacteus* and *D. confragosa* and their co culture showed significant enhanced levels of all the lignocellulolytic enzymes during the incubation period. The enhancement of the lignocellulolytic enzyme activity was observed in the concentration of 1%, 2%, 3%, 4%, 5%, 6%, 8% and 10 % and the most pronounced effect with all the enzymes were observed in 4% concentration after which the enzyme activity declined though declined was still higher than the enzyme activity observed in the control. The effect of different concentration of banana peels on lignocellulolytic enzyme production is represented in Table 3.

LiP activity was found to be absent in media supplemented with banana peels as a substrate for *I. lacteus*. LiP activity in *D. confragosa* was negligibly enhanced

from 2.56 EU/L/Min to 2. 76 EU/L/Min from 1% to 4% of banana peels after 4% decline in the LiP activity was found up to 10% but in 10% also 2.51 EU/L/Min LiP activity was noted which is slightly more than the control (Figure 1 B)

Conc of Banana peels	Fungal isolate	Enzyme					
		LiP EU/L/Min	MnP EU/L/Min	Lac EU/L/Min	AAO EU/L/Min	Xylanase EU/ml/Min	Cellulase EU/ml/Min
Cont	IL	0	0.75±0.06	1.94±0.012	44.12±0.13	5.26±0.49	0
	DC	2.47±0.03	1.75±0.06	1.07±0.017	24.42±0.11	12.83±0.90	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.92±0.04	2.18±0.07	51.25±0.10	7.79±0.12	0
	DC	2.56±0.09	1.80±0.04	2.15±0.12	33.38±0.12	13.78±0.12	0
	IL+DC	3.33±0.06	2.28±0.09	6.19±0.07	56.83±0.16	25.70±0.12	0
2%	IL	0	0.94±0.06	2.45±0.07	59.04±0.17	8.97±0.12	0
	DC	2.63±0.06	1.84±0.04	3.06±0.07	44.83±0.13	15.72±0.10	0
	IL+DC	3.20±0.06	2.57±0.06	7.09±0.04	66.31±0.11	27.91±0.12	0
3%	IL	0	1.11±0.04	2.70±0.10	68.45±0.21	10.54±0.12	0
	DC	2.70±0.06	1.95±0.06	4.14±0.07	57.08±0.13	16.88±0.13	0
	IL+DC	3.45±0.06	2.76±0.06	7.36±0.07	68.48±0.14	29.99±0.21	0
4%	IL	0	**1.63±0.06**	**3.65±0.06**	**81.22±0.19**	**13.34±0.12**	0
	DC	**2.76±0.06**	**2.09±0.04**	**6.48±0.06**	**77.14±0.11**	**20.73±0.12**	0
	IL+DC	**3.76±0.06**	**2.95±0.06**	**10.26±0.06**	**84.15±0.18**	**32.04±0.19**	0
5%	IL	0	1.15±0.05	3.31±0.04	77.10±0.19	12.17±0.11	0
	DC	2.64±0.06	2.00±0.06	6.18±0.06	75.96±0.11	19.36±0.12	0
	IL+DC	3.39±0.06	2.81±0.04	9.33±0.05	71.38±0.25	31.97±0.11	0
6%	IL	0	1.04±0.06	3.02±0.07	70.67±0.10	10.72±0.11	0
	DC	2.57±0.06	1.94±0.06	5.60±0.07	73.55±0.16	18.41±0.12	0
	IL+DC	3.01±0.06	2.57±0.06	8.46±0.06	70.55±0.11	31.44±0.17	0
8%	IL	0	0.94±0.06	2.46±0.07	67.88±0.10	8.96±0.10	0
	DC	2.55±0.09	1.87±0.04	5.02±0.07	67.75±0.14	17.28±0.10	0
	IL+DC	2.93±0.09	2.44±0.06	7.95±0.06	69.19±0.15	30.60±0.10	0
10%	IL	0	0.87±0.06	2.12±0.06	57.06±0.17	7.67±0.12	0
	DC	2.51±0.06	1.88±0.06	4.48±0.06	46.26±0.17	16.81±0.10	0
	IL+DC	2.83±0.06	2.19±0.06	7.62±0.08	61.34±0.16	30.49±0.22	0

Table 3 Effect of different concentrations of Banana peels on ligninolytic enzyme activity

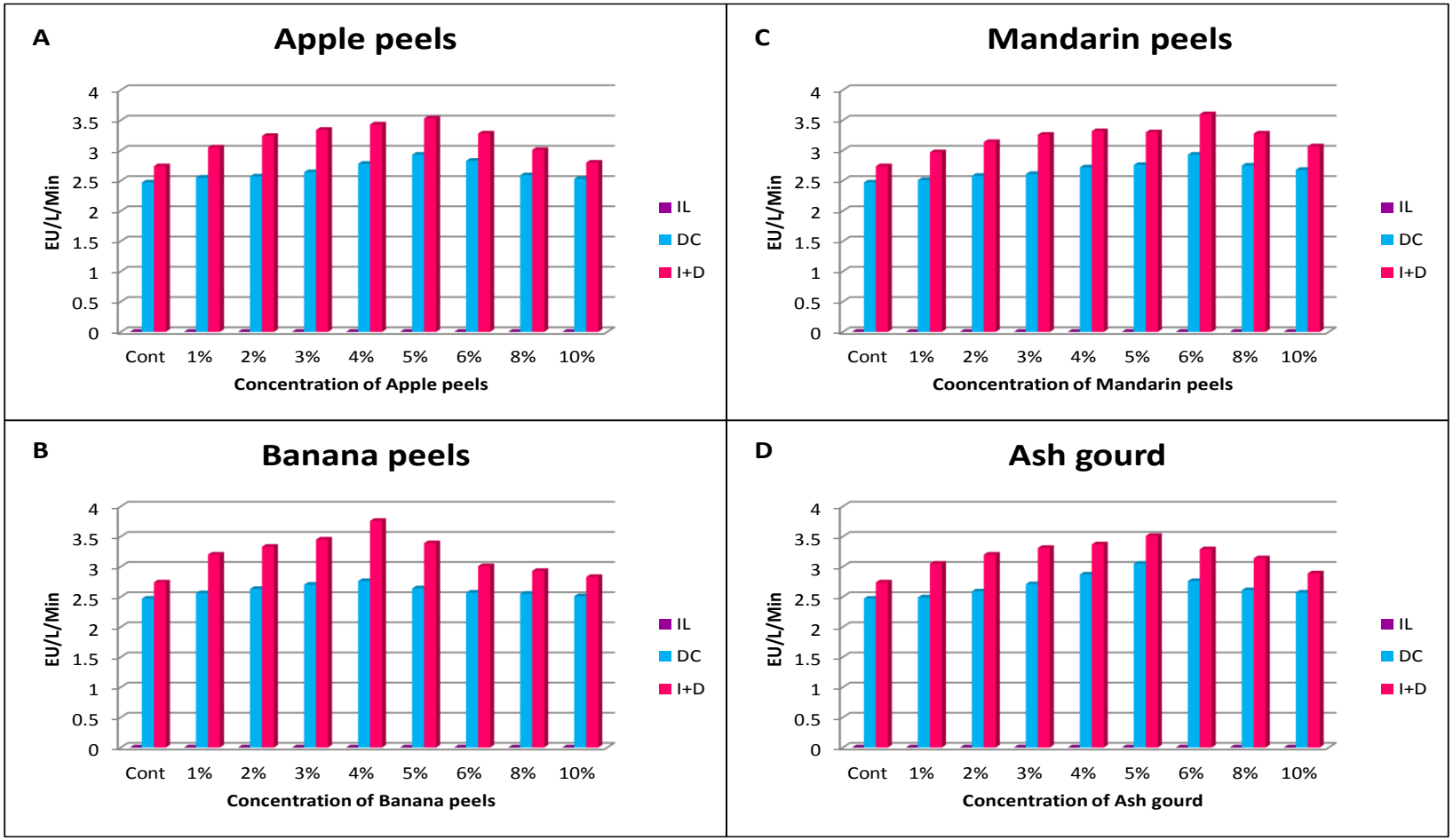


Fig. 1 Effect of different substrate concentrations on the activity of LiP

The co culture of *I. lacteus* and *D. confragosa* also showed maximum enhancement of LiP activity (3.76 U EU/L/Min) in the 4% concentration of banana peels which gradually decreased up to 2.81 EU/L/Min in 10% banana peels but in 10% also the LiP activity found slightly higher than observed in the control 2.74U EU/L/Min (Figure 1 B).

MnP activity in *I. lacteus* showed maximum enhancement in 4% banana peels (1.63 EU/L/Min). With an increase in the concentration of banana peels the enhancement in the MnP activity declined up to 10%. In 10% substrate concentration also 0.87 EU/L/Min MnP activity was noted which is still higher than the activity noted in the control 0.75 EU/L/Min. In *D. confragosa* 4% concentration was found to be the best concentration which showed 2.09 EU/L/Min after which a decline in the activity was noted up to 1.88 EU/L/Min in 10% concentration which is also higher than the observed in the control.

4% banana peels showed maximum enhancement in co culture of *I. lacteus* and *D. confragosa*. Co culture showed 2.95 EU/L/Min MnP activity after that the production of MnP enzyme was declined up to 10% concentration. In 10% also 2.19 EU/L/Min MnP activity noted which was found to be higher than 1.97 EU/L/Min observed in the control (Figure 2 B).

Laccase activity was enhanced in both the mono cultures as well as in co culture in lower concentration of banana peels. Culture bottles inoculated with 4% banana peels showed maximum level of Laccase activity 3.65, 6.48, 10.26 EU/L/Min in the cultures of *I. lacteus*, *D. confragosa* and *I. lacteus* + *D. confragosa* respectively (Figure 3 B). Further increase in the concentration of banana peels resulted in the lower production of Laccase enzyme. However the laccase production in 10% banana peels was still higher than the control.

AAO enzyme activity showed notably enhancing effect in monocultures and co culture of fungi when banana peels were used as the substrate. The maximum activity recorded with 4% concentration of banana peels in *I. lacteus*, *D. Confragosa* and Co culture of *I. lacteus* and *D. Confragosa* were 81.22, 77.14 and 84.15 EU/L/Min respectively where as the higher concentration of banana peels up to 10% resulted in lowering the AAO enzyme activity but it was found to be more than the observed in the control (Figure 4 B).

Xylanase activity was enhanced best in 4% concentration of banana peels. Maximum level of xylanase activity 13.34 EU/ml/Min, 20.73 EU/ml/Min, and 32.04 EU/ml/Min in the culture extracts of *I. lacteus*, *D. Confragosa* and Co culture of *I. lacteus* and *D. Confragosa* respectively. The xylanase activity was found to be decline in the higher concentration of banana peels but 10% banana peels still proved to be favourable for the production of xylanase as it resulted in 7.67, 16.81, 30.49 EU/ml/Min in *I. lacteus*, *D. Confragosa* and Co culture of *I. lacteus* and *D. Confragosa* respectively. These results are still higher when compared with those obtained with the control (Figure 5 B).

Similar to the apple peels results with banana peels were found to be highly promising for the AAO and xylanase activity. Amongst the studied enzymes AAO and Xylanase production was found to be significantly high when medium was supplemented with banana peels as the substrate. AAO enzyme activity was enhanced maximum in *D. confragosa* 215.88% and 84.08% increase in *I.lacteus* and 71.31% increase in the enzyme activity found in the coculture. Similarly xylanase enzyme also shows 153.61% and 61.57% increase in *I .lacteus* and *D. confragosa* respectively where as 30.42% increase in the enzyme activity was found in the coculture. AAO and xylanase showed more enhancements as compared to laccase and other lignin degrading enzymes, in both monocultures and coculture.

Concentration of Banana peels	Fungal isolate	Enzyme					
		LiP	MnP	Lac	AAO	Xylanase	Cellulase
Cont	IL	0	0.50±0.04	1.26±0.010	44.12±0.13	18.11±1.03	0
	DC	1.76±0.06	1.00±0.06	0.99±0.019	24.42±0.11	13.86±1.32	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.62±0.06	1.91±0.05	51.25±0.10	20.88±0.17	0
	DC	1.86±0.07	1.08±0.05	1.85±0.10	33.38±0.12	14.25±0.12	0
	IL+DC	3.33±0.06	2.28±0.09	6.19±0.07	56.83±0.16	25.70±0.12	0
2%	IL	0	0.75±0.06	2.17±0.07	59.04±0.17	21.20±0.15	0
	DC	1.90±0.06	1.13±0.06	2.71±0.05	44.83±0.13	16.42±0.19	0
	IL+DC	3.20±0.06	2.57±0.06	7.09±0.04	66.31±0.11	27.91±0.12	0
3%	IL	0	0.90±0.05	2.33±0.05	68.45±0.21	23.47±0.16	0
	DC	2.00±0.06	1.19±0.06	3.30±0.09	57.08±0.13	17.87±0.15	0
	IL+DC	3.45±0.06	2.76±0.06	7.36±0.07	68.48±0.14	29.99±0.21	0
4%	IL	0	**1.08±0.09**	**2.93±0.08**	**81.22±0.19**	**27.21±0.17**	0
	DC	**2.13±0.06**	**1.29±0.08**	**3.72±0.08**	**77.14±0.11**	**22.60±0.18**	0
	IL+DC	**3.76±0.06**	**2.95±0.06**	**10.26±0.06**	**84.15±0.18**	**32.04±0.19**	0
5%	IL	0	0.96±0.09	2.46±0.08	77.10±0.19	24.28±0.12	0
	DC	2.03±0.06	1.26±0.05	3.53±0.07	75.96±0.11	20.87±0.29	0
	IL+DC	3.39±0.06	2.81±0.04	9.33±0.05	71.38±0.25	31.97±0.11	0
6%	IL	0	0.90±0.04	2.14±0.07	70.67±0.10	24.10±0.15	0
	DC	1.98±0.04	1.17±0.04	3.300±0.07	73.55±0.16	19.65±0.13	0
	IL+DC	3.01±0.06	2.57±0.06	8.46±0.06	70.55±0.11	31.44±0.17	0
8%	IL	0	0.77±0.09	1.91±0.08	67.88±0.10	23.47±0.16	0
	DC	1.94±0.06	1.08±0.04	2.70±0.06	67.75±0.14	18.98±0.14	0
	IL+DC	2.93±0.09	2.44±0.06	7.95±0.06	69.19±0.15	30.60±0.10	0
10%	IL	0	0.66±0.09	1.84±0.06	57.06±0.17	21.81±0.15	0
	DC	1.84±0.09	1.05±0.06	2.46±0.06	46.26±0.17	17.34±0.15	0
	IL+DC	2.83±0.06	2.19±0.06	7.62±0.08	61.34±0.16	30.49±0.22	0

Table 4 Effect of different concentrations of Banana peels on ligninolytic enzyme activity after 20 days of incubation period

Results of enhanced lignocellulolytic enzyme activity after 20 days of incubation period was represented in the following Table 4. LiP activity was found to be absent in *I. lacteus* whereas coculture indicated 1.76 fold enhanced LiP activity compared to monoculture of *D. confragosa*. Coculture showed approximately 2.75 fold enhanced MnP activity, 3.50 fold laccase activity compared to *I. lacteus* whereas 2.28 fold and 2.75 fold enhancement in MnP and laccase activity respectively noticed compared to monoculture of *D. confragosa*. Single fold enhancement in AAO and xylanase were noticed in coculture compared to monocultures of *D. confragosa* and *I. lacteus*. These results clearly indicated 4% banana peels proved to be best concentration and after 20 days of incubation period highly significant enhancement in coculture was noticed in all the lignocellulolytic enzyme activities.

(c) Influence of Mandarin peels (MP) on enzyme production

To select the most effective concentration of mandarin peels for the enhancement of lignocellulolytic enzymes 3% MEB media was supplemented with different concentrations of mandarin peels (1%, 2%, 3%, 4%, 5%, 6%, 8% and 10%). Supplementation of different concentration of mandarin peels in the media caused variation in the lignocellulolytic enzyme activity and results showed that increasing.

As the concentration of mandarin peels in the medium increased from 1% to 10% all the lignocellulolytic enzymes were enhanced the most in 6% except laccase. Laccase activity was found to be maximum when supplemented with 8% mandarin peels in mono culture as well as in co culture (Table 5).

In 1% to 10% mandarin peels concentration LiP activity in the *D. confragosa* varied from 2.51 to 2.68 EU/L/Min and *I. lacteus* showed an absence of LiP activity where as in coculture of *I. lacteus* and *D. confragosa* it ranges from 2.97 to 3.07

EU/L/Min which was found to be higher when compared with the control which contains no mandarin peels.

Conc of Mandarin peels	Fungal isolate	Enzyme					
		LiP EU/L/Min	MnP EU/L/Min	Lac EU/L/Min	AAO EU/L/Min	Xylanase EU/ml/Min	Cellulase EU/ml/Min
Cont	IL	0	0.75±0.06	1.94±0.012	44.12±0.13	5.26±0.49	0
	DC	2.47±0.03	1.75±0.06	1.07±0.017	24.42±0.11	12.83±0.90	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.87±0.06	2.43±0.05	73.61±0.11	8.57±0.14	0
	DC	2.51±0.06	1.90±0.04	1.63±0.08	49.91±0.21	14.59±0.10	0
	IL+DC	2.97±0.09	2.00±0.07	5.50±0.25	65.41±0.15	23.07±0.10	0
2%	IL	0	1.02±0.04	2.82±0.04	79.89±0.17	9.41±0.10	0
	DC	2.58±0.06	1.93±0.04	1.86±0.05	64.26±0.11	17.79±0.15	0
	IL+DC	3.14±0.06	2.07±0.06	6.26±0.04	97.92±0.11	24.14±0.16	0
3%	IL	0	1.14±0.06	2.93±0.05	85.09±0.12	10.89±0.10	0
	DC	2.61±0.09	1.98±0.09	2.03±0.07	80.67±0.10	19.21±0.11	0
	IL+DC	3.26±0.06	2.13±0.06	6.47±0.05	119.45±0.14	27.30±0.11	0
4%	IL	0	1.20±0.06	3.65±0.06	140.25±0.13	13.74±0.12	0
	DC	2.72±0.06	2.06±0.07	2.38±0.07	88.61±0.12	20.31±0.10	0
	IL+DC	3.32±0.06	2.32±0.6	6.94±0.05	156.48±0.17	25.06±0.13	0
5%	IL	0	1.35±0.03	3.85±0.08	142.40±0.12	14.57±0.10	0
	DC	2.76±0.06	2.08±0.06	3.09±0.05	92.08±0.15	21.48±0.10	0
	IL+DC	3.30±0.07	2.62±0.04	8.45±0.08	174.61±0.12	30.08±0.16	0
6%	IL	0	**1.51±0.06**	4.25±0.05	**201.87±0.19**	**15.81±0.12**	0
	DC	**2.93±0.09**	**2.26±0.06**	5.28±0.10	**143.10±0.14**	**22.81±0.12**	0
	IL+DC	**3.60±0.09**	**2.79±0.04**	11.33±0.08	**218.29±0.13**	**39.42±0.11**	0
8%	IL	0	1.07±0.06	**4.75±0.09**	178.17±0.12	12.62±0.16	0
	DC	2.75±0.09	2.13±0.06	**7.05±0.05**	100.59±0.19	22.38±0.12	0
	IL+DC	3.28±0.09	2.40±0.07	**12.52±0.06**	181.90±0.11	26.29±0.12	0
10%	IL	0	0.86±0.09	4.42±0.07	175.41±0.11	11.03±0.14	0
	DC	2.68±0.09	2.05±0.04	6.17±0.10	96.65±0.10	22.07±0.10	0
	IL+DC	3.07±0.06	2.11±0.09	11.60±0.05	150.84±0.13	23.55±0.21	0

Table 5 Effect of different concentrations of Mandarin peels on ligninolytic enzyme activity

The maximum enhancement of LiP activity was found in 6% mandarin peels concentration in which *D. confragosa* produced 2.93 EU/L/Min and co culture of *I. lacteus* and *D. confragosa* produced 3.60 EU/L/Min (Figure 1 C).

Enhanced level of MnP activity was observed in the range of 1% to 10% mandarin peels and in 10% also MnP activity was found to be higher than observed in the control. *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* revealed the highest level of MnP activity 1.51, 2.26 and 2.79 EU/L/Min respectively in 6% concentration of mandarin peels. The activity of MnP enzyme declined with an increased concentration of mandarin peels in the medium (Figure 2 C).

The maximum laccase activity was expressed in 8% mandarin peels in both monoculture and co culture. Laccase activity enhanced from 1% to 8% concentration after which it declined in 9% and 10%. However the Laccase activity observed in 10% was still higher than the control (Figure 3 C). In 8% concentration of mandarin peels laccase activity by *I. lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were 4.75, 7.05 and 12.52 EU/L/Min respectively which represented the highest activity amongst all other concentrations used.

In case of AAO activity the comparison between 1% to 10% concentrations of mandarin peels the accumulation of AAO activity was detected in 6% after that decline was noted till 10% but the activity measured in 10% was still higher than control. In 6% concentration of mandarin peels *I.lacteus, D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were producing 201.87, 143.10 and 218.29 EU/L/Min respectively which proved to be the highest activity amongst all other concentrations used (Figure 4 C).

Concentration of Mandarin peels	Fungal isolate	Enzyme					
		LiP	MnP	Lac	AAO	Xylanase	Cellulase
Cont	IL	0	0.50±0.04	1.26±0.010	44.12±0.13	18.11±1.03	0
	DC	1.76±0.06	1.00±0.06	0.99±0.019	24.42±0.11	13.86±1.32	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.60±0.05	2.36±0.06	73.61±0.11	20.45±0.15	0
	DC	1.88±0.06	1.10±0.04	1.53±0.05	49.91±0.21	15.75±0.21	0
	IL+DC	2.97±0.09	2.00±0.07	5.50±0.25	65.41±0.15	23.07±0.10	0
2%	IL	0	0.69±0.06	2.48±0.05	79.89±0.17	22.12±0.15	0
	DC	1.94±0.06	1.24±0.04	1.79±0.05	64.26±0.11	18.52±0.11	0
	IL+DC	3.14±0.06	2.07±0.06	6.26±0.04	97.92±0.11	24.14±0.16	0
3%	IL	0	0.75±0.06	2.69±0.05	85.09±0.12	23.24±0.19	0
	DC	1.98±0.09	1.38±0.06	1.86±0.05	80.67±0.10	20.08±0.11	0
	IL+DC	3.26±0.06	2.13±0.06	6.47±0.05	119.45±0.14	27.30±0.11	0
4%	IL	0	0.81±0.06	3.00±0.08	140.25±0.13	24.75±0.11	0
	DC	2.07±0.06	1.50±0.07	2.31±0.07	88.61±0.12	22.01±0.14	0
	IL+DC	3.32±0.06	2.32±0.6	6.94±0.05	156.48±0.17	25.06±0.13	0
5%	IL	0	1.35±0.03	3.51±0.06	142.40±0.12	29.85±0.20	0
	DC	2.15±0.05	2.08±0.06	2.80±0.07	92.08±0.15	23.31±0.14	0
	IL+DC	3.30±0.07	2.62±0.04	8.45±0.08	174.61±0.12	30.08±0.16	0
6%	IL	0	**1.04±0.09**	3.81±0.07	**140.74±0.15**	**32.31±0.20**	0
	DC	2.26±0.09	**1.78±0.06**	4.89±0.05	**129.14±0.14**	**24.03±0.21**	0
	IL+DC	**3.60±0.09**	**2.79±0.04**	11.33±0.08	**218.29±0.13**	**39.42±0.11**	0
8%	IL	0	0.81±0.06	**4.14±0.09**	178.17±0.12	22.20±0.19	0
	DC	2.11±0.09	1.46±0.09	**6.91±0.05**	100.59±0.19	22.42±0.13	0
	IL+DC	3.28±0.09	2.40±0.07	**12.52±0.06**	181.90±0.11	26.29±0.12	0
10%	IL	0	0.77±0.09	3.88±0.06	175.41±0.11	21.20±0.16	0
	DC	2.07±0.09	1.36±0.04	5.90±0.05	96.65±0.10	22.84±0.10	0
	IL+DC	3.07±0.06	2.11±0.09	11.60±0.05	150.84±0.13	23.55±0.21	0

Table 6 Effect of different concentrations of Mandarin peels on ligninolytic enzyme activity after 20 days of incubation period

The results of xylanase underlines that the most optimum concentration of mandarin peels suitable for xylanase production found to be was 6% after which clear

decline in the xylanase activity was noted up to 10% but the activity reported in 10% concentration was higher as compared to control. *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were producing 15.81, 22.81 and 39.42 EU/ml/Min respectively in 6% of mandarin peels which was the highest activity amongst all other concentrations (Figure 5 C). Amongst all the ligninolytic and xylanolytic enzymes studied a significant activity of AAO and xylanase has been observed in both the mono and cocultures.

Table 6 represented enhancement in lignocellulolytic enzyme activity after 20 days of incubation period for monoculture and coculture both. Coculture showed 1.5 to 1.6 fold enhancement in all the enzyme activities compared to monoculture of *D. confragosa* whereas nearly threefold enhancement in MnP and laccase enzyme activity and 1.55 to 1.22 fold enhancement in AAO and xylanase enzyme activity were observed compared to monoculture of *I. lacteus*.

These results revealed that 6% mandarin peels was best for all the lignocellulolytic enzyme activity except laccase which enhanced the best in 8% mandarin peels and 20 days proved to be best incubation period.

(d) Influence of Ash gourd (AG) pulp on enzyme production

To elucidate the role of different concentration of Ash gourd pulp on the lignocellulolytic enzyme production MEB medium was supplemented with 1%, 2%, 3%, 4%, 5%, 6%, 8% and 10 % ash gourd and after desired incubation period the culture filtrates were collected and enzyme activity was assayed. All the concentration tested ensured the significant accumulation of lignocellulolytic enzyme activity when compared with the control medium.

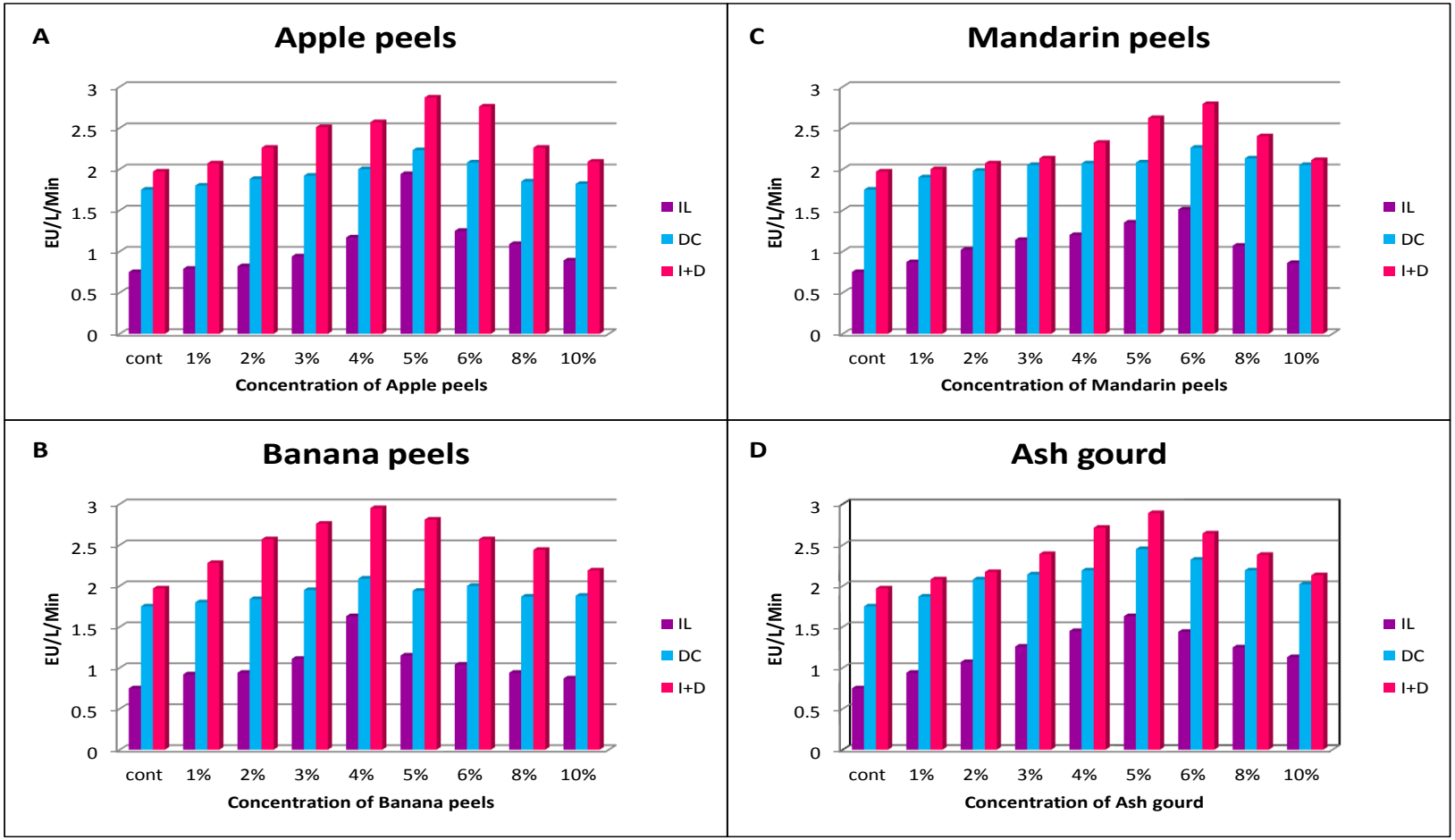


Fig. 2 Effect of different substrate concentrations on the activity of MnP

The maximum enhancement in the lignocellulolytic enzyme activity was recorded in 5% after which decline in the enzyme production was noted up to 10% which was still higher than obtained in the control which was not provided with ash gourd pulp (Table 7).

Maximum LiP, MnP and laccase activity was revealed when media was supplemented with 5% ash gourd used in monoculture and co culture. *I. lacteus* showed an absence of LiP activity throughout the experiment where as *D. confragosa* displayed maximum accumulation of LiP activity in media containing 5% ash gourd concentration after which a decline in the activity was noticed and in co culture of *I. lacteus* and *D. confragosa* also 5% ash gourd resulted in the maximum production of LiP enzyme. As the concentration of ash gourd increased from 5% to 10% decrease in the enzyme activity was observed which was still higher compared to the control (Figure 1 D).

In mono culture of *I.lacteus* MnP activity was enhanced from 0.94 EU/L/Min to 1.63 EU/L/Min in the range of 1% to 5% concentration of ash gourd after that MnP activity was declined up to 1.13 EU/L/Min in 10% ash gourd. *D. confragosa* showed maximum MnP enzyme production 2.45 EU/L/Min in media supplemented with 5% ash gourd pulp which was decreased up to 2.02 EU/L/Min in 10% but higher than control 1.75 EU/L/Min. The co culture of *I.lacteus* and *D. confragosa* favoured the highest MnP activity in 5% 2.89 EU/L/Min which further decline in 10% ash gourd up to 2.13 EU/L/Min (Figure 2 D).

I. lacteus, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* indicated the highest level of laccase activity 4.94, 7.32 and 12.74 EU/L/Min respectively in 5% concentration (Figure 3D). In 5% concentration of ash gourd AAO activity indicated by *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D.*

confragosa were 125.02, 85.53 and 143.06 EU/L/Min respectively which represented the highest activity amongst all other concentrations used (Figure 4 D).

The results of xylanase showed that 5% ash gourd was the most optimum concentration for xylanase production after that decline in the xylanase activity was noted up to 10% but it was higher as compared to control. *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were producing 17.23, 23.17 and 40.96 EU/ml/Min respectively in 5% of ash gourd pulp which was the highest activity observed amongst all other concentrations (Figure 5 D).

The activity of LiP, MnP and laccase, AAO and xylanase enzyme declined even after the concentration of ash gourd increased in the medium up to 10%. Though the activity showed a decline it was more than the control (ie without ash gourd supplementation).

Compared to *D. confragosa*, *I. lacteus* showed more xylanase and AAO activity and the coculture produces more than monoculture with ash gourd, maximum AAO activity is observed with 5% concentration which is fourfold increase. 10% concentration shows a decline in the activity, but still it is four fold more than the control in which ash gourd is not supplemented to the medium. Xylanase activity was increased nearly double fold as compared to control in monoculture and coculture both.

Monoculture and coculture when incubated for 20 days significant enhancement in all the lignocellulolytic enzyme activity were obtained which was represented in Table 8. Results indicated that approximately 1.5 to 1.8 fold enhancement in all the lignocellulolytic enzyme activities were noticed in coculture compared to monoculture of *D. confragosa* whereas nearly threefold enhancement in MnP and laccase enzyme activity and single fold enhancement in AAO and xylanase enzyme activity were observed.

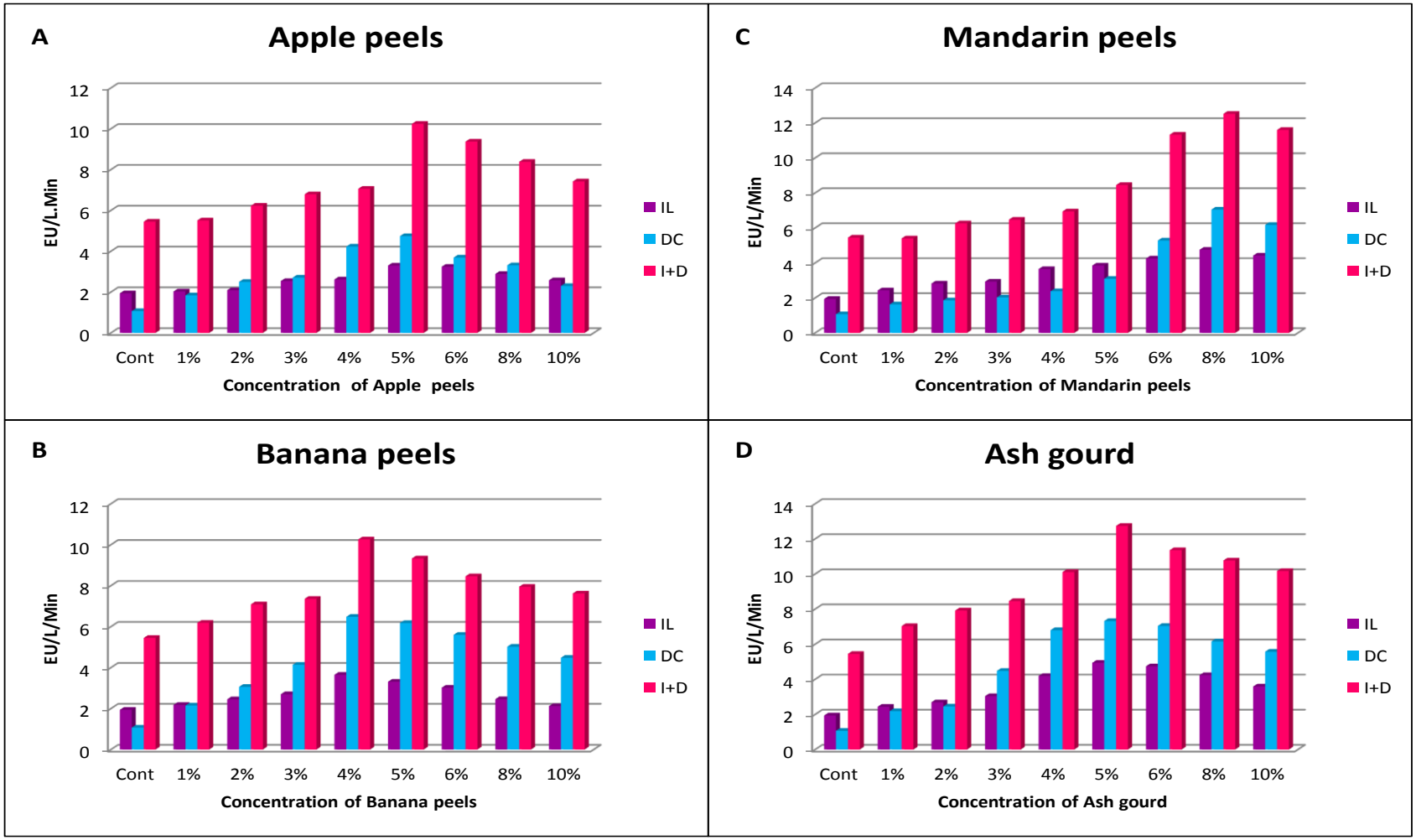


Fig. 3 Effect of different substrate concentrations on the activity of Laccase

Conc of Ash gourdpulp	Fungal isolate	Enzyme					
		LiP EU/L/Min	MnP EU/L/Min	Laccase EU/L/Min	AAO EU/L/Min	Xylanase EU/ml/Min	Cellulase EU/ml/Min
Cont	IL	0	0.75±0.06	1.94±0.012	44.12±0.13	5.26±0.49	0
	DC	2.47±0.03	1.75±0.06	1.07±0.017	24.42±0.11	12.83±0.90	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.94±0.06	2.44±0.05	44.10±0.13	10.00±0.10	0
	DC	2.49±0.04	1.87±0.06	2.19±0.06	34.81±0.14	14.33±0.12	0
	IL+DC	3.05±0.09	2.08±0.06	7.03±0.06	56.24±0.11	27.13±0.16	0
2%	IL	0	1.07±0.06	2.69±0.05	60.56±0.11	10.94±0.10	0
	DC	2.59±0.09	2.08±0.06	2.45±0.08	59.21±0.11	15.84±0.10	0
	IL+DC	3.20±0.06	2.17±0.04	7.92±0.05	60.72±0.18	29.24±0.11	0
3%	IL	0	1.26±0.06	3.04±0.09	66.31±0.13	12.20±0.12	0
	DC	2.71±0.06	2.14±0.06	4.48±0.08	72.28±0.18	16.69±0.12	0
	IL+DC	3.31±0.04	2.39±0.06	8.46±0.07	73.05±0.15	32.10±0.17	0
4%	IL	0	1.45±0.06	4.19±0.08	93.61±0.13	15.20±0.12	0
	DC	2.87±0.06	2.19±0.06	6.80±0.05	88.0±0.11	19.61±0.11	0
	IL+DC	3.37±0.09	2.71±0.06	10.11±0.05	97.80±0.16	34.98±0.18	0
5%	IL	0	**1.63±0.06**	**4.94±0.05**	**125.02±0.10**	**17.23±0.12**	0
	DC	**3.05±0.07**	**2.45±0.06**	**7.32±0.05**	**85.53±0.11**	**23.17±0.11**	0
	IL+DC	**3.51±0.06**	**2.89±0.06**	**12.74±0.10**	**143.06±0.10**	**40.96±0.11**	0
6%	IL	0	1.44±0.06	4.74±0.06	107.23±0.15	16.48±0.14	0
	DC	2.76±0.06	2.32±0.06	7.04±0.05	84.15±0.15	22.06±0.13	0
	IL+DC	3.29±0.09	2.64±0.06	11.36±0.08	112.19±0.12	36.34±0.17	0
8%	IL	0	1.25±0.06	4.24±0.04	79.78±0.12	15.89±0.15	0
	DC	2.61±0.04	2.19±0.06	6.16±0.04	78.42±0.11	21.36±0.10	0
	IL+DC	3.14±0.06	2.38±0.06	10.77±0.10	109.33±0.11	29.25±0.19	0
10%	IL	0	1.13±0.06	3.59±0.05	76.29±0.11	13.02±0.13	0
	DC	2.57±0.06	2.02±0.06	5.57±0.06	74.85±0.12	20.96±0.12	0
	IL+DC	2.89±0.06	2.13±0.06	10.17±0.07	99.96±0.10	27.72±0.17	0

Table 7 Effect of different concentrations of Ash gourd pulp on ligninolytic enzyme activity

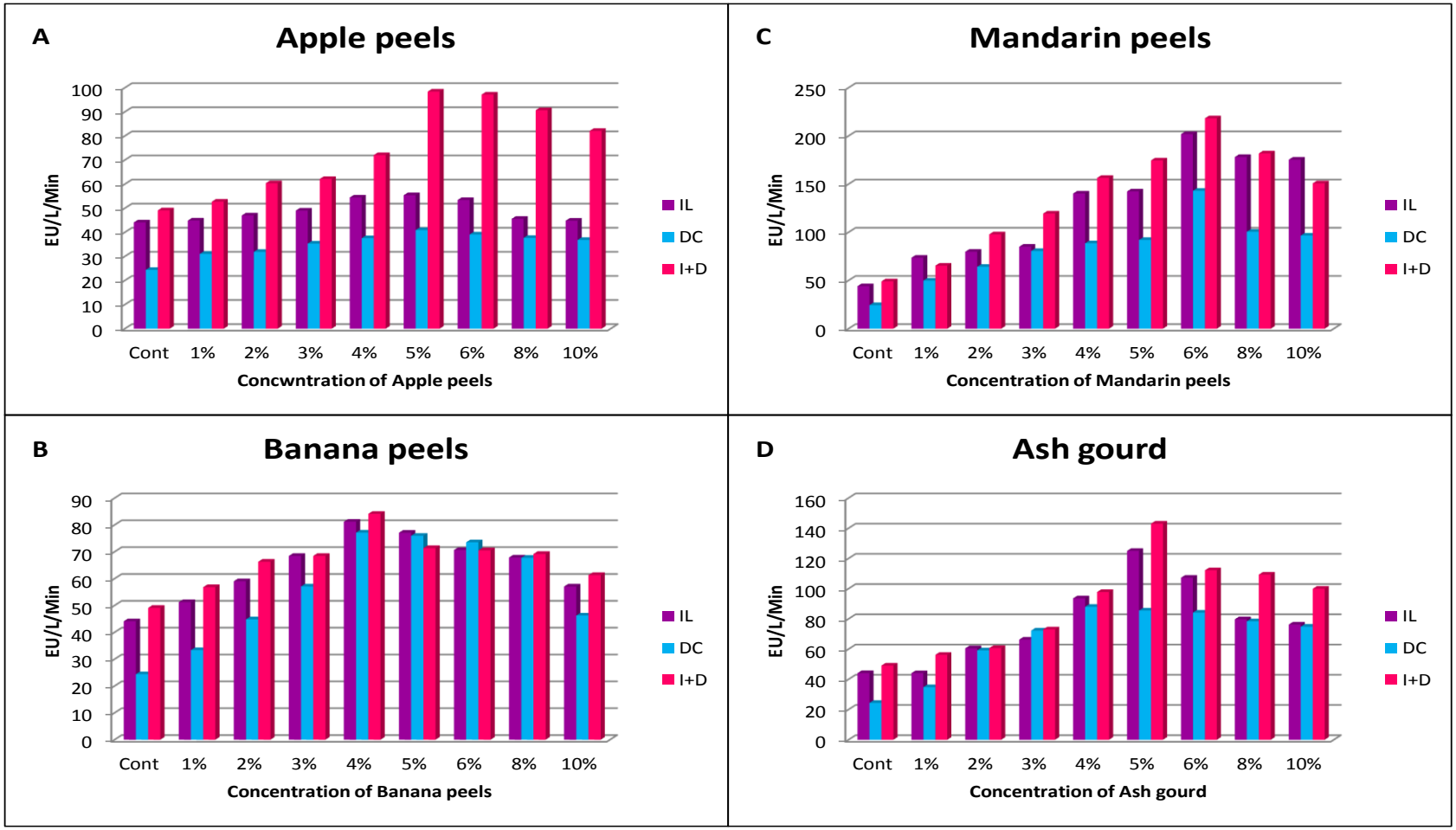


Fig. 4 Effect of different substrate concentrations on the activity of AAO

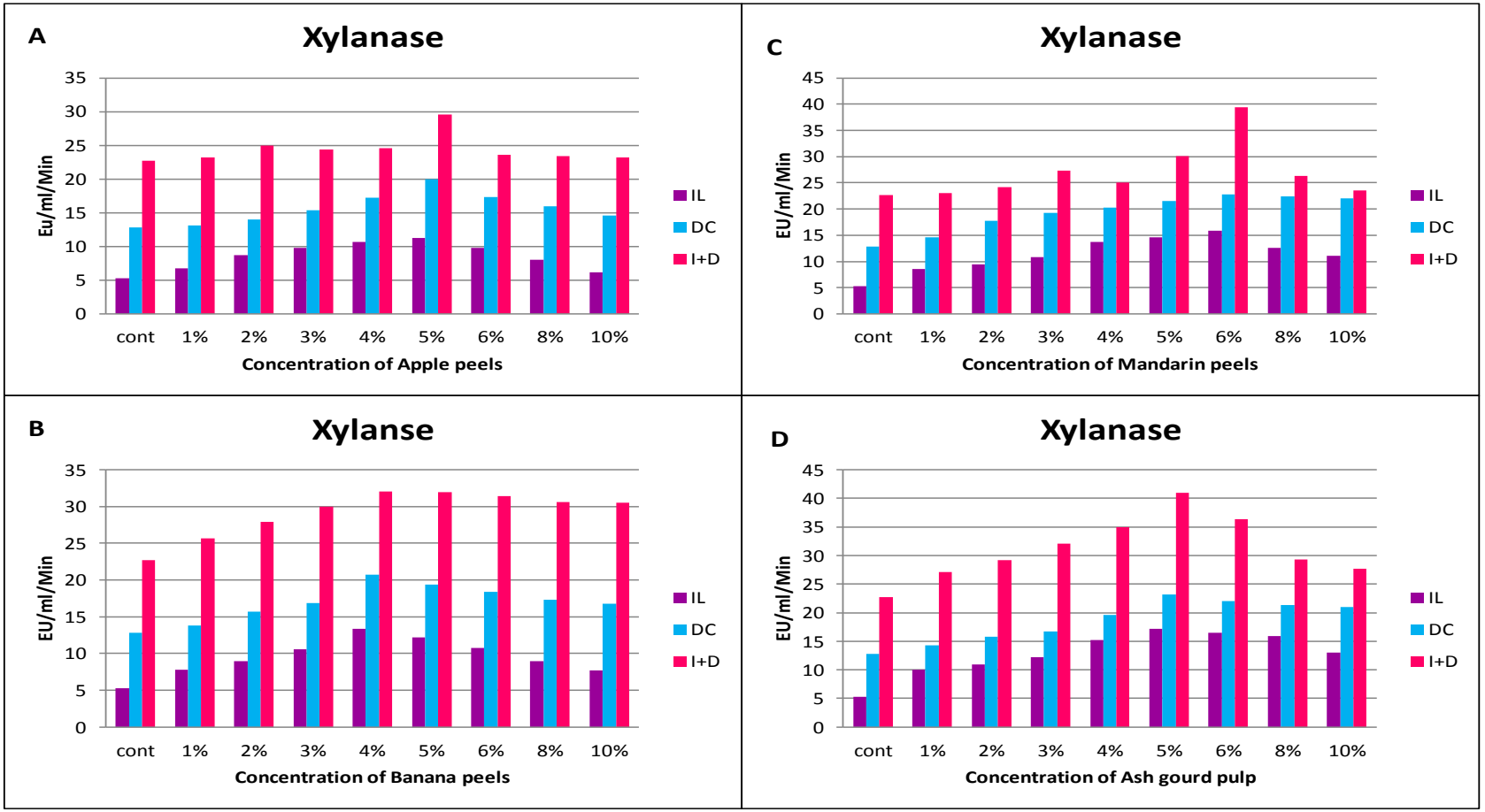


Fig. 5 Effect of different substrate concentrations on the activity of Xylanase

Concentration of Ash gourd	Fungal isolate	Enzyme					
		LiP	MnP	Lac	AAO	Xyl	Cellulase
Cont	IL	0	0.50±0.04	1.26±0.010	44.12±0.13	18.11±1.03	0
	DC	1.76±0.06	1.00±0.06	0.99±0.019	24.42±0.11	13.86±1.32	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.64±0.04	2.10±0.05	44.10±0.13	21.13±0.11	0
	DC	1.90±0.05	1.26±0.06	2.08±0.08	34.81±0.14	15.28±0.10	0
	IL+DC	3.05±0.09	2.08±0.06	7.03±0.06	56.24±0.11	27.13±0.16	0
2%	IL	0	0.75±0.06	2.43±0.09	60.56±0.11	23.19±0.11	0
	DC	2.01±0.06	1.39±0.06	2.25±0.08	59.21±0.11	16.67±0.14	0
	IL+DC	3.20±0.06	2.17±0.04	7.92±0.05	60.72±0.18	29.24±0.11	0
3%	IL	0	0.87±0.06	2.75±0.06	66.31±0.13	25.39±0.12	0
	DC	2.08±0.06	1.50±0.06	4.19±0.08	72.28±0.18	19.90±0.12	0
	IL+DC	3.31±0.04	2.39±0.06	8.46±0.07	73.05±0.15	32.10±0.17	0
4%	IL	0	0.94±0.06	3.89±0.06	93.61±0.13	28.36±0.17	0
	DC	2.19±0.06	1.59±0.09	6.23±0.07	88.0±0.11	24.17±0.16	0
	IL+DC	3.37±0.09	2.71±0.06	10.11±0.05	97.80±0.16	34.98±0.18	0
5%	IL	0	**1.02±0.09**	**4.36±0.05**	**125.02±0.10**	**34.66±0.13**	0
	DC	**2.38±0.06**	**1.88±0.06**	**7.04±0.07**	**85.53±0.11**	**26.20±0.11**	0
	IL+DC	**3.51±0.06**	**2.89±0.06**	**12.74±0.10**	**143.06±0.10**	**40.96±0.11**	0
6%	IL	0	0.96±0.07	4.20±0.06	107.23±0.15	34.12±0.14	0
	DC	2.26±0.06	1.69±0.06	6.43±0.05	84.15±0.15	25.30±0.21	0
	IL+DC	3.29±0.09	2.64±0.06	11.36±0.08	112.19±0.12	36.34±0.17	0
8%	IL	0	0.90±0.06	4.10±0.04	79.78±0.12	29.66±0.13	0
	DC	2.14±0.06	1.50±0.06	6.16±0.04	78.42±0.11	24.29±0.16	0
	IL+DC	3.14±0.06	2.38±0.06	10.77±0.10	109.33±0.11	29.25±0.19	0
10%	IL	0	0.82±0.06	3.27±0.05	76.29±0.11	28.41±0.12	0
	DC	2.00±0.06	1.38±0.06	5.32±0.06	74.85±0.12	22.39±0.11	0
	IL+DC	2.89±0.06	2.13±0.06	10.17±0.07	99.96±0.10	27.72±0.17	0

Table 8 Effect of different concentrations of Ashgourd pulp on lignocellulolytic enzyme activity after 20 days of incubation period

It was very clearly depicted from the results that 5% Ashgourd pulp proved to be best substrate and 20 days was proved to be the best incubation period as significantly higher enhancement in all the enzyme activities were obtained.

3.4.3 Discussion

There has been growing interest in studying the lignocellulolytic enzymes of white rot fungi, not only from the stand point of comparative biology but also with the hope to find better enzyme producers for use in various biotechnological applications. Owing to various biotechnological applications, studies on such enzymes producing organisms have been intensified in recent years.

Lignocellulolytic enzyme production by wood rotting white rot fungi is a common phenomenon involving the interaction between composition of media for the enzyme production and physiology of the fungal isolate used (Bakkiyaraj *et al* 2013). The increasing demand for these enzymes requires their production to be economic which can be achieved by using inexpensive raw materials and chemical inducers to make the entire process cost effective. (Terron *et al* 2004). There are many inducers which can enhance the production of enzymes (Assavanig *et al* 1992, Grazillo *et al* 1998, Lu *et al* 1996, Nyanhongo *et al* 2002, Aslam and Ashger *et al* 2011) as enhancers molecules resembles the structure derived from lignin molecule (Marbach *et al* 1985, Farnet *et al* 1999).

In the present study *I. lacteus, D. confragosa* and their coculture found to be capable of producing all the major lignocellulolytic enzymes. To study the effects of different chemical enhancers, the experiments were carried out at 25˚C temperature and pH 5 for the desired incubation of time. In order to select the best enhancer concentration of ethanol, veratryl alcohol, xylidine, yeast extract and peptone comparative lignocellulolytic enzyme production was studied in various concentrations of different inducers.

In the present study Ethanol in 4% concentration, Veratryl alcohol in 12mM and 16mM concentration, Xylidine in 30µM concentration proved to be best inducers

for all the enzymes in both mono cultures and coculture except ethanol which gives best enhancement in 1% in DC. In *I. lacteus* LiP activity was not enhanced by any of the chemical inducers used. 4% ethanol, 12mM Veratryl alcohol 30µM Xylidine proved to be best enhancers and can be used for the further bio pulping experiment.

In case of *I. lacteus* and *I. lacteus + D. confragosa* AAO activity was enhanced only when the media was supplemented with veratryl alcohol as an inducer where as not enhanced by ethanol and xylidine. Laccase enzyme activity was enhanced the most when the media was supplemented with veratryl alcohol in case of *D. confragosa* and not enhanced by other inducer. When the media was supplemented with veratryl alcohol the enhancement could be achieved by using a relatively low concentration (12mM) in comparison to earlier studies (40mM) where enhancement was occurred (Dekker and Barbosa 2001).Some authors have suggested that the increase in enzyme production in the presence of veratryl alcohol is due to the fact that it protects the enzyme from H_2O_2 dependent inactivation (Tonon and Odier, 1988).

In *I. lacteus* MnP activity enhanced stimulated highest (128.00%) when 4% ethanol was supplemented in the medium and moderately enhanced by 30µM xylidine (101.33%) and least by 12mM veratryl alcohol (92.01%) which completely contrasted with xylanase. Laccase enzyme activity was enhanced the highest by30µM xylidine (184.02%) and least by 12mM veratryl alcohol (15.46%) where as 4% ethanol enhanced moderate (34.53%) laccase activity. Xylanase activity was enhanced the most by 12mM veratryl alcohol (105.70%) and 105.13% by 30µM xylidine and 4% ethanol enhanced only 101.90% enhancement in xylanase activity which is similar to the results found in laccase activity. AAO enzyme was enhanced 39.37% only when veratryl alcohol was present as an inducer and not enhanced by xylidine and ethanol

where as LiP activity was not enhanced by any of the inducers used in the present study.

In *D. confragosa* enhancement of LiP activity was highest (77.73%) in 12mM veratryl alcohol and 4% ethanol (76.11%) and the enhancement was minimum in xylidine (41.17%). MnP enzyme activity was enhanced the most (65.14%) with ethanol and least by xylidine (8.57%) while veratryl alcohol showed (43.42%) intermediate enhancement. Laccase enzyme activity was enhanced (14.95) only when the media was supplemented with 12mM veratryl alcohol and not by any other inducers. AAO activity was stimulated maximum (92.87%) in 30µM moderately (21.45%) by 12mMveratryl alcohol and least by 1% ethanol (15.76%). Similar pattern of enhancement in xylanase was also observed where 49.34% stimulation was occurred by 30µM xylidine followed by 16.45% by veratryl alcohol and 15.14% by 1% ethanol. The enhancement observed in xylanase and AAO was completely in contrast to LiP and MnP.

In coculture of *I. lacteus* and *D. confragosa* LiP activity was stimulated 69.70% in veratryl alcohol containing medium, moderately (64.96%) enhanced by ethanol and least by xylidine (35.03%). MnP activity enhanced maximum 67.42% in 30µM xylidine and least in 12mM veratryl alcohol 37.05% and moderate by 4% ethanol 49.74%. Laccase enzyme activity was enhanced maximum in 30µM xylidine followed by 4% ethanol 25.50% and 24.03% by 12mM veratryl alcohol which is exactly similar to the enhancement observed in *I. lacteus*. Maximum enhancement of AAO enzyme activity was noticed only in 12mM veratryl alcohol which was not stimulated by any other inducers which is also as similar observed in *I. lacteus*. Xylanase enzyme activity was enhanced maximum in 16mM veratryl alcohol (90.21%) followed by 30µM xylidine (44.70%) and 4% ethanol (10.62%).

In *I. lacteus* and coculture of *I. lacteus* + D. *confragosa* maximum enhancement in all the enzymes occurred in xylidine and veratryl alcohol where as *D. confragosa* showed maximum enhancement when the media was supplemented with veratryl alcohol.

Many reports are available on enhancement of laccase activity in fungal cultures can be achieved by the addition of different aromatic compounds as inducers to the media (Iqbal *et al* 2011, Patel *et al* 2009, Prasad *et al* 2005). The addition of xylidine to the culture media resulted accumulation of laccase production but at very high concentrations xylidine could be toxic to organisms, leading to the reduction in cell growth and enzyme production (Janusz *et al* 2006, Prasad *et al* 2005).

In the present study it was observed that 30 µM xylidine concentration found to be optimum for ligninolytic enzymes in both monoculture and co culture. The obtained results were in contrast to Prasad *et al* (2005) as they have suggested that 1.0 mM xylidine is optimum for ligninolytic enzyme production which was quite higher than used in the present study. The difference in the optimum concentration for enhancement of enzymes was observed due to capability of fungal isolate to use xylidine as excess amount of xylidine proved to be toxic (Patrick *et al* 2011).

Xavier *et al* (2007) compared effects of different inducers concentrations for the induction of laccase in *Trametes versicolor*. They found that best concentration of xylidine to induce laccase was 30µM, providing the maximum laccase activity 1583U/L in *T. versicolor*. In the same study 2mM veratryl alcohol produced 345U/L of laccase indicating maximum laccase enhancement occurred when xylidine was provided in the medium. The present study is in accordance as 30µM xylidine reported to produce maximum enhancement in laccase activity 5.51EU/L/Min in *I. lacteus* and 6.90 EU/L/Min in *I. lacteus + D. confragosa* which found to be very low

compared to *T. versicolor* of their study and 16mM veratryl alcohol produced 2.2U/L, 1.23U/L and 6.76U/L in *I. lacteus*, *D. confragosa*, and *I. lacteus* + *D. confragosa* respectively indicating xylidine gives better enhancement compared to veratryl alcohol.

Both the inducers veratryl alcohol and xylidine containing aromatic ring and resulting in improved production of laccase as well as other lignocellulolytic enzymes also. The results obtained in the present study are showing different order compared to Lee *et al* 1999 where veratryl alcohol found to be the most effective inducer compared to xylidine and ethanol.

In the study of Lee *et al* (1999) ethanol was also reported as an inducer of laccase even if it is not an aromatic compound. In the present study also addition of ethanol concentration was tested and 1% ethanol showing maximum enhancement in *D. confragosa* while 4% ethanol provide the most induction in *I. lacteus* and *I. lacteus* + *D. confragosa* which was completely in accordance with Lee *et al* 1999 as *T. versicolor* found to be enhanced most in 4% concentration. Their study determined laccase activity was not inhibited by ethanol in the range of 0% to 5% as it did not affect the enzyme activity directly. Ethanol is responsible to change the membrane permeability if provided in the concentration above 40g/L and inhibits the cell growth may lead to decrease in the enzyme activity.

Elisashvili *et al* (2006) studied the effect of various inducers/stimulators to enhance the lignocellulolytic enzyme production of *Pleurotus dryinus* IBB-903 and the result indicated that 1mM xylidine addition increased the laccase up to 49% and 42% increase in MnP enzyme activity. In the present study 30µM xylidine showed 101.33% enhancement of MnP activity and 184.02% enhancement in laccase activity with *I. lacteus* which is quite in higher amount than their study at lower concentration.

In the same study they have analyzed the concentration of ethanol (2.5% and 5%) as a carbon source and result indicated laccase and xylanase enzyme were inhibited where as in the present study 1% ethanol stimulated all the enzymes except laccase in *D. confragosa* and 4% ethanol concentration showed enhancement in all the enzymes except AAO in *I. lacteus* and coculture of *I. lacteus* + *D. confragosa* which is completely contradictory to their investigations.

Although *I. lacteus, D. confragosa* and *I. lacteus* + *D. confragosa* showed differences in lignocellulolytic enzyme production in response to various concentration of chemical inducers xylidine appeared to be the most convenient inducer for enhanced enzyme production followed by veratryl alcohol and ethanol for both the monocultures and coculture.

In the study of Nyanhongo *et al* (2002) effect of various inducers viz Ferulic acid, Veratryl alcohol, xylidine, gallic acid, copper sulphate, syringic acid and caffeic acid were tested on laccase production in *Trametes modesta* and the maximum laccase induction was observed in xylidine followed by veratryl alcohol which is exactly similar to the present study.

Alcantara *et al* (2007) investigated the effect of xylidine, veratryl alcohol and copper sulphate on enhanced laccase production of *Coriolopsis rigida* and results showed that the maximum enhancement of laccase was observed in 10mM xylidine followed by 20mM veratryl alcohol and 2mM $CuSO_4$ exactly the same result was also observed in the present study.

Many previous reports have shown that nature and concentration of nitrogen sources play a crucial role in regulating lignocellulolytic enzyme production in white rot basidiomycetes (Zakariashvili and Elisashvili 1993, Sun *et al* 2004, Rosales *et al* 2005, Galhaup *et al* 2002). The present study was further aimed to investigate the

effect of different concentration of nitrogen source on the enzyme production so yeast extract and peptone were selected as the nitrogen source and in order to optimize the best concentration various concentrations (0.1g/L, 0.2g/L, 0.4g/L, 0.6g/L and 0.8g/L) were checked for monocultures as well as cocultures.

The results of present study indicated that when yeast extract and peptone were supplemented as nitrogen source in the media, growth of fungi was accompanied in monocultures as well as in coculture. All fungi grew well and enhanced significant enzyme activities when additional nitrogen was present in the medium. By gradually increasing concentration from 0.1g/L to 0.8g/L enzyme activity also stimulated and once the maximum enhancement of activity occurred it tend to decline in 0.8g/L. the maximum enhancement of all the enzyme activities in 0.6g/L concentration of yeast extract and 0.4g/L in peptone.

LiP activity was not enhanced in *I .lacteus* even the media was supplemented with additional nitrogen sources but in *D. confragosa* it enhanced 31.57% by 0.6g/L yeast extract and 22.67% by addition of 0.4g/L peptone. In coculture of *I. lacteus* and *D. confragosa* it enhanced up to 32.84% and 18.61% in 0.6g/L yeast extract and 0.4g/L peptone respectively.

In *I. lacteus* MnP enzyme activity was enhanced the most (109.3%) with 0.6g/L yeast extract and least 58.66% with 0.4g/L peptone. In *D. confragosa* MnP activity was stimulated almost at the same rate 18.85% and 18.87% with addition of 0.6g/L yeast extract and 0.4g/L peptone respectively. Coculture of *I. lacteus* and *D. confragosa* maximum MnP enhancement was achieved (47.71%) in 0.6g/L and 18.78% with 0.4g/L peptone.

Laccase activity was enhanced very little (5.60%) with 0.4g/L peptone and not enhanced with addition of yeast extract in the monoculture of *D. confragosa*. In *I.*

lacteus it enhanced 8.24% and 22.16% in yeast extract and peptone respectively where as in coculture it enhanced 23.48% in yeast extract and 9.54% in peptone.

AAO activity was not enhanced in *I. lacteus* and its coculture when yeast extract was supplemented in the medium whereas 12.85% increased in *I. lacteus* with peptone. In *D. confragosa* 13.39% increase in AAO activity was noticed with 0.6g/L yeast extract and 6.83% with 0.4g/L peptone.

Xylanase activity was enhanced moderate in coculture of *I. lacteus* + *D. confragosa* 73.85% with 0.4g/L peptone concentration. In monoculture of *I. lacteus* 106.65% and 110.45 % increase in xylanase activity was noticed in yeast extract and peptone respectively. *D. confragosa* showed 17.38% and 47.93% increase in xylanase activity with peptone and yeast extract respectively.

In general, the yield of ligninolytic and hydrolytic enzymes were enhanced by supplementation of medium with an additional nitrogen source. However comparison of the specific activities of the tested monocultures and cocultures evidenced that yeast extract proved to be better nitrogen source than peptone.

Kanwal and Reddy (2011) reported *Morchella crassipes* exhibited various growth patterns depending on the carbon and nitrogen sources used. Peptone and sodium nitrate served as the best nitrogen sources for the growth of fungus. Yeast extract showed less enhancement in *M. crassipes* which resembles to the present study, but this strain showed an absence of LiP and MnP which are present in *D. confragosa* and enhanced significantly with the yeast extract.

In the studies of Hou *et al* (2004) enhancement of laccase production by *Pleurotus ostreatus* 32 using various carbon and nitrogen sources were evaluated. The results indicated when 0.5g/L peptone and yeast extract were supplemented in the media laccase production was enhanced up to 143U/ml and 129U/L respectively

which was quite higher than the results obtained in the present study, might be due to the variation in the culture medium and conditions of the fungal isolate used.

Most of the studies were carried out on enhancement of ligninolytic enzymes and it was noted that the activity of ligninolytic enzymes of white rot fungi was stimulated in nitrogen rich media. The results of present studies indicated that nitrogen sources have a great influence on the production of xylanase also.

Qinnghe *et al* (2004) conducted study for optimization of the culture condition for xylanase in *Pleurotus ostreatus* using 5% wheat bran and 5% corn cob as a carbon source and Ammonium nitrate, Ammonium sulphate, yeast extract, tryptone, peptone and beef extract as nitrogen sources. The result indicated xylanase was enhanced maximum when peptone was added as a nitrogen source followed by beef extract and yeast extract. The results of present study indicated peptone was the best nitrogen source for the enhancement of xylanase enzyme in monoculture and coculture both followed by yeast extract which is exactly same as their study. In the same study various concentrations of peptone were also tested (0.20, 0.50, 0.80, 1.20%) to determine the additional levels of peptone on xylanase enhancement and xylanase activity was noticed maximum 19.98U/ml when 0.80% peptone was provided in the medium. In the present study *I. lacteus* showed 11.07EU/ml/Min in 0.6g/L peptone and coculture showed 39.43EU/ml/Min in 0.4g/L of peptone concentration which is higher compared to their study. Enhancement in the xylanase enzyme activity may be possible due to the fact that organic nitrogen contains various amino acids which can be directly absorbed by mycelia and leads to higher biomass production.

Study conducted by Tallapragada and Venkatesh (2011) described the effect of various carbon and nitrogen sources on the xylanase activity in *Aspergillus niger* under submerged fermentation and the results indicated that 14.37 units/ml xylanase

was produced with 2g/L yeast extract which was considered the best compared to peptone, ammonium and beef extract. The result of present study also indicated yeast extract and peptone as good nitrogen sources for xylanase activity and in agreement as 0.6g/L yeast extract produce 10.87, 18.98 and 29.57 EU/ml/Min in *I. lacteus*, *D. confragosa* and *I. lacteus* + *D. confragosa* respectively which was higher compared to their result because of different fungal isolate use in the present study.

The results of optimization experiments indicated 4% Ethanol, 12mM Veratryl alcohol, 30µM Xylidine concentration proved to be best inducers and yeast extract at 0.6g/L and peptone at 0.4g/L concentration as nitrogen source for all the enzymes in both mono cultures and coculture so can be selected for further experiments of biopulping where effect of supplementing the optimal conditions of enhancers concentration with *Eucalyptus* wood blocks and pulp were analyzed and compared with the normal enzyme production.

Cellulose, hemicellulose and lignin together form the complex lignocellulolytic structure. The complexity of hemicellulose, lignin, crystalline structure of cellulose and pectin make lignocellulolse highly insoluble and provide plant cell wall resistance to attack. Therefore delignification is required to demonstrate the lignin mesh and further liberate cellulose and hemicellulose from lignocellulose.

The white rot basidiomycetes played crucial role in the process of lignocellulose degradation by producing ligninolytic and hydrolytic enzymes simultaneously (Elissetche *et al* 2007, Sun *et al* 2004). The synergistic enzyme degradation mechanism helps these fungi to hydrolyse woody material and almost simple sugars for their metabolism and growth. Key extracellular enzymes of the

fungal ligninolytic system are laccase and two hemicellulose containing peroxidases: Lignin Peroxidase and Manganese Peroxidase.

In order to increase the production of lignocellulolytic enzymes several approaches need to be explored. Amongst all, supplementation of stimulators or inducers to the cultivation medium for significant enhancement of enzymes appeared to be most effective. However, many reports are available on addition of lignocellulosic materials to the nutrition medium stimulate the secretion of target enzymes in basidiomycetes fungi (Rosales *et al* 2005, Kapich *et al* 2004, Tsiklauri *et al* 1999).

In the present study the potential of different lignocellulosic materials like apple peels, banana peels, mandarin peels and ash gourd pulp with their various concentrations on the enzyme activity of white rot fungi in monocultures and coculture were studied. All the lignocellulosic materials promoted excellent growth of fungi. The data represented in Table 1 to 4 showed that the fungi exhibited different responses to the substrates used and enzyme yield significantly depended on the concentration of each substrate. The extracellular enzyme activities produced during fermentation of substrates varied among the monoculture and coculture of fungi.

Evaluation of lignocellulolytic enzyme activity showed that fruit residues proved to be the appropriate substrates for hydrolytic enzyme production as well as ligninolytic enzyme production simultaneously (Elisashvilli *et al* 2008, Rosales *et al* 2005, Songulashvili *et al* 2006). Fruit residues proved to be an appropriate substrate due to the presence of free mono and disaccharides, organic acids which ensured abundant growth of basidiomycetes fungi (Tsiklauri *et al* 1999, Elisashvilli *et al* 1992). In addition to this they also contain water soluble aromatic compounds like

flavones and flavonols which tend to induce ligninolytic enzyme synthesis (Elisashvilli *et al* 2006).

A substitution of apple peels in the concentration of 5%, banana peels in 4% concentration, mandarin peels at 6% and ash gourd pulp at 5% concentration caused the maximum enhancement of all enzyme activities. The enzyme activity increased with increase in the lignocellulosic substrate concentration because the content of soluble carbohydrates, aromatic compounds and micro elements increased during media sterilization which promotes better growth of fungi and enzyme production. Once the enzyme activity reached the peak after that gradual decline in the activity was noticed up to 10% which was still higher than that of enzyme activity in the control. Further increase in the substrate concentration after optimum concentration, the enzyme activity slowed down at higher concentration probably due to higher viscosity of medium, decreased aeration and mass exchange as indicated by Elisashvilli *et al* 2006. Cellulase activity was found to be absent in control as well as the medium supplemented with lignocellulosic materials.

A substitution of various concentrations of lignocellulosic materials in MEB medium caused significant increase in all the enzyme activity of monocultures and coculture except LiP in *I. lacteus* which was not stimulated by addition of any lignocellulosic material.

LiP enzyme activity by *D. confragosa* was enhanced maximum when medium was supplemented with Ash gourd pulp, moderately by Mandarin peels and least by banana peels. MnP activity was enhanced the most by ash gourd pulp in both the monocultures but in coculture banana peels showed maximum enhancement followed by ash gourd pulp while apple peels and mandarin peels provides the least

enhancement. In *I. lacteus* least enhancement of MnP enzyme activity was reported by apple peels which showed moderate enhancement in *D. confragosa*.

The highest enhancement of laccase and xylanase enzyme activity was observed by ash gourd pulp followed by mandarin peels, banana peels and apple peels in monoculture and coculture both. The least enhancement in laccase and xylanase was reported when the media was supplemented with apple peels and banana peels showed moderate enhancement in both the enzymes as compared to ash gourd pulp and mandarin peels.

AAO enzyme activity was induced the most when mandarin peels and ash gourd pulp were used as a substrate in monoculture and coculture both. In both the monocultures banana peels caused moderate enhancement and apple peels caused least enhancement where as in coculture banana peels provides least enhancement of AAO and apple peels was responsible for moderate enhancement.

Elisashvili *et al* (2006) reported mandarin peels to be an appropriate substrate for lignocellulolytic enzyme production as compared to tree leaves from the study in which various concentrations of mandarin peels and tree leaves (1%, 2%, 4%, 6%and 8%) were used as substrates for lignocellulolytic enzyme production by *Pleurotus dryinus*. Results indicated 2%, 4% and 6% mandarin peels concentration best for MnP, xylanase and laccase activity respectively. Results of the present study revealed 6% mandarin peels concentration to be optimum for all the enzymes except laccase which was enhanced to the maximum when supplemented with 8% mandarin peels concentration. Variation in the optimum concentration of the substrate for the maximum enzyme production may be reasoned out probably due to use of different fungal isolates and their metabolism which is directly related to the enzyme production.

Banana peels supplementation provided higher amount of xylanase and laccase activity compared to apple peels which was in accordance with the study of Elisashvili *et al* (2008) in which effect of different lignocellulosic materials like tree leaves, wheat straw, banana peels and apple peels concentration of 4g/L on the lignocellulolytic enzymes of seven different fungal isolates namely *Cerrena maxima* IBB 681, *Funalia trogii* IBB 146, *Trametes pubescens* IBB 663, *Tremetes versicolor* IBB 897, *Coriolopsis polyzona* 38443, *Pycnoporus coccineus* 38527, *Pleurotus ostreatus* 2191were carried out. Amongst all the seven fungi tested except *T. pubescens* and *P. ostreatus* laccase were stimulated more in banana peels as compared to apple peels. In case of xylanase also except *C. maxima* and *T. versicolor* all the five fungi produced more amount of enzyme in banana peels than apple peels. In the same study it was also noted that maximum xylanase activity was obtained when the media was supplemented with mandarin peels followed by banana peels and apple peels and MnP activity was noticed highest in banana peels which was exactly similar to the findings of the present study in case of xylanase and MnP. Laccase activity was enhanced the most in banana peels followed by apple peels and mandarin peels. This result was completely opposite to the present study. As in the present study the maximum enhancement of laccase was observed in mandarin peels followed by apple peels and banana peels probably due to difference in the composition of cultivation media and the fungal culture isolate.

Elisashvili *et al* (2009) in their study reported to have mandarin peels and wheat bran caused the maximum accumulation of laccase amongst the seven different substrates evaluated. Similar observations were also reported in other studies conducted with various white-rot fungi (Mikiashvili *et al* 2005, Songulashvili *et al* 2006, 2007, Osma *et al* 2007) suggesting that mandarin peels can be used as a suitable

growth substrates for accumulation of laccase activity in basidiomycetes. Here also the results revealed that maximum laccase activity was observed when ash gourd pulp used as a substrate followed by mandarin peels.

Mikiashvili *et al* (2005) studied the influence of different carbon sources on production of ligninolytic activity of *Trametes versicolor* for which medium was supplemented with three different concentrations of mandarin peels (2%, 3%, 4%) and the results indicated 4% mandarin peels to have produced the maximum activity of laccase (5250 U/ L) and MnP (54 U/L) compared to the other carbon sources used. In the present study also 6% mandarin peels produced moderate enhancement of Laccase and MnP that difference in the concentration in which the maximum enhancement was noticed may be probably due to the difference in the fungal strain and the cultivation medium.

In the study of Elisashvili *et al* (2008) *Lentinus edodes* and *Pleurotus* species were compared for their lignocellulolytic enzyme activity produced in SSF and SF of different plant raw material. Amongst them in the submerged fermentation three different species of *Pleurotus* viz *P. dryinus* IBB 903*, P. ostreatus* 2175 *and P. ostreatus* 2191 produce the maximum amount of laccase activity when mandarin peels were provided as a substrate followed by apple peels and banana peels. Results of the present study equated with the same kind of observations as Mandarin peels were showing best enhancement in laccase activity and after that apple peels and banana peels were noted to show maximum enhancement in laccase enzyme activity.

Songulashvili *et al* (2007) evaluated the effect of mandarin peels and residues of ethanol production on laccase and MnP enzyme activity of 18 different basidiomycetes fungi. Results indicated that *Ganoderma adspersum* 845 and *Ganoderma lucidum* 447 to have shown accumulation of laccase enzyme activity up

to 27000 U/L in the in the culture media supplemented with mandarin peels where as *Ganoderma applanatum* showed no enhancement in laccase activity till 10th day but with further incubation period on the 14th day 200 U/L laccase activity indicating that the substitution with mandarin peels as a substrate could have influence the laccase enzyme activity. MnP activity ranged between 30U/L to 2160 U/L amongst which the maximum MnP activity (2160 U/L) was detected in the culture of *Pleurotus robustus* when supplemented with mandarin peels as a substrate. Equivalent results were noted in the present study where laccase activity was found to be enhanced when mandarin peels were used as a substrate for *I .lacteus* and *D. confragosa* in monoculture and coculture both.

Ash gourd contain higher amount of carbohydrates, especially xylan and pectin (Mazumdar *et al* 2004, 2005). Ash gourd pulp was used as a substrate for the first time in the present study and result indicated that in monocultures all the enzymes activity enhanced especially AAO and xylanase when ash gourd pulp was used as a substrate and in co culture LiP and MnP were moderately enhanced where as laccase as well as xylanase were enhanced the most when the medium was supplemented with ash gourd pulp.

As a result of novel approach to use the lignocellulosic material for inducing enzyme activity both the oxidative as well as hydrolytic enzymes enhanced significantly when mandarin peels and ash gourd pulp without addition of other chemical enhancers due to good growth of fungi in monocultures and coculture. Utilization of such inexpensive lignocellulosic materials provide an opportunity to obtain high yield of lignocellulolytic enzymes in the simple medium which played major role in the degradation of lignin without supplementing expensive enhancers to the culture medium. The selection of lignocellulosic material adequate for the growth of fungus and target enzyme synthesis may lead to develop an efficient technology for scaling up the lignocellulolytic enzyme production.

3.5 BIO PULPING EXPERIMENT

From the results of previous experiments conducted on the effect of various conditions (incubation period, temperature, pH), different chemicals used as enhancers (Ethanol, Veratryl alcohol, Xylidine, Yeast extract, Peptone) and lignocellulosic substrates (Apple peels, Banana peels, Mandarin peels, Ash gourd pulp) to enhance the different lignocellulolytic enzyme activities, the optimum temperature, pH and best concentrations of chemical enhancers as well as lignocellulosic materials were further used to evaluate and optimize the enzyme activity in bio pulping using raw material (*Eucalyptus* block) and pulp obtained after delignification stage from J. K. Paper industry. The best conditions selected for the optimization experiment were 20days incubation period, coculture of *Irpex lacteus* + *Daedaleopsis confragosa*, 25˚C temperature and 5 pH. On the basis of experiment on enhancement in enzyme activity concentrations 1% ethanol, 16mM veratryl alcohol, 30µM xylidine, 0.4g/l yeast extract, 0.6g/l peptone were selected as best chemical enhancers and 5% apple peels, 4% banana peels, 6% mandarin peels and 5% ash gourd pulp as best lignocellulosic material were selected for further study.

3.5.1 Optimization of enzyme activities using chemical enhancers

The chemical enhancers were substituted in 3% Malt extract broth (MEB) along with *Eucalyptus* blocks and pulp The experimental samples were autoclaved at 121˚C and 15 Psi pressure for 45 minutes in the culture bottles. After cooling the medium was inoculated with 9 mm disc of 10 days old fungal culture under aseptic condition. All the culture bottles were incubated at 25±1˚C for the desired incubation period and after completion of incubation period the experimental culture bottles were taken out. The mycelium were broken with laboratory hand blender and filtered through Whatman paper No.1 disc. The culture filtrates were used to evaluate enzyme

activity and remaining wood blocks and pulp were used to analyze weight loss, lignin, cellulose and hemi cellulose. In this study the maximum enzyme activity was considered as highest, minimum enzyme activity as lowest and the enzyme activity obtained in between maximum and minimum as moderate enzyme activity.

(a) Biopulping experiment using *Eucalyptus* wood blocks as substrate

All the chemical enhancers under the optimum conditions when substituted with *Eucalyptus* wooden blocks increased the lignocellulolytic enzyme activities as shown in the following Table1.

In the control 2.74EU/L/Min LiP activity was obtained and it enhanced with substrate *Eucalyptus* wood blocks up to 3.34EU/L. Amongst the chemical enhancers, maximum enzyme activity (4.79 EU/L/Min) was noted when 16mM veratryl alcohol was substituted in the medium followed by 4% ethanol (4.64EU/L/Min). LiP enzyme activity was moderately enhanced by 0.6g/L yeast extract (3.99EU/L/Min) and 30µM xylidine (3.91 EU/L/Min) and with 0.4g/L peptone showed 3.72EU/L/Min LiP enzyme activity as can be clearly depicted in Figure1A.

Enzyme	Control wood	Optimum Concentration of Various chemicals				
		Eth (1%)	VA (16mM)	XY (30µM)	YE (0.6g/L)	PEP (0.4g/L)
LiP EU/L/Min	3.34±0.09	4.64±0.06	**4.79±0.09**	3.91±0.09	3.99±0.09	3.72±0.09
MnP EU/L/Min	2.38±0.06	3.01±0.06	3.51±0.06	**3.64±0.09**	3.01±0.06	2.95±0.06
Laccase EU/L/Min	6.17±0.06	6.96±0.06	7.06±0.04	**7.29±0.10**	7.01±0.08	6.77±0.08
AAO EU/L/Min	56.25±0.16	53.39±0.10	**64.29±0.11**	59.90±0.17	54.97±0.16	41.28±0.10
Xylanase EU/ml/Min	23.17±0.11	32.55±0.16	48.11±0.15	**57.76±0.17**	44.31±0.18	42.25±0.13
Cellulase EU/ml/Min	0	0	0	0	0	0

Table1 Effect of chemical enhancers and *Eucalyptus* wood blocks on enzyme activities in coculture of *I. lacteus* and *D. confragosa*

In control 1.97EU/L/Min MnP activity was noted which enhanced up to 2.38EU/L/Min on addition of wood blocks to the media. MnP activity enhanced also found in the media supplemented with 30µM xylidine (3.64 EU/L/Min) followed by 16mM veratryl alcohol (3.51 EU/L/Min). 1% ethanol and 0.6g/L yeast extract showed exactly same amount of MnP enzyme production 3.01EU/L/Min. 0.4g/L peptone showed least MnP enzyme activity (2.95EU/L/Min) as shown in Figure 1 B.

Laccase activity in the control was 5.45EU/L/Min and enhanced to 6.17EU/L/Min when supplemented with wood blocks as substrate. The highest activity of laccase was noted in 30µM xylidine followed by 16mM veratryl alcohol (7.06EU/L/Min) and 0.6g/L yeast extract (7.01EU/L/Min). The lowest laccase enzyme production was obtained in 1% ethanol (6.96EU/L/Min) and 0.4g/L peptone (6.77EU/L/Min) in the coculture (Fig. 1 C).

Results in the Table 1 showed that 16mM veratryl alcohol proved to be the best chemical enhancer for AAO enzyme activity. AAO enzyme activity was enhanced in 16mM veratryl alcohol (64.29 EU/L/Min) and 30µM xylidine (59.90 EU/L/Min) while the other chemical enhancers studied did not enhance the AAO activity in coculture (Fig.1 D).

Xylanase activity in MEB medium was 22.68EU/ml/Min and on addition of wood blocks it enhanced up to 23.17EU/ml/Min. Xylanase enzyme activity was found to be maximum (57.76EU/L/Min) in 30µM xylidine, 48.11EU/ml/Min in 16mM veratryl alcohol. 0.6g/l yeast extract concentration and 0.4g/L of peptone brought about 44.31 EU/ml/Min and 42.25EU/ml/Min xylanase activity and 1% ethanol showed (32.55EU/ml/Min) the least enzyme activity (Fig.1 E).

Cellulase activity was found to be absent in the medium even when supplemented with wood blocks as a substrate to the medium or stimulated even after addition of the various optimum concentrations of chemical enhancers to the media (Fig.1 F).

Concentration of chemical enhancers	% weight loss	% lignin loss	% cellulose loss	% Hemicellulose loss
Control	7.13±0.28	24.27±1.70	20.78±1.41	25.04±1.19
Eth (1%)	10.02±1.60	46.84±2.18	18.40±1.99	24.29±4.32
VA (16mM)	8.99±1.01	46.99±2.91	14.41±1.31	26.48±2.63
XY (30µM)	**10.28±1.59**	**52.85±1.83**	**19.03±1.67**	24.74±3.88
YE (0.6g/L)	9.97±1.13	52.83±1.16	13.46±1.49	**32.11±4.71**
PEP (0.4g/L)	9.31±1.65	49.22±1.17	18.62±3.18	25.43±4.09

Table 2 Effect of chemical enhancers and *Eucalyptus* wood blocks on wood components

Results of biochemical analysis for various organic compounds of wood after pretreatment with fungi under optimized conditions are depicted in Table 2.

Both the fungi *I. lacteus* and *D. confragosa* in coculture completely colonized the wood blocks in control and the other experimental bottles in which chemical enhancers were added in optimum concentration. However differences in their potential to decompose the wood blocks were observed. The loss in the weight, lignin, cellulose and hemicellulose content present in the wood blocks were noticed due to the action of fungal lignocellulolytic enzymes activity as shown in Fig.2.

The results of bio chemical analysis indicated that all wood components showed maximum loss when the medium was supplemented with 30 µm xylidine in *I. lacteus* and coculture except for hemicellulose. Maximum loss of Hemicellulose was noted when supplemented with 0.6g/L Yeast extract supplemented with in coculture.

I. lacteus+ D. confragosa caused the maximum dry weight loss (10.28%) in 30µm xylidine followed by (10.02%) in 1% ethanol where as minimum weight loss (8.99%) was noticed in 16mM veratryl alcohol. Supplementation of 0.6g/L yeast extract and 0.4g/L peptone showed 9.97% and 9.31% loss respectively as shown in Fig.2 A.

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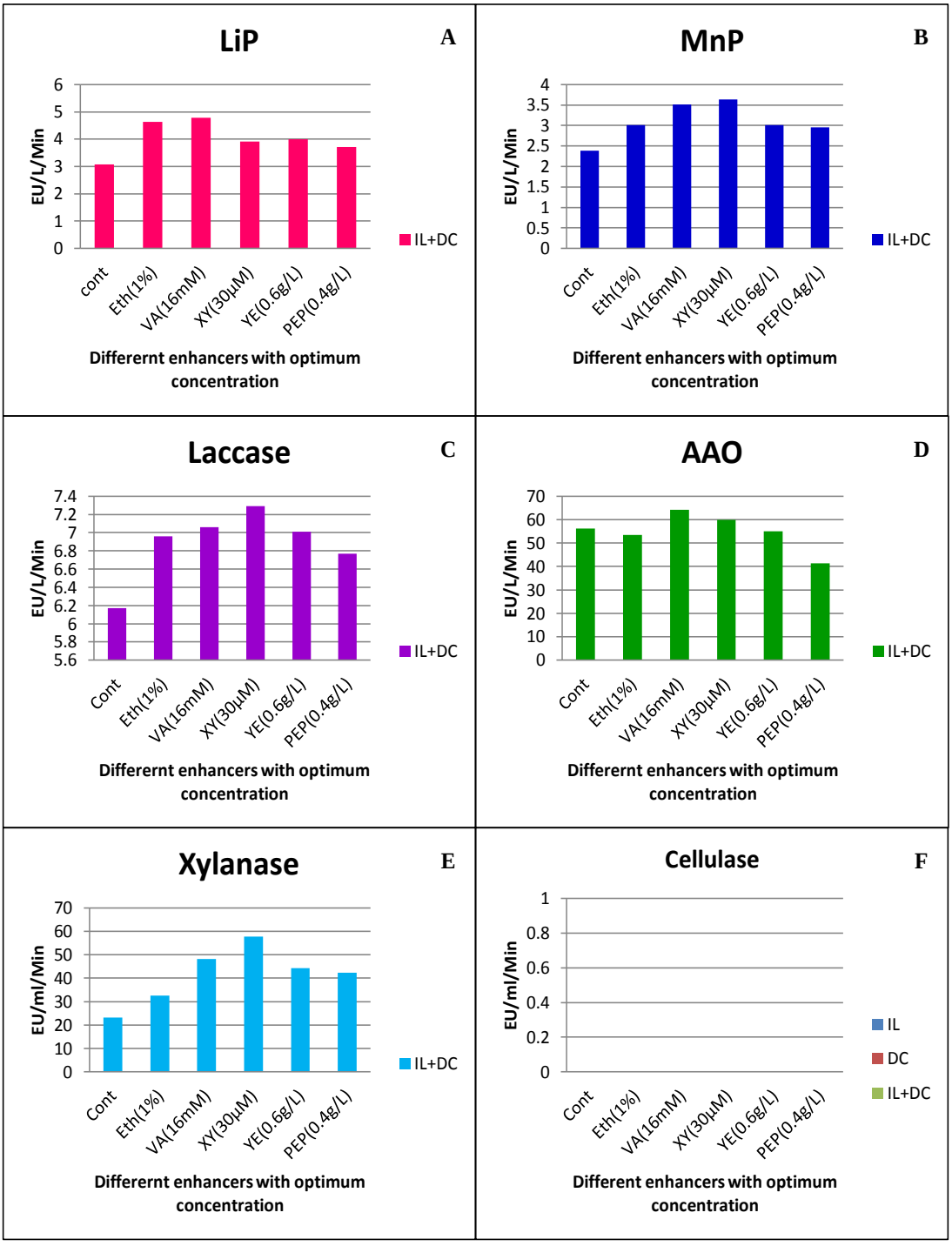

Fig. 1 Effect of enhancers + *Eucalyptus* wood blocks on the lignocellulolytic enzyme activity

Results obtained in the coculture showed two fold enhancements in the loss of lignin content compared to control where the highest lignin loss was found in 30µm and 0.6g/L yeast extract 52.85% and 52.83% respectively. 0.4g/L Peptone showed moderate lignin loss 49.22% and lowest in 1% ethanol 46.84% (Fig. 2 B).

Percent loss of cellulose was also increased along with the lignin loss when chemical enhancers were supplemented in the medium. Significant loss of cellulose was noted in co culture of both the fungi but it was less as compared to control which is actually beneficial for the biopulping process. Maximum loss of cellulose was noted in the media provided with 30µM xylidine (19.03%) followed by 1% ethanol and 0.4g/L peptone depicting loss amounting to 18.40% and 18.62 % respectively. The minimum loss of cellulose was noticed in the medium supplemented with 16mM veratryl alcohol and 0.6g/L yeast extract 13.41% and 14.46% respectively as shown in the Fig.2 C.

The maximum loss of hemicellulose was recorded in coculture of *I. lacteus* and *D. confragosa* with 0.6g/L yeast extract (32.11%). Concentrations of 16mM veratryl alcohol and 0.4g/L peptone showed moderate hemicellulose loss 26.48% and 25.43% respectively where as 24.74% and 24. 29% hemicellulose loss were noted with 30µM xylidine and 1% ethanol supplemented medium (Fig. 2 D).

(b) Biopulping experiment using pulp as substrate

Pulp obtained from J.K. paper industry after oxygen delignification stage used as substrate for the experiment. The results revealed significant enhancement in the enzyme activity and simultaneous loss in wood components occurred on addition of enhancers (Table 3).

LiP activity in control was 2.74EU/L/Min and enhanced up to 3.37EU/L/Min when media was supplemented with the pulp as a substrate.

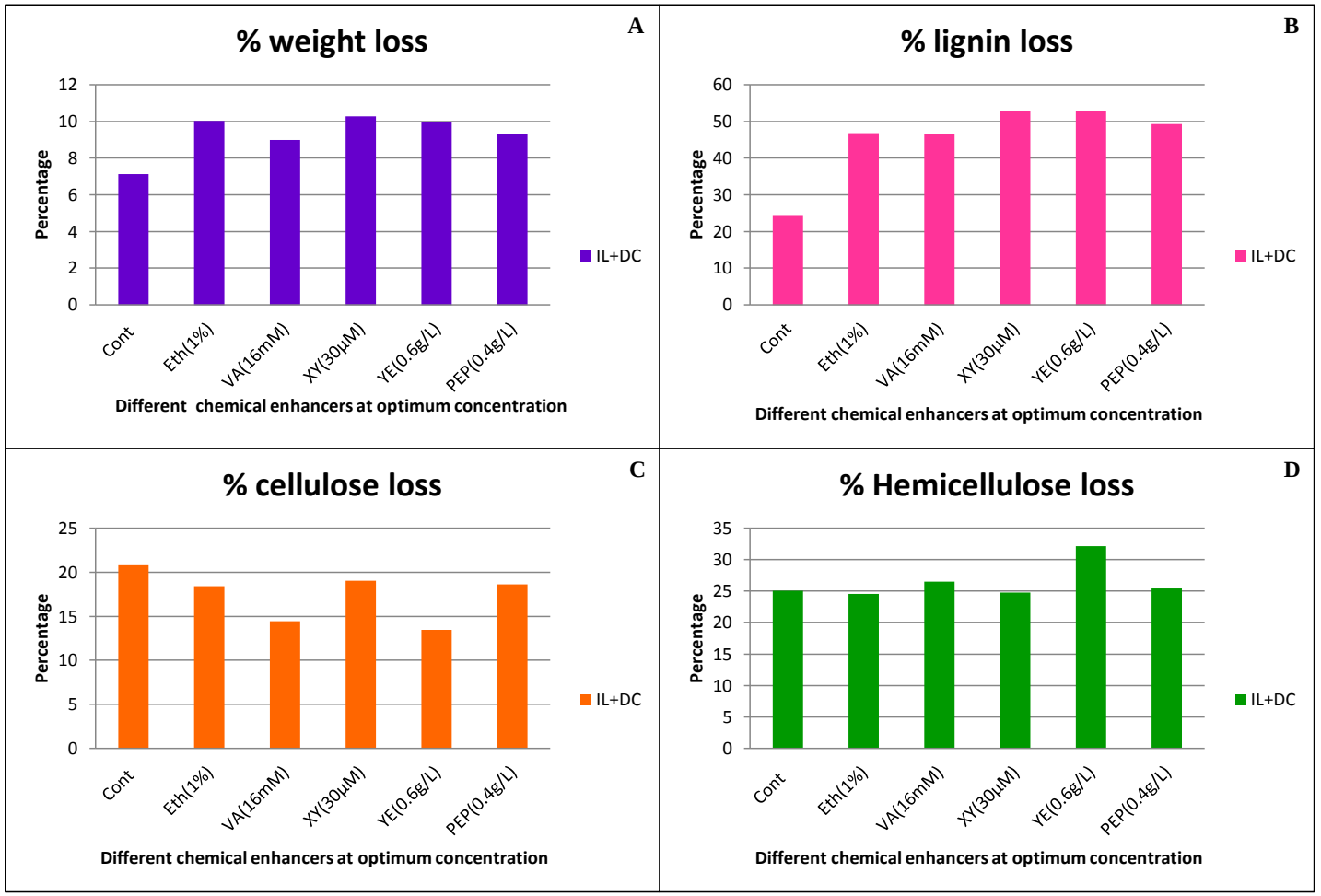

Fig. 2 Effect of *Eucalyptus* wood blocks+ chemical enhancers on wood components

Enzyme	Control Pulp	Optimum Concentration of Various chemicals				
		Eth (1%)	VA (16mM)	XY (30µM)	YE (0.6g/L)	PEP (0.4g/L)
LiP EU/L/Min	3.37±0.09	4.56±0.09	**4.71±0.06**	3.83±0.06	3.80±0.09	3.57±0.06
MnP EU/L/Min	2.19±0.06	2.64±0.06	2.36±0.09	**2.90±0.09**	2.17±0.09	2.72±0.09
Laccase EU/L/Min	5.63±0.07	6.90±0.07	6.76±0.06	**6.96±0.07**	6.82±0.04	6.66±0.06
AAO EU/L/Min	54.95±0.21	60.45±0.20	**67.19±0.10**	51.22±0.14	64.29±0.16	65.67±0.18
Xylanase EU/ml/Min	22.53±0.19	29.98±0.20	44.53±0.15	**59.84±0.19**	38.80±0.14	37.60±0.14
Cellulase EU/ml/Min	0	0	0	0	0	0

Table 3 Effect of chemical enhancers and pulp on enzyme activity in coculture of *I. lacteus* and *D. confragosa*

The highest LiP activity was noticed in (4.71 EU/L/Min) in 16mM veratryl alcohol and 1% ethanol (4.56 EU/L/Min). Moderate LiP activity was noticed in 30µM xylidine (3.83 EU/L/Min) and 0.6g/L yeast extract (3.80 EU/L/Min). 0.4g/L peptone represented the least Lip production (3.57 EU/L/Min) in coculture of *I. lacteus* and *D. confragosa* as shown in Fig.3 A.

Normally in the control 1.97EU/L/Min MnP activity was noticed which enhanced 2.38EU/L/Min with addition of pulp. The coculture of both fungi showed significant enhancement in MnP production with 30µM xylidine (2.90 EU/L/Min), 0.4g/L peptone (2.72 EU/L/Min), 1% ethanol (2.64 EU/L/Min), 16mM veratryl alcohol (2.36 EU/L/Min)and0.6g/L yeast extract (2.17 EU/L/Min) amongst which xylidine enhanced the maximum enzyme activities and yeast extract the least enhancement in MnP activity as shown in Fig.3 B.

In coculture laccase activity in MEB medium was 5.45 EU/L/Min which was found to be enhanced and provided 6.17EU/L/Min activity in the media which contain

pulp as a substrate. Laccase activity was enhanced maximum in 30µM xylidine (6.96 EU/L/Min), followed by 1% ethanol (6.90 EU/L/Min), 0.6g/L yeast extract (6.82 EU/L/Min), 16mM veratryl alcohol (6.76 EU/L/Min) and 0.4g/L peptone (6.66 EU/L/Min) as shown in Fig.3 C.

AAO enzyme activity was found to be maximum in coculture of *I. lacteus* + *D. confragosa* when 16mM veratryl alcohol was supplemented in culture media. In coculture of *I. lacteus* and *D. confragosa* 16mM veratryl alcohol, 0.4g/L peptone and 0.6g/L yeast extract were found to report the highest AAO activity 67.19 EU/L/Min, 65.67 EU/L/Min and 64.29 EU/L/Min respectively. 60.45 EU/L/Min and 51.22 EU/L/Min AAO activity was noted with 4% ethanol and 30µM xylidine as represented in Fig. 3 D.

Xylanase activity in the MEB medium was 22.53EU/ml/Min which enhanced up to 23.17 EU/ml/Min when pulp was provided as the substrate. In coculture 1% ethanol was found to show the least xylanase activity. The highest activity of xylanase was noticed in 30µM xylidine (59.84EU/ml/Min) followed by 16mM veratryl alcohol (44.53EU/ml/Min). Moderate xylanase activity was noticed in 0.6g/L yeast extract (38.80EU/ml/Min), 0.4g/L peptone (37.60EU/ml/Min) and 4% ethanol found to be showing the least enzyme activity (29.98EU/ml/Min) Fig. 3 E.

Cellulase activity was not induced even after addition of pulp to the media along with the different chemical enhancers at optimum concentration (Fig. 3 F).

It was very clear from the results of bio chemical analysis that compared to the control all wood components showed a loss on addition of chemical enhancers to the medium in optimum concentration. Both the monocultures and coculture degrade lignin and hemicellulose extensively without much loss of cellulose as can be clearly depicted from the following Table.4.

Concentration of chemical enhancers	% weight loss	% lignin loss	% cellulose loss	% Hemicellulose loss
Control	7.51±0.40	33.85±1.62	14.94±1.41	27.73±1.63
Eth (1%)	11.7±1.5	**49.29±1.05**	12.75±2.04	27.11±4.55
VA (16mM)	8.70±1.5	44.07±2.18	**15.55±3.72**	24.74±4.31
XY (30µM)	8.65±1.43	45.74±1.42	12.44±1.58	**28.23±3.57**
YE (0.6g/L)	**12.3±1.3**	45.41±2.71	14.04±1.96	27.79±0.79
PEP (0.4g/L)	11.65±1.05	40.95±3.05	14.57±1.82	24.81±0.10

Table 4 Effect of chemical enhancers and pulp on wood components

It was observed that in coculture 16mM veratryl alcohol and 30µM xylidine were reported to show minimum weight loss. Coculture showed the excellent weight loss in 0.6g/L yeast extract (12.3%) and 1% ethanol as well as 0.4g/L peptone were found to represent moderate weight loss 11.70 % and 11.65% respectively. 16mM veratryl alcohol and 30µM xylidine were showing 8.70% and 8.65% which was minimum amongst all chemical enhancers used (Fig.4 A).

To determine the decay of wood, loss of lignin was measured and it was noticed that in coculture the loss of lignin was maximum when the medium was supplemented with 1% ethanol and lowest in 0.4g/L peptone. Coculture represented maximum loss of lignin with 1% ethanol (49.29%) followed by 30µM xylidine (45.74%), 0.6g/L yeast extract (45.41%) and 16mM veratryl alcohol (44.07%) where as 0.4g/L peptone (40.95%) showed the minimum loss of lignin as shown in Fig.4 B.

Percent cellulose loss did not enhance much on addition of chemical enhancers compared to control. In coculture 16mM veratryl alcohol showed highest loss of cellulose (15.55%) which was not very significantly high than the control and with rest of the enhancers cellulose loss was not enhanced. Loss of cellulose was decreased compared to control with enhancers other than veratryl alcohol Fig. 4C.

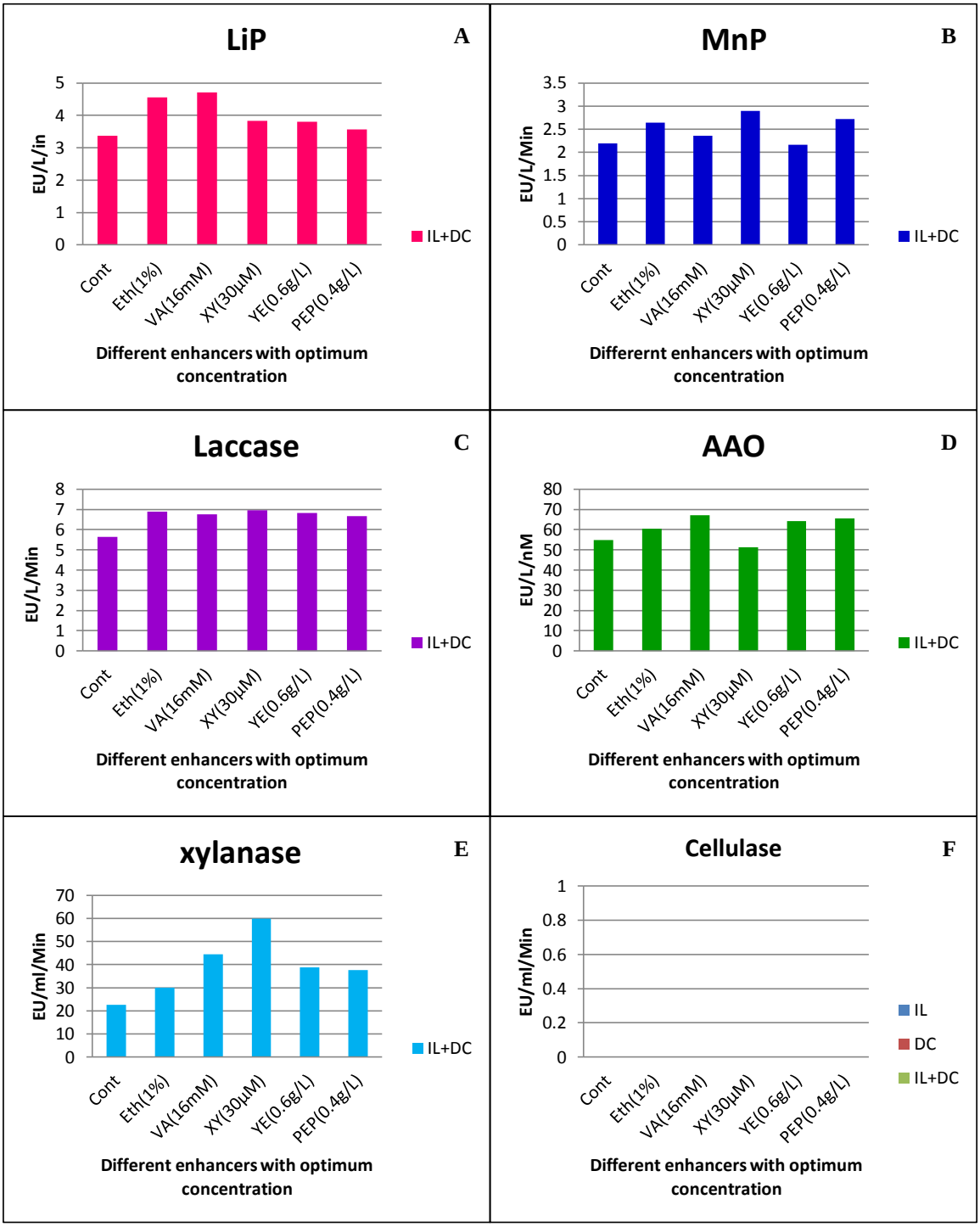

Fig. 3 Effect of enhancers + pulp on the lignocellulolytic enzyme activity

In coculture 30µM xylidine was showed the maximum hemicellulose loss (28.23%). In 1% ethanol, 16mM veratryl alcohol, 0.6g/L yeast extract showed 27.11%, 27.745 and 27.79% hemicellulose loss respectively which was almost similar to control. The least loss was when media was supplemented with 0.4 g/L peptone (24.81%) which was lesser to control as shown in Figure 4 D.

3.5.2 Optimization of enzyme activities using lignocellulosic material as substrate

From the previous experiment on enzyme activities and its enhancement with chemical enhancers and lignocellulosic substrates results clearly indicated coculture/mixed culture to be more potential enzyme producers compared to monocultures and also incubation period required was reduced. It was clearly depicted form the optimization experiment that when the wood blocks were substituted in the medium along with the chemical enhancers provides maximum enzyme activity and wood components loss. So the final biopulping experiment was conducted with the optimum culture (coculture) and the optimum conditions under which it produced the maximum enzyme activities.

(a) Bio pulping using *Eucalyptus globulus* wood blocks as substrate

When lignocellulosic materials were supplemented along with the substrate *Eucalyptus globulus* wood blocks quantitative variations were noticed in the enzyme activity (Table 5).

LiP activity and MnP activity did not enhance significantly on addition of four different lignocellulosic materials. Amongst all the substrates used ash gourd pulp proved to be the best for the enhanced production of laccase and xylanase enzyme activity where as mandarin peels proved the best producer of AAO enzyme activity. Cellulase activity was found to be absent in control as well as the medium in which various substrates were added.

Enzyme	Control wood	Optimum Concentration of lignocellulosic materials			
		AP (5%)	BP (4%)	MP (6%)	AG (5%)
LiP EU/L/Min	3.34±0.09	4.27±0.06	**4.77±0.06**	4.52±0.06	4.24±0.09
MnP EU/L/Min	2.38±0.06	3.07±0.06	**3.95±0.06**	3.57±0.06	3.70±0.06
Laccase EU/L/Min	6.17±0.06	7.30±0.05	7.54±0.04	6.38±0.07	**13.05±0.06**
AAO EU/L/Min	56.25±0.16	104.26±0.14	126.55±0.14	**236.00±0.25**	187.29±0.13
Xylanase EU/ml/Min	23.17±0.11	34.09±0.14	39.04±0.19	42.23±0.15	**48.40±0.16**
Cellulase EU/ml/Min	0	0	0	0	0

Table 5 Effect of lignocellulosic substrates and *Eucalyptus* wood blocks on enzyme activity

Coculture represented 4.77 EU/L/Min LiP activity in 1% banana peels followed by 6% mandarin peels (4.52 EU/L/Min), 5% apple peels (4.27 EU/L/Min) and 5% ash gourd pulp (4.24 EU/L/Min) as shown in Fig. 5 A which was slightly higher than the control as 3.34EU/L/Min LiP activity was noticed in the control with pulp as a substrate.4% banana peels showed highest activity of MnP in coculture (3.95 EU/L/Min) where as 5% ash gourd pulp were representing the highest 3.70 EU/L/Min. 5% apple peels and 6% mandarin peels showed 3.07EU/L/.Min and 3.57EU/L/Min respectively which was still higher compared to control and nearly single fold increase was observed (Figure 5 B).

Laccase activity was enhanced maximum in 5% ash gourd pulp. The maximum laccase activity 13.05 EU/L/Min was obtained in 5% ash gourd pulp indicated two fold enhancement compared to the control. 5% apple peels and 5 % banana peels represented 7.30EU/L/Min and 7.54EU/L/Min respectively. The lowest laccase activity (6.38EU/L/Min) was noticed with 6% mandarin peels which proved to be still higher than the control (Figure 5 C).

Highest AAO activity was noticed with 6% mandarin peels and lowest with 5% apple peels. AAO activity was found to be enhanced nearly 5 fold when 6% mandarin peels were provided to the medium. The highest AAO activity (236.00EU/L/Min) was noticed in 6% mandarin peels followed by 5% ash gourd pulp which showed (187.29EU/L/Min). 5% apple peels and 4% banana peels were showing 104.26EU/L/Min and 126.55EU/L/Min respectively which is also nearly two fold enhancement (Figure 5 D).

Xylanase activity was also found to be enhanced two fold in coculture of both the fungi. The highest xylanase activity was obtained in 5% ash gourd pulp. The highest xylanase activity (48.40EU/ml/Min) was observed in 5% ash gourd pulp and lowest 34.09EU/ml/Min in 4% banana peels (Figure 5E).

Cellulase activity was not induced even after addition of lignocellulosic substrates to the medium. It was found to be absent in all the media along with the control Figure 5 F.

The data presented in Table 6 indicated that up on addition of lignocellulosic materials to the culture medium showed an enhancement in the degradation of wood as compared to control in which no substrate was provided other than the wood blocks.

In all the media supplemented with different lignocellulosic materials 5% ash gourd pulp was showing the maximum and 5% apple peels was showing minimum weight loss of the wood blocks in coculture of *I. lacteus* and *D. confragosa*.

Amongst the three main components of wood (Lignin, Cellulose and Hemicellulose) loss of cellulose was found to be only around 20% in the control which did not enhance with any of the lignocellulosic substrate supplementation.

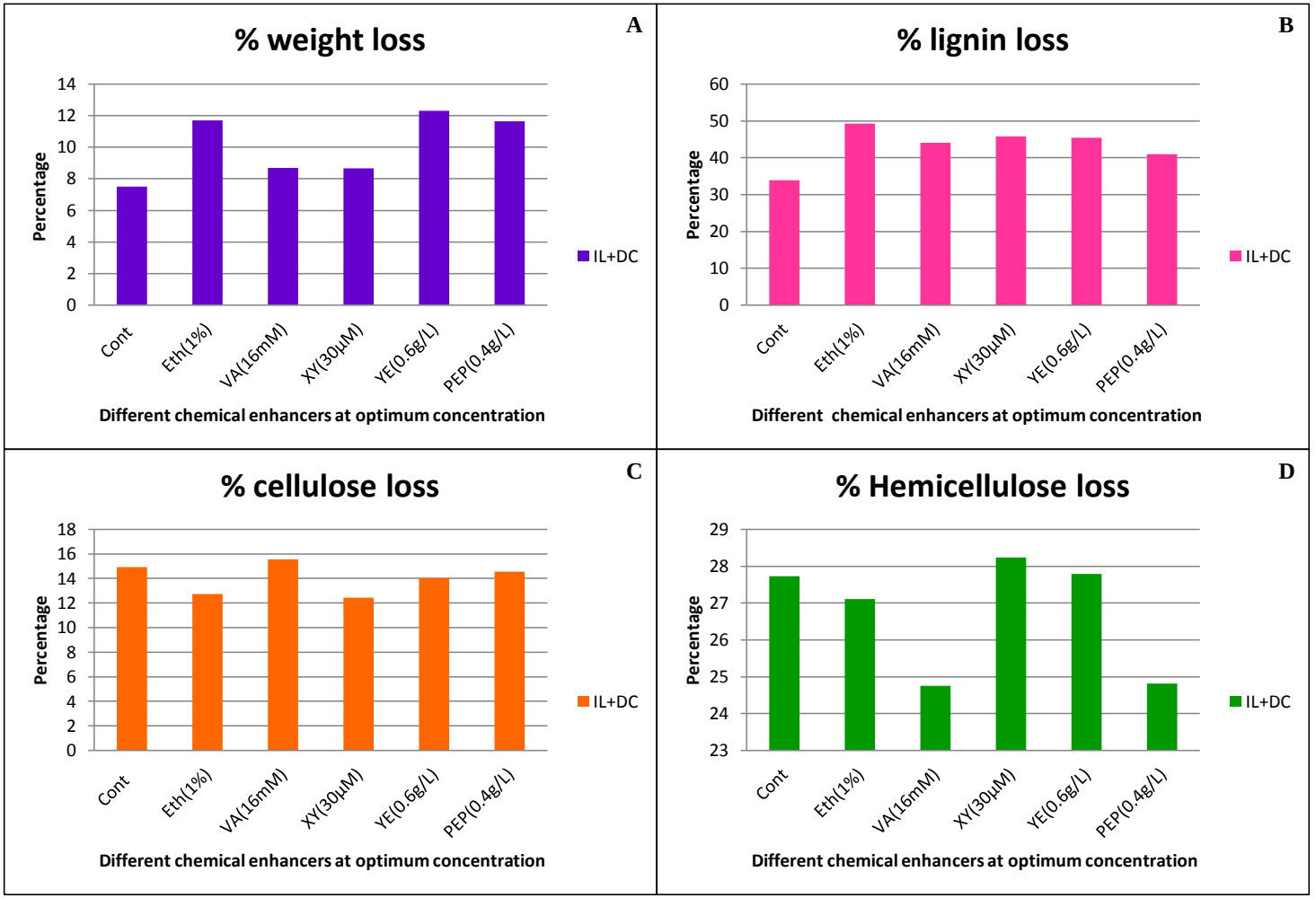

Fig. 4 Effect of pulp + chemical enhancers on wood components

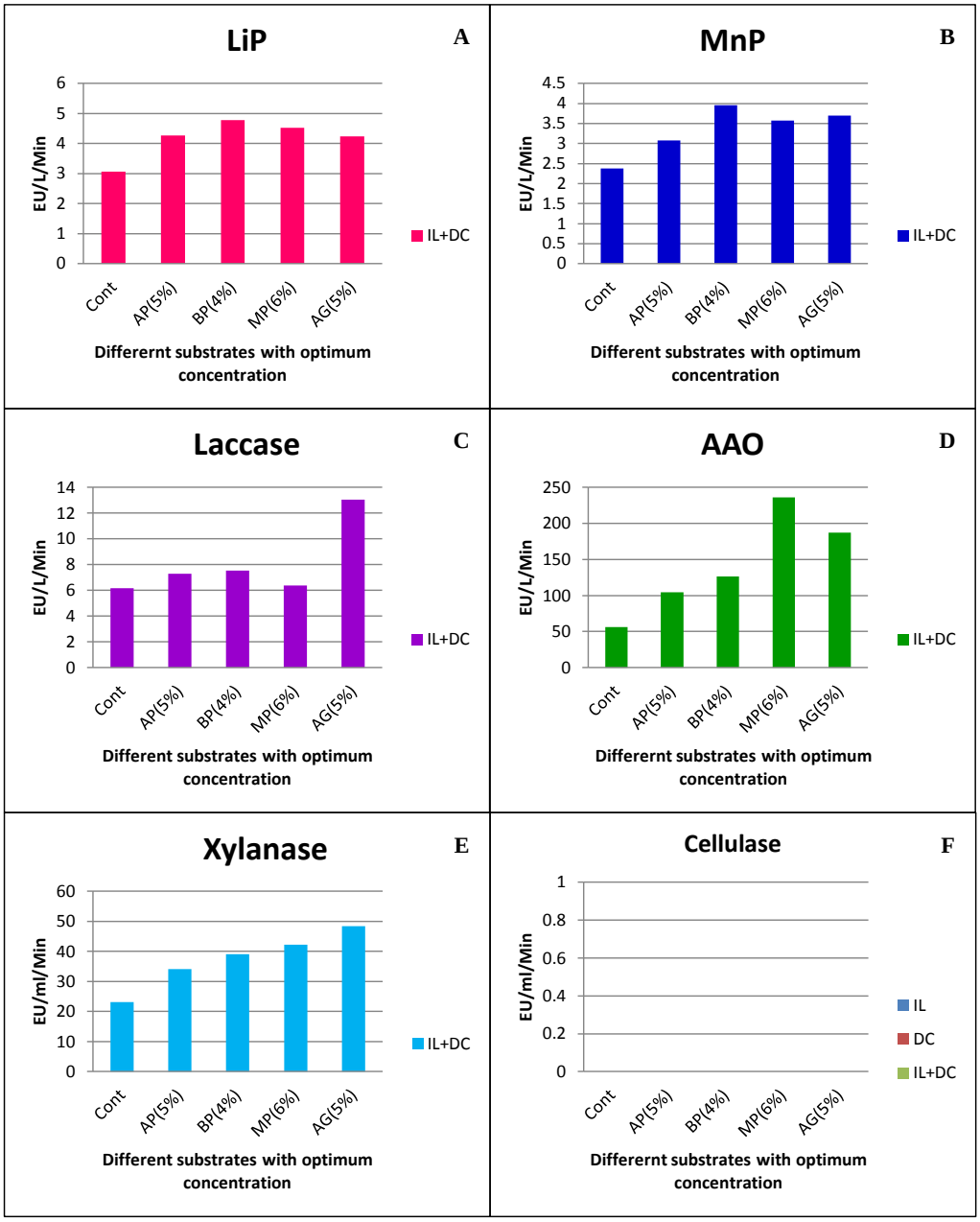

Fig. 5 Effect of substrates + *Eucalyptus* wood blocks on the lignocellulolytic enzyme activity

Concentration of chemical enhancers	% weight loss	% lignin loss	% cellulose loss	% Hemicellulose loss
Control	7.13±0.28	24.27±1.70	20.78±1.41	25.04±1.19
AP (5%)	3.67±0.49	51.07±1.56	18.12±1.64	24.46±2.36
BP (4%)	4.12±0.70	51.63±2.32	14.30±1.90	**28.95±2.75**
MP (6%)	6.33±0.38	**56.35±2.36**	14.51±3.16	27.89±1.67
AG (5%)	**6.86±0.68**	52.42±2.36	**18.43±3.13**	25.50±1.10

Table 6 Effect of lignocellulosic substrates and *Eucalyptus* wood blocks on wood components

Loss of lignin increased two fold with addition of all the studied lignocellulosic substrates (Apple peels, banana peels, mandarin peels and ash gourd pulp) with the maximum percent loss with the supplementation of 6% mandarin peels. This result indicated potential use of the substrate mandarin peels for the biopulping in paper and pulp industry where loss of lignin with minimum loss of cellulose is a priority.

Medium supplemented with 5% ash gourd pulp brought about maximum weight loss in the wood blocks (6.86%). Followed this by 6% mandarin peels (6.33%), 4% banana peels (4.12%) and 5% apple peels (3.67%) shown in Fig.6 A. It is important to note that here when the media was supplemented with all the lignocellulosic substrates the weight loss was less as compared to control in which no amount of substrate was provided. It may be due to the reason that the lignocellulosic material contain large amount of soluble carbohydrates which were used by the fungus instead of using complex sugars present in the wood blocks.

In coculture of both the fungi the highest lignin loss was found in 6% mandarin peels (56.35%) followed by 5% ash gourd pulp (52.42%) where as 5% apple peels and 4% banana peels were showing almost same lignin loss 51.07% and

51.63% respectively as shown in Fig. 6 B. Minimum lignin loss found coculture was also almost double than the lignin loss obtained in the control medium (Figure 6 B).

Cellulose loss was noticed highest in 5% ash gourd pulp and lowest in 4% banana peels. Coculture of *I. lacteus* and *D. confragosa* showing 18.43% loss of cellulose in medium with the 5% ash gourd pulp which was found to be lesser than the cellulose loss obtained in control medium as shown in Fig.6 C. Cellulose loss noticed in coculture was lesser than the control indicating 5% ash gourd pulp to be used as substrate for betterment of biopulping significantly.

Hemicellulose loss was found to be lesser than the control because of higher loss of cellulose in the medium. The highest hemicellulose loss was recorded in 4% banana peels up to 28.95% in coculture of both the fungi and lowest was found with 5% apple peels (24.46%) which was slightly lesser as compared to control medium (Figure 6 D).

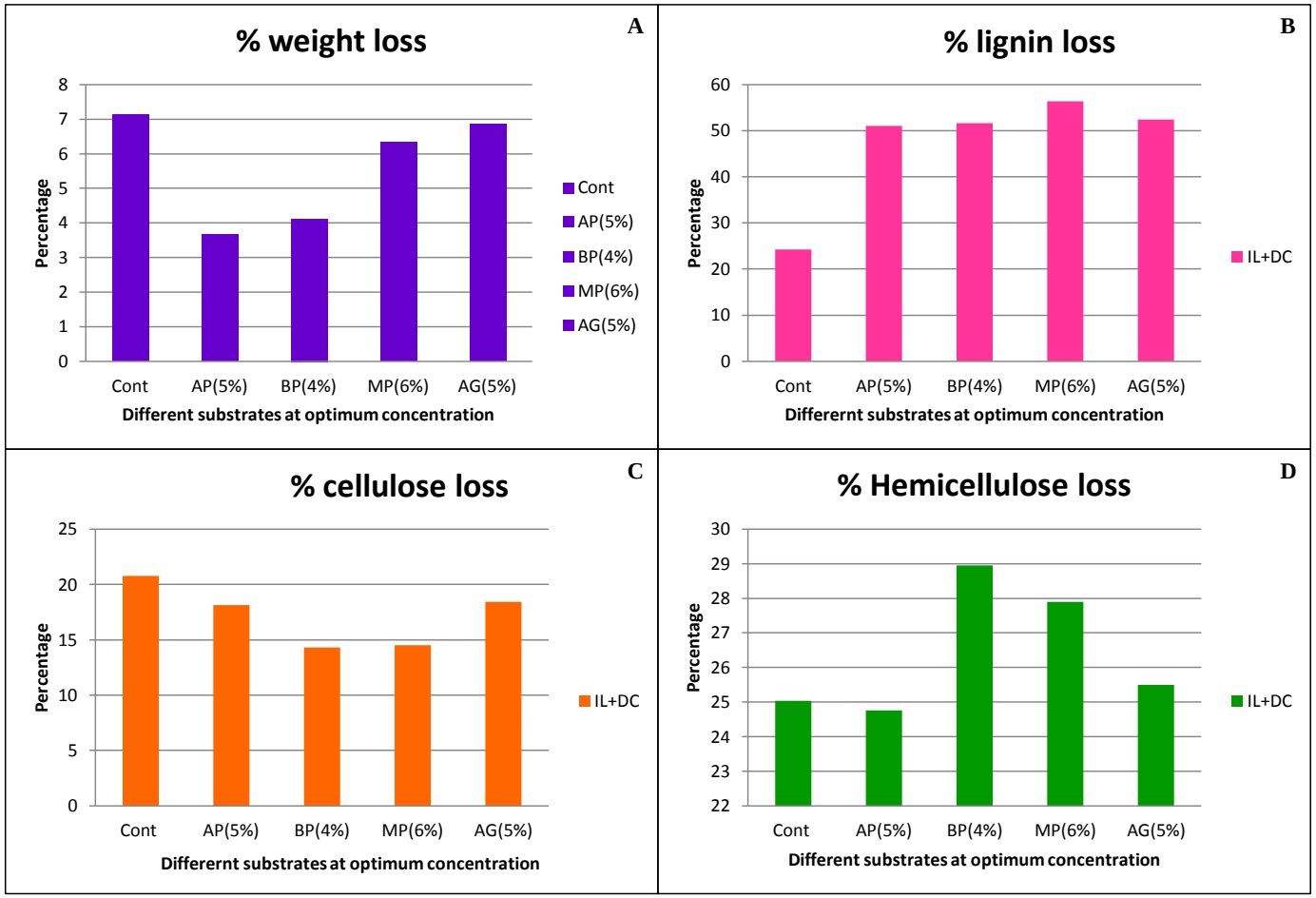

Fig. 6 Effect of *Eucalyptus* wood blocks+ chemical enhancers on wood components

3.5.3 Properties of untreated and treated *Eucalyptus* wood blocks obtained under optimized condition

In the experiment of biopulping it was noticed that when *Eucalyptus* wood blocks were treated with coculture of *I. lacteus* and *D. confragosa* supplemented with 5% ash gourd pulp provided highest enhancement of all the enzyme activities. Loss of lignin and hemicellulose significantly was also higher compared to the other enhancers studied. To study the morphology of fibers shape of fibers, shape of lumen, fiber ends shape were obsereved under the light microscope. A total of 25 randomly selected fibers were measured for both treated and nontreated fibers. Fiber dimensions and their derived values of fungal pretreated and untreated wood blocks were evaluated, compared with each other and is represented in the following Table 7.

Fiber dimensions	**Untreated *Eucalyptus* fiber**	**Pretreated *Eucalyptus* fiber**
Fiber length (mm)	1.424 ±0.157	1.418±0.183
Fiber diameter (µm)	4.89±0.49	4.59±0.79
Lumen diameter (µm)	2.77±0.70	2.62±0.83
Cell wall thickness (µm)	2.12±.73	2.04±0.35
Slenderness ratio	291.20	308.93
Flexibility coefficient	56.64	57.08
Rigidity coefficient	43.35	44.44
Runkel ratio	1.61	1.55

Table 7 Fiber dimensions and derived values of fungal treated and untreated *Eucalyptus* wood fibers

Quality of fiber is very important in the paper industry. Fiber morphological features to be paid attention include shape of fibers, shape of lumen, shapes of fiber ends, surface markings and irregularities of fiber walls. Fiber morphological features like length, width, curls and fines

have dominating influence on the quality of pulp as they are directly related to improve inter fiber bonding and strength of paper (Moral *et al* 2010). Long fibers have more fiber joints and can create a stronger network by forming bigger flocs compared to shorter fibers. Similarly fiber wall thickness also play an important role in pulping and paper making process as the fiber with thinner cell wall collapse easily than fiber with thicker cell wall indicating thin cell wall containing fibers are beneficial for paper making (Johanson 2009). Thin walled fibers bonds better in structure of sheet and results in stronger dense and smoother sheets of paper. The fibers of *Eucalyptus* wood blocks having ribbon like shape containing uneven width with no dislocations with cross marking on their surface. Results in Table 7 revealed that there is no significant difference in fiber dimensions and the derived values (indices) of fungal treated and untreated fibers indicating fungal treatment did not change fiber quality.

3.5.4 Discussion

Eucalyptus was one of the most important woods being used as a raw material for making paper so in the bio pulping experiment it was used as one of the substrates in the form of *Eucalyptus* wood blocks. The other substrate used was pulp which was obtained after oxygen delignification stage from the J.K. paper industry. These both the substrates were subjected for the bio pulping experiment along with all the optimized conditions. The obtained results were indicating enhanced production of lignocellulolytic enzymes upon addition of chemical enhancers in optimum concentration compared to control in which enhancers were not supplemented. As the secretion of enzymes increased degradation of wood blocks and pulp were also enhanced as compare to control.

Both the substrates *Eucalyptus* wood blocks and pulp showing enhancement of all the lignocellulolytic enzyme activity as compared to control (MEB) medium. All the

lignocellulolytic enzymes resulted in slightly higher enzymes activity with the substrate wood blocks except LiP. LiP activity was found to be little higher in pulp compared to wood blocks. 3.34EU/L/Min and 3.37EU/L/Min LiP activity was noticed in the media containing wood blocks and pulp respectively. This indicates the difference of only 0.3EU which was very negligible.

All the lignocellulolytic enzymes activity showing single fold enhancement up on addition of wood blocks as substrate. Cellulase enzyme activity was found to be absent throughout the whole experiment of biopulping as it was not stimulated with both the substrates and any of the enhancers.

Normally LiP activity noticed in MEB medium was 2.74EU/L/Min.16mM veratryl alcohol with wood blocks provide nearly 1.5 fold enhancement and 4.79EU/L/Min LiP activity was noticed which was highest amongst all the chemical enhancers studied. 4.71EU/L/Min LiP activity was noticed in the medium containing 16mM veratryl alcohol with pulp as a substrate.

MnP activity in the medium with wood blocks represented 3.64EU/L/Min which indicates 1.5 fold increase up on addition of 30µM xylidine where as with 16mM veratryl alcohol 3.51EU/L/Min MnP activity was obtained which can also be considered nearly 1.5 fold enhancement. In the culture media containing pulp as a substrate and 30µM xylidine. MnP enzyme activity was enhanced from 2.19EU/L/Min to 2.90EU/L/Min which was 1.25 fold enhancement in the MnP activity compared to control.

The highest laccase activity was noticed in the media supplemented with wood blocks and 30µM xylidine. Laccase activity showing single fold enhancement as it increase from 6.17EU/L/Min to 7.29EU/L/Min. similarly single fold enhancement was also noticed with the substrate pulp.

AAO activity enhanced single fold in 16mM veratryl alcohol both the substrates wood blocks and pulp which was highest amongst all the chemical enhancers. AAO activity was enhanced from 56.25EU/L/Min to 64.29EU/L/Min and 67.19EU/L/Min in wood blocks and pulp respectively indicating slightly higher AAO activity in the pulp which was not very significant.

Similar to AAO enzyme activity xylanase activity was enhances 2.5 fold with both the substrates and here also slightly higher xylanase activity was noticed in pulp compared to wood blocks. Xylanase activity enhanced from 23.17EU/ml/Min to 57.76EU/ml/Min and 59.84EU/ml/Min respectively in the wood blocks and pulp.

Results of enzyme activity noticed that LiP, MnP and Laccase activity was higher in the medium supplemented with the wood blocks whereas AAO and xylanase activity was slightly higher in the medium supplemented with pulp as a substrate. Comparison of results indicated there is not very much significant difference between the enzyme activity of substrate wood blocks and pulp was observed. 1- 2.5 fold enhancement in the enzyme activity was obtained depend upon chemical enhancers. Different lignocellulolytic enzymes were showing enhancement with various chemical enhancers these enhanced enzymes have direct effect on lignin degradation so lignin degradation can be considered as valuable parameter to check the effect of lignocellulolytic enzyme activity on biopulping.Both the substrates were subjected for biochemical analysis further in which wood components such lignin, cellulose and hemicellulose loss along with the dry weight loss of wood were analysed.

Results of biochemical analysis revealed that loss of all the components of wood was significantly higher in the wood blocks compared to pulp. Maximum weight loss in the wood (10.28%) was noticed in the media supplemented with 30µM xylidine. In the same medium 52.85% loss of lignin was noticed which was highest amongst all the other enhancers studied.

Cellulose loss was also noticed simultaneously along with the loss of lignin. Highest cellulose loss noticed was 19.03% in the media containing 30µM xylidine as an enhancer which was lower compared to the control without any enhancer.

The highest hemicellulose loss 32.11% was noticed in media containing 0.6g/L yeast extract in which cellulose loss was minimum. In the media containing 30µM xylidine 24.74% hemicellulose loss was noticed which was slightly lower than control. It may be because the cellulose loss in the medium with 30µM xylidine was highest and Hemicellulose was calculated by subtracting alpha cellulose from the holocellulose.

From the results of lignocellulolytic enzyme activity and biochemical analysis it was very clear that 30µM xylidine and 16mM veratryl alcohol proved to be the best chemical enhancers and can be supplemented for the efficient bio pulping.

As most of the lignocellulolytic enzymes were enhanced maximum when wood blocks were used as a substrate and due to enzymatic action wood blocks were degraded more compared to pulp which was used as an another substrate. So wood blocks were subjected further to evaluate the effect of optimum concentration of various lignocellulosic materials on enzyme activity and degradation.

When wood blocks were further subjected to the various lignocellulosic material as enhancers all the enzyme activity was enhanced significantly except cellulase which was not stimulated even after addition of lignocellulosic materials at optimum concentration.

It can be concluded from the results of lignocellulolytic enzyme activity and wood component loss in bio pulping experiment that 30µM xylidine as chemical enhancer and 5% ash gourd pulp as lignocellulosic material supplemented to the medium along with the wood blocks

significantly enhanced the biopulping which was directly benefited to the industry and ultimately to the environment.

LiP and MnP enzyme activity was enhanced up to 1.5 fold when 4% banana peels were substituted to the medium as enhancer which found to be best amongst all the other lignocellulosic material used. LiP activity enhanced from 3.34EU/L/Min to 4.77EU/L/Min whereas MnP activity was noticed to enhance from 3.34EU/L/Min to 3.95EU/L/Min. 5% ash gourd pulp showing 4.24 LiP activity and 3.70EU/L/Min MnP activity which was also 1.25 and 1.5 fold higher compared to control indicating not very significant difference was noticed.

Laccase activity was found to be highest in the media containing 5% ash gourd pulp. It enhanced from 6.17 EU/L/Min to 13.05EU/L/Min which showed two fold enhancement in the laccase activity. Highest AAO activity was noticed in the media containing 6% mandarin peels. It enhanced from 56.25EU/L/Min to 236EU/L/Min which was significantly fourfold enhancement in AAO activity whereas 5% ash gourd pulp resulted in 187.29EU/L/Min and showed more than threefold enhancement.

Xylanase enzyme activity was highest (48.40EU/ml/Min) in the media supplementing with 5% ash gourd pulp showing two fold enhancement in the enzyme activity. 6% mandarin peels also showing nearly two fold increase in the xylanase activity and showed 42.23EU/ml/Min. These results of enhancement in lignocellulolytic enzyme activity indicated 2-4 fold enhancement which was significantly higher compared to the enhancement obtained with the various chemical enhancers.

Results of biochemical analysis showed highest loss of lignin was observed in 6% mandarin peels (56.35%) followed by 5% ash gourd pulp (52.42%). In the media containing 5% ash gourd pulp 18.43% cellulose loss was obtained which was the highest amongst all other

lignocellulosic substrates but it was still lower compared to control. In the same medium 25.50% hemicellulose loss was noticed however highest hemicellulose loss (28.95%) was observed in 4% banana peels which was higher than observed in control.

Saparrat *et al* (2008) studied ligninolytic enzyme ability of white rot fungus *Grammothele subergentea* LPSC no 436 strain and its biotechnological applications. Using solid state fermentation extracellular ligninolytic enzyme activity ability to degrade synthetic dyes and *Eucalyptus globulus* wood chips was evaluated. Results indicated that after 15 and 30 days of incubation period 291.9mU/g wood and 336.6mU/g wood laccase enzyme activity was noticed, 5.5% and 8.8% weight loss was reported and 1.1% and 13.5% loss in the lignin was noticed. The results of present study revealed 10.28% weight loss and 52.85% lignin loss after 20days of incubation period when the media was supplemented with 30µM xylidine along with the *Eucalyptus globulus* wood blocks and MEB medium which was higher compared to their study in lesser incubation period due submerged liquid media along with chemical enhancer and coculture of *I. lacteus* + *D. confragosa* and the liquid media.

The study conducted by Pointing *et al* (2000) to optimize laccase production of *Pycnoporus sanguineus* using submerged culture process reported 12 fold increase in the laccase enzyme activity upon addition of wood in the high carbon low nitrogen medium as a substrate but when wood was supplemented along with the 20µM xylidine in the medium results indicated 3 fold decrease in the laccase enzyme activity may be due to media composition as it contained high amount of carbon to support growth of fungi. In the present investigation results obtained were completely in contrast as laccase activity seems to be increased highest with 30 µM xylidine and wood blocks.

Characteristics of wood like basic density, fiber length, chemical composition and whiteness are very much important for the paper industry to make the pulp (Senisterra *et al* 2000). Many researchers have already studied the fiber properties of various lignocellulosic waste materials like wheat straw (Deniz *et al* 2004), corn stalk and tobacco straw (Eroglu *et al* 1992), Rye straw (Usta and Eroglu 1987), *Eucalyptus grandis* (Dutt and Tyagi 2011) and suggested the potential use of this in paper insustry.

In the present study also fiber properties of fungal pretreated and untreated (Normal) *Eucalyptus* wood blocks were evaluated. The results depicted that the fibers are long and having pointed ends and thin walls which are preferentially used in the paper and pulp industry (Ververis *et al* 2004). Long fibers containing extensibility of the bonding sites which increased strength properties to the paper sheet. Short and thick fibers do not produce good surface contact and fiber to fiber bonding (Ogbonnaya *et al* 1997).

Padhiar *et al* (2014) evaluated fiber properties of five different wood species (*Mangifera indica*, *Syzygium cuminii*, *Bombax ceiba*, *Eucaluptus globulus*, *Pithecellobium dulce*) amongst which *Eucaluptus globulus* having very good derived values specially slenderness ratio which is in agreement with the present study as slenderness ratio in the present study was found to be 291.20. Slenderness ratio more than 70 is considered to be satisfactory pulp tear indices and bursting strength for printing and writing purposes (Cappelletto *et al* 2000, Law *et al* 2001). In the same study fiber dimensions and their derived values of other 15 grass species were also carried out and showed that among the grasses length of the fibers is more in *Phragmites karka, Vetiveria zizanioides, Saccharum spontaneum, Imperata cylindrica* and *Sorghum halepense.* Similarly fibers with large lumen and thin walls tend to flatten to ribbons during papermaking with enhanced interfiber bonding between fibers and consequently have good strength

characteristics. Study was conducted to evalualte and compatre the fiber properties of grass species and wood species to be potentially used in the process of making paper.

Elasticity coefficient/Flexibility coefficient of a fibrous material if lowers than 70 is important for good quality of pulp in the paper production (Bektas *et al* 1999). If the fibers containing elasticity coefficient between 50-70 such fibers can easily be flat and give good paper with high strength properties. Here also *Eucalyptus* fibers of sound wood showed elasticity coefficient 56.64 and treated fiber showed 57.08 which falls in the range suggested by (Bektas *et al* 1999) proved to be good for making paper. Higher rigidity coefficient gives lower paper strength properties like lower burst, tear and tensile indexes (Bektas *et al* 1999). The lower runkel ratio containing fibers are considered to be better material for paper making (Lessard and Chouinard 1980).

Dutt and Tyagi (2011) evaluated eleven different *Eucalyptus* species for their morphological, chemical and pulp and paper making characateristics for getting high pulp yield of good strength. *Eucalyptus grandis* showed 0.92mm fiber length, 2.80µM cell wall thickness and low runkel ratio 0.52 which is in agreement with the present study where *Eucalyptus globulus* represented 1.4mm fiber length, cell wall thickness 2.12µM and 1.61 runkel ratio indicating length of fiber is slightly higher compared to *E. grandis* which also leads to higher runkel ratio where as cell wall thickness is lower compared to *E. grandis*. Long and thin walled fibers exhibit plastic formation and provide more surface contact for binding of fibers with each other. Fiber diameter and cell wall thickness governs fiber flexibility as thin walled fibers affect bursting and tensile strength and folding capacity of paper.

In the present study fibers of fungal treated *Eucalyptus* wood blocks showed slenderness ratio more than 70, flexibility coefficient less than 70, lower rigidity coefficient and lower runkel

ratio indicating cocultured fungal pretreatment for 20 days incubation period in the biopulping experiment did not alter the fiber properties of the wood and can be used for the enhancement of biopulping process.

Comparison from the results of enzyme activity and bio chemical analysis revealed that 5% ash gourd pulp and 6% mandarin peels were proved to be good substrate but if we compare the results of chemical enhancers and lignocellulosic material it was very clear that lignocellulosic materials are cheaper substrates and results in significantly higher enhancement in enzyme activities as well as wood degradation so it can be substituted/admired for the enhancement of biopulping process.