INTERNATIONAL JOURNAL OF RESEARCH AND SCIENTIFIC INNOVATION (IJRSI)
ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue II February 2023

Tomato fruits as a Potential source of Pectinolytic Enzyme Production by Pathogenic Fungi

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Received: 07 January 2023; Revised: 21 January 2023; Accepted: 01 February 2023; Published: 28 February 2023

Abstract: - Microbes are well known source for the production of extracellular enzymes and industrial important secondary metabolites. Since fruits contain pectin, hence any microorganism that will involve in their spoilage must naturally possess cell wall destroying ability. This study was aimed at isolation and characterization of pectinase producing fungi from Tomato fruits. Tomato fruits were collected from Bodija. Market, Ibadan, Nigeria. Spoilage fungi were isolated and characterized with standard techniques. Six (6) fungi were isolated from the fruits: *Aspergillus niger*, *Penicillium nigricans*, *Rhizopus stolonifer*, *Fusarium oxysporium* and *Trichoderma harzanium*. The fungi were screened for the pectinolytic enzyme production on solid media. Among the isolates, *Aspergillus niger*, *Penicillium nigricans*, *Rhizopus stolonifer* and *Fusarium oxysporium* were selected for pectinase production using solid state fermentation (SSF) with Orange peel. Crude enzyme production was optimized with pH, temperature, substrate concentrations and incubation period. All the isolates produced maximally at pH 7, 1.5 substrate concentration , 50 ^{0}C and 72Hr incubation except *Penicillium nigricans* at pH(5) and temperature of 40^{o}C. These organisms can further be studied for their enzymes to be purified and characterized.

Keywords: pectinase; *Aspergillus niger*; *Penicillium nigricans* ; extracellular enzymes; fermentation

I. Introduction

Vegetables constitute commercially and nutritionally important indispensable food commodity. Vegetable naturally play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. Vegetables are widely distributed in nature. One of the limiting factors that influence the fruit economic value is the relatively short shelf-life period, caused by pathogen's attack (Droby, 2006; Zhu, 2006).

Tomato (*Lycopersicon esculentum*) is the most important vegetable worldwide. Tomato is an annual plant, which belongs to the Solanaceae family, which includes other well-known species such as Potato, Tobacco, Pepper and Egg plants (aubergine) and can reach a height of over two meters. It is a berry fruit grown mainly in soil (Frazier and Westhoff, 2004). Tomato is essential mainly for its dietary needs and can be consumed in diverse ways. It can be cooked as vegetable, as an ingredient in many dishes and sauces; in the making of stew, fruit juices and can be eaten raw in salads (Masefield *et al*., 2002). Tomato is grown in gardens and irrigation schemes in West Africa. In Nigeria, most tomatoes are grown in the northern parts of the country (Erinle, 2007) and there is no record of any systematic or organized traditional storage method for vegetables and fruits. They are usually sold immediately after harvesting. They are packed in baskets, cardboard boxes, or wooden crates ready for transportation to the markets. Tomato is the most perishable vegetable during handling, transportation and storage. This is because tomato contains large amount of water that makes them susceptible to spoilage by the action of microorganisms such as fungi, bacteria and protozoans. Estimates have shown that about one third of the produce is lost before reaching the consumer (Erinle, 2007), this loss has been attributed to a number of factors which include physical (mechanical breakage, bruises), physiological and also damages caused by pathological agents (Thompson and Kelly, 2000). Market value of the tomato are mainly reduced by these factors. The spoilage of food by microorganisms should not be viewed as a sinister plot on the part of the microbes deliberately to destroy foods but as a normal function of these organisms in the total ecology of all living organisms (Watt and Merrill, 2000). It has also been estimated that 20% of all fruits and vegetables harvested for human consumption are lost through microbial spoilage causing one or more of 250 market diseases (Watt and Merrill, 2000). Spoilage of fresh tomato usually occurs during storage and transit and while waiting to be processed. Frazier and Westhoff, (2004) reported that fruits and vegetables after picking continue to respire and as a result heat is produced, this heat is known as 'vital heat', the amount which varies with amount of fruits and vegetables. Vegetables that respire more thereby generating most heat are those that perish/spoil most rapidly (Wheeler, 2005). The resulting respiration of the fruits in addition to the normal ripening process complicates an independent discussion on microbiological spoilage of fruits in general and tomato in particular. *Fusarium oxysporium* is a fungus that cause wilt disease on tomato. Fusarium is more common in replant

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fields and gardens and are also destructive on greenhouse tomatoes and in commercial stake tomatoes. Fusarium wilt is considered a warm temperature disease.

Enzymes are proteins that speed up the rate of reaction. Pectinases or pectinolytic enzymes are today one of the upcoming enzymes of the commercial sector. It has been reported that microbial pectinases account for 25% of the (Jayani *et al*., (2005) global food enzymes sales. Pectinase is a group of enzymes consisting of lipase, hydrolyses, lyase and it is able to breakdown pectin and plant cell wall. Primarily, these enzymes are a heterogeneous group of related enzymes that hydrolyze the pectic substances. Pectinolytic enzymes are widely distributed in higher plants Sharma *et al*., (2011), and microorganisms (Akinyele *et al*., 2017). They are of prime importance for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage (Reda *et al*., 2008). They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials (Jayani *et al*., 2005). Different types of pectinase enzymes vary in pectin degrading capacity, some degrade pectin to a long polysaccharide of sugars that forms a gel. Pectin forms the center of the plant cell wall. When pectin is degraded by pectinase, the cell walls become weaker. For instance, during fruit ripening, the enzyme polygalacturonase helps certain fruit, such as tomatoes, become soft and edible (Reda *et al*., 2008). Pectin is complex negatively charged heteropolysaccharide and is major component of cell wall of plants. Pectic substances contribute 0.5- 4% of fresh weight of plant materials. (Jayani *et al*., 2005). Pectin are bio-converted into its simpler and high value end products by pectinases based on substrate specificity and specific catalytic mechanism (Kholi and Gupta, 2015). Pectinases are commonly employed in food industry for clarification of fruit juices, preparation of functional foods, fruits and vegetable processing, animal feed industry, processing of wine and fermentation of tea, coffee and cocoa beans (Kashyap *et al*., 2001; Sharma *et al*., 2017;Deng *et al*., 2019).

Most fruits and vegetables are subjected to attack and spoilage by microorganisms, fungi inclusive and these plant produces are discarded alongside with the pathogens. However, these pathogens can be of great importance by employing them in production of products of industrial importance. This work investigates the potentiality of tomato fungal pathogens in the production pectinolytic enzyme.

II. Materials and Methods

Sources of Samples and sample Size

Tomato fruits were purchased from two different markets in Ibadan North Local government area of Oyo State, Nigeria. Six (6) samples were collected from these markets (Bodija and Shasha market). Three samples were randomly selected from three different traders in different location in each market.

Isolation of Fungal and Identification of Fungal

The diseased tomato samples were first washed under a running tap water, then dipped into 1% Sodium Hypochlorite to surface sterilize for three seconds and rinsed in three changes of sterile distilled water. They were then blot dried by using sterile blotting paper. For fungal isolation, direct planting method was used. A sterile scalpel was used to cut 3 mm × 3 mm section of tissue from the tomato moving from the healthy portion to the decayed portion where the pathogens are likely to be more active. The pieces were dried using sterile filter paper and the dried infected tissues were directly plated on sterile PDA. They were incubated in the laboratory at room temperature (25°C) for 5 days. After incubation, different colonies were selected and subcultured onto PDA plates containing pectin and incubated at 25 ◦C for 3–5 days. Fungal colonies with distinct morphology were selected and subcultured repeatedly to obtain pure culture by point inoculation fungal colonies. Pure cultures of microorganisms were then inoculated on slants and stored at 4 ^{0}C for further study. Identification of Fungal isolates was according to Akinyele *et al*. (2020).

Pectinase Screening Assay

The isolates were preliminarily screened for pectinase synthesis using pectinase screening agar medium (PSAM) containing (g/L): Tryptone 10, Citrus Pectin 5, Sodium Chloride 5, Yeast Extract 5, and Agar 15. The pH of the medium was adjusted to 5.5 ± 0.5 before sterilization and then autoclaved with a temperature of 121◦ C for 15 minutes. Finally, 20–25 ml of media was poured on sterile Petri dishes in the microbiological hood and allowed to solidify at room temperature. Isolates were inoculated into the media and incubated at 30 ^{0}C for 3-5 days. At the end of the incubation period, the plates were flooded with 50 mM Potassium iodide-iodine solution. A clear halo zone around the colonies indicates the ability of an isolate to produce pectinase (Beg *et al*, 2000).

Enzymatic Index (EI) was calculated for each fungal culture by following formula:

Enzymatic Index = Zone Size of Hydrolysis/ Fungal growth on the plates

Enzyme Production

Solid State Fermentation for Enzyme Production

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Solid-state fermentation (SSF) was carried out on *Aspergillus niger*, *Penicillum citrinum*, *Rhizopus nigrificans* and *Fusarium oxysporum* using a 250 mL Erlenmeyer flask containing 5g of sterilized substrate of orange peel (since the pectin used is from citrus) and 5ml of distilled water. The flask was sterilized at 121ºC for 15mins and cooled to room temperature. It was then inoculated with the fungal isolates, mixed well. After inoculation, 10 mL of nutrient solution, composed of 0.1% NH_4NO_3; 0.1% $NH_4H_2PO_4$; 0.1% $MgSO_4$ x $7H_2O$, was added to each of the flasks, incubated at 30ºC for 120 hours. At the end of the incubation period, the flask was taken out and each content were extracted with 25mL sterile distilled water at 24h intervals the solid fermented material, corresponding to one Erlenmeyer flask, was mixed with 30 mL of distilled water, stirred for 40 min, filtered and centrifuged. The supernatant was used as crude enzyme solution. (Martin *et al*., 2002). The pectinase activity in the fermented broth was monitored by using the dinitro salicylic acid (DNS) assay method as described by Miller, 1959. One unit of pectinase was defined as the amount of enzyme that released Iμmol reducing sugar as glucose equivalent per minute in the reaction mixture under the specified assay conditions

Effect of Various Factors on Enzyme Production

Effect of Incubation period on Enzyme Activity

To study the effect of incubation period, solid state fermentation was carried out and DNS assay was performed after 48hours to 120 hours. For estimation of optimum incubation time, the enzyme assay was carried out at optimum pH, substrate concentration and temperature in different incubation time (48, 72, 96 and 120 hours).

Effect of Temperature on Enzyme Activity

For temperature assessment, 1 mL of enzyme suspended on sodium acetate buffer (0.1 M, pH 4.2) was added to 1 mL of 0.3% (w/v) pectin solution in sterile tubes. The tubes were incubated from 30 to 60ºC for 15 min. After incubation, 2 mL of the DNS reagent was added and incubated in a boiling water bath for 15 min. The enzyme activity was monitored by the DNS assay method.

Effect of pH on Enzyme Activity

The effect of pH on enzyme activity was tested using sodium acetate buffer (0.1 M; pH range, 3 –5), sodium phosphate buffer (0.1 M; pH range, 5 –7) and Tris-HCl buffer (0.1 M; pH range, 7 –9). Tubes containing 0.5 mL respective buffers were mixed with 0.5 mL of the enzyme. Then, 1 mL of 0.3% (w/v) pectin solution was added and all the tubes were incubated at 30ºC for 10 min. Afterward, 2 mL of the DNS reagent was added and incubated in boiling water for 15 min. The enzyme activity was measured by DNS assay method. The best pH for enzyme activity was determined by carrying out the enzyme assay at optimum temperature in different pH levels (3, 5, 7 and 9).

Effect of Substrate Concentration on Enzyme Activity

Pectin substrates of different concentrations (0.5%, 1.0%, 1.5% and 2.0%) were prepared in different tubes to evaluate the effect of substrate concentration on enzyme activity. One mL of enzyme suspended in acetate buffer (0.1 M, pH 4.2) was mixed with 1 mL of the respective substrate concentrations. Determination of the pectinase activity was done by the DNS assay method.

III. Results and Discussion

Table 1: Identification of the Fungal Isolates

Sample ID	Fungal Isolates	Fungi Description
A	*Trichoderma hazanium*	It has 1-2 concentric rings with green conidia production. The conidia were denser in the centre towards the margins. It has a branched-conidiophores that duster into fascicles. It also has broad/straight flexuous branches.
B	*Aspergillus niger*	Blackish-brown often with yellow mycelium. Reverse greenish-yellow to yellow-orange. Its heat globose, splitting with age. Its metulac is long closely packed, and brownish.
C	*Penicillum citrinum*	The diameter of the colony is 2.3 to 3.2cm. The texture is radially sulcate

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		floccose in center and velutinous at margins, it has yellow exudates on surface. It is Greenish-blue green while it's pale to brown in reverse. The penicillin is biverticillate, philades ampulliform. It has a medium sized collula. The conidia is spherical, smooth and greenish.
D	*Rhizopus nigrificans*	These phycomycetes bear its spore in sporangia and grow fast on Acid-potato-Dextrose Agar (APDA). It is white or pale grey mycelium. The young mycelium at the border of the colony consists of characteristically branched cells of large diameter. The sporangiophores arise in groups of two or three from a tangle of rhizoids and they are not branched.
E	*Fusarium oxysporum*	The texture is floccose and whitish-cream in colour. chlamydospores are abundant and usually single on hyphae. The reverse is pale to bluish violet in colour.

Plate 1: Pectinase assay of *Aspergillus niger*

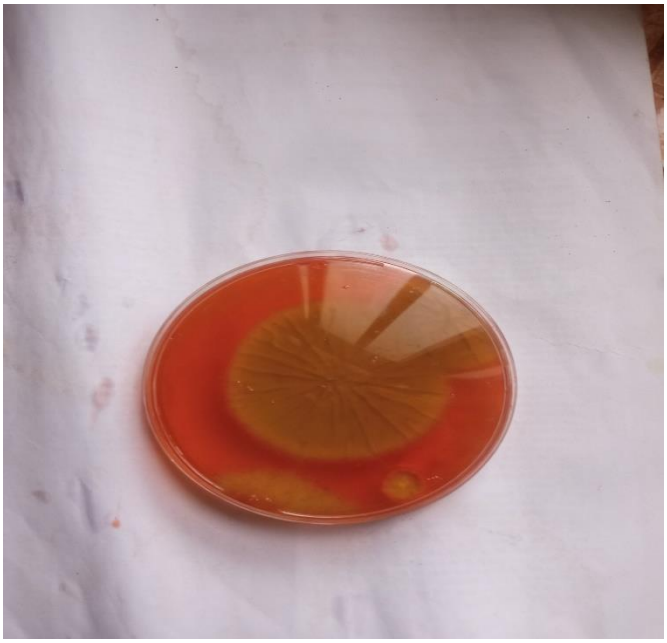

Plate 2 : *Fusarium oxysporum*

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Table 2: Qualitative Assay of Pectinase from Fungal Isolates

Sample ID	Fungal Isolates	Enzyme activity (mm)
A	*Trichoderma harzanium*	5
B	*Aspergillus niger*	11
C	*Penicillum citrinum*	6
D	*Rhizopus nigrificans*	5
E	*Fusarium oxysporum*	3

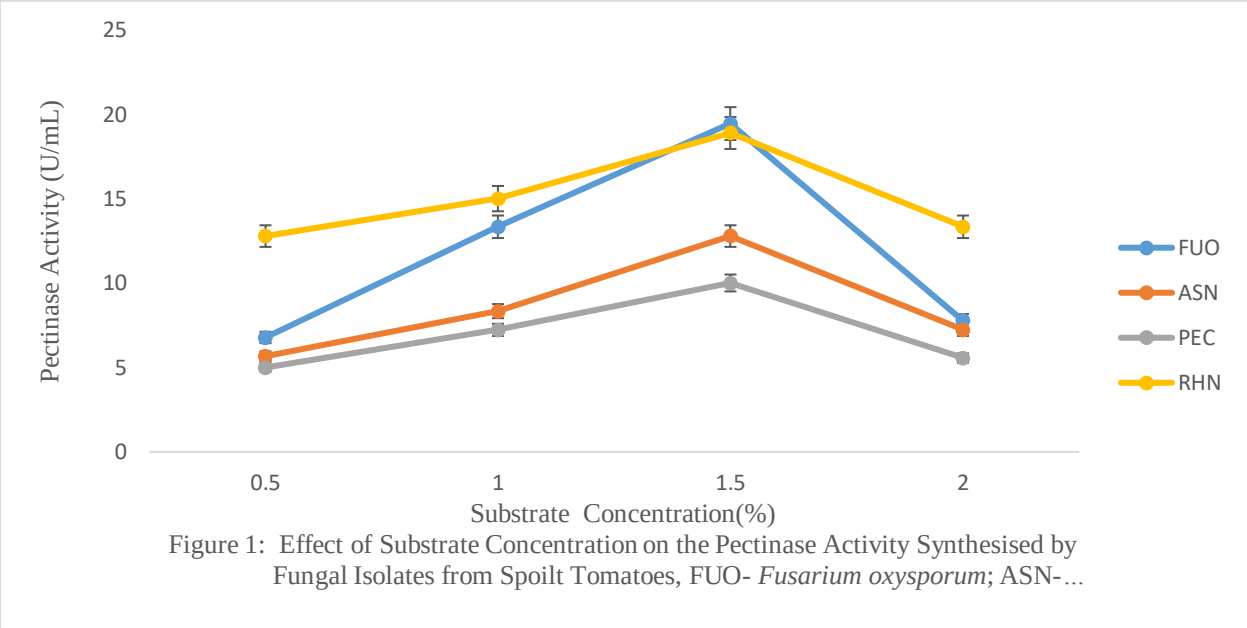

Figure 1: Effect of Substrate Concentration on the Pectinase Activity Synthesised by Fungal Isolates from Spoilt Tomatoes, FUO- *Fusarium oxysporum*; ASN-...

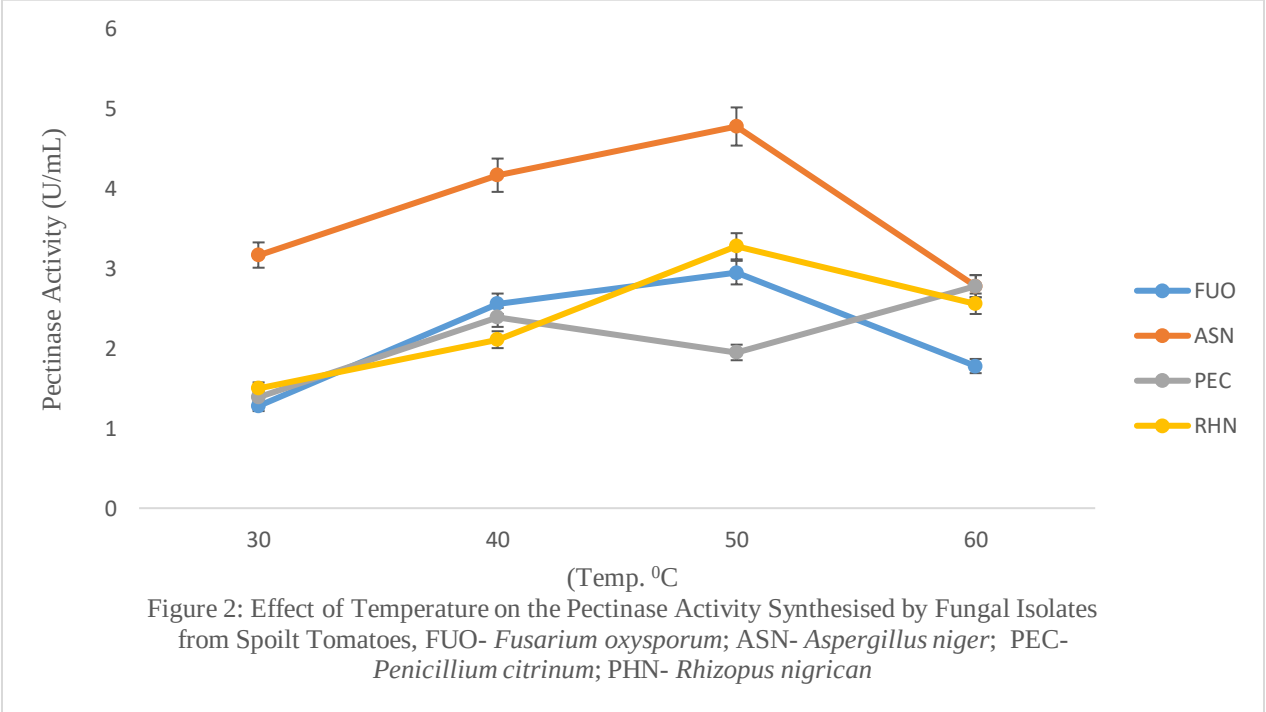

Figure 2: Effect of Temperature on the Pectinase Activity Synthesised by Fungal Isolates from Spoilt Tomatoes, FUO- *Fusarium oxysporum*; ASN- *Aspergillus niger*; PEC- *Penicillium citrinum*; PHN- *Rhizopus nigrican*

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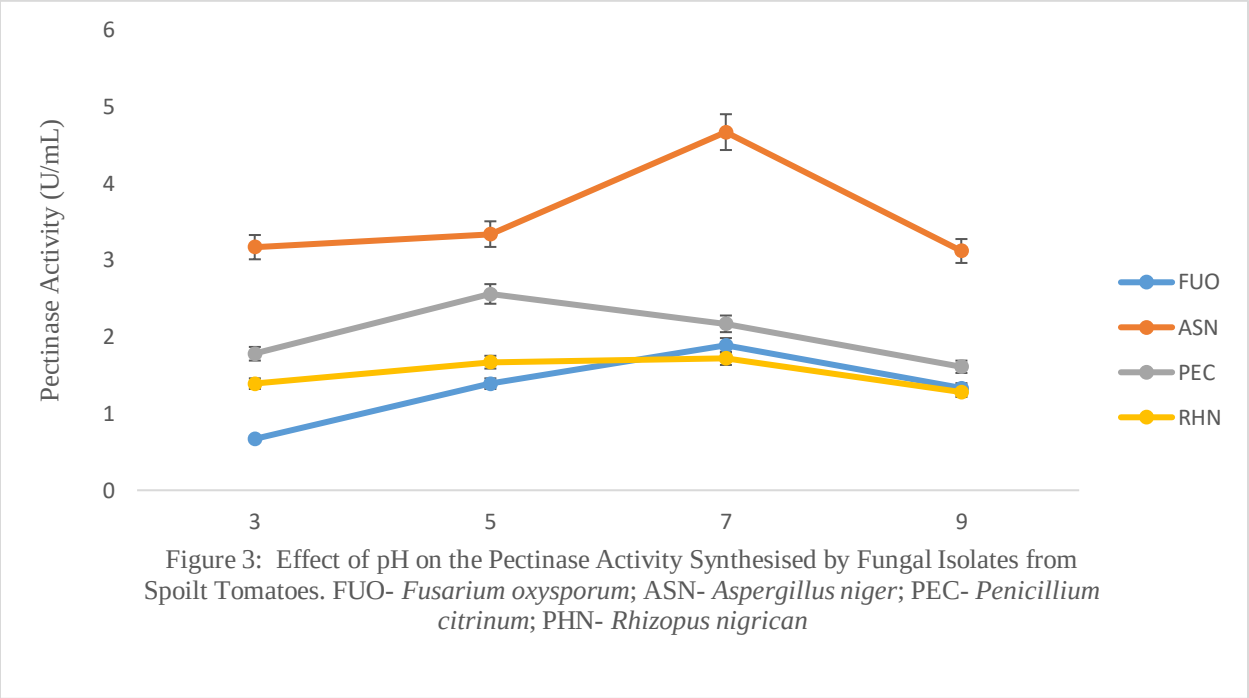

Figure 3: Effect of pH on the Pectinase Activity Synthesised by Fungal Isolates from Spoilt Tomatoes. FUO- *Fusarium oxysporum*; ASN- *Aspergillus niger*; PEC- *Penicillium citrinum*; PHN- *Rhizopus nigrican*

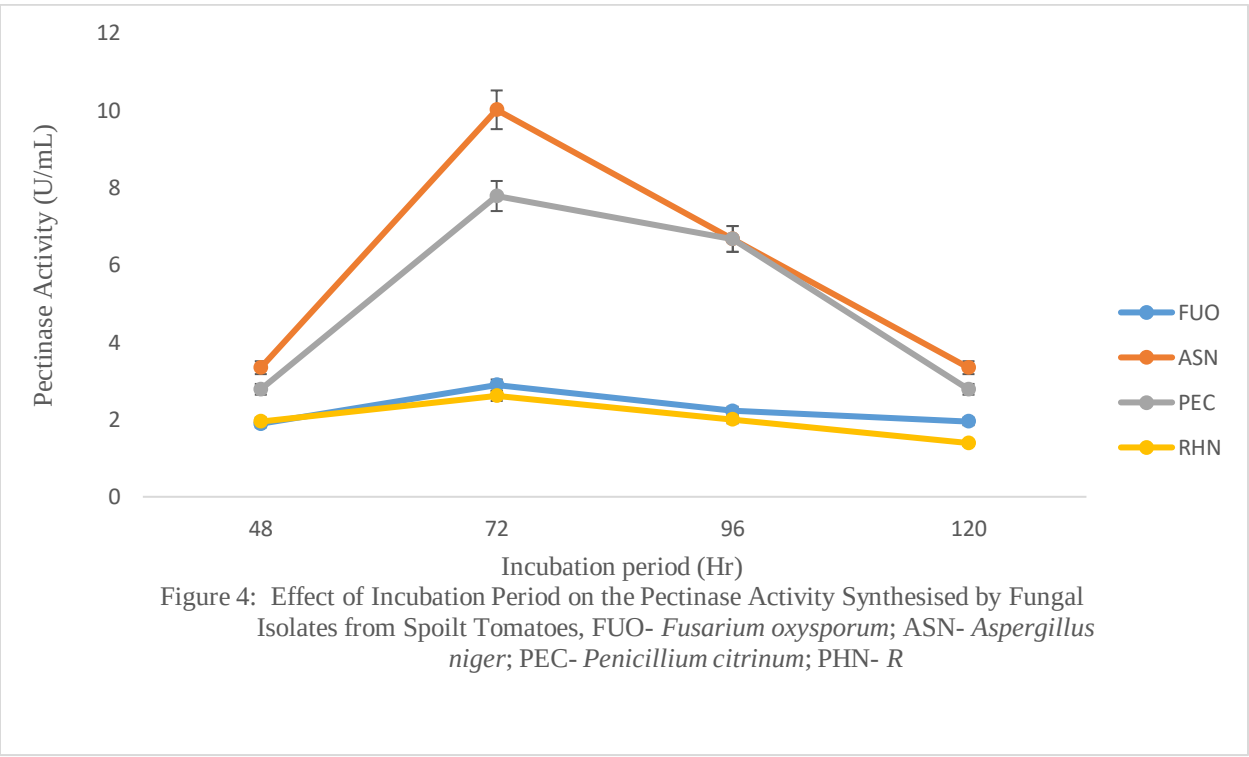

Figure 4: Effect of Incubation Period on the Pectinase Activity Synthesised by Fungal Isolates from Spoilt Tomatoes, FUO- *Fusarium oxysporum*; ASN- *Aspergillus niger*; PEC- *Penicillium citrinum*; PHN- *R*

Discussion

Pectinolytic enzymes are of significance in the current biotechnological era with their embracing applications in food industries, alcoholic beverages and tea fermentation (Sharma *et al*., 2011). In this study, fungi *Aspergillus niger*, *Penicillium nigricans*, *Fusarium oxysporium*, *Rhizopus stolonifer* and *Trichoderma harzanium* were isolated from spoilt tomato and this is comparable to the findings of Yusuf *et al*., (2020), who identified the same organisms from his study. He stated that *Aspergillus* spp is one of the major fungi responsible for the production of volatile compound in spoilt tomato. Akinmusire, (2006), reported

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that *Rhizopus* spp were associated with tomato spoilage. Wogu and Ofuase, (2014), also reported isolation of *Aspergillus* spp, *Penicillium* sp and *Fusarium* spp from spoilt tomato fruits.

The fungi isolated in this study were used for screening and production of pectinase, *Aspergillus niger*, *Fusarium oxysporium*, *Penicillium nigricans* and *Rhizopus stolonifer* were selected for further studies. Among the four isolate, A. *niger* produced the highest zone of hydrolysis (11mm). This observation is supported with the study of Patil and Dayanand, (2010), who had mentioned about hydrolysis around colony indicating the zone by *Penicillium* spp is also supported with the report of Sudeep *et al*., (2020), who chose *Aspergillus niger* because it was found to be a potent strain for the enzyme production. *Aspergillus niger* has the highest pectinase produced at 72 hours incubation period. This observation is in agreement with the report of Abdullah *et al*., (2018); Rashmi *et al*., (2008), who reported that the highest enzyme activity of *A. niger* at 48-72 hours after which the enzyme production declined. Plautela *et al*., (2005), stated that the cause of decrease in enzyme production after certain time interval during incubation might be due to the exhaustion of essential supplements and/ or accumulation of toxic metabolites in the culture medium. This observation was noted with *Rhizopus stolonifer*, *Fusarium oxysporium* and *Penicillium nigricans* for incubation period. The shorter fermentation period of 48-72hrs among fungi could be advantageous for production of pectinase at industrial scale (Plautela *et al*., 2005; Sudeep *et al*., 2020)).

In this study, all the isolates except *Penicillium* sp. have their maximal activities at 50^0C. This attribute in of great importance to withstand heat generated during industrial fermentation. This is observation has equally been reported for fungal isolates (Natalia et al., 2004). Similar to Sudeep *et al*. (2020), who reported highest enzyme activity at the second temperature used in his study (30^oC), the highest enzyme activity reported in this study was the second temperature (40 ^{o}C) and he stated that at a higher temperature, enzyme production declined. Al-Najada *et al*., (2012), reported that enzyme is usually denatured at higher temperature resulting to decreased activity. Previous studies from Singh and Mandal, (2012); Khatri *et al*., (2015), have also stated that the maximum yield of pectinase enzyme from the members of genus *Aspergillus* spp occur at the temperature range of 30^oC- 50^oC. The results in our study also conform with these earlier observations as the highest pectinase production of *Aspergillus niger* is at 50 ^{o}C. With 1.5 % substrate concentration the strains produced maximum pectinase, the enzyme production was found to be decreased when the substrate concentration was more than 1.5%. This observation has been observed by Sudeep *et al*., (2020). Biz *et al*., (2014); Patil and Chaudari, (2010; Akinyele *et al*., 2020), reported that a higher substrate concentration increases the viscosity of culture media and creates nutrient rich environment. Higher nutrients and substrate in the fermentation media inhibit microbial growth lowering enzyme production. Akhter *et al*., (2011), also stated that lower substrate concentration is effective for enzyme production. The requirement of low substrate concentration might be cost effective for large scale production of pectinase enzyme. The enzyme produced from the strains was found to be active over a wide range pH (5-9) which is similar to the findings other researchers (Natalia *et al*., 2004;Sudeep *et al*., (2020), who reported active enzyme production of pH (3.2-9). Although, the optimum enzyme activity was observed at pH 5-7 indicating that the pectinase enzyme showed higher activity in the slightly acidic range. Highest activity at pH 5-7 was observed and this may be due to enzymes activity stability. Fungal pectinases are found to be stable from acidic to alkaline pH ranges (4-8) but their activity and stability may have different peaks at different pH ranges as stated by (Jayani *et al*., 2005). From these optimization data, this study found that 72 hours incubation period, 1.5% substrate concentration, 50^oC temperature and pH 5-7 could be the optimum cultural conditions for *Rhizopus stolonifer*, *Fusarium oxysporium*, *Aspergillus niger* and *Penicillium nigricans* to produce maximum pectinase enzyme.

IV. Conclusion

The present study revealed the isolation of *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium*, *Penicillium nigricans* and *Trichoderma harzanium* from spoiled of Tomato fruit. The maximum pectinase production was obtained under optimal cultivation conditions of 48 hours of incubation period 1.5% substrate concentration, 50^oC temperature and pH 5-7. The isolates exhibited production of Pectinolytic enzyme. 75% of required 50 ^{0}C for the enzyme synthesis which make the isolates good candidates for industrial application.

References

1. Abdullah, R., Farooq I., Kaleem, A., Iqtedar, M, M. And Iftikhar, T. 2018. Pectinase from *Aspergillus niger* IBT - 7 using solid state fermentation. *Bangladesh journal of Botany* 47,473- 478. doi.org/10.3329/bjb.v47i3.38714
2. Akhter, N., Morshed, M. A., Uddin, A., begun, F., Sultan., T. And Azad A. K 2011. Production of pectinase of *Aspergillus niger* cultured in solid state media *International journal bioscience*, 1,33 - 42.
3. Akinmusire, O. O., 2006. "Fungi species associated with the spoilage of some edible fruits in maiduguri, North Eastern Nigeria." *Advances in Environmental Biology*, vol. 5, pp. 157-161.
4. Akinyele A. Hafiz, Babalola A Moses, Nwokeoma Cheryl and Adesemoye A. Elizabeth. 2017. Isolation of Fungi from Sawdust and their Ability to Produce Pectinase. *FJPAS* Vol 2(1): 244-251.

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue II February 2023

5. Akinyele H.A., Taliat A.A.T. Enwerem G.C. , Dawodu O.G and Owojuyigbe O.S. 2020. LignocellulosicWaste Degradation Potential of Some Cellulolytic Fungal Strains Isolated from Putrid Fruits. *J Pure Appl Microbiol* 14(4):2585-2593. https://doi.org/10.22207/JPAM.14.4.34
6. AL-Najada, A. R., AL-Hindi R, R., Mohammed S. A 2012. Characterization of polygalacturonase from fruit spoilage *Fusarium oxysporum* and *Aspergillus tubingenesis*. *African journal biotechnology*, 11, 8527 - 8536. DOI**:** 10.5897/AJB12.355
7. Beg Q. K., Bhushan B., Kapoor M., and Hoondal G. S. 2000. "Production and characterization of thermostable xylanase and pectinase from Streptomyces sp. QG-11-3," *Journal of Industrial Microbiology and Biotechnology*. vol. 24 (6): 396–402. doi.org/10.1038/sj.jim.7000010
8. Biz, A., Frarias F. C., Motter, F. A., De Paula, D. H., Richard,P., Krieger, N.and Mitchell, D. A 2014. Pectinase activity determination : An early deceleration in the release of reducing sugars throws a spanner in the works. PLoS ONE, 9, e 109529.
9. Droby, S. (2006). Improving quality and safety of fresh fruits and natural materials. *Asiahorticulture*. 709: 45 - 51.
10. Erinle, I. (2007). Tomato diseases in the Northern States of Nigeria, *Agricultural . Extention Resource. Liaison Services, Extention. Bull* No. 31.
11. Frazier, W. C. and Westhoff D. C. (2004). Food Microbiology 3rd Edition, *McGraw-Hill Book Company*, New York, St. Louis, London, pp. 17-40.
12. Jayani, R.S., Saxena, S. and Gupta, R. 2005. Microbial Pectinolytic enzymes: a review. *Process Biochem*. 40: 2931-2944. doi.org/10.1016/j.procbio.2005.03.026
13. Khatri, B.P., Bhattarai, T., Shrestha, S. and Maharjan, J. 2015. Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolated from Manaslu Conservation Area, Gorkha, Nepal. Springerplus, 4, 488. doi.org/10.1186/s40064-015-1286-y
14. Kohli, P. and Gupta R. 2015. Alkaline pectinases: A review. *Biocatalysis and Agricultural Biotechnology*. 4: 279-285. doi.org/10.1016/j.bcab.2015.07.001
15. Martins E.S., Silva D., Da Silva R., Gomes E. (2002). Solid state production of thermostable pectinases from thermophilic *Thermoascus aurantiacus***.** *Process Biochemistry*. 37 (9): 949-954. doi.org/10.1016/S0032-9592(01)00300-4
16. Masefield, G.B., Willis, M., Harrison, S.G. and Nicholson, B.E. (2002).The Oxford Book of Plants. *Oxford University Press*. Pp. 124-129.
17. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959,31, 426–428.
18. Natalia Martin, Simone Regina de Souza, Roberto da Silva and Eleni Gomes. 2004. Pectinase Production by Fungal Strains in Solid-State Fermentation Using Agro-Industrial Bioproduct. *Brazilian Archives of Biology and Technology*. Vol.47, 5 : 813-819. doi.org/10.1590/S1516-89132004000500018
19. Patil, N.P. and Chaudhari, B.L. 2010. Production and purification of pectinase by soil Isolate *Penicillium* app and search for better agro residue for its SSF. *Recent Research science and technology* 2, 36 - 42.
20. Phutela, U., Dhuna,V., Sandhu, S. and Chadha, B.S. 2005. Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposting orange peels. *Brazillian Journal of Microbiology*. 36, 63–69. /doi.org/10.1590/S1517-83822005000100013
21. Rashmi, R., Murthy, K., Sneha, G.; Shabana, S.; Syama, A.; Radhika, V. 2008. Partial purification and biochemical characterization of extracellular pectinase from *Aspergillus niger* isolated from groundnut seeds. *Journal of Applied Bioscience* 9, 378–384.
22. Reda, A., Bayoumi, Hesham, M., Yassin, Mahmoud, A., Swelim, Ebtsam, Z. and Abdel. 2008. Production of bacterial pectinase from agro industrial wastes under solid state fermentation condition *Journal of Applied Sciences Research*, 4: 1708-1721.
23. Sharma N.R, Sasankan A.,Singh A.,Soni G. 2011. Production of polygalacturonase and pectin methyl esterase from agro waste by using isolates of *Aspergillus niger*, *Insight Microbiology* 1(1):1-7,ISSN 2041-0387. 10.5567/IMICRO-IK.2011.1.7
24. Singh, S., Mandal, S.K. 2012. Optimization of processing parameters for production of pectinolytic enzymes from fermented pineapple residue of mixed *Aspergillus* species. *Jordan J. Biol. Sci*. 5, 307–314.
25. Sudeep, K.C., Jitendra, U., Dev, R.J., Binod, L., Dhiraj, K.C, Bhoj, R.P., Tirtha, R.B., Rajiv, D. Santosh, K., Niranjan, K. and Vijaya, R. 2020. Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. *Mdpi journal of fermentation*, 6, 59. doi.org/10.3390/fermentation6020059
26. Thompson, H.C. and Kelly, W.C. (2000).Vegetable Crops.5th Ed.*McGraw-Hill book company. London.*Pp. 471-500.
27. Wogu M.D., Ofuase O. 2014. Microorganisms responsible for the spoilage of tomato fruits, *lycopersicum esculentum*, sold in markets in Benin City, Southern Nigeria. *Scholar's Academic Journal of Bioscience*, Vol. 2 (7): 459-466.

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue II February 2023

28. Watt, B.K., and Merril, A.L. (2000).Composition of Foods - raw, processed, prepared.*'Agricultural Handbook No. 8 Washington D.C. Agricultural Research Service, USDA.*
29. Wheeler, B.E.J. (2005). An Introduction to Plant Diseases. *John Wiley and Sons Ltd. London, New York., Sydney, Toronto.*Pp. 185.
30. Yusuf L., Agieni G. A and Olorunmowaju A. I. 2020. Isolation and Identification of Fungi Associated With Tomato (*Lycopersicon esculentum* M.) ROT. *Sumerianz Journal of Agriculture and Veterinary*. Vol. 3, (5): 54-56.
31. Zhu, S. J. (2006). Non-chemical approaches to decay control in postharvest fruit. In Nourdaie B, Ngrig, S. (Eds), Advance in Postharvest Technology for Horticultural crops. Arteand Signpast, Trwandrum, India. Pp. 297-313.