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Study Of Cellulase Produced By Pathogenic Fungi In *Carica Papaya*

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1. Abstract:

Extracellular enzymes are involved in the fungal pathogenesis in plants. Currently, culture media, data analyses, and data report related to extracellular enzymes produced *in vitro* conditions are different and therefore, lack standardization. This work with the potato-dextrose-agar (PDA) medium combined with a specific compound to produce extracellular enzymes through pathogenic fungi (*Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularialunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum, and Rhizopus stolonifer*), as well as to analyze and report enzyme data based on different criteria. The assay was randomized, with three factors (culture media, isolates, and enzymes). The studied enzymes were cellulase and pectinase. The normal media detected more enzymes and was more precise compared to the PDA medium plus specific compound. We suggest the normal media culture to study enzyme production, as well as the criteria mentioned to assess and report the data related to enzyme activities.

Keywords: *Carica papaya*, cellulase, occurrence, cellulose, substrate

2. Introduction:

Pathogenic fungi are known to produce cellulase which can degrade the cellulose in papaya (Kuhad et al., 2011). Cellulase play an important role in the infection process, entry, proliferation and can causing significant damage to the host plants(Lebeda et al., 2001). Therefore, understanding the production of cellulase by pathogenic fungi in papaya is important for developing strategies to control fungal infections. The hydrolytic enzymes produced by the fungi provide a genetic basis of the adaptability to thrive in the environment where the nutrients are not readily available for the growth(Walker & White, 2017). This renders the fungi to express secondary metabolites that can readily degrade the carbon source in the fruits to convert it to energy for itself. There is diversity of the presence of the genes coding for the hydrolytic enzymes and the expression varying with the environmental factors(Schaller et al., 2005). The study of the production of the hydrolytic enzymes can be done to characterize the pathogenicity of the fungal isolates(El_Komy et al., 2015).

The extracellular proteins that fungi release have the power to macerate tissues and break down cell wall constituents.Therefore, they must include the enzymes that correlate to the different kinds of glycosidic connections found in the polysaccharides found in cell walls. Extracellular enzymes are crucial to fungus not just for digestion but also frequently for the pathogenic process, as they can help the pathogen get past the host's built-in defences while also producing soluble by-products that can be ingested and used as food (Griffin, 1996).

Studies of enzymeproduction by a pathogenic fungus arecomplicated by the presence of plant,particularly by the presence of plantenzymes and microbial enzyme inhibitors.Studies of enzyme therefore, the most practical technique to research the synthesis of an enzyme by a fungi is to research the production of its enzymes on artificial growth conditions devoid of plant material or plant-produced enzyme inhibitors. The goal of this study was to ascertain how pathogenic fungi produce cellulase and pectinase enzyme in relation to growth circumstances and medium composition.

3 Materials and methods:

3.1 Collection of samples:

The papaya plants were collected from Aurangabad fruit market (19.8762° Latitude, 75.3433° Longitude). Two infected fruits of papaya from each location, and a total of 5 locations were selected for sampling. The infected patches of *Carica papaya* fruit samples were used to isolate pure cultures of pathogenic fungi. The fungal isolates were sampled from the papaya fruit peel on the places showing pathogenic symptoms to avoid redundant sampling.

3.2 Isolation of fungi:

Small pieces measuring 5 mm^2 each of infected tissue, were peeled off from these fruits with the help of a sterile sharp knife. The pieces were separately transferred to sterile potato dextrose agar plates (PDA) and incubated at 28 °C for seven days (Ugwuanyi & Obeta, 1991). Petri dishes was observed daily, and the distinct colonies of fungi were picked. The isolated fungi were purified using single spore technique (Leyronas et al., 2012) and the pure colonies of fungal isolates were maintained on PDA slants.

3.3 Identification of fungi:

The fungi occurring on each and every diseased tissue portion in plates were identified preliminary on the basis of sporulation characters like asexual or sexual spores and or fruiting structure with the help of microscope. In some cases the infected tissues were stained by cotton blue and Lactophenol (McCleary & Glennie-Holmes, 1985) and observed under compound microscope. The identification and further confirmation of the fungi were made by preparing slides of fungal growth and observing them under compound microscope. The identification was made with the help of manuals recommended by (Barnett & Hunter, 1972) and symptoms were confirmed by Koch's postulates. Pure culture of these fungi were prepared and maintained on PDA agar slants.

3.4 Production of cellulase and assay

The fungi were grown on a liquid medium containing CMC - 10 g, KNO_3 - 0.25%, KH_2PO_4, - 0.1%, and $MgSO_4$.7H20 - 0.05%, pH - 5.0, in order to produce cellulase. In 100 ml conical flasks, 25 ml of the medium was then added and autoclaved at 15 1bs pressure for 15 minutes. After cooling, the flasks were each individually inoculated with test fungus generated from cultures that had been grown on PDA slants for 7 days. The flasks were inoculated for 6 days at 25 °C and with a diurnal periodicity of light. The contents of the flasks were collected on the 7th day by filtering them with Whattmanfilter paper No. 1. The filtrates were collected and labelled as crude enzyme preparation; in pre-sterilized bottle.Different nutritional sources such as Carbon (0.5%), Nitrogen (0,25%), Phosphorus, Sulphur (0.5%), Vitamins (100 ppm), Amino acids (100 ppm) and antibiotics (100 ppm) were given to above basal medium.

Assay-(Wagh & Bhale, 2014)cup-plate technique was applied for the enzyme assay. The assay medium, which comprises 2% difco agar and 1% CMC, was added to petri dishes (20 mL per plate) and allowed to solidify. With a pre-sterilized cork borer, a 6 mm diameter cup or cavity was made in the centre. The cup was placed in an incubator for 48 hours at room temperature with 0.1 mL culture filtrate. Flooding the plates with a 3% lead acetate solution (10–15mL/plate) developed the activity zone. After 30 minutes, it was easy to discern milky white activity zones when the lead acetate solution was removed with distilled water. The zone diameter was measured in mm.

4 Results:

4.1 Occurrence of fungi:

Isolation of pathogenic fungi from 5 cultivars of infected papaya fruits from the Aurangabad region using PDA. Morphological identification of the fungi using fungal hyphae and spore structures showed the isolates to be *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularialunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum, Rhizopus stolonifer and penicillium islandicum.*

4.2 Celluase production:

4.2.1 Effect of substrate and non-substrate media on cellulase production

The production of cellulase by all fungi, including *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularialunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum, and Rhizopus stolonifer,* was observed. However, in comparison to non-substrate media, substrate medium was where all fungi produced the most cellulase. According to the findings, *Penicillium digitatum, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, and Fusarium equiseti* produced the most cellulase, followed by *Fusariummoniliforme, Curvularialunata, and Rhizopus stolonifera.* The effect of different substrate and non-substrate media are shown in figure 1.

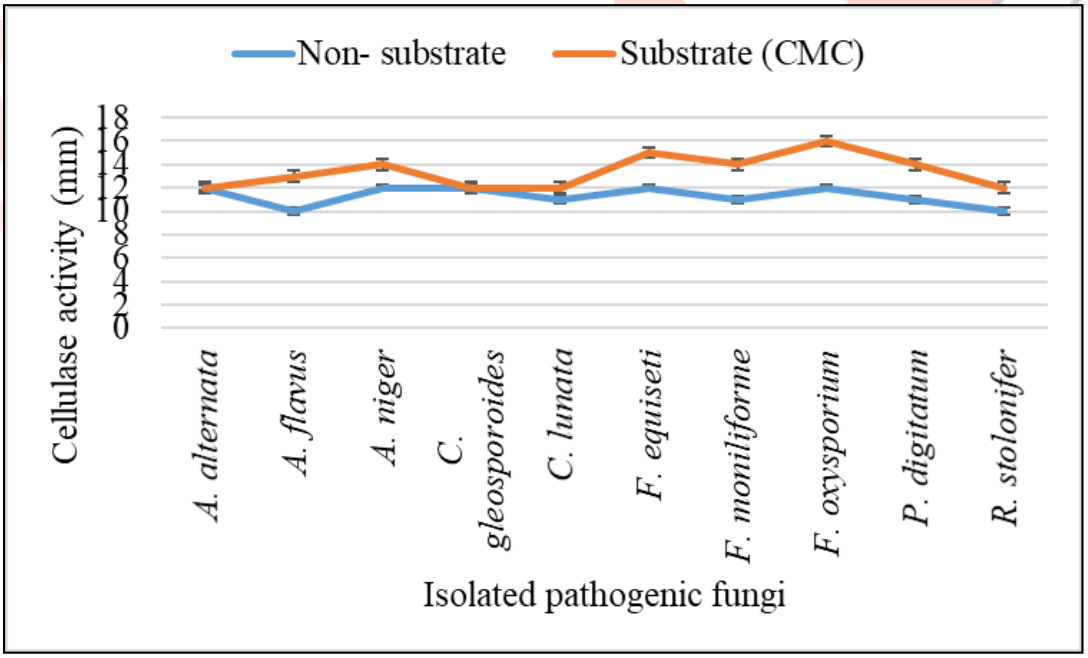

Figure 1 Effect of substrate and non-substrate media on cellulase production

4.2.2 Effect of nutritional source:

a. Effect of carbohydrates on cellulase production:

Six different carbohydrate sources were supplemented individually in the basal medium to test their effects on the production of cellulose. All of the tested fungi, with the exception of *Aspergillus niger and Colletotrichum gloeosporioides*, produced the most cellulase when given xylose instead of glucose and the least when given fructose. Among the disaccharides, sucrose was found to stimulate the production of cellulase in all ten fungi. All of the investigated fungi had their cellulase activity reduced by CMC and starch.The effect of different carbohydrates on cellulase productionare shown in figure 2.

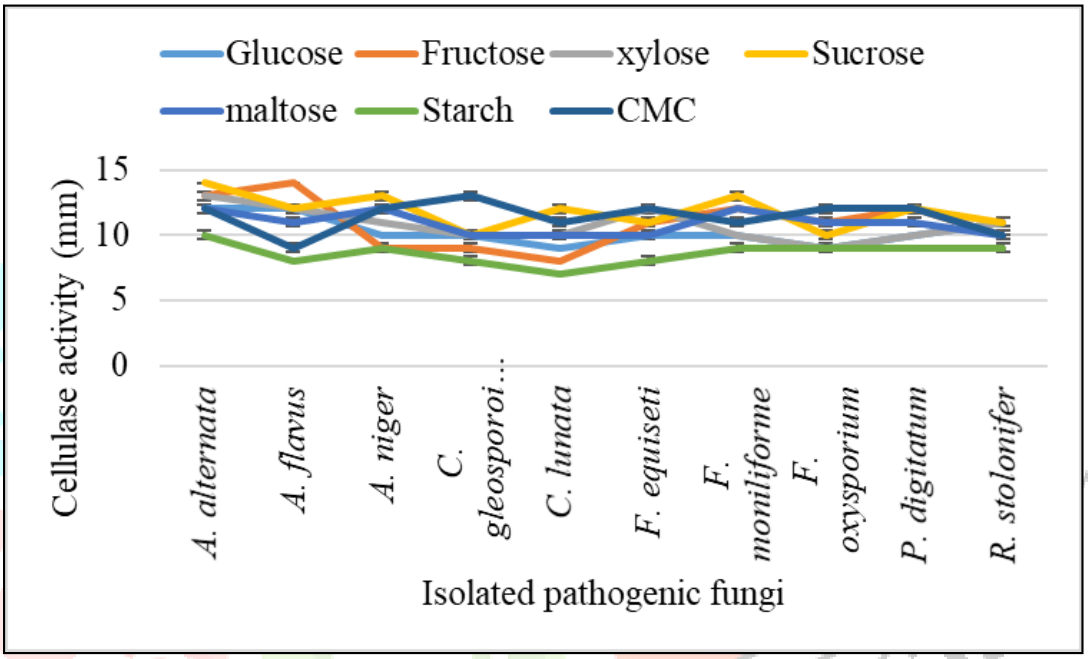

Figure 2 Effect of carbohydrates on cellulase production

b. Effect of nitrogen source on cellulase production:

Among the numerous sources of nitrate, nitrite forms, ammonium forms, amide forms, and organic forms were each added individually to the basal medium at a concentration of 0.25%, and their impact on the generation of cellulase was noted. The control was a base medium containing potassium nitrate. It was found that the maximum amount of cellulase was produced *by Curvularialunata, Penicillium digitatum, Aspergillus niger, Colletotrichum gloeosporioides, and Fusarium equiseti* when nitrogen sources like sodium nitrate were used, whereas *Aspergillus flavus, Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme and rhizopusstolonifer* were found inhibitory for cellulase production (figure 3). While in sodium nitrite, *Aspergillus niger, Fusarium oxysporum, Aspergillus flavus* were showed maximum production of cellulase, whereas *Colletotrichum gloeosporioides, Rhizopus stolonifer, Alternaria alternata, Fusarium equiseti, and Fusarium moniliforme* inhibited the production of the cellulase enzyme. In amide forms, urea stimulated the production of the cellulase enzyme in *Aspergillus niger, Fusarium equiseti, and Penicillium digitatum,* whereas it inhibited the enzyme's production in *Aspergillus flavus, Alternaria alternata, Colletotrichum gloeosporioides, Fusarium*

oxysporum, and Fusarium moniliforme. Additionally, it was discovered *that Aspergillus niger, Penicillium digitatum, Aspergillus flavus, and Fusarium moniliforme* produced the highest levels of cellulase when exposed to gelatin, whereas *Colletotrichum gloeosporioides, Fusarium equiseti, and Rhizopus stolonifer* inhibited the production of cellulase activity. *Aspergillus flavus, Alternaria alternata, and Penicillium digitatum* all produced the greatest amounts of cellulase when exposed to peptone, however *Fusarium oxysporum and Rhizopus stolonifer* decreased the activity of the cellulase enzyme. Comparatively to other fungi, *Alternaria alternata, Curvularialunata, Fusarium equiseti, and Rhizopus stolonifer* produced the most whereas *Aspergillus flavus, Fusarium oxysporum and Penicillium digitatum* inhibited the production of cellulase activity.

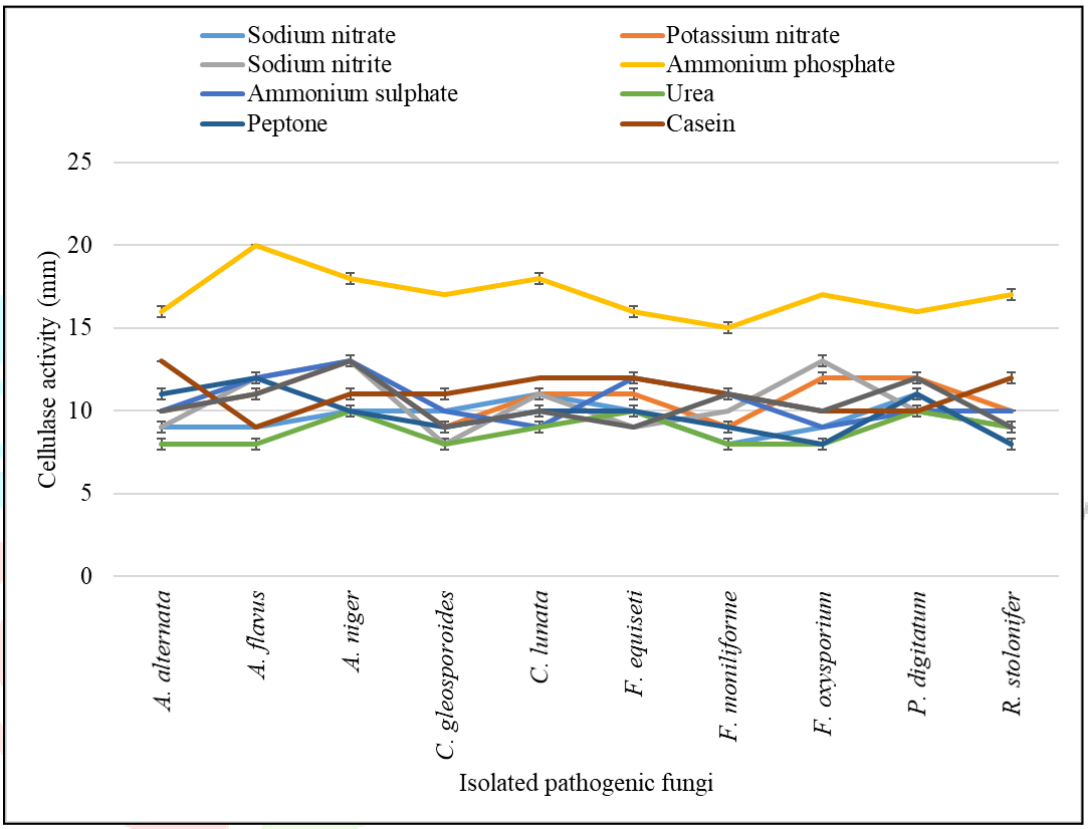

Figure 3 Effect of different nitrogen sources on cellulase production

c **Effect of phosphorus sources on cellulase production**

When dipotassium hydrogen phosphate was replaced with five different phosphorus sources, their effects on cellulase production were examined. *Aspergillus niger, Curvularialunata, and Fusarium moniliforme* were found to be inhibited by sodium dihydrogen phosphate whereas *Rhizopus stolonifer and Alternaria alternata* produced the most cellulase when sodium dihydrogen phosphate was present (figure 4). Disodium hydrogen phosphate was shown to suppress the synthesis of cellulase in *Aternariaalternata, Curvularialunata, and Fusarium oxysporum* while stimulating the production of cellulase in *Aspergillus flavus and Penicillium digitatum.*

While Alternaria alternata, Colletotrichum gloeosporioides, and Fusarium moniliforme produced less cellulase when exposed to potassium hydrogen phosphate, *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, and Fusarium equiseti* produced the most cellulase when exposed to the compound. While ammonium phosphate increased cellulase synthesis in *Aspergillus niger, Aspergillus flavus, Fusarium equiseti, and Fusarium moniliforme*, ammonium showed inhibiting for cellulase development in *Curvularialunata and Fusarium oxysporum*. Ammonium biphosphate inhibited the growth of *Curvularialunata, Fusarium moniliforme, Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Fusarium equiseti, and Penicillium digitatum, whereas Aspergillus flavus, Rhizopus stolonifer, and Fusarium oxysporum* produced the highest It is noteworthy that none of the phosphorus sources were discovered to be superior than dipotassium hydrogen phosphate (control) to support cellulase production in fungi.

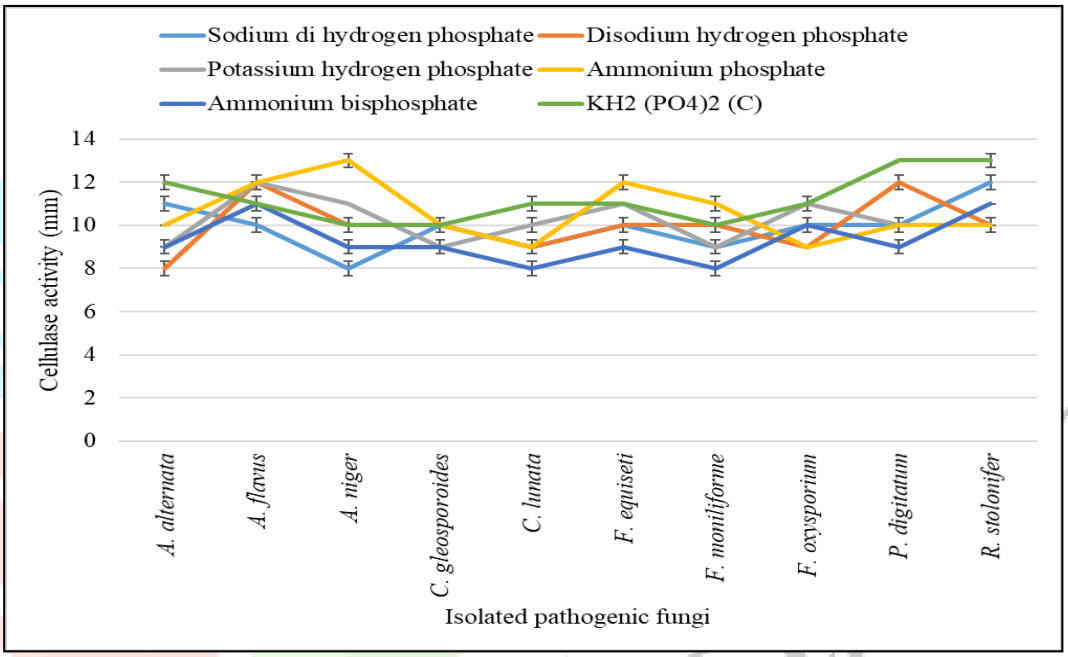

Figure 4 Effect of phosphorus sources on cellulase production (Ala is a)

d. Effect of sulphur sources on cellulase production

In order to produce cellulase, ten fungi were exposed to seven different sources of sulphur at 0.5% concentrations. It's noteworthy to notice that the production of enzymes involves all seven sources of sulphur in almost similar amounts. The formation of cellulase was hindered by ferrous and zinc sulphates, whilst the production was stimulated by the remaining sulphur sources (figure 5). The production of cellulase was found to be inhibited by sodium sulphate for *Aspergillus niger and Fusarium equiseti*, sodium thiosulphate for *Aspergillus flavus*, ammonium sulphate *for Aspergillus niger and Colletotrichum gloeosporioides,* magnesium sulphate for *Aspergillus oxysporum and Fusarium equiseti*, potassium sulphate for *Fusarium moniliforme and Fusarium equiseti* were found inhibitory for cellulase production where as ferrous sulphate and zinc sulphate inhibited cellulase production in all tested fungi except *Alternaria alternata and Fusarium oxysporum.*

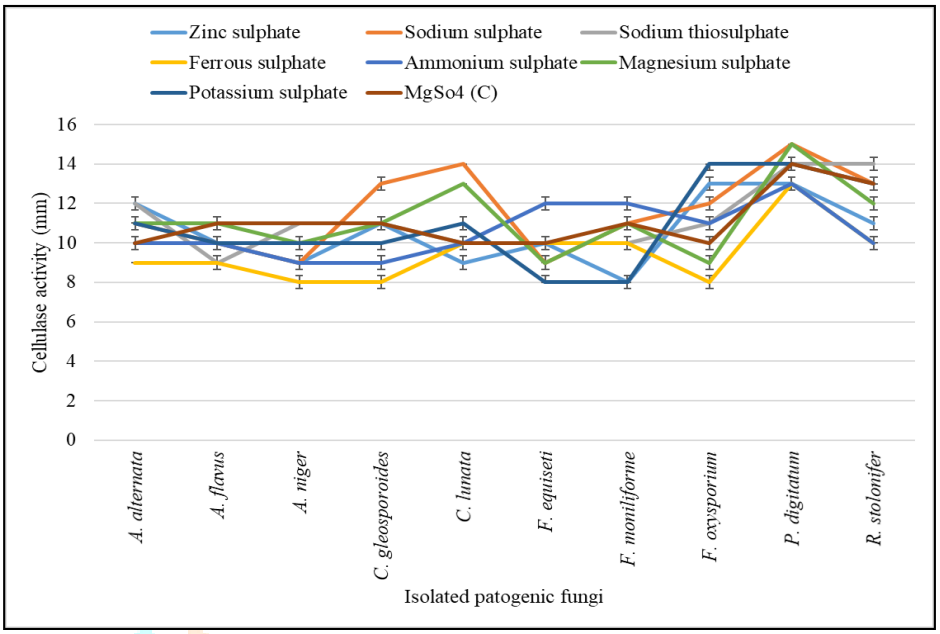

Figure 5 Effect of sulphur sources on cellulase production

e. Effect of trace elements on cellulase production

To understand how various trace elements affect the fungi that grow after harvest in terms of their ability to produce cellulase. Five trace elements were examined at a concentration of 0.01%.It was found that trace metals like Fe decreased the activity of the cellulase enzyme, whereas Mn, Ba, Cu, and Zn boosted it (figure 6). While the remaining examined fungi were stimulatory for cellulase activity, Mn delayed cellulase activity in *Fusarium moniliforme and Alternaria alternata*. In contrast to the remaining examined fungi, Ba was shown to be stimulatory for cellulase activity in *Alternaria alternata and Fusarium moniliforme*. While the remaining investigated fungi are stimulatory for cellulase activity, copper reduced the formation of cellulase in *Aspergillus niger*.

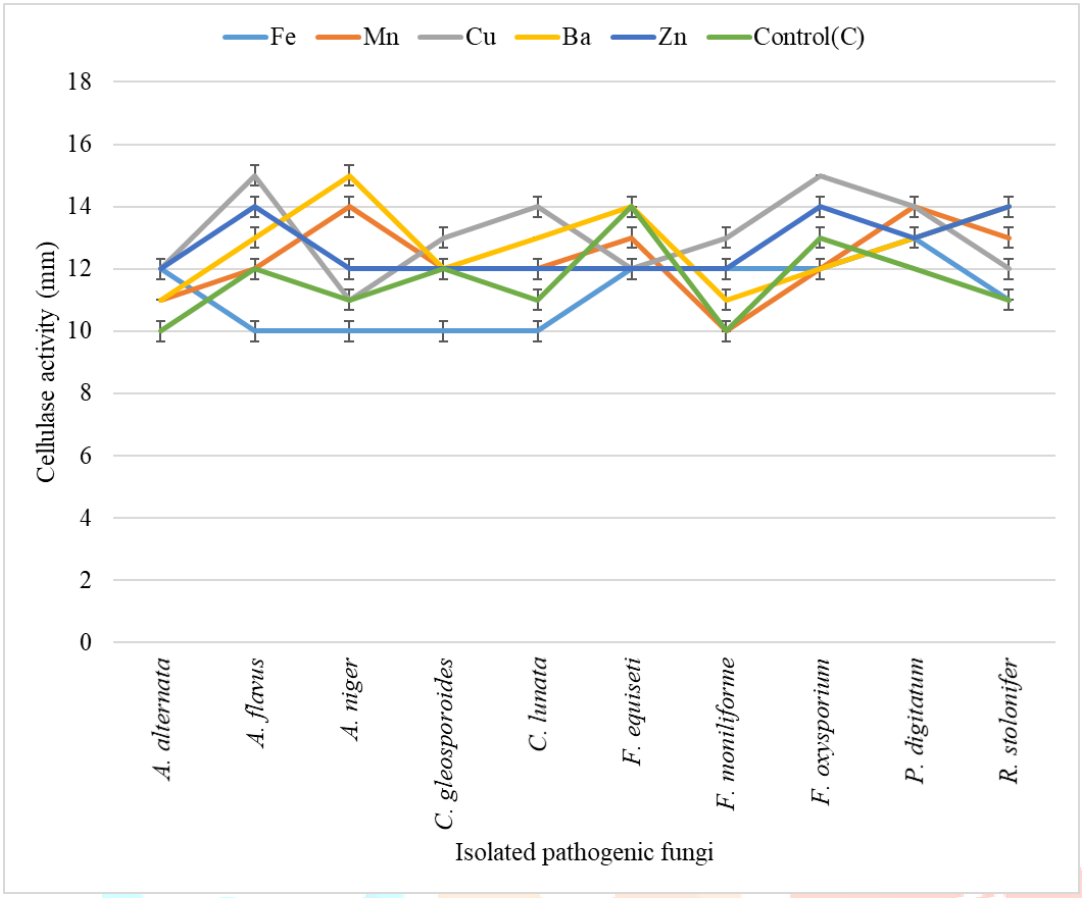

Figure 6 Effect of trace elements on cellulase production

f. Effect of amino acids on cellulase production

Five amino acids were tested with ten fungi to determine their effects at 100 ppm.

In Alternaria alternata, Curvularialunata, and Fusarium moniliforme, as well as in *Curvularialunata and Fusarium moniliforme*, it was shown that amino acids such arginine monochloride and threonine hindered enzyme function. Methionine, aspartic acid, and alanine had no impact on cellulase synthesis (figure 7).

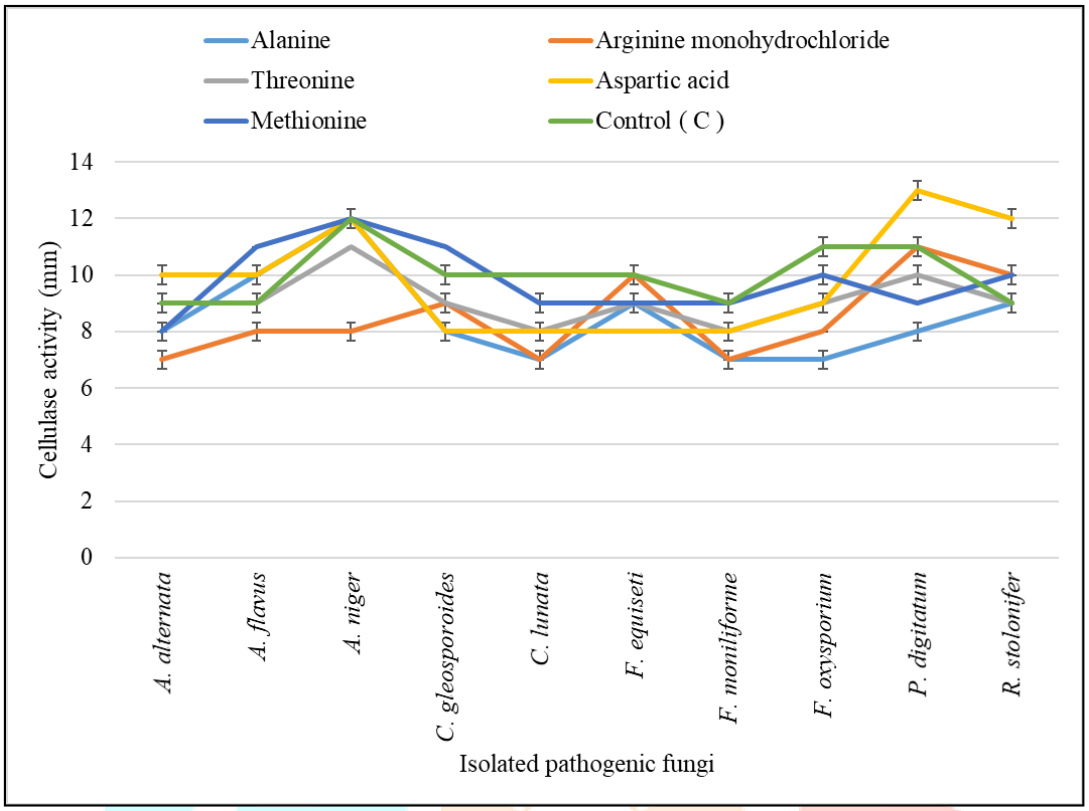

Figure 7 Effect of amino acids on cellulase production

g. Effect of antibiotics on cellulase production

These five distinct sources of antibiotics were used independently against ten fungi. The effect of antibiotics on cellulase production was examined. It was shown that the presence of griseofolin enhanced the production of cellulase in *Aspergillus niger and Penicillium digitatum*, whereas terramycin stimulated the production of cellulase in *Fusarium moniliforme and Rhizopus stolonifera* (figure 8). Streptomycin stimulated the production of cellulase in *Penicillium digitatum and Aspergillus niger*, followed *by Colletotrichum gloeosporioides and Rhizopus stolonifer*, whereas doxycyclin was shown to stimulate the production of cellulase *in Penicillium digitatum and Aspergillus niger*. It's noteworthy to observe that all of the examined fungi had cellulase activity suppressed by ampicillin.

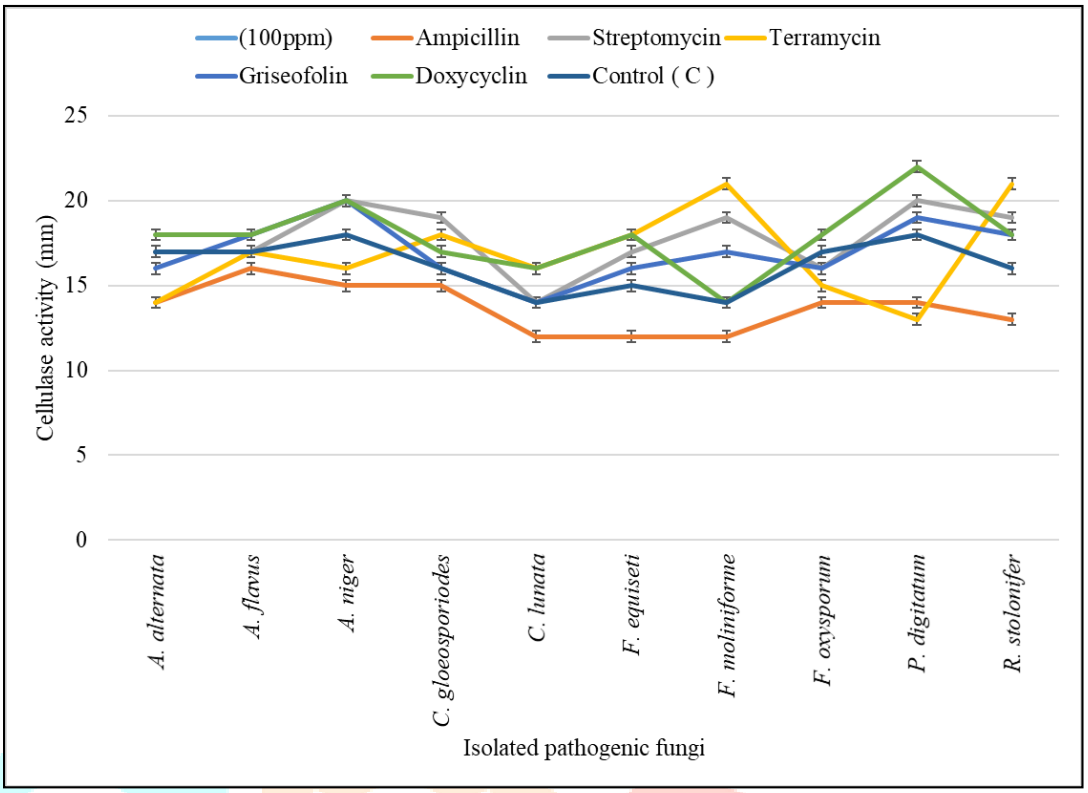

Figure 8 Effect of antibiotics on cellulase production

h. Effect of fungicide on cellulase production

Five fungicides were tested with ten fungi in order to examine the effects of fungicide at a dosage of 100 ppm. The results showed that fungicides such as capton proved inhibitory in *Fusarium moniliforme*, dithane M-45 proved inhibitory in *Fusarium oxysporum*, benomyl proved inhibitory in *Aspergillus niger, Curvularialunata, and Fusarium moniliforme*, dinocap proved inhibitory in *Fusarium moniliforme, and Fusarium equiseti*, while dithane I-78 inhibited the cellulase enzyme production in *Aspergillus flavus, Fusarium equiseti and Fusarium oxysporum*(figure 9).

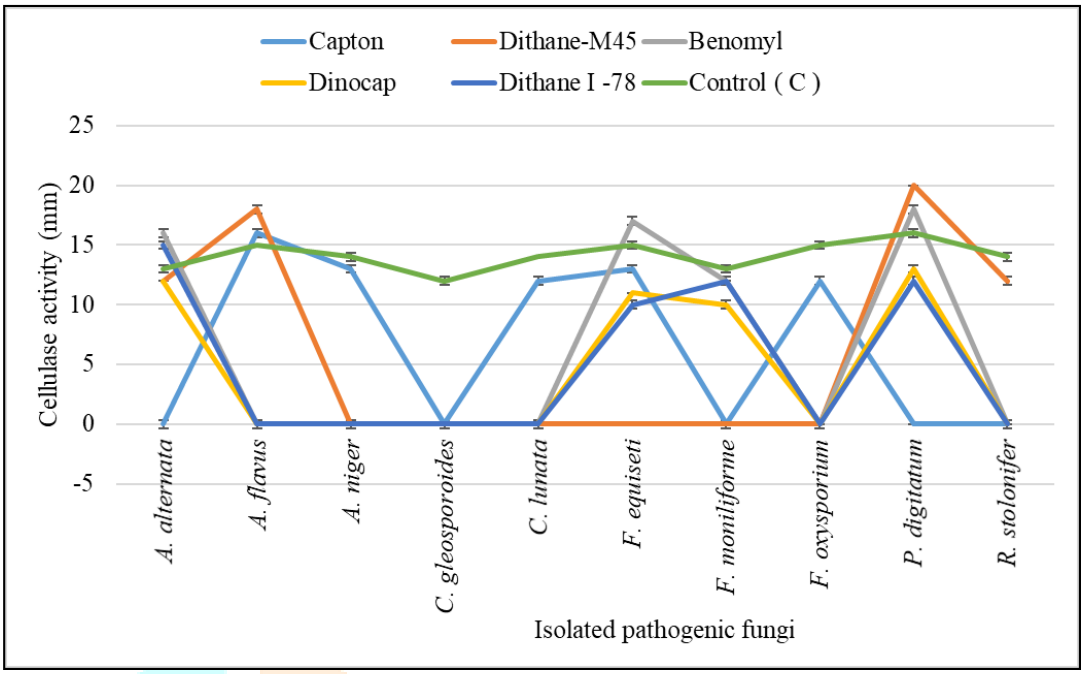

Figure 9Effect of fungicide on cellulase production

3.Physical factors

a. Effect of illumination of light on cellulase production

Ten fungi production studies were conducted in connection to various light illuminations. The results show that *Alternaria alternata, Fusarium moniliforme, Aspergillus flavus, Colletotrichum gloeosporioides, and Penicillium digitatum* were active in continuous light for cellulase production while *Alternaria alternata, Fusarium moniliforme, and Rhizopus stolonifer* were very inefficient in continuous dark for cellulase production. However, alternate light and dark illumination was proved to be favourable for cellulase activity of in all fungi (figure 10).

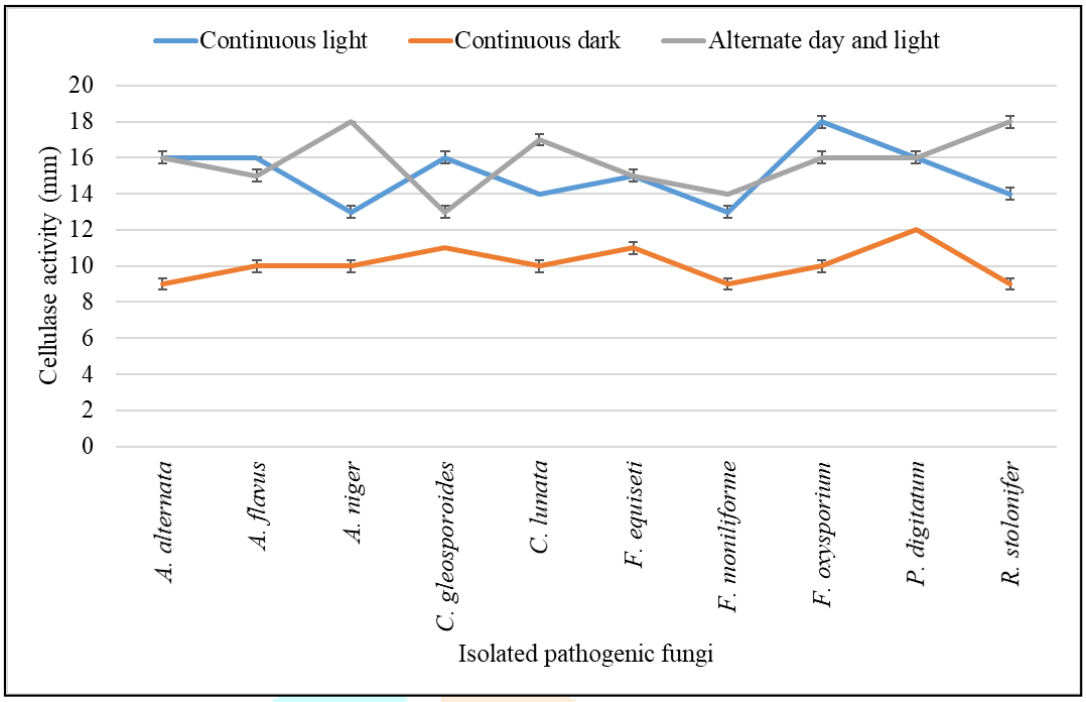

Figure 10Effect of illumination of light on cellulase production

b. Effect of incubation period on cellulase production

The culture filtrates of test fungi from 5 to 25 days of incubation were tested for cellulase activity in order to determine the best time for production.Out of the 10 fungi, the results revealed that *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium moniliforme, and Penicillium digitatum* were unable to generate cellulase at 5th day whereas other test fungi demonstrated cellulase synthesis after 5 days of incubation. Cellulase production rapidly rose till it reached 10th day. It's noteworthy to notice that post-harvest fungi boost cellulase synthesis up to 15 to 20 days after harvest, but on 25 days there was little to no difference (figure 11).

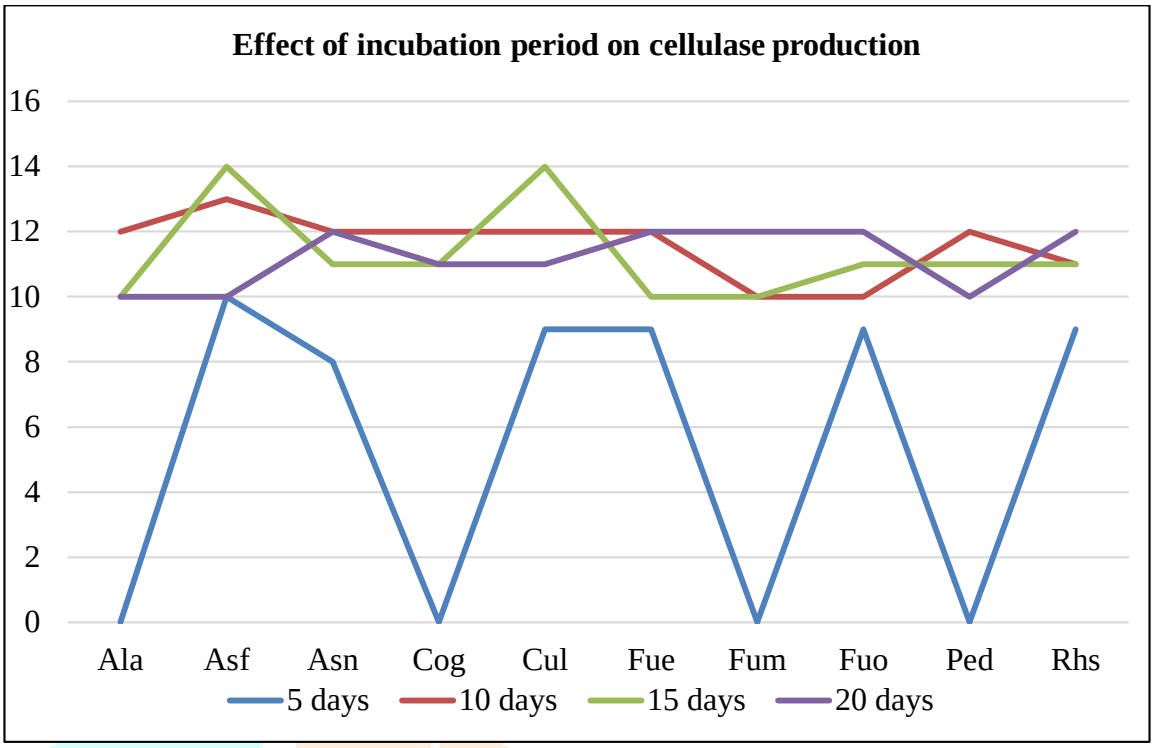

Figure 11Effect of incubation period on cellulase production

c. Effect of temperature on cellulase production

Six different temperatures were used to study the post-harvest fungi's ability to produce cellulase.The results showed that other tested fungi, with the exception of*Curvularialunata and Penicillium digitatum*, were unable to produce cellulase at low temperatures (10°C) and at higher temperatures (20°C), with the exception of *Aspergillus flavus, Aspergillus niger, Curvularialunata, and Fusarium oxysporum*(figure 12). All fungi were shown to produce more cellulase when the temperature was between 20 and 35°C, but as the temperature rose, so did the activity of the cellulase enzyme.

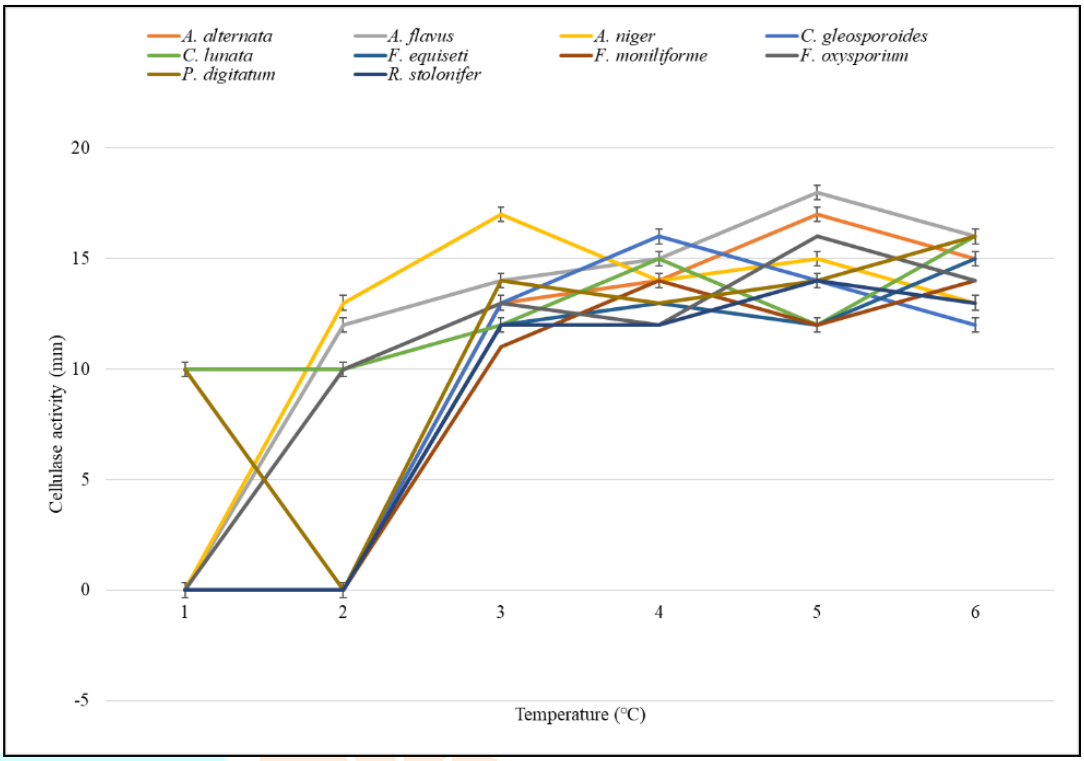

Figure 12 Effect of temperature on cellulase production

d. Effect of pH on cellulase production

The findings of the cellulase production study, which involved growth of the fungi on glucose nitrate medium at twelve various pH levels ranging from 3.0 to 8.5 can be seen in the graph below (figure 13).Except for *Curvularialunata*, it was found that none of the test fungi developed cellulase enzyme at pH 3.5. All of the investigated fungi had their best pectinase activity between pH 5.0 and 7.0. Both *Alternaria alternata and Fusarium moniliforme* were discovered to be totally inhibitory for cellulase synthesis at pH 4.0 and pH 4.5, respectively. Maximum cellulase production occurred at 6.0 pH in *Penicillium digitatum, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium oxysporum, and Rhizopus stolonifer*, but not in *Curvularialunata, Aspergillus flavus, Fusarium moniliforme, Fusarium oxysporum, and Rhizopus stolon, Penicillium digitatum* were produced maximum cellulase activity at 6.5 pH.

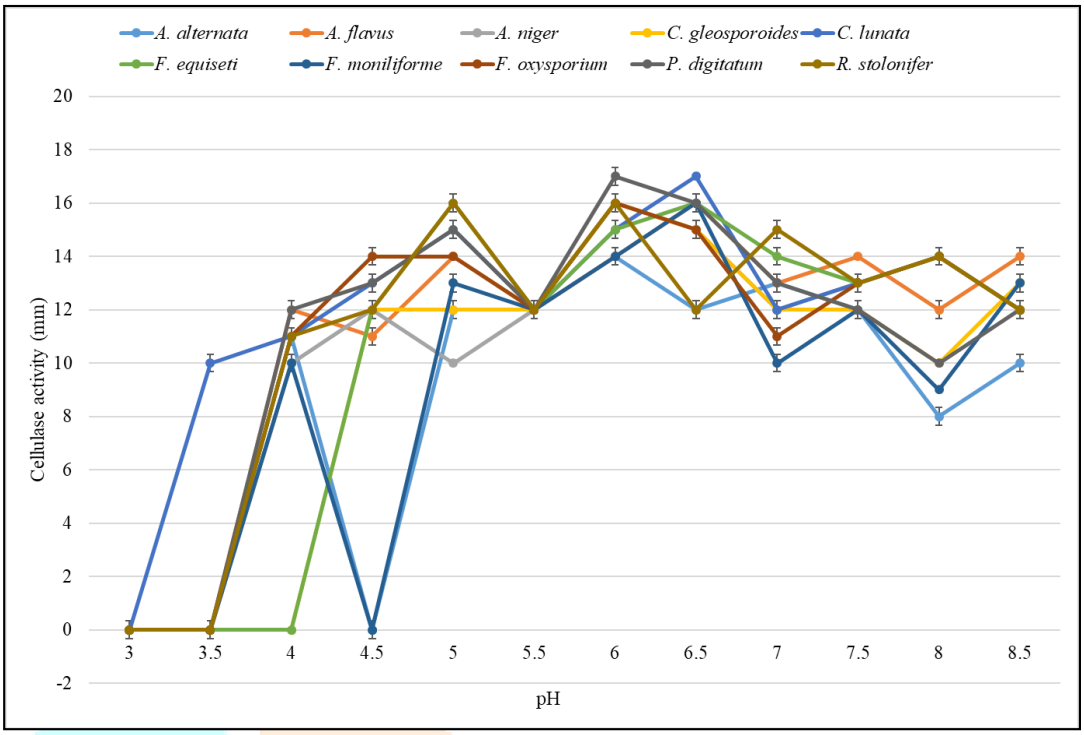

Figure 13Effect of pH on cellulase production

4 Discussion:

Ten fungi *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularialunata, Fusariumequiseti, Fusarium moliniforme, Fusarium oxysporum Penicillium digitatum and Rhizopus stolonifer* were isolated from papaya were used to study the cellulase production and ability under the influence of physical and nutritional factors. It is observed that substrate media favours cellulaseproduction as compared to non-substrate media. Which clearly indicates that (Osagie & Obuekwe, 1991), (Nadar et al., 2018), (Kulkarni et al., 2018)also reported that maximum production of hydrolytic enzymes in substrate media as compare to non-substrate media.

Glucose stimulated cellulase action of *Alternaria alternata, Aspergillus flavus and Rhizopus stolonifer* where as it inhibited the same *in Aspergillus niger, Colletotrichum gloeosporioides, Curvularialunata, F.equiseti, F. moniliforme, F. oxysporum and Penicillium digitatum.*

Nitrogen sources like sodium nitrate favours maximum production of cellulase where as it inhibits the same in *Aspergillus flavus, Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, and Rhizopus stolonifer*. (Gangasagar et al., 2022)reported inhibitory nature in potassium nitrate and sodium nitrate on amylase activity in *Alternaria alternata*. Recently, (Channya et al., 2019)found similar results in *Aspergillus niger, Fusarium oxysporum and Penicillium notatum.*

Ferrous sulphate and zinc sulphate inhibited the cellulase production in all post-harvest fungi. Sodium sulphate inhibited the cellulase action *in Aspergillus niger and Fusarium equiseti.* Recently, (Gadgile & Chavan,

2009)found that ferrous sulphate, zinc sulphate and copper sulphate significantly retard the cellulase production of *Aspergillus niger, Colletotrichum gloeosporioides, Penicillium sp., and Rhizopus stolonifer.*

Folic acid and riboflavin retard cellulase action while Ascorbic acid stimulated cellulase production in *Alternaria alternata, Aspergillus flavus, Fusarium oxysporum, and Penicillium digitatum*. Thiamine induced cellulase action in *Aspergillus niger, Fusarium equiseti and Rhizopus stolonifer*. Pyridoxine stimulated cellulase action in *Rhizopus stolonifer, Aspergillus flavus and Penicillium digitatum*. (Bhikane, 1988)found that thiamine and nicotinic acid stimulate a protease action in *Aspergillus flavus* while pyridoxin inhibited the same in *Curvularialunata, Fusarium oxysporum, and Rhizopus stolonifer*.

It was found that Fe inhibited cellulase action in all pathogenic fungi while Mn and Ba inhibited cellulase action in *Alternaria alternata and Fusarium moniliforme* whereas remaining all tested fungi are stimulatory for cellulase activity. Cu inhibitory for cellulase production in *Aspergillus niger.*

Amino acid like Arginine monochloride and threonine inhibited cellulase action in *Alternaria alternata, Curvularialunata, and Fusarium moniliforme*. Alanine, aspartic acid and methionine did not affect significantly for the production of cellulase action. Ampicillin retards cellulase action in all pathogenic fungi while streptomycin, doxycyclin, and terramycin induced cellulase action in some pathogenic fungi. (Patel & Sankhavara, 2017) found similar finding about impact of antibiotic at 100 ppm concentration in different seed borne fungi. Recently (Gadgile & Chavan, 2009)reported that almox DT significantly inhibited pectinase activity of *Penicillium spp. and Aspergillus flavus.* Fungicides like capton, diathane M-45, benomyl, dinocap and diathane I-78 retard the cellulase production of all tested fungi. It was also reported that benomyl, dinocap and diathane I-78 totally retard cellulase production of *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium oxysporumCurvularialunata, and Rhizopus stolonifer*. Impact of physical factors like illumination of light, incubation period, temperature and pH on cellulase production was studied. Alternate light and dark stimulated cellulase action in all tested fungi. Maximum cellulase activity of all pathogenic fungi was found in between 15-20" days of incubation period. Temperature range between 20-35°C is more suitable for cellulase production(Osagie & Obuekwe, 1991), (Nadar et al., 2018), (Kulkarni et al., 2018)reported similar findings about the effect of incubation period, temperature, pH and light on hydrolytic enzyme of fungi.

5. Conclusion:

If the cellulase is supplied in the nutritive media, fungi prefer to grow on it and the cellulase enzyme is also produced by the fungi. It was concluded that starch, ammonium sulphate, ammonium biphosphate, ferrous sulphate, folic acid, arginine and ampicillin hold back the production of cellulase activity due to pathogens such nature of reticence of these may be useful to control the spoilage of fruits by fungi. If the cellulose is supplied in the nutritive media, fungi prefer to grow on it and the cellulase enzyme is also produced by the fungi.

References

Barnett, H. L., & Hunter, B. B. (1972). Illustrated genera of imperfect fungi. *Illustrated genera of imperfect fungi*.(3rd ed).

Bhikane, N. (1988). Studies on Seed borne fungi of some legumes. *Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS) India*.

Channya, F., Asama, P., & Anjili, S. (2019). Effect of Khaya Senegalensis Bark and Oil on Post-Harvest Fungal Agents of Groundnut Seeds Rot in Adamawa State, Nigeria. *Journal of Plant Science and Phytopathology*, *3*(2), 076-080.

El_Komy, M. H., Saleh, A. A., Eranthodi, A., & Molan, Y. Y. (2015). Characterization of novel Trichoderma asperellum isolates to select effective biocontrol agents against tomato Fusarium wilt. *The Plant Pathology Journal*, *31*(1), 50.

Gadgile, D., & Chavan, A. (2009). Impact of nutritional sources on the activity of enzyme cellulase produced by post-harvest fungi isolated from mango fruits. *BIOINFOLET-A Quarterly Journal of Life Sciences*, *6*(3), 227-229.

Gangasagar, P. Y., Ingale, S. L., Kurhe, A. B., & Patki, U. S. (2022). *Studies on Species Diversity of Mycoflora and Biocontrol related to Vegetables in Nanded District* (Vol. 1). Ashok Yakkaldevi.

Griffin, D. H. (1996). *Fungal physiology*. John Wiley & Sons.

Kuhad, R. C., Gupta, R., & Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme research*, *2011*.

Kulkarni, N., Vaidya, T., & Rathi, G. (2018). Optimization of cellulase production by Aspergillus species under solid state fermentation. *Pharm Inn J*, *7*(1), 193-196.

Lebeda, A., Luhová, L., Sedlářová, M., & Jančová, D. (2001). The role of enzymes in plant-fungal pathogens interactions/Die Rolle der Enzyme in den Beziehungen zwischen Pflanzen und pilzlichen Erregern. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 89-111.

Leyronas, C., Duffaud, M., & Nicot, P. C. (2012). Compared efficiency of the isolation methods for Botrytis cinerea. *Mycology*, *3*(4), 221-225.

McCleary, B., & Glennie-Holmes, M. (1985). Measurement of (1→ 3),(1→ 4)-β-D-glucan in barley and malt. *J. Inst. Brew*, *91*, 285-295.

Nadar, S. S., Rao, P., & Rathod, V. K. (2018). Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Research International*, *108*, 309-330.

Osagie, I., & Obuekwe, C. (1991). Extracellular hydrolytic enzyme production by pathogenic strains of Fusarium oxysporum f. sp. elaeidis. *Mycological Research*, *95*(1), 116-122.

Patel, R. L., & Sankhavara, C. (2017). Biodiesel production from Karanja oil and its use in diesel engine: A review. *Renewable and Sustainable Energy Reviews*, *71*, 464-474.

Schaller, M., Borelli, C., Korting, H. C., & Hube, B. (2005). Hydrolytic enzymes as virulence factors of Candida albicans. *Mycoses*, *48*(6), 365-377.

Ugwuanyi, J. O., & Obeta, J. A. (1991). Incidence of heat-resistant fungi in Nsukka, Southern Nigeria. *International journal of food microbiology*, *13*(2), 157-164.

Wagh, P., & Bhale, U. (2014). Potentials of nutritional factors on production of cellulose enzyme by post-harvest fungal pathogens on sapodilla fruit. *Current Biotica*, *7*(4), 256-265.

Walker, G. M., & White, N. A. (2017). Introduction to fungal physiology. *Fungi: biology and applications*, 1-35.