International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 21-08-2019; Accepted: 23-09-2019
www.botanyjournals.com
Volume 4; Issue 6; November 2019; Page No. 134-140

Induction of xylanase and pectinase enzymes of *Aspergillus* by *Mentha* deproteinised leafy broth

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Abstract
Under the current study, an investigation on the xylanase and pectinase enzymes of fungi *Aspergillus Niger* and *Apergillus flavus* was done under the influence of Deproteinized juice (DPJ).
Xylanase and Pectinase are important extra-cellular enzymes produced by fungi in order to breakdown the complex organic matter around them into simpler forms which they then use as nutrients for their growth. In recent years, these enzymes have been identified to have large scale industrial applications. Since these enzymes are hard to obtain, a large amount of research has been focused into this area where methods are being developed in order to enhance production of extra-cellular fungal enzymes. Deproteinized juice of *Mentha veridis* has been used in the current study as a liquid culture media on which fungi were grown in order to enhance the production of xylanase and pectinase enzymes. The Deproteinized juice of *Mentha viridis* has been seen enhancing the biomass of *Aspergillus flavus*, and also the enhancement in the activity of xylanase enzyme of both fungi. However, pectinase enzyme activity of *Aspergillus flavus* had been reduced. Mycotoxins were found enhancing by the influence of *Mentha* DPJ.

Keywords: *Mentha*, aflatoxins, mycelia, PDB, *Aspergillus*, cup plate assay

Introduction

During the preparation of Leaf Protein Concentrate (LPC) suggested by Prof. N. W. Pirie [1] by the Process of Green Crop Fractionation (GCF), a juice is formed by squeezing the pulp of fractionated leafy crop. This juice is heated at 90°C to coagulate the proteins to form the curd and the curd gets precipitated. The supernatant formed is isolated and mostly disposed randomly, called as Deproteinized juice (DPJ). This DPJ is used in research because it consists of carbohydrates, minerals, vitamins, non-protein nitrogen etc. In previous researches, it was used to grow economically conventional industrial fungi and can also be used as the source of metabolites [2] and can also be used for single cell protein production by yeast fermentation and its secondary metabolites [3]. In the previous investigation, the production of organic citric acid was analyzed from the culture filtrate of *Aspergillus Niger* cultivated on DPJ from Lucerne (Alfalfa). There was decrease in Invertase enzymes by yeast fermentation using DPJ. It was found that DPJ enhances productivity of alcohol by yeast Fermentation [4]. When different flours were added in DPJ for fungal growth, there was enhancement in the mycelial growth as compared with control as well as in productivity of enzyme amylase. In earlier findings DPJ used to study the activity of fungal enzymes grown on it *viz*., proteases, cellulases and lipases [5]. This is a novel Industrial approach. Activity of the enzyme nitrate reductase varies when the DPJ employed from various plants and treated as soil conditioner for growth of various plants. Radish DPJ also enhanced the rate of seed germination and retarded the dormancy.

During this present investigation, an experiment was conducted in which DPJ was used as a medium to grow fungi and observe the effect of DPJ on the production of fungal enzymes [6]. In earlier studies glucose nitrate medium was used as the control. Now, these fungi were grown on PDB as a control to compare with DPJ to know if there is any significant change in the production of extra-cellular enzymes. It was investigated whether *Mentha viridis* deproteinized Juice influences as antifungal [7, 8].

Fungi are ecologically involved in the degradation of a variety of complex materials, a property attributed to a battery of enzymes produced by these microorganisms. Fungi have received considerable attention as a source of large-scale production of industrially useful enzymes. Fungal based enzyme technology has come a long way and today the emphasis is on exploring naturally occurring fungal strains for a variety of enzymes, notably hydrolases of carbohydrates, proteins and fats. Amylase, Protease, Pectinase, Cellulase and lipase are among the diverse enzymes manufactured by fungal fermentation. Fungal enzymes which are investigated in this study are, Xylanase and Pectinase. *Aspergillus Niger* and related species have been in commercial use for the manufacture of pectolytic enzymes. These are enzymes that degrade pectin. These are the cell wall degrading enzymes [9, 10].

Research on pectinase enzyme has been studied in more pathogens and in more detail than any other wall depolymerase enzyme. The original rationale for the study of pectinase enzyme was that they are able to cause tissue maceration, the characteristic symptom of soft-rot diseases. Recently, research on pectinase enzyme has received new impetus from the demonstration that pectinase and pectic fragments induce numerous physiological effects in plants [11]. The role of pectin degradation in pathogenicity of *C. carbonum* will not be answerable until a strain unable to grow on pectin can be constructed. Pectinase's ability to break down pectin provides it with many specific benefits. These benefits primarily affect the digestive system, including the support of intestinal bacteria, colon function and support with seasonal conditions.

Xylans are major components of the hemicelluloses of land plants, and many microorganisms produce Endo-3-1, 4-

xylanase and P-xylosidase [12]. Commercial xylans used as assay substrates are not pure β-1, 4-xylan, any enzymes that can cleave the contaminating linkages will appear in reducing sugar assays as "xylanases". Second, despite the names that scientists give them, wall depolymerizing enzymes are rarely if ever completely specific. Some xylanase enzymes and cellulose enzymes, for example, are structurally related and can have reciprocal activity [13]. Hence, it is more difficult to make a definitive conclusion about the pathogenic importance of any particular enzymatic activity as opposed to any particular gene. Better digestion of plant-based foods which may help increase the availability of nutrients. Potentially increase xylan-based prebiotics to support healthy intestinal bacteria. Help reduce gas or intestinal discomfort from eating some difficult-to-digest plant foods such as beans, cereals, and fibrous vegetables.

Aflatoxins are polyketide compounds synthesized by secondary metabolic pathway in *Aspergilli*. As they are carcinogenic, teratogenic and mutagenic in nature, it is a real challenge to prevent and detoxify these compounds. The polyketide biosynthetic machinery imparts the potential to secrete aflatoxins in *Aspergillus flavus* isolates. This pathway also contributes to structural and functional diversity of various other polyketides produced by different *Aspergillus* species.

Aspergillus species are the main source of aflatoxins in the environment. These species are ubiquitous and universal in distribution. The high ecological, biological and metabolic diversity of *Aspergillus* species led to exploration of secondary metabolites among these species.

Aspergillus species influence human and animal health directly and indirectly with a significant economic impact on the society. *A. flavus* and *A. parasiticus* are the two major species that produce aflatoxins. Several mycotoxins are reported from several other mycotoxigenic fungi of which the aflatoxins are the most toxic and damaging polyketides. Economically important crops such as maize, rice, cottonseed, peanuts, and spices are all susceptible for contamination of aflatoxin. *A. flavus* is the major contributor of aflatoxin in pre and post-harvest agricultural food and feed. It is a major global challenge to manage *Aspergillus* infections in humans and aflatoxin contamination in crops and other food products.

Approximately 25% of the crops are considered contaminated by mycotoxins of which aflatoxin B_1 is the major toxin (FAO). Microbial enzymes, involved in the degradation and transformation of plant cell-wall polysaccharides, have found many biotechnological applications [14]. Ligninases, hemicellulases, cellulases, pectinases and amylases are the enzymes which are required to degrade not only the plant biomass to its completion but also have found applications in different industries and pharmaceutical preparations [15]. Hemicellulose (xylan and its derivatives), a heterogeneous group of hexoses, pentoses and some other sugars can be converted into fermentable sugars by xylanases [16]. Cellulose is the most abundant biopolymer present on this planet and can be degraded to glucose when different types of cellulases act in synergy [17]. Pectin, the constituent of middle lamella in some plants [18] comprises of galacturonic acid and some other compounds that are frequently degraded by microbial pectinases [19]. Beside these carbohydrolases, proteases are another important group of industrial enzymes that is widely used in detergent, baking and some other industries. Fungi are ecologically involved in the degradation of a variety of complex materials, a property that is attributed to a battery of enzymes produced by these microorganisms. Fungal enzymes have been used in enzyme-technology industries for decades [20] and hence there is an ever-increasing demand for the isolation and screening of new fungal isolates. These enzymes can degrade *in situ* plant cell-mass completely, however, the ecological interactions among these microbial agents and organic matter disintegration are poorly understood. Although, many consortia of microorganisms have been developed in laboratories for the degradation of complex plant materials, still there is and will remain a demand for new microorganisms which can secrete large amount of hydrolytic enzymes to decompose plant biomass. Keeping in view the importance of fungal enzymes, present study was initiated to isolate and screen the indigenous fungal strains and to explore their hydrolytic potential for their possible future applications. In view of using plant nutrients more efficiently,

Pirie [1] advocated the process of Green Crop Fractionation (GCF). The process of Green Crop Fractionation involves the extraction of proteins from green leaves for use in human and animal nutrition. In the process of Green Crop Fractionation two products are obtained, one being LPC i.e. Leaf Protein Concentrate and the other is DPJ i.e. Deproteinized Juice. While LPC (Leaf Protein Concentrate) is used as a protein and vitamin A supplement in human and animal nutrition, Deproteinized Juice is considered as a byproduct of this process. In order to make the process more efficient and economical and also to avoid local environmental bio-pollution, means to utilize the byproduct i.e. DPJ need to be developed.

Deproteinized juice prepared from *Allium cepa* has shown to inhibit the growth of the fungi *Trichoderma*, while the DPJ from other forages have shown to favor the seed germination, plant growth and mycelia biomass of fungi and hydrolytic enzymes like protease, cellulose, amylase and lipase [21].

Further studies could contribute to the utility of DPJ in the following ways. It is predicted that there will be a significant difference obtained in the results of the studies done on the enzymes of fungi grown on Potato Dextrose Broth and Deproteinized Juice. There will be variations found in enzyme zones by different DPJ as compared with PDB.

As *Mentha viridis* is an antibiotic species it can inhibit the growth of fungi, there could be a reduction in the amount of fungal mycelia (Biomass), when grown on DPJ of *Mentha* or the fungi might be able to grow on it normally, if so then a quantitative analysis on the fungal biomass will be needed. The fungi will be grown on liquid mediums i.e. Potato Dextrose Broth and Deproteinized Juice. The culture filtrates obtained used as a source of secondary metabolites and therefore to study the fungal pectolytic, xylanic enzymes. There could be an increase or reduction in the amount of fungal enzymes when treated and cultured in DPJ as compared to PDB.

In Japan, Prof. Oshima has contributed a lot on the studies of Deproteinized Juice. It was observed by the researchers that DPJ can be a suitable source of fertilizers which can be provided to crop plants. Its judicious use as a higher concentration may be phytotoxic.

The work at all these places indicated suitability of leaf

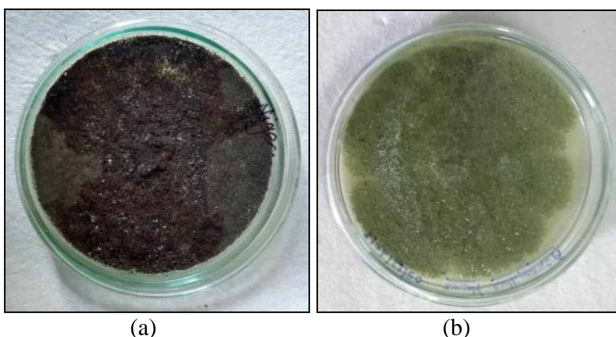
protein in human nutrition and they have also stated that there is a need for studies on DPJ for its proper utilization and to avoid pollution due to its random disposal. During the present investigation, use of the fungus grown on the natural medium prepared from the leafy extracts called as Deproteinized Juice was compared with fungi grown on Potato Dextrose Broth. Fungal mycelia were collected, weighed and its culture filtrates were collected for the purpose to analyzing the secondary metabolites. Deproteinized leaf juice was selected from non-leguminous species and it was the antibiotic and antacid herb called *Mentha* or “Pudina”. Comparative studies were done of the biomass and enzyme assay of the fungi grown on Deproteinized Juice and Potato Dextrose Broth which was used as control.

Formerly, Deproteinized juice prepared from *Allium cepa* has shown to inhibit the growth of the fungi *Trichoderma*. While the DPJ from other forages have shown to favor the seed germination, plant growth and mycelia biomass of fungi and hydrolytic enzymes like protease, cellulase, amylase and lipase [5]. Presently other enzymes are taken into consideration. Depending upon past studies and research conducted on the effect of DPJ on Fungal growth the following results can be expected, the data presented here indicate that *Aspergillus* is a highly diverse group of naturally occurring fungi. Members of this genus are well known for their potential to degrade plant biomass and industrial applications since ancient times [22]. Since *Aspergilli* were also tested as the host of some heterologous proteins there is a possibility to subject some of these isolates to genetic manipulation for developing a strain capable of complete degradation of plant-based biomass.

These culture filtrates after the growth of the fungi on DPJ and PDB were also utilized for the purpose to detect the production of aflatoxin content by the fungi *Aspergillus flavus* will be reduced by thin layer chromatography [23]. The detoxification of fungi *Aspergillus* was experimented by utilizing the DPJ of antibiotic herb *Mentha viridis* [24, 25].

Materials and Methods

Obtaining fungal cultures



(a) (b)

Fig 1: Pure Cultures of (A) *Aspergillus Niger* and (B) *Aspergillus flavus.*

Fungal cultures required for this particular study i.e. *Aspergillus flavus* and *Aspergillus niger* were obtained by inoculating infected seed and soil (Warcup’s method) samples on a Solid Agar Plate medium, the medium was also suspended with the antibiotics streptomycin/Rose Bengal to discourage bacterial growth showed in figure 1. The agar plate was then incubated for around 5-7 days at 30°C. The fugal colonies obtained were then identified. Pure cultures of the fungi of interest were obtained on PDA plate by sub-culturing.

Media Used

(1) Potato Dextrose Broth (PDB)

For preparing 1000ml of Potato Dextrose Broth medium the components required were Potato, 200 g, Dextrose 20 g, and distilled water 1000 ml. In order to prepare 1000 ml of Potato Dextrose Broth one must first peel, chop and weigh 200gms of potato. This 200gms of potato is then heated in distilled water (800-1000ml) and the potato starch is allowed to be extracted from the potato into the water. Once the potato is heated in distilled water, it is filtered and 20gms of Dextrose is then added to the filtrate. The Dextrose is allowed to dissolve. The volume is adjusted to 1000ml. Then the pH of the media is checked and adjusted to 5.4-5.8. The media i.e. PDB is then sterilized in an autoclave at around 120°C and at 15 lbs pressure; this is done in order to kill all contaminants. This sterilized media can then be used inoculate and grow desired fungi.

(2) Deproteinized Juice (DPJ) of *Mentha viridis*

Deproteinized Juice is obtained from GFC i.e. Green Crop Fractionation. In Green Crop Fractionation the weighed plant material is first macerated, after maceration the plant material is then pressed completely in order to extract pulp. The pulp that is obtained as a result of pressing is then heated at 95°C, as a result of which, proteins in the pulp coagulate to form a curd referred to as Leaf Protein Concentrate (LPC). The coagulated Leaf Protein Concentrate obtained is then isolated by filtration. The filtrate which is obtained is called as Deproteinized Juice. This Deproteinized juice can further be sterilized in an autoclave at 120°C and at 15 lbs pressure and can then be used as a medium to grow desired fungi.

Fig 2: DPJ of *Mentha viridis* and Potato Dextrose Broth as control.

Inoculation of desired fungal strains on sterilized PDB and DPJ Culture Media:

Fungal strains were obtained by isolating them from seed and soil samples, using techniques such as Warcup’s method, Agar plate method. Once fungal cultures are obtained, desired fungi were identified; isolated and pure cultures of the same are established. All the above mentioned procedures were conducted under aseptic conditions. Once the pure cultures of desired fungal strains were made available, then they were inoculated on Potato dextrose Broth (PDB) and Deproteinized juice (DPJ) culture mediums. Both media were provided with the antibiotic Streptomycin to avoid bacterial growth. PDB is used as a

control. Fungal inoculation was also done under aseptic conditions. The inoculated media were then incubated for 7 days at 30°C. The fungi were allowed to grow. After 7 days, the fungal mycelia matt and the culture filtrates were separated using Whatmann Filter paper. Further investigations were done on the fungal mycelia and culture filtrates.

Collection of fungal biomass

Once the fungal mycelium grows on the Deproteinized Juice and on Potato Dextrose Broth mediums for a certain amount of time (7 days), they are then filtered using Whatmann Filter paper, the weight of the filter paper is noted.

After filtering the fresh weights of the fungal mycelia grown on DPJ and the fungal mycelia grown on PDB are taken, the fungal mycelia are then dried at 80°C for two hours in a hot air oven and the Dry weights are then noted, the weight of the filter paper is subtracted from this, which gives us the value of the dry weight of fungal mycelium. Following which the fresh weights and the dry weights of the fungal mycelia grown on DPJ and PDB are then compared. The culture filtrates are taken to study the secondary metabolites of the fungi grown on DPJ and PDB by Cup-plate assay method.

Xylanase and Pectinase enzyme protocol of cup plate method

Culture filtrates of Deproteinized Juice (DPJ) and Potato Dextrose Broth (PDB) containing fungal Xylanase and pectinase enzymes were obtained by inoculating *A. Niger* and *A. flavus* for 7 days. The fungal mycelia were filtered and culture filtrates were used for Xylanase and Pectinase enzymatic study.

This investigation was done by Cup-plate method also known as well-diffusion method [26].

For Xylanase enzyme, a solid media containing Agar as solidifying agent and Xylan as sole carbon source was used. The media was poured in a plate and allowed to solidify, after which wells were made in the centre of the plate. The DPJ and PDB culture filtrates were inoculated in a well in the centre of the plate. After waiting for 24 hours zones were observed around the well caused by the digestion activity of Xylanase enzyme present in the culture filtrate to the solid media. The size of the zones were noted and comparisons were done.

For Pectinase enzyme, a solid media containing Agar as solidifying agent and Pectin as sole carbon source was used. The media was poured in a plate and allowed to solidify, after which wells were made in the centre of the plate. The DPJ and PDB culture filtrates were inoculated in a well in the centre of the plate. After waiting for 24 hours zones were observed around the well caused by the digestion activity of Pectinase enzyme present in the culture filtrate to the solid media. The sizes of the zones were noted and comparisons were done. The size of the zone directly corresponds to the amount of Enzyme present in the Culture filtrate.

Thin layer chromatography for detection of mycotoxins-

For isolation of mycotoxins from the culture filtrate, 50ml culture filtrate was taken and 150ml methanol was added to it. The extract was filtered through Whatmann No.1 filter paper and the filtrate was collected. The filtrate was the methanol extract.

30ml of methanol extract was mixed with 60ml, 20% warm ammonium sulphate along with 30ml hexane in separating funnel. The contents were shaken vigorously for 20 seconds. The lower layer in the separating funnel was collected and 5ml of methyl chloride was added to it. The mixture was again taken in a separating funnel and shaken vigorously for 20 seconds. The methyl chloride extract was collected separately and evaporated to dryness in a water bath. The residue obtained was dissolved in 0.5ml mixture of benzene and acetonitrile in a ratio of 98:2 v/v. This extract was used for the chromatographic separation of mycotoxins.

The extract was spotted on the TLC plates coated with silica gel prepared in acetone and the plates were developed in a solvent system containing a mixture of acetone and chloroform in a ratio of 12:88 v/v. The plates were examined under UV light (254nm) to observe Fluorescent blue, purple and green spots. Their R_F values were calculated.

Results and Discussion

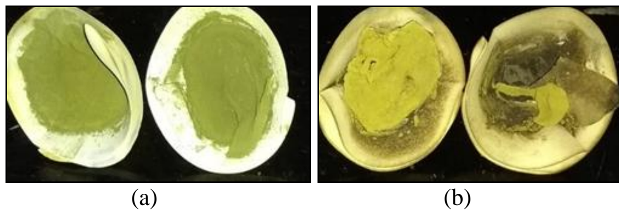
For the purpose of the secondary metabolites assessment, DPJ culture media was used for fungal growth and for comparison PDB was used (shown in figure 2). Both the fungi *Aspergillus flavus* and *Aspergillus Niger* were grown on DPJ and for comparison on PDB as control, in aseptic conditions, at room temperature (shown in figure 3 and 4). The mycelium of the fungi were filtered by Whatmann filter paper after 7 days and dried in a hot air oven (shown in figure 5 and 6).

Fig 3: Growth of *Aspergillus flavus* on *Mentha* DPJ and Potato Dextrose Broth.

Fig 4: Growth of *Aspergillus Niger* on *Mentha* DPJ and Potato Dextrose Broth.

Table 1: Measurement of the dry mycelia weight of the fungi grown on DPJ as compared with control of PDB.

No.	Culture Medium.	*Aspergillus flavus* Mycelial dry weight (gm)	*Aspergillus niger* Mycelial dry weight (gm)
1.	PDB	0.452	0.528
2.	DPJ	0.481	0.447



(a) (b)

Fig 5: Mycelial collection by filtration of *Aspergillus flavus* grown on (A) PDB (B) Deproteinized juice of *Mentha viridis* by Whatmann filter paper.

(a) (b)

Fig 6: Mycelial collection by filtration of *Aspergillus Niger* grown on PDB and Deproteinized juice of *Mentha viridis* by Whatmann filter paper.

Table 1, Indicates the appreciable amount of mycelial growth of *Aspergillus flavus* fungi on *Mentha viridis* DPJ despite *Mentha* being a well-known growth inhibitor of fungi. The mycelial weight of *A. flavus* was found to be 452mg on PDB while it was observed to be enhanced by *Mentha* DPJ at 481mg. *Mentha viridis* retarded the growth of the other fungi i.e. *Aspergillus niger* as compared to Potato Dextrose Broth (PDB) medium which was used as a control for comparison. Table 1, indicates that dry weight of *Aspergillus Niger* in DPJ media was 447mg, less as compared to 528mg in dry weight of fungal mycelia grown on PDB. Therefore, the results prove that *Mentha viridis* DPJ does not possess the efficacy of reducing the growth of aflatoxin creating *Aspergillus flavus* fungi. But *Mentha viridis* DPJ has been found to be retarding the growth of *Aspergillus Niger* fungi. The graphical representation of mycelia dry weight of both the fungi is illustrated in the figure 7.

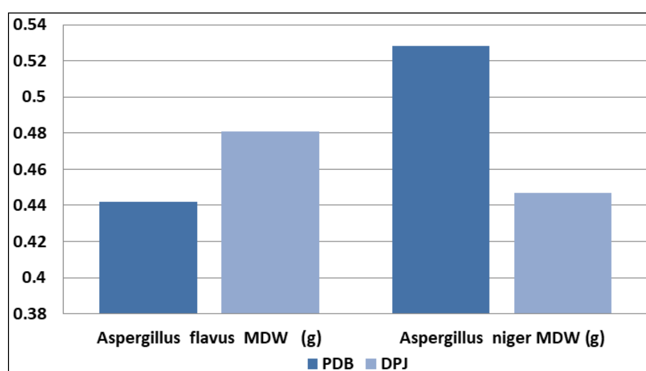


Fig 7: Graphical presentation of the mycelia dry weight (MDW) of *Aspergillus flavus* and *Aspergillus Niger* on *Mentha* DPJ as compared with PDB.

Table 2: Measurement of the zone of enzyme Xylanase by cup-plate method using the culture filtrates of *Aspergillus Niger* and *Aspergillus flavus* grown on DPJ and PDB.

No.	Fungi	Zone in cm (PDB)	Zone in cm (DPJ)
1.	*Aspergillus niger*	0.3	0.5
2.	*Aspergillus flavus*	0.3	0.4

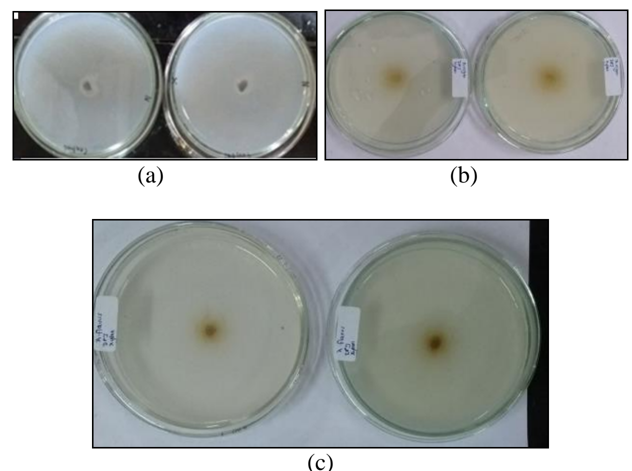
Deproteinized Juice from *Mentha viridis* was found to be enhancing the activity of hydrolytic enzyme Xylanase of both fungi i.e. *Aspergillus flavus* and *Aspergillus Niger* which is indicated in table 2. The zone of enzyme Xylanase, of *A. Niger* and *A. flavus* culture filtrates of DPJ are 0.5cm and 0.4cm respectively which is a clear enhancement as compared to the zones obtained from the culture filtrates of PDB which are 0.3cm in size in the case of both fungi. Therefore the result proves that DPJ is a significant medium, when used for the growth of fungi and production of secondary metabolites as compared with the ordinary Potato Dextrose Broth (PDB) medium used in the laboratories.

Table 3: Measurement of the zone of enzyme Pectinase by cup-plate method using the culture filtrates of *Aspergillus Niger* and *Aspergillus flavus* grown on DPJ and PDB.

No.	Medium	*Aspergillis flavus* Zone (cm)	*Aspergillus niger* Zone (cm)
1.	PDB	0.7	1.9
2.	DPJ	1.53	1.4

The results of Pectinase enzyme yield were found to be appreciable when DPJ was used as culture filtrates for fungi *Aspergillus flavus* as compared to *Aspergillus Niger*. As the growth of *A. flavus* was found to be enhanced on *Mentha* DPJ, there was also the enhancement in the enzyme yield i.e. zone size of 1.53cm? As indicated in table 3. As there was a retardation of the mycelial growth of *A. Niger* fungi on *Mentha* DPJ, also a reduction in the activity of the enzyme pectinase in its culture filtrate i.e. 1.4cm zones as compared to the 1.9cm zones obtained from PDB.

Figure 8, indicates that the culture filtrates of the fungi grown on DPJ show enhanced enzyme activity of Xylanase and pectinase as compared to the usual medium Potato Dextrose Broth utilized for the growth of the fungi. Despite *Mentha viridis* being an antibiotic, when its Deproteinized Juice can be utilized for the growth of the fungi *Aspergillus flavus*.



(a) (b)

(c)

Fig 8: Illustration of the petriplates showing the enzyme activity of Xylanase and pectinase each by cup-plate assay method from the culture filtrates of (A) Fungi grown on PDB (B) *Aspergillus niger* grown on DPJ and (C) *Aspergillus flavus* grown on DPJ. The petriplates showing brown zones are from the culture filtrates of fungi grown on DPJ.

Aspergillus fungi grown on the DPJ and PDB, their culture filtrates were utilized for the purpose of the investigation of

toxins by the process of thin layer chromatography (TLC). There were toxins isolated by this method.

Table 4: Retention factor (R_F) values of the Thin Layer Chromatography from the culture filtrates of *Aspergillus flavus* fungi grown on PDB and DPJ from *Mentha* leaves of Aflatoxins.

No.	R_f of Aflatoxins of *Aspergillus flavus* from PDB	R_f of Aflatoxins of *Aspergillus flavus* from DPJ
1.	0.071	0.1

Table 4, indicates the enhancement of the R_f value of the aflatoxins from the culture filtrate of *Aspergillus flavus* fungi grown on DPJ as compared with the fungi grown on PDB by Thin Layer Chromatography (TLC) method. As DPJ favors more growth of fungi the quantity of aflatoxins was also found to be more. Therefore this result proves that there was no efficacy of *Mentha* DPJ to reduce the content of aflatoxins shown graphically in figure 10.

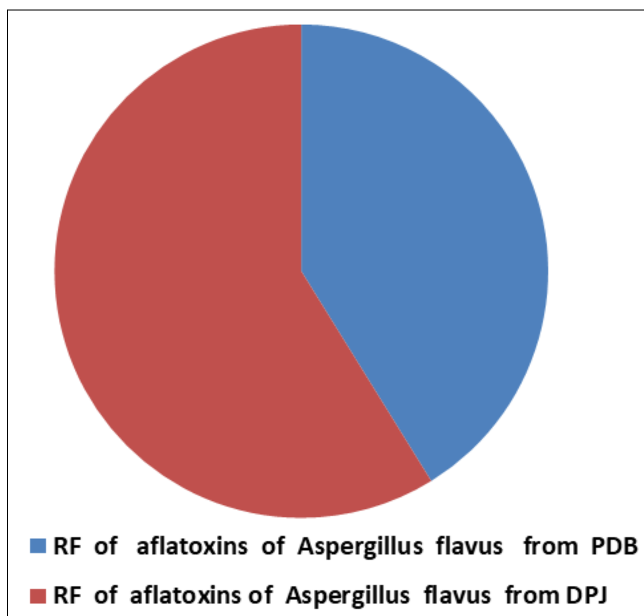


Fig 10: Pie graph showing the enhancement of content aflatoxin in the culture filtrate of *Aspergillus flavus* grown on DPJ as compared with PDB.

Conclusion

Therefore, from the above investigation it can be concluded that the Deproteinized leaf extract prepared from *Mentha viridis* favors the biomass of *Aspergillus flavus*, but not *Aspergillus Niger*. DPJ made from *Mentha viridis* plant has the efficacy of inhibiting the growth of *Aspergillus Niger* fungi, but not the carcinogenic aflatoxin producing *Aspergillus flavus* fungi. The activity of Hydrolytic enzymes *viz*., Xylanase and Pectinase was also observed to be effected by the usage of Deproteinized Juice of *Mentha viridis* as a liquid culture media. *Mentha* DPJ induced increased activity of enzyme xylanase in the culture filtrates of both the fungi. While in case of pectinase, *Mentha* DPJ was found to be reducing this enzyme activity of fungi *Aspergillus flavus*. Instead reducing DPJ favoured the aflatoxin content due to its growth inducing efficacy.

Acknowledgements

The authors thanks to Ruparel College, University of Mumbai to provide laboratory facilities and wish to express deep sense of gratitude to Professor and Head, Dr. A. M. Mungikar, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India. Authors also indebted to Dr. N. S. Suryawanshi, Head, Department Of Botany, Pendharkar College, Dombivli, Kalyan, Maharashtra, India and Miss Pallavi Pandey for cooperation in the research.

Conflict of Interest

The authors do not have any potential conflict of interest.

References

1. Pirie NW. Leaf Protein and Other Aspects of Fodder Fractionation. Cambridge University Press, London, 1978, 118-123.
2. EDB Josephine, IU Sayyed. Deproteinized leaf juice as a medium for fungal growth and for Production of protease. Geobios. 2005; 32:282-284.
3. Rajesh K Jadhav. Yeast utilizing deproteinised leaf juice (DPJ) as a medium for growth and production of metabolites. Plant Archives. 2018b; 18(2):1716-1720.
4. Jadhav RK, Deshmukh Sayali. Study of yeast quality enhancement by forage Deproteinized juice (DPJ) fermentation. Journal of Experimental Sciences. 2018; 9:9-12.
5. Rajesh Khanduji Jadhav. *In vitro* screening of cell wall degrading enzyme productivity from fungal culture filtrates on Deproteinized plant fluid by cup plate assay. Fungal Territory. 2018; 1(2):5-9.
6. Jadhav Rajesh Khanduji. Evaluation and detection of Brassicaceae leafy whey phytohormones efficacy for exploiting as a broth medium obtaining optimistic culture of fungi *in vitro*. Innovative In Agriculture. 2019; 2(1):01-05. DOI: https://doi.org/10.25081/ia.2019.v2.1116
7. El-Khateeb, Ayman Y, Elsherbiny A Elsherbiny, Louis K Tadros, Safaa M Ali, Hassan B Hamed. *et al.* Phytochemical Analysis and Antifungal Activity of Fruit Leaves Extracts on the mycelial Growth of Fungal Plant Pathogens, J Plant Pathol & Microb, 2013; 4:9.
8. Ramesha A, Srinivas C. Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata L.* and *Plumeria obtusifolia L.* Pelagia Research Library European Journal of Experimental Biology. 2014; 4(2):35-43.
9. Ralph A Dean, William E. Timberlake. Production of Cell Wall-Degrading Enzymes by *Aspergillus nidulans*: A Model System for Fungal Pathogenesis of Plants the Plant Cell. 1989; 1:265-273.
10. Jonathan D. Walton Deconstructing the Cell Wall. Plant Physiol. 1994; 104(11):13-1.
11. Rolf A Prade, Dongfeng Zhan, Patricia Ayoubi, Andrew J. Mort. Pectins, Pectinases and Plant-Microbe Interactions, Biotechnology and Genetic Engineering Reviews. 1999; 16(1):361-392. DOI: 10.1080/02648725.1999.10647984 T
12. Dolamani Amat, Livleen Shukla, Surender Singh, Bioprospecting of Xylanolytic Fungi Isolated from Degraded Corn Cobs for Xylooligosaccharides (XOs) Production. Journal of pure and Applied Microbiology. 2016; 10(4):2687-2696.
13. Annie Deborah Harris, Ramalingam C. Xylanases and its Application in Food Industry. Journal of Experimental Sciences. 2010; 1(7):01-11.
14. De Vries RP, Visser J. Aspergillus enzymes involved in

degradation of plant cell wall polysaccharides. Microbiology and Molecular Biology Reviews, 2001, 497-522. http://dx.doi.org/10.1128/MMBR.65.4.497 - 522.2001

15. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiol. Molec. Rev. 1998; 62:597-635.
16. Gawande PV, Kamat MY. Production of Aspergillus xylanase by lignocellulosic waste fermentation and its application. J Appl. Microbiol. 1999; 87:511-519.
17. Yang YH, Wang BC, Wang QH, Xiang J, Duan CR. Research on solid state fermentation on rice chaff with a icrobial consortium. Colloids Surfaces, 2004; 34:16. https://doi.org/10.1016/j.colsurfb.2003.10.009
18. Muhammed Sohail, amina naseeb, Sikander Khan Sherwani, Shahida Sultana, Sahiba Aftab, Salim Shahzad. *et al*. Distribution Of Hydrolytic enzymes among native fungi: Aspergillus, The predominant genus off hydrolase producer. Pakistan Journal Of Botany. 2009; 41(5):2567-2009.
19. Fawole AB, Odunfa SA. Pectolytic moulds in Nigeria. Lett. Appl. Microbiol. 1992; 15:266-268.
20. Dalboge H. Expression cloning of fungal enzyme genes; a novel approach for efficient isolation of enzyme genes of industrial relevance. FEMS Microbiology Reviews. 1997; 21(29):42. https://doi.org/10.1111/j.1574 6976.1997.tb00343.x
21. Jadhav Rajesh K, Mestry Nimisha. Protease inhibition by *Allium cepa.* L. forage deproteinised juice (DPJ) in *Trichoderma viride.* Mycology and Plant Pathology. 2018; 48(3):156-166.
22. Adeniran AH, Abiose SH. Amylolytic potentiality of fungi isolated from some nitrogen agricultural wastes. Afr. J. Biotechnol. 2009; 8:667-672.
23. Vijayanandraj S, Brinda R, Kannan K, Adhithya R, Vinothini S, Senthil K. *et al*. Detoxification of aflatoxin
24. B1 by an aqueous extract from leaves of *Adhatoda vasica* Microbiological Research. 2014; 169(4):294-300.
25. Sandosskumar R. Muthusamy Karthikeyan, Sathish Mathiyazhagan, Velazhahan R. Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B1 by the medicinal plant zimmu (Allium sativum L. × Allium cepa L.). World Journal of Microbiology and Biotechnology. 2007; 23(7):1007-1014. DOI: 10.1007/s11274-006-9327-x.
26. Velazhahan R. Bioprospecting of Medicinal Plants for Detoxification of Aflatoxins. Int J Nutr Pharmacol Neurol Dis [serial online]. 2019; 7:60-3. : http://www.ijnpnd.com/text.asp?2017/7/3/60/209424.
27. Bacharach AL, Cuthbertson WFJ. The cup-plate method in microbiological assay, with special reference to riboflavine and aneurine. Journal Analyst, 1948, 867.