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Bioconversion of lemon peel wastes for pectinase production using *Fusarium solani* under liquid state fermentation and its application

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Abstract:

Pectinase is an important developing industrial enzymethat is used in the food and fermentation industry. pectinase is produced from bacteria, fungi,and yeast which are cheap and eco-friendly. Among thesemicrobes,fungal pectinase can produce from locally available agro-industrial waste like lemon, orange,mosambi, and sapota peel and degrading soil. In the present study,I have obtained 8 fungal isolatesfrom serial dilution technique in that 4 isolates show better zone formation in pectinolytic screening activityso further Optimizationstudies was carried out in czapdox and sabouraud dextrose broth medium,pH,carbon,nitrogen source, and fermentation period. among the 4 isolates,*Fusarium solani*is superior to otherisolates.Liquid state fermentation was carried out for enzyme production, further partial purification of the crude enzyme is done with chilled acetone ina centrifuge. This present study shows the partial purification of pectinase enzyme obtained from*Fusarium solani*isolate fromfruit waste of sapota soil which is used in the application of sewage wastewater treatment.

Keywords:Agrowaste, *Fusarium solani*, pectinase,partial purification, sewage water treatment.

Introduction:

Industrially, microbial pectinase is used in a variety of applications and it fulfillstheir global demand. Pectins are heteropolysaccharides that containα(1- 4)linked D-galacturonic acid residue and it hasrhamnose on the main sign followed by arabinose, galactose, and xylose on the side chain(1).Pectins are present in the middle lamellae andprimary cell wall of higher plants which is made up of calcium and magnesium pectate (2). Pectinase is a depolymerizing enzyme that degrades the pectic substance by breaking polygalacturonic acid into monogalacturonicacid(3). Pectinase is produced from fungal genera like *Aspergillus*, *Trichoderma*, *Fusarium*, and *Penicillium*species(4-7). Diverse sources for pectinolytic isolates in nature are peels or rinds of fruits, rotten vegetables, and their dump yards(8).substates used to isolate fungal species are orange bagasse(9-13),wheat bran(14-17)jack fruit(18),grape pomace (19-21)corn corb (22)sugarbeet pulp(23) .The soil sample

containorange, mosambi,and sapota which were serial diluted based on the concentration. Further subculturing can be done to obtain the pure culture of the fungal isolates. In a sterilized environment, fermentation is done in a conical flask with the inoculum andmedium.Submerged and solid-state fermentation is employed to produce pectinase.Submerged fermentation is more favorable than solid-state fermentation because it has a regulated process, good sanitation, minimal risk of infection, reduced product contamination, and high enzyme production(24). .optimization studies were carried out based on the medium, carbon source, nitrogen source,pH, and fermentation periodto enhancepectinase production.

The effects of the growing human population include scarcity of food and water as well as the loss of natural resources.The amount of wastewater emitted increases in proportion to the rate of urbanization.Over 80% of wastewater discharged globally is improperly treated, leading to diseases and other health issues.Food and agro-industrial waste that is mixed with streams and lakes to cause pollution to create activated sludge, a waste product of treated water.To overcome these,microbial pectinase enzymescan use in sewage water to break the pectin material efficientlyand prevent pollution.This research aims to produce pectinase enzyme produced from *Fusarium solani*from fruit waste of sapota soil which is a cheap,cost-effective andeco-friendly product.

Methodology:

Preparation of Raw materials:

Fruit peels and Waste of mosambi, orange, lemon, sapota, and papaya were collected from nearby fruit shops.The starter culture was prepared by taking 25 gof fruit waste mixed with soil in a pot. Daily few amounts of water were sprinkled. After one month the soil degraded the fruit sample which was used as inoculum‘precursors’

Isolation of fungal microbes:

1000ml of czapek dox Agar medium($NaNO_3$-2g,k_2HPO_4-0.50g,$MgSO_4$-0.25g,KCl-0.25g,$FeSO_4$-0.01g, sucrose-30g,Agar-15g, pH-7.3) was prepared in a 1l conical flask. Sterilized with autoclave and pour it into a Petri plateand leave it for 15min to solidify. Fungal isolates were isolated using the serial dilution plate technique (25). Take 1g of soilinoculumwith 9ml of water,from thatserial dilution was made in the concentration of 10^1,10^2,10^3. Pour 1ml of each Seriallydiluted sample into a Petri plate containing medium. After five days, the development of fungal spores was observed.

Maintenance and growth:

After the fungal growth is formedin plates,furthersubcultureis repeated to get pure culture.so the culture plate should be kept in a hot-airincubator at 30 ℃ for 5 days. after obtaining the pure culture it was covered with paraffin paper for future use this was labelled as ‘mother cultureplates’.

Identification of the microorganism:

Slide culture technique is followed, in this PDA medium was prepared and pour it in a Petri plate and wait for 15 minutes to solidify. This PDA media was cut into squareswith help of knives and scalpel and placed over the slides. Take a small pinch off fungal spores with help of an inoculation loop and place it above the PDAsquares and cover it with a cover slip. Maintainthe slides at 30 ℃ for 5 days for the growth of fungal spores. Then the slides were

stained with lactophenol cotton blue to view the fungal structures, a binocular microscope was used and the photographs were taken fungal isolates were identified with the help of microbiology department in PSG college of arts &science,Coimbatore, and with the help of thebook described by (26-27).

Screening for pectinolytic activity:

To detect thepectin activity of the fungal isolates in a czapek dox medium containing pectin. flooding culture plates with freshly prepared Iodine-Potassium iodide solution is used (Iodine-1.0g, Potassium iodide-5.0g dissolved in 330ml of distilled water)(28). After 15 minutes translucent halo ring-like region appeared, where pectin is degraded. It forms a zone around the microorganism. Based on zone formation, pectinase-producing isolates were identified.

Inoculum preparation:

Fungal spores are maintained on culture plates. It was taken andgrown in a 100 ml conical flask containinga czapek dox medium. 50 ml ofsterilized 0.9% of isotonic NaCl solution was poured ona fungal spore in a flask and shaken vigorously to release the spore into the saline solution. Thisfungal spore suspension is used asan inoculum for further optimization studies. (29).

Optimization studies:

It was carried out in a 100 ml conical flask for the production of pectinase enzymein liquid state culture.Forthat medium, pH, carbon source,and nitrogen equivalentwas taken.

Liquid state Fermentation:

In conical flasks of 250ml each, 100ml of Sabourad dextrose broth and Czapek dox broth were taken. To adjust pH in analkaline nature dissolved NaOHpellets were used,for an acidic naturediluted HCL is used.Carbon source and nitrogen equivalents were added and it was sterilized in an autoclave at 120°C for 20 minutes.Each sample contained 2% fungal spore suspension, which was poured into the culture flask.For 3-6 days, the culture flasks were shaken in a mechanical shaker at 150 rpmto get the crude enzyme.

Partial purification:

The crude enzyme produced during fermentation was partially purified using the technique described by (30).The culture filtrate was treated with three volumes of chilled acetone and left to stand for 15 minutes after being cooled at 4°C for 30 minutes.The precipitate was formed when the crude enzyme was centrifuged at 10,000 rpm for 10 minutes. This precipitate was mixed with 10ml of sodium acetate buffer to get a partially purified enzyme.

Enzyme assay:

Protein estimation:

Determine the protein content in partially purified enzyme followed by the method(31) bovine serum albumin is used as standard.

Application of partially purified enzyme in sewage water treatment:

Sewage water was collected from the PSG College of Arts & Science sewage treatment plant in Coimbatore.The initial TDS and pH of the water were recorded.To observe enzyme activity, different enzyme concentrations (3, 5, and 10%) were taken and added to sewage

water.It was then shaken for three hours in a mechanical shaker at150rpm.The TDS and pH value of the enzyme water mixture was then recorded.The concentration at which the enzyme was effective was noted.

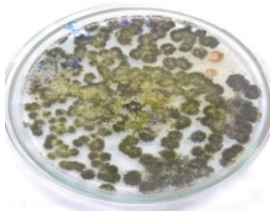
Results and Discussion:

Enzymesproduced by microbes are used in a wide range of applications.Pectinase is an important enzyme used in the food industry, the leather and textile industries, wastewater treatment, and the tea and coffee fermentation industries.Pectin is primarily found in fruit and vegetable waste..In this study, I conducted four different trials using pectin-rich samples and isolate eight fungal isolates: *Fusarium solani*, *Aspergillus Niger*, *Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicilliumcitrinum*,*Rhizopus oryzae*, *and Fusarium oxysporum*.screening of pectinolytic activity was done in all isolates in that 4 isolates show maximumzone formation in *Fusarium solani*,*Aspergillus fumigatus*, *Aspergillus flavus*,*and Aspergillus niger*.optimization studies were done in medium, pH, carbon, nitrogen equivalent Since *Fusarium solani* performed better than the others, liquid state fermentation was carried out for pectinase enzyme production.the enzyme was partially purified and it is used in sewage water treatment applications.

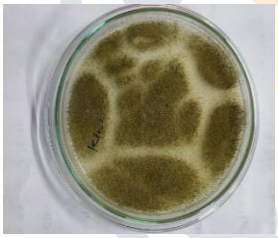
Isolation of Fungal isolates from pectin rich samples

4 trailswere carried out from the pectin-rich sample 4genera and 8 species offungal isolates were obtained.

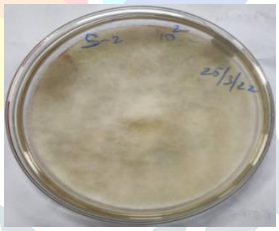
Plate 1: Fungal Isolates:



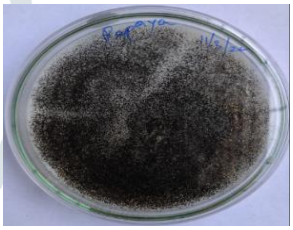
Fusarium solani



Aspergillus fumigatus

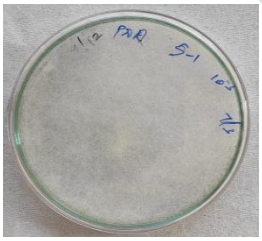


Aspergillus flavus

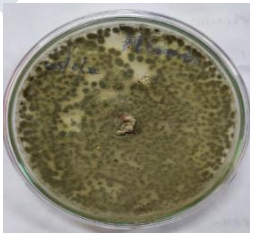


Aspergillus niger

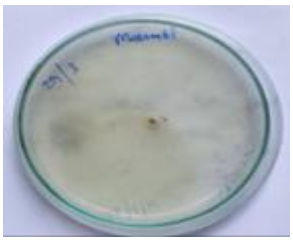
Penicillium citrinum



Aspergillus oryzae



Rhizopus oryzae

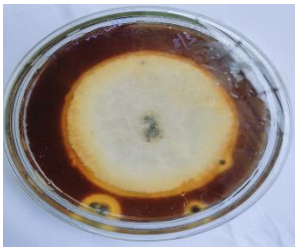


Fusarium oxysporum

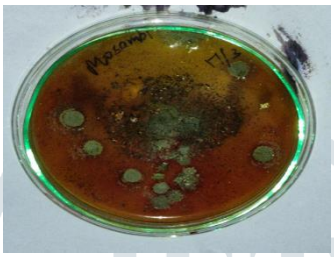
Screening for pectinolytic activity:

Todetect the pectin activity in the culture medium flooding the culture plates with freshly prepared iodine solution is used, after 15 min the solution formsa halo region in which the pectin in the medium gets degraded, among 8 isolates 4 only showa better zone formation in that*Fusarium solani* shows the maximum zone formation of 5.5(mm)followed by, *Aspergillus fumigatus, Aspergillus flavus*. *Aspergillus niger.*

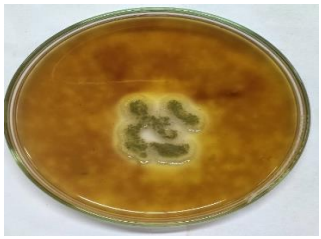
Plate 2:Pectinolytic Activity of Isolates



Fusarium solani



Aspergillus fumigatus



Aspergillus flavus

Aspergillus niger

Optimization of pectinase producing fungal isolates in culture media:

From the culture screening for pectinolyticactivity was done out of 8isolates 4 only shows the better zone formation so further optimizationwas done in 4 fungal isolates they *are Fusariumsolani*, *Aspergillus flavus*,*Aspergillus fumigatus*, *Aspergillus niger*.czapekdox andsabouraudbroth medium is used. Among these*fusarium solani* show effective production in sabouraud mediumfollowed by *Aspergillusfumigatus* and *Aspergillus flavus*.*Aspergillus niger* shows the least performance in czapek dox and sabouraud medium.The results are shows in (Figure1)

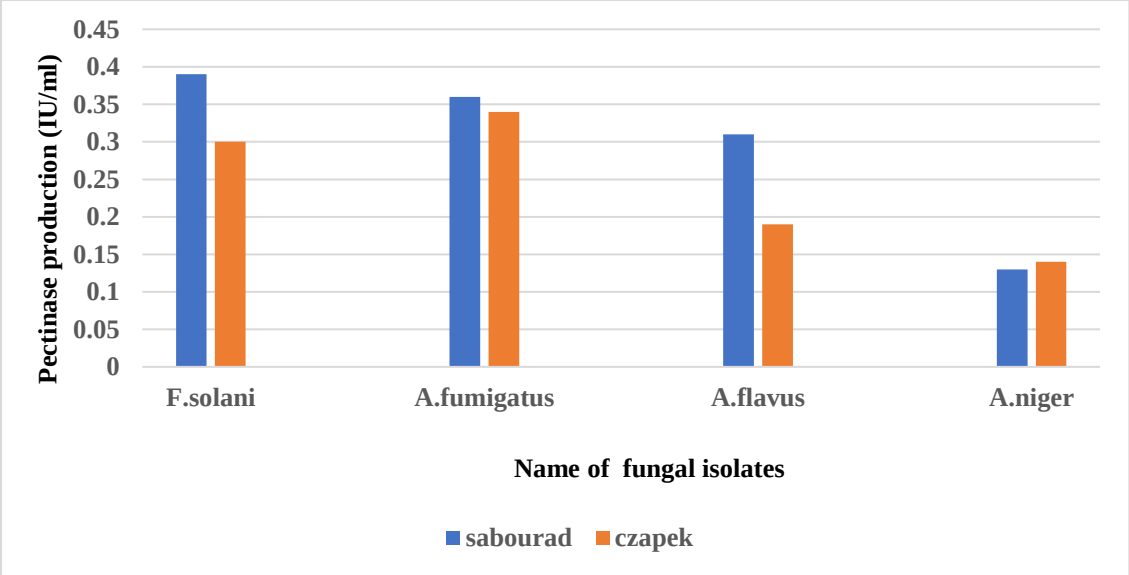


FIGURE NO 1: Optimization of pectinase producing fungal isolates in culture media

Optimization of pH on pectinase producing fungal isolate

The pH variations for pectinase production at 4.5,5,5.5 were carried out for *Fusarium solani, Aspergillus flavus, and Aspergillus fumigatus*. This***Fusarium solani*** **shows maximum pectinaseproduction at pH 4.5 this is the first report**followed by *Aspergillus fumigatus* at pH5 and *Aspergillus flavus*at 5.5 in sabouraud mediumand the pH carried out for *Aspergillus niger*at 6,6.5 and 7 in czapekdox medium.the results are shows in (Figure2 and 3)

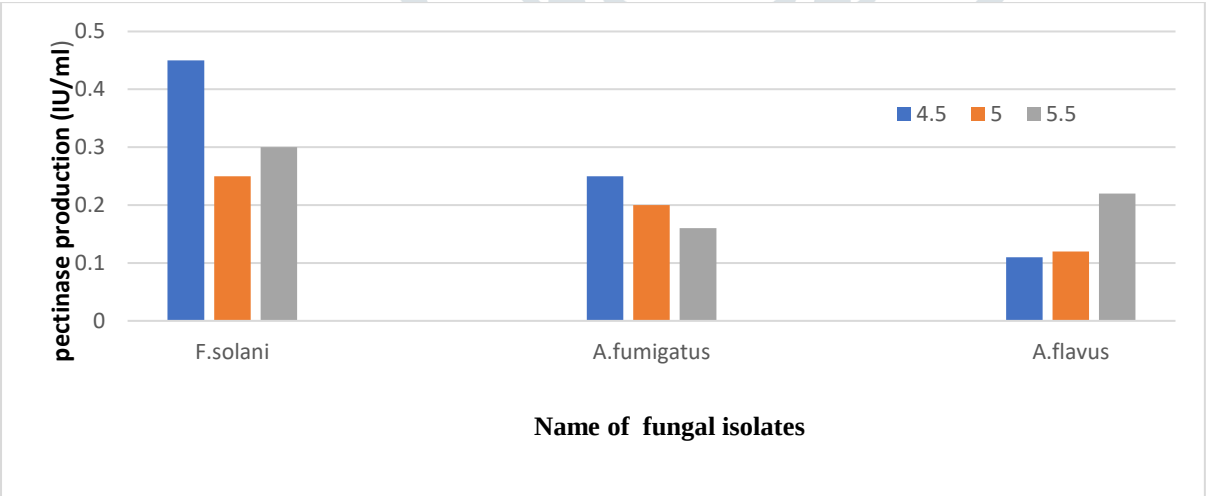


FIGURE NO.2: Optimization of pH on pectinase producing fungal isolates in sabouraud media

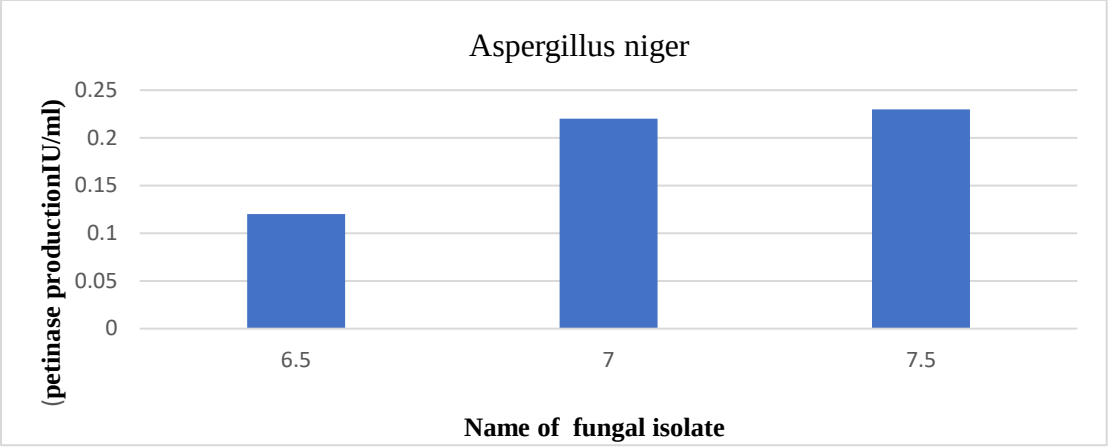


FIGURE NO 3: Optimization of pH on pectinase producing fungal isolates in Czapek dox medium

Optimization of Carbon source on pectinase producing fungal isolates:

4 different carbon sources were used for the production of the pectinase enzyme. They arelemon peel,orange peel, mosambi peel, and rice bran. Among the 4 carbon sources lemon peel proved to be more effective in pectinase production in the growth of *Fusarium solani***this is the first report in which maximum pectinase was produced,**followed by*Aspergillus flavus, Aspergillus Niger, Aspergillus fumigatus***.**The results are shows in (Figure 6).

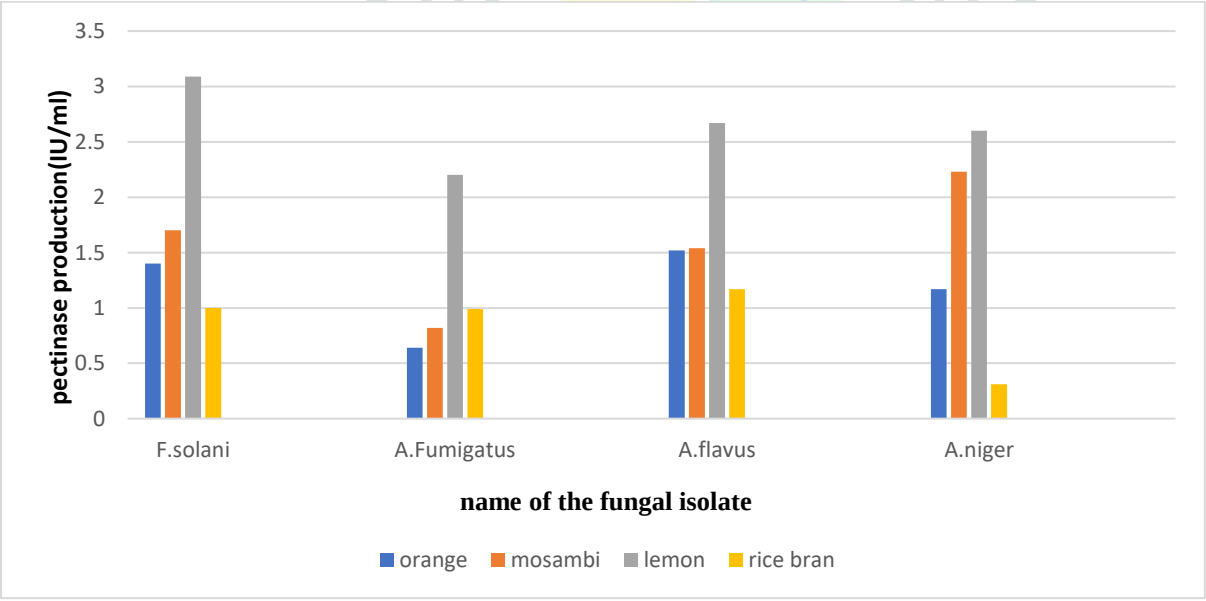


Figure 4:Optimization of Carbon source on pectinase-producing fungal isolates:

Optimization of Nitrogen equivalents on pectinase producing fungal isolates:

To enhance pectinaseproduction in czapek dox medium sodium nitrate was used and peptone was used as nitrogen equivalents in sabouraud medium. In **peptone at a concentration of 1.5% *Fusarium solani*shows the high potential production of pectinase this is the first report**and *Aspergillus niger* shows good results in sodium nitrate at 0.3%.The results are shows in (Figure5 and 6)

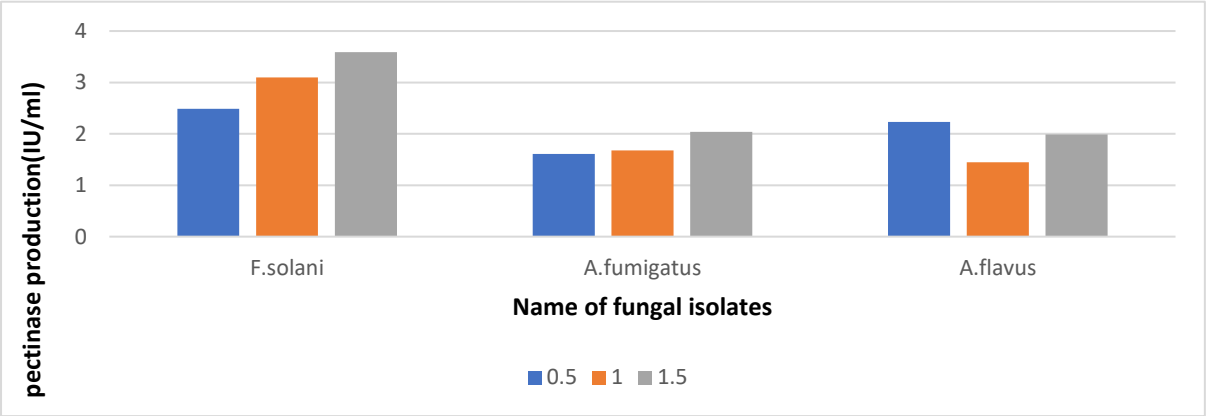


FIGURE NO 5: Optimization of Nitrogen equivalents on various pectinase producing fungal isolates in sabouraud agar medium

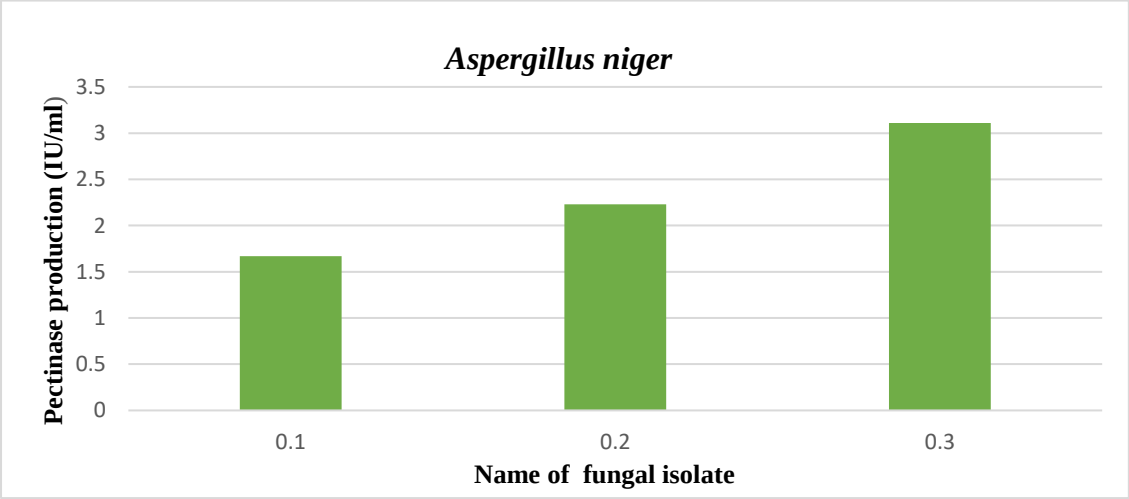


FIGURE NO.6: Optimization of Nitrogen equivalents on pectinase producing fungal isolates in Czapek dox medium

Optimization of Fermentation period on pectinase producing fungal isolates:

liquid state fermentation was performed in a 100 ml conical flask to produce the pectinase enzyme.It determines how well enzymes are generated.The culture organisms *F.solani*, *A.fumigatus*, *A.flavus*, and *A.niger* were inoculated into the conical flask that contained the

appropriate media.It was done on the third and sixth days.The maximum enzyme synthesis was seen in ***Fusarium solani* on day six**, while *Aspergillus fumigatus* showed the least production on day three. The results are shows in (Figure 7)

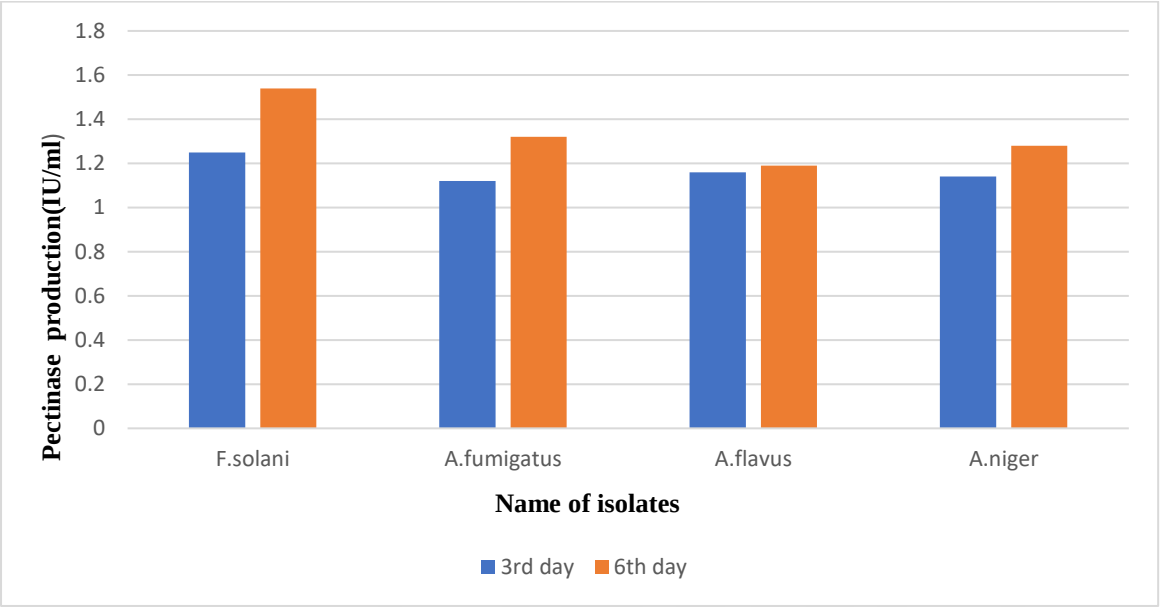


FIGURE NO.7: Optimization of Fermentation period on pectinase producing fungal isolates

Application of enzyme in sewage water treatment:

The crude enzymeobtained from *Fusarium solani* is partially purified.to determine the activity of enzyme initial TDs, and the pH of the sewage water was noted. After treating thepartiallypurified enzyme in the sewage water at the concentration of (3%, 5%, 10%). 5 % shows the best TDS of 1770ppm and pHof 7.9the resultsare shows in (Figure8 and 9)

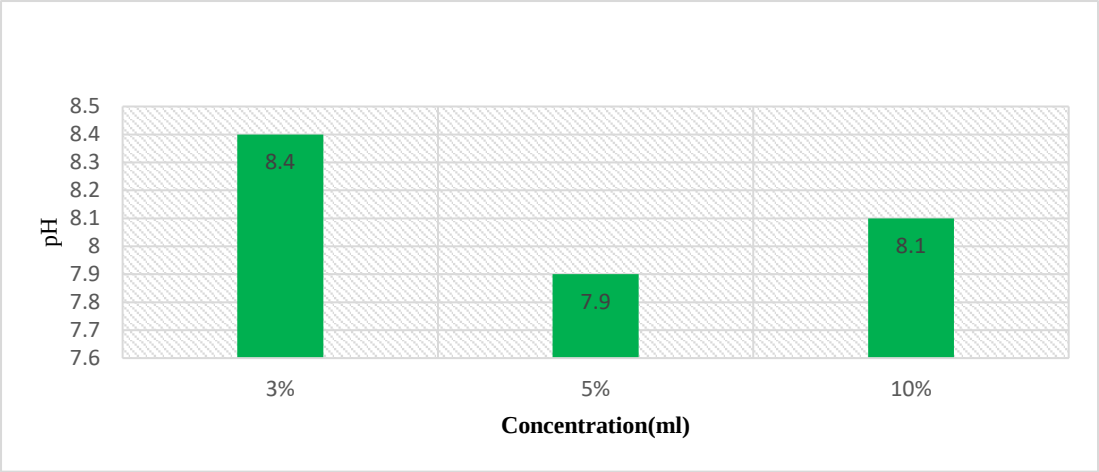


FIGURE NO8: pH values on various concentrations of enzyme in sewage water

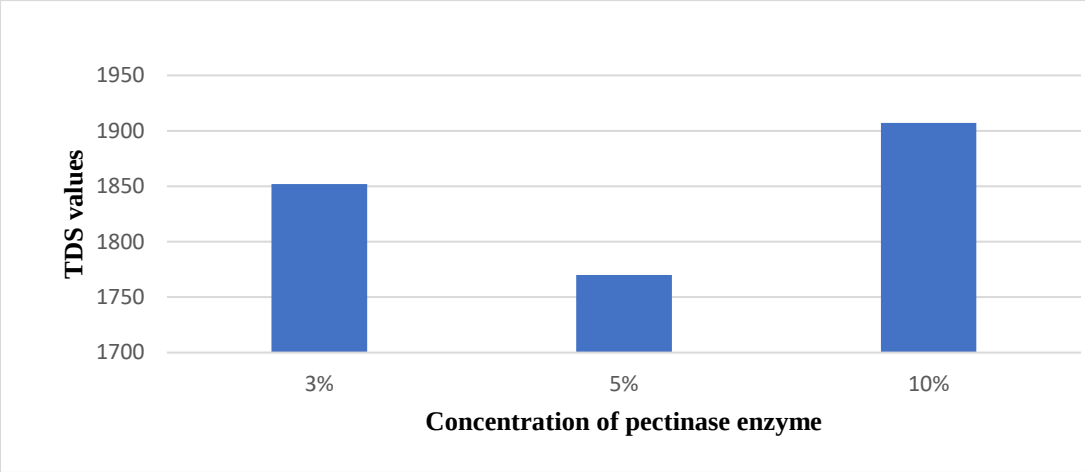


FIGURE NO.9: TDS values on various concentrations of enzyme in Sewage water

Protein estimation of thepartially purified enzyme:

To determine the protein content of the enzyme protein estimation.Sample at a concentration of 0.2% shows ahigh protein content.The resultareshows in (Figure 10)

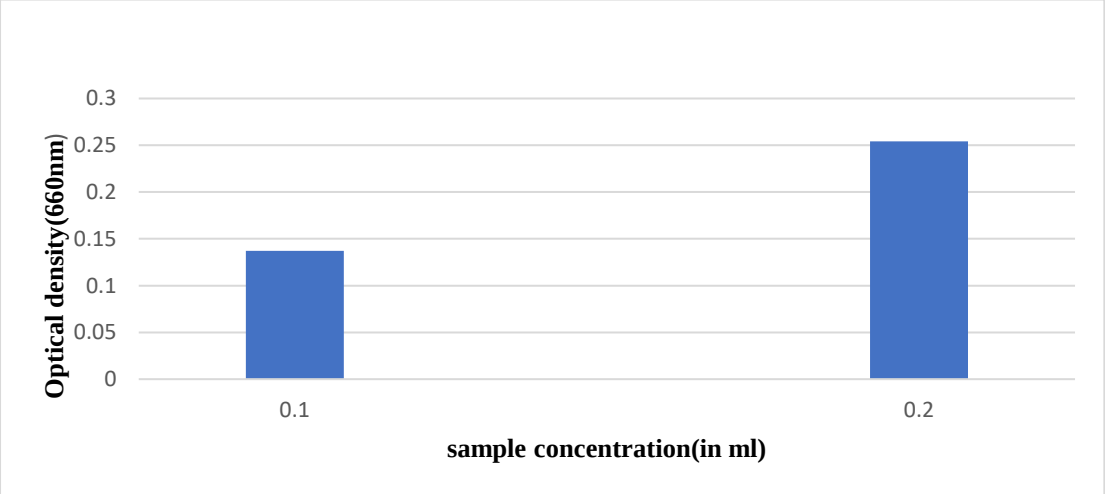


FIGURE NO.10: Estimation of protein in partially purified pectinase enzyme

Conclusion:

Microbial enzymes are required due to the expanding population.Pectinase is one of the enzymes that meet the global need.Commercial pectinase is expensive; however, by employing agro-waste, we can reduce production costs.The pectinase enzyme used in this work was synthesized from decaying sapota soil fruit waste, which is a cheap and easily available eco-friendly product.To my knowledge, no research proof has been discovered, and this is the first report.It has been proven that the pectinase enzyme produced can be

effectively used in sewage water treatment. In the future, this enzyme could be applied at the protein molecular level in the field of bioinformatics.

Acknowledgment:

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