International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 123-128 www.ijprajournal.com ISSN: 2249-7781

Production of Protease from Moringa Oleifera Leaves by Aspergillus Flavus in Solid State Fermentation.

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Date Of Submission: 20-03-2021 Date Of Acceptance: 05-04-2021

ABSTRACT: The present research work is about optimization and production of protease enzyme by Moringa oleifera leaves as substrate in a solid state fermentation using microorganism Aspergillus flavus. Proteases are large and complex group of enzymes that plays an essential role in nutritional and regulatory activities. Proteases are important for physiological functions in living organisms; they assist in breakdown of protein food materials into amino acids, which the body can utilizes for energy, and also plays an important role in essential processes, such as blood clotting, cell division. The process occurs on a non-soluble material that acts both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent is known as Solid-state fermentation. Optimization of protease enzyme conditions like incubation time, incubation temperature, pH, inoculum level and moisture content were measured. The fermentation time of 48hrs and the temperature of 28^0C, pH 5, inoculum level of 14%v/v and moisture content of 70%v/w were observed optimum for the production of Protease. Different carbon and nitrogen sources were screened for their influence on enzyme yield; they are glucose, maltose, sucrose, fructose and lactose used as supplements. Among these supplements sucrose gave better yield at 1%w/w. 0.4% w/w of potassium nitrate as nitrogen source was observed maximum for the production of protease enzyme.

Keywords: Moringa oleifera leaves, Aspergillus flavus, Solid-state fermentation.

I. INTRODUCTION

Proteases are large and complex group of enzymes that plays an essential role in nutritional and regulatory activities. Proteases are important for physiological functions in living organisms; they assist in breakdown of protein food materials into amino acids, which the body can utilizes for energy, and also plays an important role in essential processes, such as blood clotting, cell division. Protease enzymes are present in a wide variety of sources such as plants, animals and microorganisms and they are mainly produced by bacteria and fungi. Microbial proteases are enzymes, that catalyze total hydrolysis of proteins[1,2]. These are the most influential industrial enzymes and about 60% of the total enzymes market in the world [3,4]. Protease enzymes are absolutely used in food industries, leather, meat processing, cheese making, detergents, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds. They are also having medical and pharmaceutical applications. Microbial proteases are primarily extracellular and can be secreted in the fermentation medium[5,6]. The process occurs on a non-soluble material that acts both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent is known as Solid-state fermentation. Solid-state fermentation having many applications like superior volumetric productivity, use of simpler machinery, use of inexpensive substrates, simpler downstream processing, and lower energy requirements when compared with submerged fermentation[7,8]. Fungi generate a wide variety of proteolytic enzymes than bacteria. They have a potential growth under varying environmental conditions such as time, temperature, pH and moisture content etc, utilizing a wide variety of substrates as nutrients[9,10]. Nutrients can affect the growth of microorganisms indirectly by affecting the availability of nutrients or directly by action on the cell surfaces[11]. Keep inview, the importance of protease enzyme and the advantage of solid state fermentation, the present investigation is proposed to study the production and optimization of protease enzymes from cheaply and abundantly available raw materials under solid state fermentation.

II. MATERIAL AND METHODS

Substrate: Moringa oleifera leaves was collected in our village, Sathupally dried naturally and powdered, packed and stored until further use.

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Microorganism: Aspergillus flavus was used for the optimization and production of protease enzyme using Moringa oleifera leaves as substrate. Potato dextrose agar medium was prepared and it was used for the maintenance and sub culturing of the microorganism.

Preparation of Inoculum: streaking is done on freshly prepared potato dextrose agar slants from the old cultures of Aspergillus flavus and incubated them at 28^0C for 3 days.

Development of Inoculum: 10ml of sterile distilled water were added to the 3 days old potato dextrose agar slants, 1ml of suspension may contain approximately 10^5 -10^6 spores/ml, and from this 1ml suspension was inoculated into each flask and incubated.

Fermentation condition: Solid-state fermentation was accomplished in 250ml conical flask which containing 5g of substrate with 5ml of production medium (g/l). KNO_3 2.0, $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1.0, $ZnSO_4.7H_2O$ 0.437, $FeSO_4.7H_2O$ 1.116, $MnSO_4.7H_2O$ 0.203, pH 5.0 and it was sterilized in an autoclaved at 121^oC for 20 min. After sterilization, the flasks were inoculated with 1.0ml of spore solution and initially it was incubated at 30^0C for six days. After 24hrs, cultures were extracted with 100ml of distilled water by orbital shaker at 150rpm for 1hr. Then it was filtered, the filtrate obtained was centrifuged at 8000rpm for 10min at room temperature. The supernatant solution used as crude enzyme extract.

Determination of Enzyme Activity:

Enzyme assay: Protease activity was measured according to the modified Anson's method. 1ml of the supernatant solution was taken in a 100ml conical flask and 1ml of pH 7.0 phosphate buffer was added. 1ml of the substrate (2% Hammersten's casein pH 7.0) was added to the buffer solution and incubated at 37^0C for about 10minutes in a water bath. At the end of 10minutes, 10ml of 5N Trichloroacetic acid solution was added to terminate the reaction. The precipitated casein was filtered off and 5ml of the filtrate was taken in a test tube. To this filtrate 10ml of 0.5N NaOH solution and 3ml of folin ciocalteu reagent were added. Final readings were observed in spectrophotometer at 750nm. Blank samples were made by addition of Trichloroacetic acid solution. The optimization of process parameters like incubation time, temperature, inoculums size, pH, moisture content, carbon source and nitrogen sources on the production of protease was studied.

III. RESULTS AND DISCUSSION:

Protease enzyme has a number of applications in various food, leather, meat processing and cheese making industries. These are economically used in domestic detergents for the digestion of pertinacious stains of fabrics. It has been noted that the production of extracellular proteases are by different microorganisms can be strongly influenced with the culture conditions. So, it has become essential to understand the nature of proteases and their catalytic potentiality under different parameters. To optimize the effect of time on enzyme production, the medium was incubated at different time intervals and the maximum protease activity was perceived at 48hrs. After 48hrs, it was decline due to lack of nutrient materials. Protease enzyme production at different time intervals is shown in the figure 1.

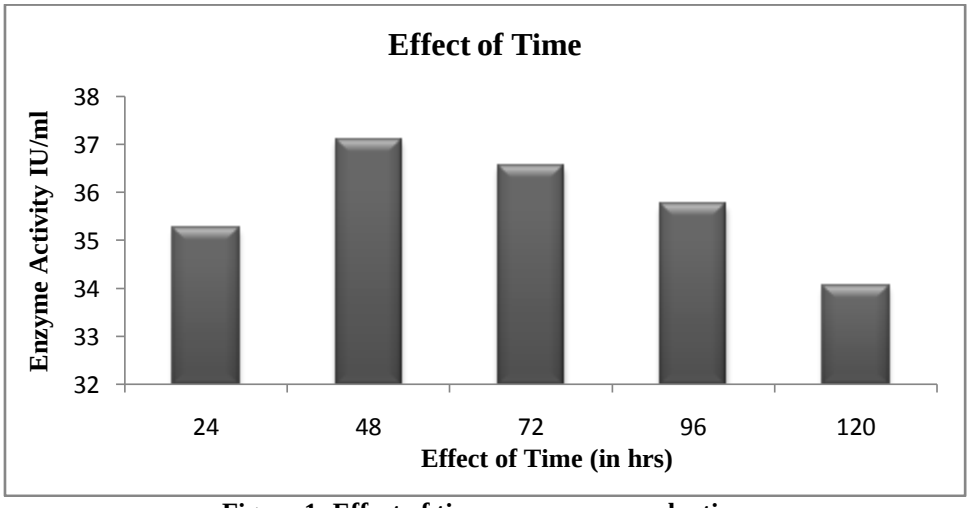

Figure 1: Effect of time on enzyme production

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The temperature of the substrate was very critical in Solid state fermentation because it ultimately affects the growth of the microorganism. The maximum amount of protease was observed at 28^0c temperature Fig.2.

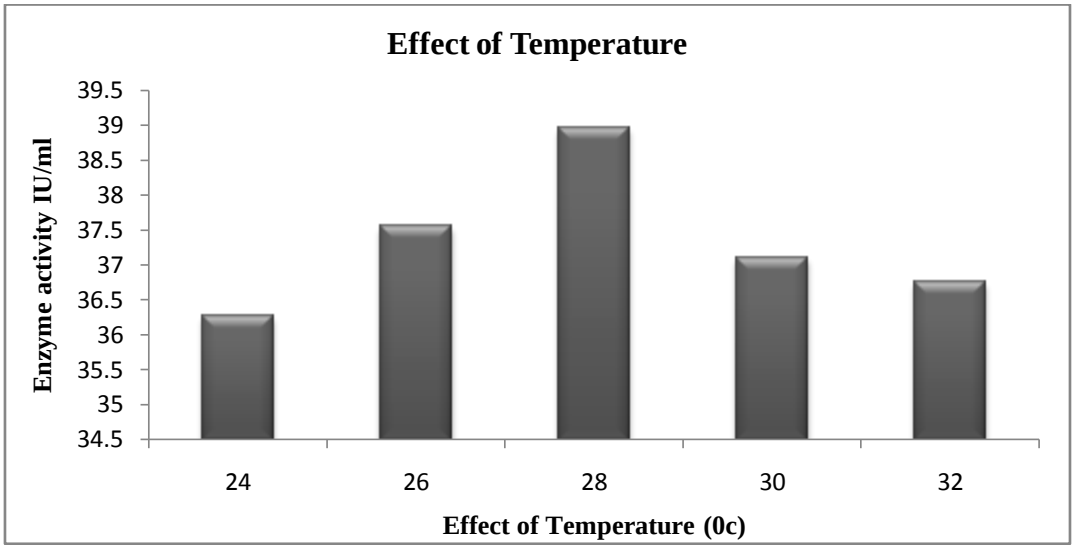

Figure 2: Effect of temperature on enzyme production

To determine the effect of pH, the nutrient medium was makeup with different pH ranges 3, 4, 5, 6, and 7.0. The maximum production of Protease was recorded at pH 5 figure 3.

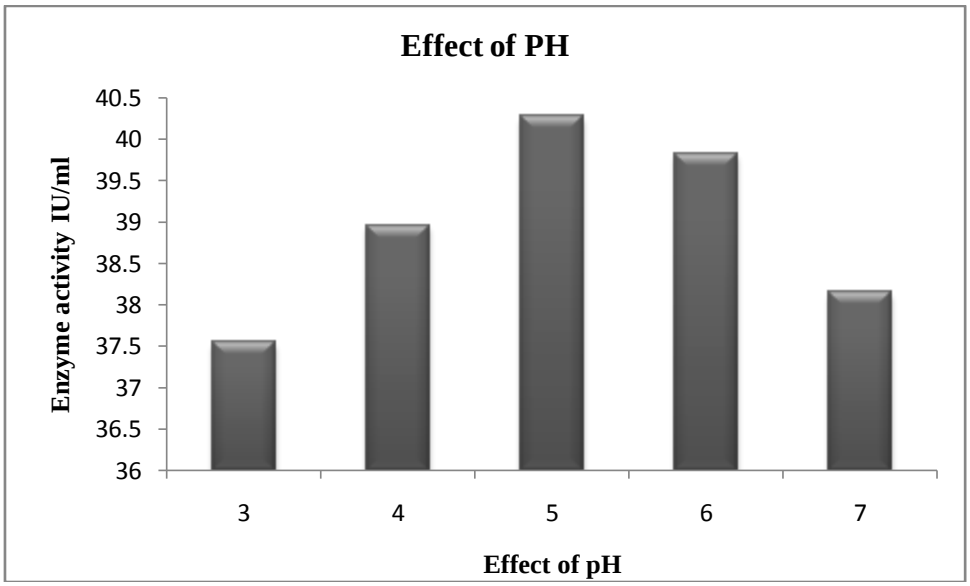

Figure 3: Effect of pH on enzyme production

Different inoculum levels were make ready for the production of enzyme 10%, 12%, 14%, 16%, 18%, 20% and 22% v/v. The maximum amount enzyme production was observed at 14%v/v of inoculum figure 4.

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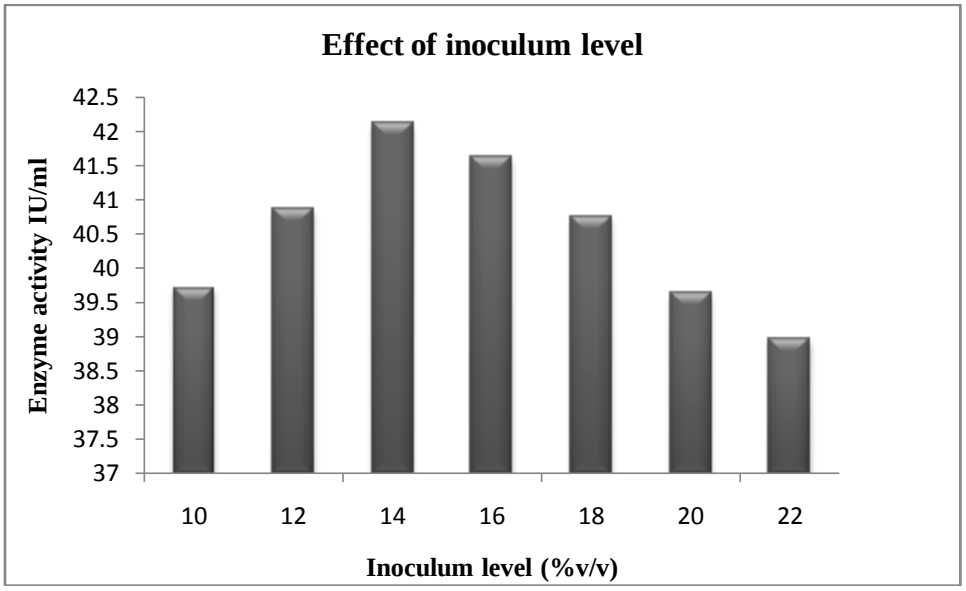

Figure 4: Effect of inoculum level on enzyme production

Moisture content is very crucial parameter for the production of enzymes in Solid state fermentation. High moisture content results in decreasing substrate porosity, which prevents oxygen penetration. This may assist bacterial contamination. Different moisture content 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w were prepared and placed in each conical flask. The maximum enzyme activity was observed at 70% v/w. figure 5.

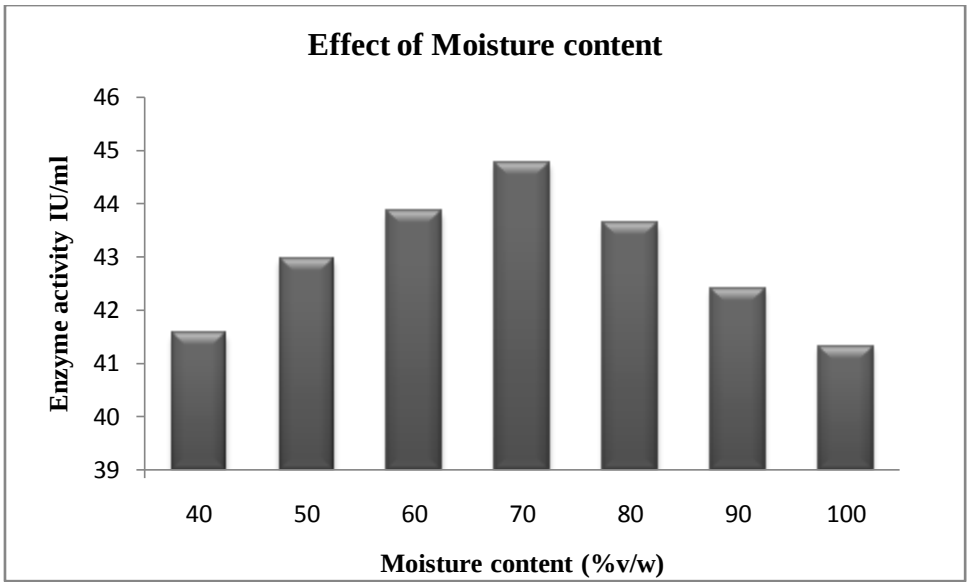

Figure 5: Effect of moisture content on enzyme production

Five different carbon sources were screened to get maximum production of protease enzyme which includes sucrose, maltose, glucose, fructose, and lactose. The results designate that sucrose supplementation gave remarkable improvement in enzyme production than other supplementations. Production medium was made with different concentrations of sucrose like 1, 2, 3, 4, 5 and 6 %w/w. The result indicates that maximum enzyme production was observed at 3% w/w of sucrose concentration figure 6.

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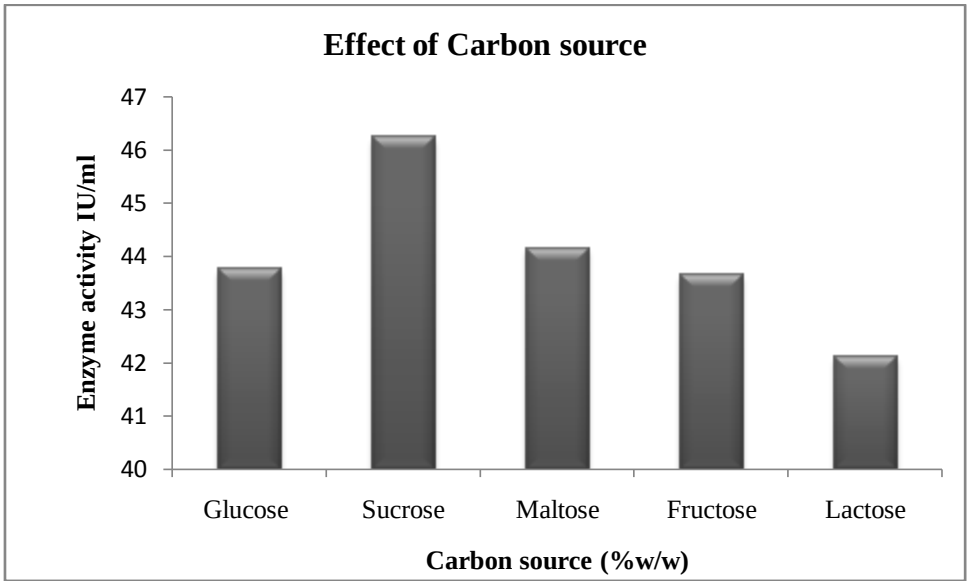

Figure 6: Effect of carbon source on enzyme production

To determine the effect of nitrogen on protease enzyme, the production medium was prepared with different concentrations of potassium nitrate like 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.7% w/w were dispersed in 250ml conical flasks. The result indicates that maximum enzyme production was observed at 0.4% w/w concentration figure 7.

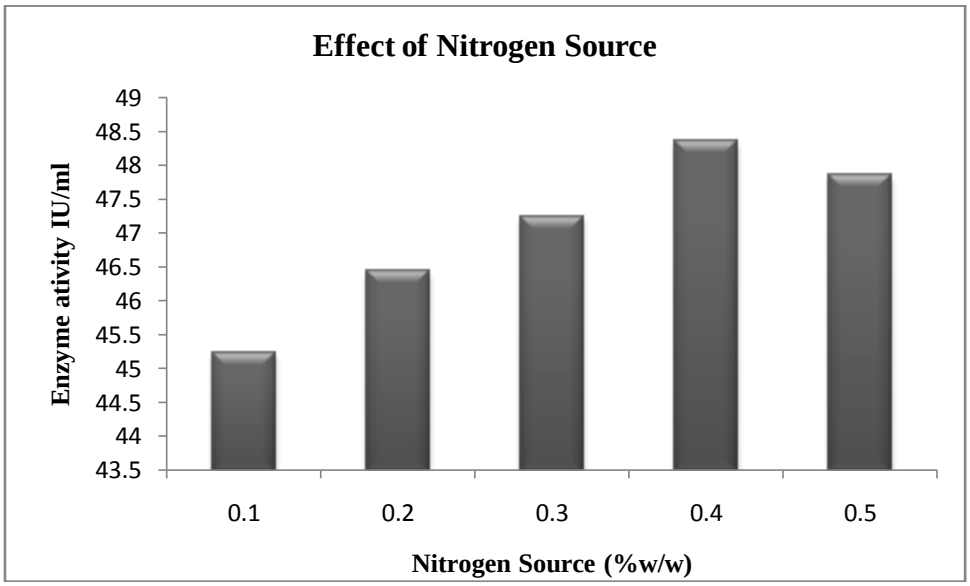

Figure 7: Effect of nitrogen source on enzyme production

IV. CONCLUSION:

Finally we concluded that Aspergillus flavus is a promising agent for industrial application since it gave a significant Protease (48.36 IU/ml) activity in Moringa oleifera leaves under solid state fermentation. As Moringa oleifera leaves are low cost substrate, easily available raw material and showing suitability for solid state cultivation of microbes, the lab-scale study on protease production from Moringa oleifera leaves as major substrate might give the basic information of further development for large scale protease production.

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ACKNOWLEDGEMENT:

The authors express their sincere thanks to the management, Mother Teresa Pharmacy College, Sathupally, Telangana for providing the necessary facilities to carry out the research work.

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