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Research Article

Comparative Responses of Food-Borne *Aspergillus niger JX 442527 and Penicillium minioluteum JX 442528* to Selected Plant Extracts

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ABSTRACT

The methanolic leaf extracts of Carica papaya, Chromolaena odorata, Alcohorneae laxiflora and Elaeis guineensis were investigated for antifungal activity against *Penicillium minioluteum* JX 442528 and *Aspergillus niger* JX 442527 in the mycology laboratory of Babcock University, Ilishan remo.. Also, common agro-wastes (wheat bran, rice bran and corn cobs) served as treatments as components of bioreaction for *Penicillium minioluteum* and *Aspergillus niger* both in submerged and solid state fermentations. Fungal growth inhibition was recorded with extracts from Carica and Chromolaena spp Extract of Elaeis guineensis extract has the lowest antifungal effect on *Penicillium minioluteum*. The solid state fermentation has higher enzyme activity compared to the submerged fermentation. Highest production for the 4 enzymes assayed for was observed on wheat bran media, using the SPSS statistical package. For industrial enzyme production, these cultural characteristics could therefore be critical.

Keywords: *Aspergillus niger, Penicillium minioluteum*, food, plant extracts.

INTRODUCTION

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. Fungi can exploit marginal living conditions in large part because they produce unusual enzymes capable of performing chemical reactions. Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available and the high yields possible. Also the ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of micro organisms make their exploitation attractive. Pathogenic fungi are known to inhabit food materials just as numerous filamentous fungi naturally thrive on plant wastes because they can penetrate the dead plant matter and utilize the cell wall components as growth substrates (Grant & Long, 1981).

Approximately 90% of all industrial enzymes are produced in submerged fermentation (SmF), often using genetically modified microorganisms (Holker et al., 2004). In this respect, SmF processing offers an insurmountable advantage over Solid state fermentation (SSF). Interestingly, fungi, yeasts and bacteria that were tested in SSF in recent decades exhibited different metabolic strategies under conditions of solid state and submerged fermentation. (Gouda, 2000).

Agricultural, agro industrial and domestic wastes including sugarcane baggase, orange baggase, wheat bran, rice bran, corn cob, seeds, fruit peels, vegetable peels and effluents from the paper industry have increased as a result of rising population and industrialization (Da silva *et al.*, 2005). These residues also represent an alternative and cheaper source for microbial growth, aiming at the production of single cell protein (SCP) or industrial enzymes.

The aim of this study is to evaluate fungi metabolism of some local natural plants which have potential for treating infectious diseases and also production of metabolites (enzymes) by fungi grown on agricultural wastes under submerged and solid state fermentation.

MATERIALS AND METHODS

Test Fungi

The fungal species used in this study (*Aspergillus niger and Penicillium minioluteum)* were collected from the Microbiology Laboratory of Fountain University, Osogbo, Nigeria. They were earlier identified by the USDA, Louisiana, New Orleans, USA. They were originally isolated from food sources in Nigeria. The organisms were maintained on potato dextrose agar slant at 4^{o}C.

Subsrates

The substrates used were corn cobs, rice bran and wheat bran collected as wastes from feedmills and dumps.

Solid State Fermentation Medium

Agro residues were obtained and air dried for 1 month. Thirty grams (30g) of the dried samples were kept separately in a 250 mL conical flask and then moistened with 30 mL of distilled water. The common basal medium contained di potassium hydrogen phosphate; 0.2g, magnesium sulfate; 0.1g, sodium nitrate; 0.6g, potassium chloride; 0.1g, ferrous sulfate; 0.0002g and agar; 2g. The media were then autoclaved at 121 ^{o}C for 15 min and used as corn cob agar, rice bran agar and wheat bran agar.

Broth Fermentation Medium

Agro residues were obtained and air dried for 1 month. Six gram (6g) of the dried samples (corn cobs were crushed after drying) was weighed into 200ml of water, boiled at 100oC for 90 minutes and sieved using muslin cloth and each filtrate was stored in a 250mL conical flask. To each was added the following: di potassium hydrogen phosphate; 0.2g, magnesium sulfate; 0.1g, sodium nitrate; 0.6g, potassium chloride; 0.1g, ferrous sulfate; 0.0002g, and water. The media were then autoclaved at 121oC for 15 minutes and used as corn cob, rice bran and wheat bran broth.

CONTROL CULTURE MEDIUM

Modified Czapek- dox medium was used in this study consisting of 6.0g of D- glucose as the main carbon source and the addition of the following: the basal medium was as stated earlier, then autoclaved at 121^{o}C for 15 minutes and used as control medium for comparative analysis.

Inoculation Of Fungi On Extracts And Subtrates

Agar plugs (6mm) of 5 –day old cultures on potato dextrose agar of the test isolates served as inocula on the prepared solid and liquid media. The broth cultures were incubated at 25 ℃ with continuous agitation at 100 Osci/min on the rotary flask shaker, while those in solid state were incubated at 25 ℃ without agitation. Change in pH was noted after 14 days of incubation and enzymes were carried out.

Preparations Of Plant Extract

The leaves of *Carica papaya*, *Elaeis guineensis*, *Alcohorneae laxiflora* and *Chromolaena odorata* were air dried for three weeks and blended to powder. Methanol was added to the blended samples for extraction in the ratio of 1:10 w/v and maintained on the orbital shaker at 300rpm for 20 hours.The mixture was then filtered using Whattman No1 filter paper. The filtrate was put into the water bath at 60^{o}C for the methanol to evaporate to dryness. The plant extracts were then weighed.15ml of Potato Dextrose agar was dispensed into 42 McCartney bottles and sterilized. The extract was diluted with methanol under the concentration of 100 ppm each.100micrometer of each of the 4 extracts was added to the 6 McCartney bottles each containing sterilized media (6x 4= 24 bottles). For comparative analysis, acetic acid and ethanol was used.100 micrometer of each of acetic acid and ethanol was pipetted into 9 sterilized McCartney bottle each (9x2=18).

Phytochemical Screening Of The Fungi Used

Phytochemical screening was carried out according to Harbone (1973) for the presence of alkaloids, tannins, saponins, phenolics, glycosides and phlobatannis in the fungi extract. Ethanolic extracts of the fungi was prepared by scrapping the fungal growth on a Potato Dextrose Agar (PDA) slant into 100 ml of ethanol. Chemical tests were carried out on the extract using standard procedure to identify the constituent by Sofowora (1993).

Fungus-Plant Extract Relation

The agar diffusion technique on Potato Dextrose Agar (PDA) was adopted in demonstrating the fungus-extract relation. The 4 plants were prepared and applied by the disc diffusion method, with the control having none of the test plants. Triplicates of these were incubated at 25^0c for 7days.

Solid Wastes As Substrates For Test Organisms

Four media which contains sterilized wheat medium, rice bran medium, corncob medium and Czapek dox agar served as possible enhancing substrates for *A. niger* and *Penicillium minioluteum*. 6mm cork borer disc of inoculum of 4 day old culture on Potato Dextrose Agar PDA was seeded into its corresponding substrates. Inoculation was done for 14 days and observation were taken.

Inoculation Of Organism On The Broth

Four sterile 200ml media which contain sterilized wheat medium, rice medium, corncob medium and Czapek Dox broth were done in duplicates for *A. niger* and *Penicillium minioluteum.* 6mm cork borer disc of inoculum of 4 day old cultures on Potato Dextrose Agar (PDA) were carefully seeded into the corresponding broths and incubation was for 14 days on a rotary shaker (100 rpm) at 25^oC. At the end of 14days, the mycelial dry weight of each was measured by filtering the broth through a Whatman No1 filter paper and air drying the residue (mycelia mass) to a constant weight for 24 hours. The filtrates were stored at 10^oC for enzyme analysis.

Enzyme Assay

The filtrate (extract) was then centrifuged at a speed of 3000rpm for 10mins and the supernatant was collected in a sterile bottle for analysis. Enzyme assay were carried out by the methods of Lakshmi and Narasimha 2012 (cellulase); Saqib and Whitney 2006 (endoglucanase); Chandra *et al* 2012 (xylanase); and Jensen and Olsen,1992 (amylase).

Method of the discontinuous reducing was employed in accordance with procedure of Laemmni (1970) by using 15 and 5% w/v acrylamide resolving and stacking gels respectively.

Statistical Analysis

Data obtained for submerged fermentation was compared with that of solid state fermentation with t-test using SPSS version 16.0.

RESULTS

Fungal growth on plant extract

The fungi were able to metabolize all the plant extract at 100ppm concentration. The fungi were unable to grow on the control which is acetic acid.

Table 1. Zone of inhibition (in cm) of Fungi on plant extracts.

EXTRACT	**ZONE OF INHIBITION (CM)**	
	A. niger	*Penicillium minioluteum*
Carica papaya	0.70	1.00
Chromolaena odorata	0.60	0.10
Elaeis guineensis	1.50	1.10

Alcohorneae laxiflora	2.40	1.20
Control	0.50	1.10

RESULTS and DISCUSSION

Table 1 shows the growth inhibiting potentials of *Chromolaena* and *Carica* on the 2 fungi. The least growth was recorded when *Elaies spp* was applied. The growth of fungi on the extracts was indicative of their ability to colonize and possibly incite diseases on it. Many mycotoxins are produced from oil palm products (Goto *et al* 2012). The ability to invade plant products also reflects the ease with which the enzymes necessary for such invasion is secreted by the moulds. Although *Chromolaena* is a weed, it can be exploited when fungal isolates are to be controlled as disease agents thereby attracting industrial and chemotherapeutic attention to the common weed. It is on record that plants produce many metabolites which are exploited as microbiocides, pesticides and many pharmaceutical drugs (Ogundipe *et al.,* 1998). This study demonstrated that herbal medicine can be as effective as modern chemotherapy to combat pathogenic microorganisms. Fungi are significant destroyers of foodstuff and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins. Plant extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trials (Akinyosoye and Oladunjoye 2000; Bouamama et al., 2006; Mohana and Raveesha, 2006). Saponins and alkaloids were significantly higher in quantity in *Penicillium minioluteum* than in *Aspergillus niger,* while others like terpenes, flavonoids and chalcones were detected in *Penicillium* but absent in *Aspergillus* (Table 2). With wheat bran and rice bran, *Aspergillus* easily converted the medium toward acidity, a contrast to the conduct of *Penicillium* under same conditions (Table 3). Although the 2 fungi are known contaminants of these crops in farm and store, these mechanism of medium pH conversion in this particular one is not clear.

Rice bran and wheat bran were able to support the growth of the organisms more than corn cob the former recording less weight in the organisms than even the control (Table 4)

Generally, amylase, cellulase endoglucanase and xylanase activities in *Aspergillus niger* were better in solid state than submerged regime (Fig 1,2,3,4)., except in the control of amylase (350 IU/mL) and xylanase (450 IU/mL).

This was also replicated for Penicillium (Fig 5, 6, 7, 8). However, the highest enzyme activity was obtained with wheat bran as substrate. Castilho et al., (2000) had stated that the conditions in solid-state fermentation were closer to those found in the natural habitat of filamentous fungi, which were, thus, able to grow better and excrete larger quantities of enzymes. Therefore for industrial production of these enzymes and under similar cultural conditions, involving these moulds, solid state fermentation is recommended. Substrate composition and optimization is a vital aspect of biopolymer secretion by fungi (Riley et al., 2000; Sinha et al., 2001). The use of the agro-waste in large scale fermentation will not only turn the waste into a valuable resource but also help reduce the environmental pollution due to the cellulosic biomass.

Also, pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antifungal from this plant are the future challenges. More research should be done on these filamentous fungi to provide humanity with the better understanding of their potentials industrial and public health.

Table II: Phytochemical profile of the Fungi

	FUNGI	
SAMPLE	A. *niger*	*Penicillium minioluteum*
TANINNS	**+**	**+**
PHILOBATANNINS	_	**+**
SAPONIN	**++**	**+++**
FLAVONOID	_	_
STEROIDS	_	**+**
TERPENES	_	**+**
CARDENOLIDES	_	**+**
AKALOIDS	**++**	**+++**
ANTHRAQUINONE	_	_
CHALCONES	_	**+**
PHENOL	**++**	**++**
GLYCOSIDE	**+**	**+**

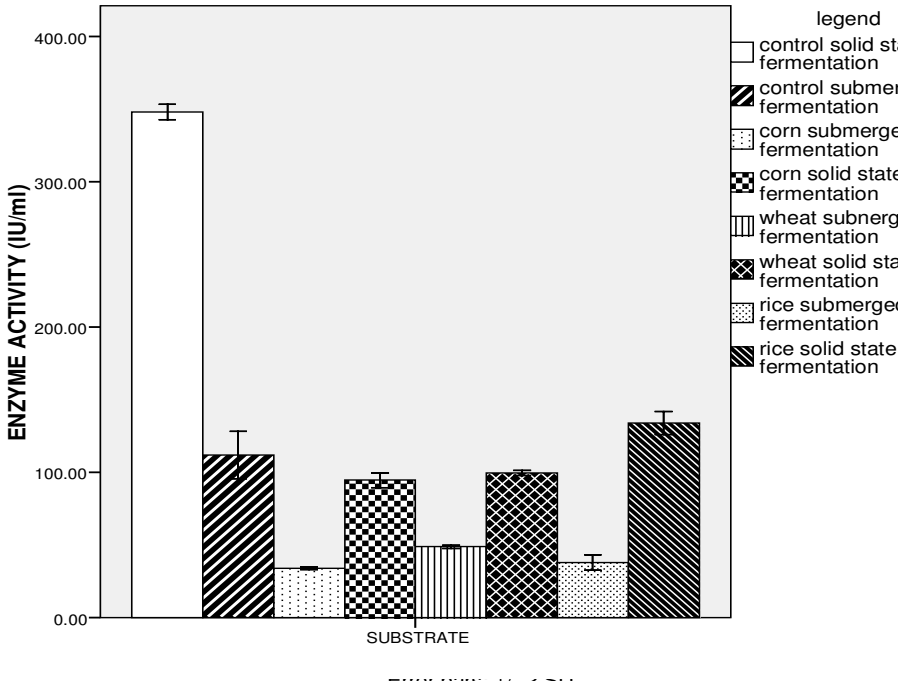
+++ = present in appreciable amount, ++ =present in a moderate amount, +=present in a minute or trace amount, - =completely absent.

Table III: Fungi and pH changes in different submerged substrates

	Aspergillus niger		*Penicillium minioluteum*	
Substrate	Initial	Final	Initial	Final
Wheat bran	6.93	4.36	6.86	7.03
Rice bran	6.74	5.65	6.86	7.34
Corn cob	7.75	8.33	7.74	8.20
Control medium	7.24	.54	6.99	7.60

Table IV: The mycelia dry weight of *Aspergillus niger* and *Penicillium minioluteum* on different substrates

Substrate	Weight(g)	
	Aspergillus niger	*Penicillium minioluteum*
Wheat bran	1.223	1.449
Rice bran	1.734	0.897
Corn cob	0.0595	0.102
Control medium	1.291	1.614



Error bars: +/- 2 SD

FIG. 1: Substrate impact on the amylase activity of *Aspergillus niger* JX 442527

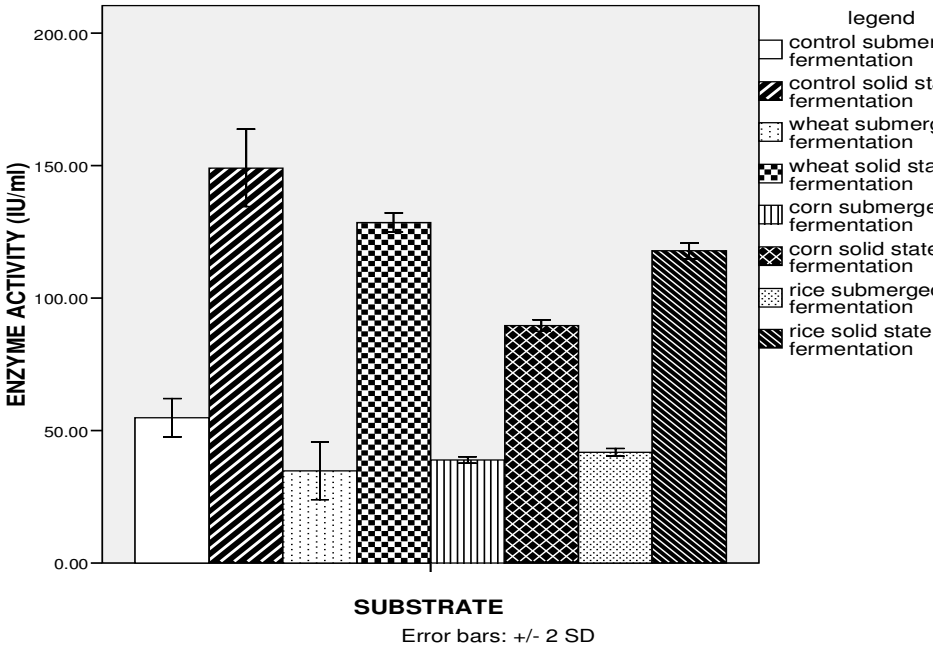


FIG. 2 Substrate impact on the cellulase activity of *Aspergillus niger* JX 442527

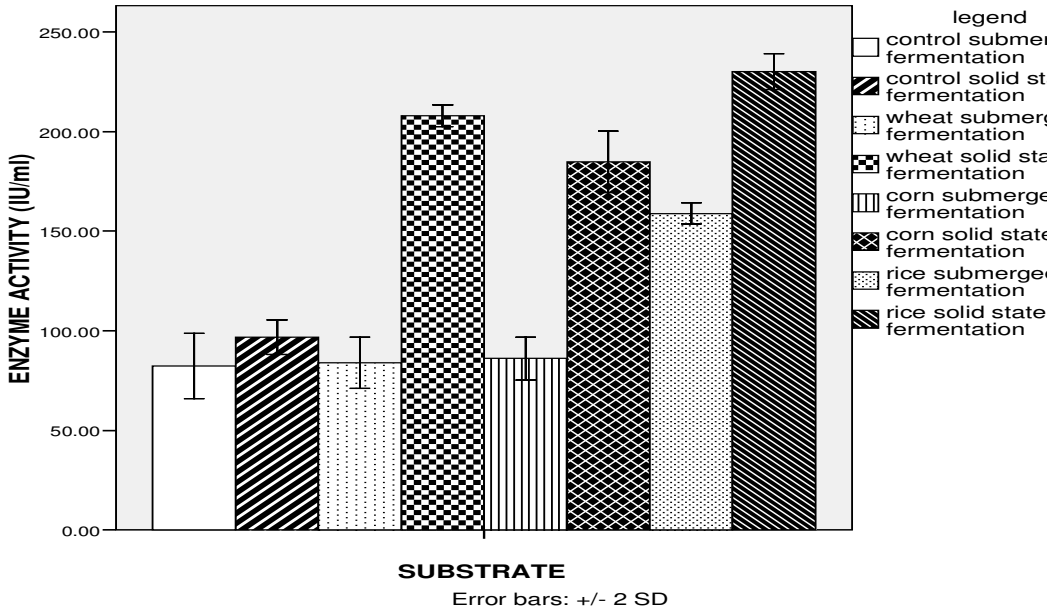


FIG. 3 Substrate impact on the endo glucanase activity of *Aspergillus niger* JX 442527

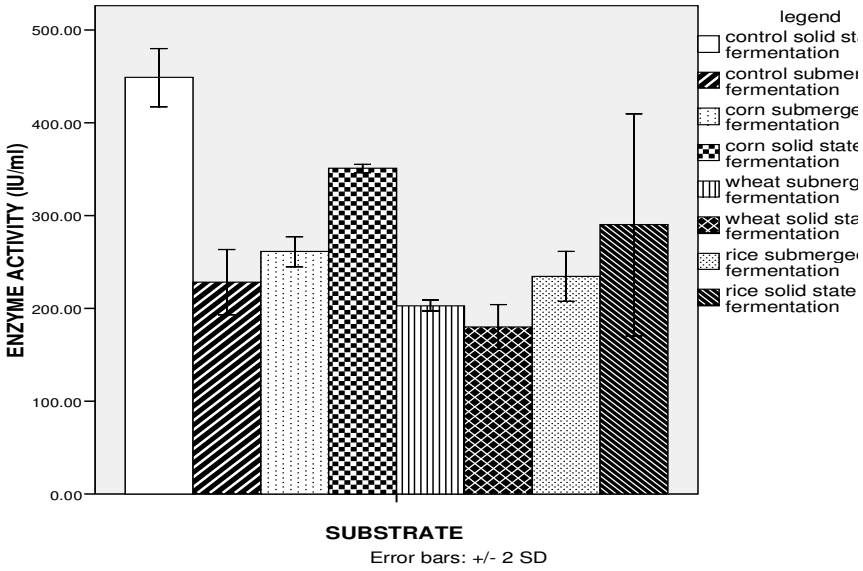


FIG.4 Substrate impact on the xylanase activity of *Aspergillus niger* JX 442527

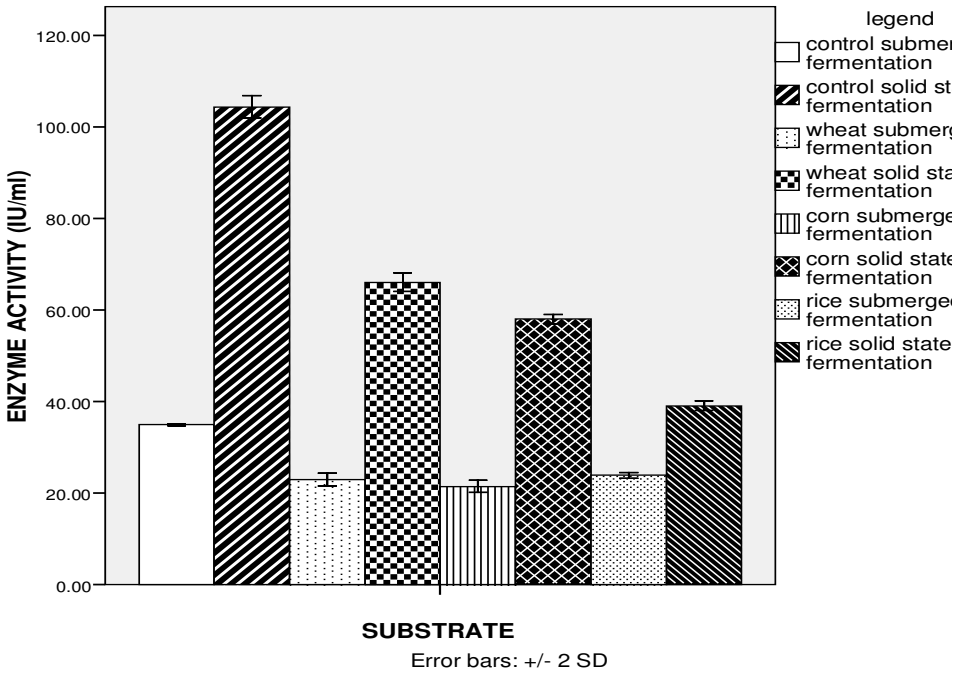


FIG. 5 Substrate impact on the amylase activity of *Penicillium minioluteum* JX 442528

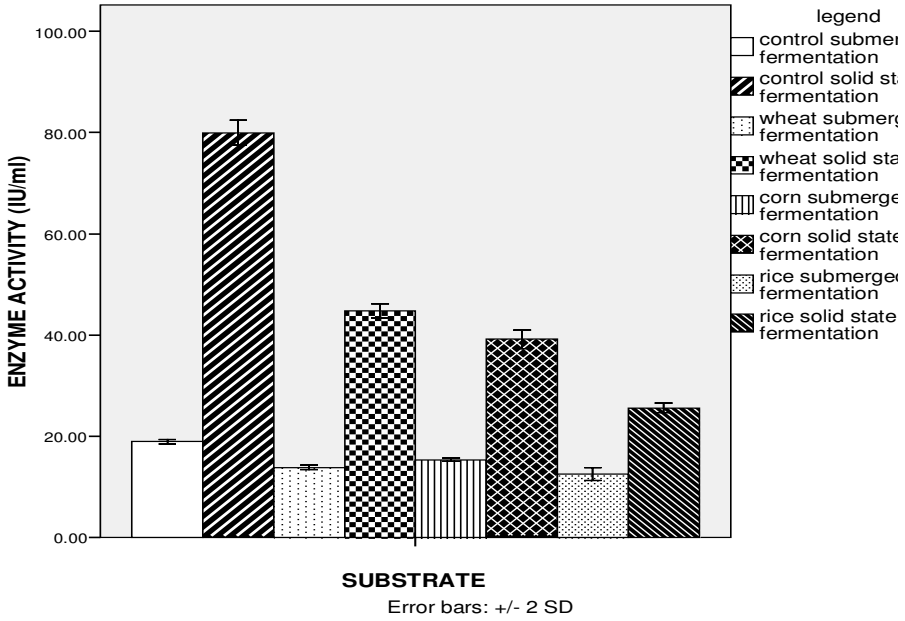


FIG. 6 Substrate impact on the cellulase activity of *Penicillium minioluteum* JX 442528

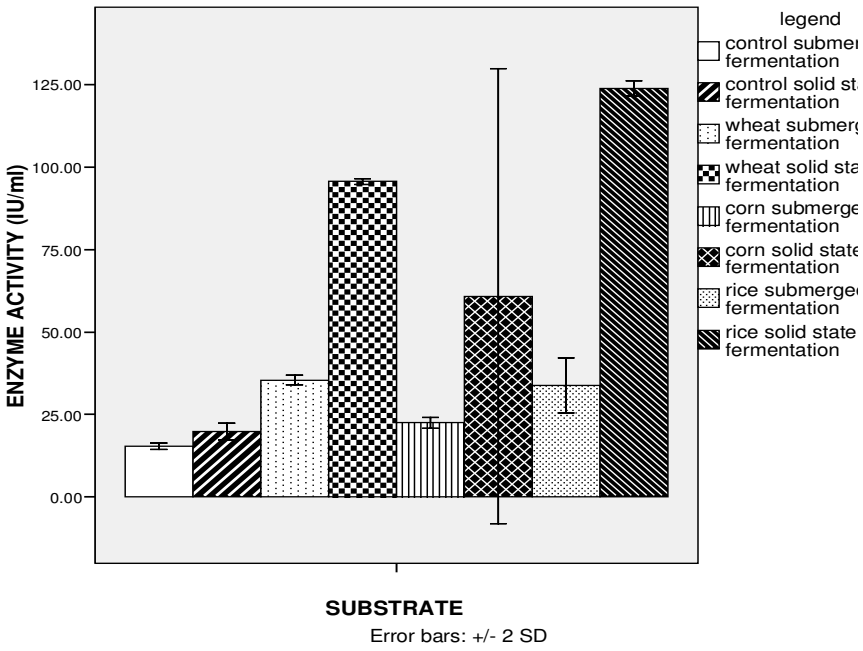


FIG. 7 Substrate impact on the endo glucanase activity of *Penicillium minioluteum* JX 44252

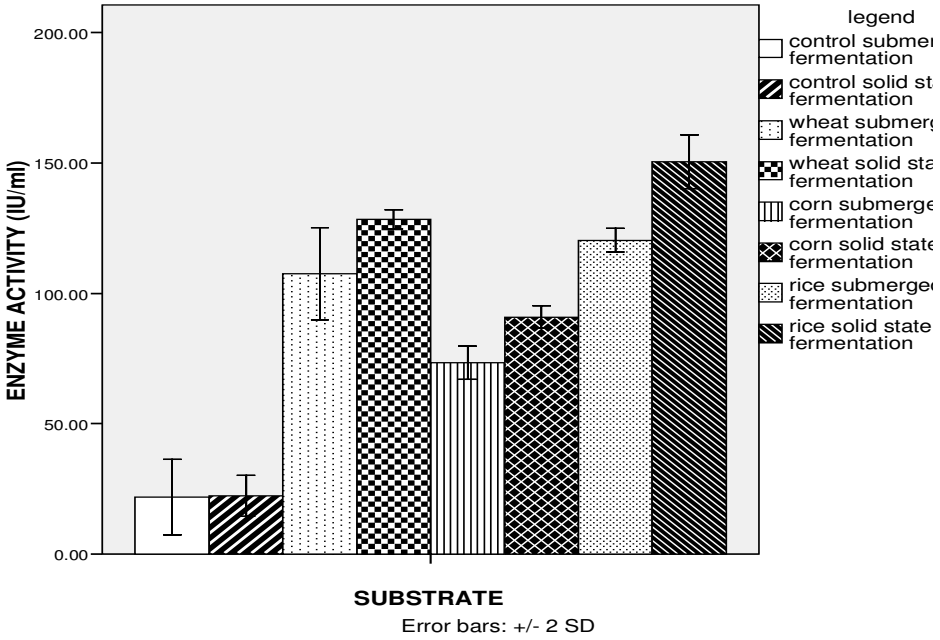


FIG. 8 Substrate impact on the xylanase activity of *Penicillium minioluteum* JX 442528

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