ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2017 ISBN 1595-689X VOL18 No. 3
AJCEM/1726 http://www.ajol.info/journals/ajcem
 https://dx.doi.org/10.4314/ajcem.v18i3.6
AFR. J. CLN. EXPER. MICROBIOL. 18 (3): 167-173

ISOLATION AND SCREENING OF FUNGAL ISOLATES FROM BAMBARA (*VIGNA SUBTERRANEA*) NUTS FOR TANNASE PRODUCTION

***Ajayi, A.A., Adeniji, O.F., Egunjobi, A., Atolagbe O.M., Onibokun, E.A., Anosike, S. O., Oniha, M.I.**

Department of Biological Sciences, Covenant University, Ota

***Corresponding author: adesola.ajayi@covenantuniversity.edu.ng**

ABSTRACT

Tannase (Tannin acyl hydrolase, EC 3.1.1.20) is an enzyme produced in the presence of tannic acid by various filamentous fungi. They are produced principally by fungi of the genus *Aspergillus* and *Penicillium*. The enzyme is used in the food and beverage industry as a clarifying agent for wines, beers and fruit juices. In Africa, billions of dollars are expended yearly on the importation of commercial enzymes for the food and pharmaceutical industries and this increases the cost of production and the finished goods. This study was carried out to isolate tannase producing fungal species using Bambara nuts as a substrate in a bid to finding alternatives to the importation of tannase. Fresh Bambara nuts were collected from different locations in Nigeria. They were cleaned, sorted and intermittently moistened with water to encourage fungal growth for fourteen days. The different fungi obtained after fourteen days were inoculated onto Potato Dextrose Agar plates and incubated at 25°C for five days. Subculturing of fungal isolates was carried out to obtain pure cultures of isolates. Tannilytic activity (hydrolysis of tannin) of isolates was assessed by inoculating them in media containing tannin. The plates were incubated at 25°C for 2-5 days after which the plates were observed and zones of hydrolysis measured. A total of eighteen isolates were obtained. They were all members of the *Aspergillus* genus. 56% (10) of the isolates were able to degrade tannin acid with mean zone of hydrolysis of 39mm ±23.7 mm (Range 10-70mm). This study established members of the *Aspergillus* genus isolated from Bambara nuts as viable fungi for application in the production of tannase. This study adds to existing reports on fungal production of tannase.

Keywords: Tannase; Fungal isolates; *Aspergillus*; Bambara nuts; *Vigna subterranea*

L'ISOLEMENT ET LA PRÉSÉLECTION DES ISOLATS FONGIQUES DE BAMBARA (VIGNA SUBTERRANEA) POUR PRODUCTION DE CHAMPIGNONS DE TANNASE.

A.A., Adeniji, O.F., Egunjobi, A., Atolagbe O.M., Onibokun, E.A., Anosike, S. O.,Oniha, M.I.

Département des Sciences Biologiques, Université du Pacte, l'Ota*

Auteur correspondant : adesola.ajayi@covenantuniversity.edu.ng

ABSTRACT

Tannase acyle hydrolase, Tanin (EC 3.1.1.20) est une enzyme produite en présence d'tannicacid par divers champignons filamenteux. Ils sont produits principalement par des champignons de l'genusAspergillus et Penicillium. L'enzyme est utilisé dans l'industrie des aliments et boissons comme aclarifying agent pour vins, bières et jus de fruits. En Afrique, des milliards de dollars sont expendedyearly sur l'importation d'enzymes commerciales pour l'industrie alimentaire et pharmaceutique andthis augmente les coûts de production et les produits finis. Cette étude a été réalisée à l'aide d'espèces de champignons produisant des isolatetannase écrous Bambara comme substrat dans le but d'findingalternatives à l'importation de tannase. Les écrous Bambara frais ont été prélevés dans differentlocations au Nigéria. Ils ont été nettoyés, triés par intermittence et humidifié avec de l'eau croissance fongique toencourage pendant 14 jours. Les différents champignons obtenus après 14 jours de wereinoculated sur Potato Dextrose Agar plaques et incubées à 25°C pendant 5 jours. Sous-offungal isolats a été réalisée pour obtenir des cultures pures d'isolats. Activité Tannilytic hydrolysisof (tanin) des isolats a été évaluée en inoculant dans des milieux contenant du tanin. Le plateswere incubé à 25°C pendant 2-5 jours après laquelle les plaques ont été observées et les zones mesurées. ofhydrolysis Un total de 18 isolats ont été obtenus. Ils étaient tous membres d'theAspergillus genre. 56 % (10) des isolats ont été capables de dégrader l'acide tannique zone ofhydrolysis avec une moyenne de 39mm ±23,7 mm (10-70 mm). Cette étude a établi les membres de theAspergillus genre isolé de Bambara écrous comme champignons viables pour l'application dans la production de tannase Cette étude ajoute aux rapports existants sur la production de champignons de tannase.

Mots-clés : Tannase ; Aspergillus isolats fongiques ; noix ; Bambara Vigna subterranea

Significant Statement

This study revealed that bambara nuts (*Vigna subterranea*) plays host to a variety of members of the genus *Aspergillus* that can be beneficial for tannase production. Previous researchers have established tannase production by microorganisms isolated from other legumes. This study hopes to diversify the use of bambara nuts for industrial enzyme production rather than consumption alone. Tannase production locally will help to reduce the overall cost of enzyme importation particularly in developing countries like Nigeria.

Statement on Conflict of Interest: "none declared"

INTRODUCTION

Tannase can be obtained from plant, animal and microbial sources (1). It is present in tannin-rich plants mainly in their fruits, leaves, branches and barks of trees (2). For animal sources, the enzyme can be extracted from bovine intestine and the ruminal mucous (3).The microbial sources of tannase are mainly from bacterial and fungal origin and this represents most enzymes used in biotechnological applications (4). Tannase can be obtained from different sources such as tea waste dump sites, agro-residue waste sites and site nearby tannery industries (5). Microbial sources of enzymes are preferred to animal and plant sources (6). Tannase is produced by a number of microorganisms like fungi (*Aspergillus, Penicillium, Rhizopus sp*), yeast (*Candida* sp), and bacteria (*Bacillus* sp) (7 ; 8). Enzymes including tannase are used in almost all industries in Nigeria and the country spends #200 billion annually on importation of industrial enzymes (9). Tannase has several industrial applications; as clarifying agent in the manufacturing of instant tea, beer, fruit juices and some wines (10), for treatment of tannin-polluting industrial effluents and agricultural waste and for the hydrolysis of gallotannin to gallic acid which is an intermediate required for the synthesis of an antifolic antibacterial drug, trimethoprim (8). Bambara (*Vigna subterranea*) commonly known as 'Okpa' in Nigeria is widespread in Africa and is related to cow pea. *Aspergillus niger* is a unique organism compared to other organisms used in individual enzyme application because of its good fermenting capabilities, wide range of enzymes produced from degradation of plant cell wall polysaccharides, high level of protein secretion, more predictable and controllable enzyme content, it is relatively harmless compared to other filamentous fungi (11). There is paucity of information on the practical uses of tannase due to insufficient knowledge of its properties, optimal uses and purification processes (12). This study is therefore an approach to screen for fungal isolates from bambara nuts for tannase production in a bid to decrease the high cost associated with importation of enzymes for industrial uses in the developing economy of Nigeria.

MATERIALS AND METHODS

Sample Collection

Bambara nuts of two different colour coats (Red and Cream) (Fig. 1) were purchased from three different locations. The locations are Ota Market – Cream Bambara nuts, Bodija Market –Cream Bambara nuts and IITA Ibadan – Red Bambara nuts. Samples were transported to the Microbiology laboratory of the Department of Biological Sciences, Covenant University, Ota Nigeria for sorting, cleaning and analysis.

Fig. 1: Cream and Red Colour Coat Bambara (*Vigna subterranean)* Nuts

Sample Processing

A total of 35g of each sample was weighed into a sterile petri dish and 5mls of sterile distilled water was sprinkled on it according to a modified method described by (13). Petri dishes containing the samples were covered up and then sealed with paper tape. Sterile distilled water was sprinkled intermittently, every other day for Fourteen days. After fourteen days, moulds growing on the samples (Fig. 2) were harvested.

Fig.2. Moulds growing on Cream Colour Coat Bambara nuts

A second set of 35g each of the three samples (Fig. 3) were weighed and soaked inside a sterilized beaker containing 40mls of sterile distilled water and sealed with paraffin for 6h. The water was drained and the nuts were transferred into sterile petridishes, covered and sealed up with paper tape for fourteen days.

Fig 3: Bambara Nuts Soaked in Sterile Distilled Water

Isolation of Fungal Isolates from Mouldy Bambara Nuts

Moulds growing on Bambara nut samples were inoculated on Potato dextrose agar plates (Fig. 4) and incubated at 28°C for 72h. The plates were further sub-cultured until pure cultures were obtained from each of the plates.

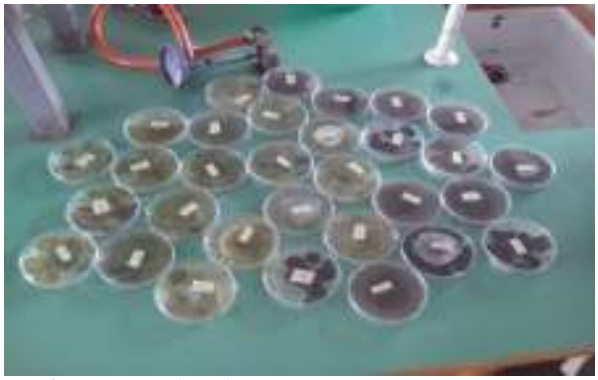

Fig. 4: Fungal Isolates on Potato Dextrose Agar Plates

Screening of Fungal Isolates for Tannase Prodution

The selection medium (g/l) consisted of $NaNO_3$-3.00, KH_2PO_4-1.00, $MgSO_4.7H_2O$-0.50, $FeSO_4.7H_2O$-0.01, Agar- 30.0 according to the method described by (13). It was autoclaved at 121°C for 15 min and supplemented with 1% tannic acid (10 ml) which was previously filter-sterilized through Whatman filter paper No1(pH- 4.0) and adjusted using 100 mM NaOH. Strains of *Aspergillus sp* isolated were then point-inoculated and incubated at 30°C for 96 h. The diameters (mm) of the colonies were measured at 24h intervals for seven days.

RESULTS

A total of 18 fungal isolates were obtained (Table 1). All the isolates were members of the genus *Aspergillus* (Table 2). Fungal isolates were obtained from soaked Bambara nuts only from two locations (Bodija Market and Ota Market) while none was obtained from IITA samples (Table 3). For the wet Bambara nuts fungal isolates were obtained from the nuts of all three locations (Table 3). All the 18 fungal isolates (*Aspergillus* genus) were screened for tannase production. The zone of hydrolysis for fungal isolates obtained from soaked bambara nuts from two locations (Bodija Market and Ota Market) is shown in Figure 5. The zones of hydrolysis for fungal Isolates obtained from wet Bambara nuts from the three sample locations is shown in Figure 6. Highest zones of hydrolysis for both wet and soaked bambara nuts (Fig.7).

TABLE 1: FUNGAL ISOLATES OBTAINED FROM BAMBARA NUTS AND LOCATION

Sample Location	Isolate Codes	Colour Change	Sample Location	Isolate Codes	Colour Change	Sample Location	Isolate Codes	Colour Change
BODIJA MARKET IBADAN	SBJ_{A11}	Brown	OTA MARKET	SOT_{B11}	Black	IITA IBADAN	$WIIT_{A11}$	Brown
	SBJ_{B11}	Brown		SOT_{B12}	Green		$WIIT_{C11}$	Brown
	SBJ_{A21}	Green		WOT_{A11}	Greenish yellow		$WIIT_{C21}$	Brown
	SBJ_{A21}	Black		WOT_{A12}	Green		$WIIT_{C22}$	Brown
	SBJ_{B21}	Greenish yellow		WOT_{B12}	Brown			
	WBJ_{A11}	Brown						
	WBJ_{B12}	Greenish yellow						
	WBJ_{C11}	Brown						
	WBJ_{C21}	Greenish yellow						

TABLE 2: MICROSCOPIC CHARACTERISTICS FOR IDENTIFICATION OF *ASPERGILLUS* SPECIES

Isolate codes	Colour Change	Shape	Surface	Conidia Surface	Identification
SBJ_{A11}	Brown	Spherical	Rough walled	Smooth	*A. tamarii*
SBJ_{B11}	Brown	Spherical	Rough walled	Smooth	*A. tamarii*
SBJ_{A21}	Green	Glubose	Finely roughened	Distinctly rough	*A. parasiticus*
SBJ_{A21}	Black	Glubose	Smooth walled	Very rough irregular	*A. niger*
SBJ_{B21}	Greenish yellow	Glubose ellipsoid	Quietly spherical	Smooth finely roughened	*A. flavus*
WBJ_{A11}	Brown	Spherical	Rough walled	Smooth	*A.tamarii*
WBJ_{B12}	Greenish yellow	Glubose ellipsoid	Quietly spherical	Smooth finely roughened	*A. flavus*
WBJ_{C11}	Brown	Spherical	Rough walled	Smooth	*A. tamarii*
WBJ_{C21}	Greenish yellow	Glubose ellipsoid	Quietly spherical	Smooth finely roughened	*A.flavus*
SOT_{B11}	Black	Glubose	Smooth walled	Very rough irregular	*A. niger*
SOT_{B12}	Green	Glubose	Finely roughened	Distinctly rough	*A. parasiticus*
WOT_{A11}	Greenish yellow	Glubose ellipsoid	Quietly spherical	Smooth finely roughened	*A.flavus*
WOT_{A12}	Green	Glubose	Finely roughened	Distinctly rough	*A. parasiticus*
WOT_{B12}	Brown	Spherical	Rough walled	Smooth	*A. tamarii*
$WIIT_{A11}$	Brown	Spherical	Smooth walled	Smooth slightly rough	*A. nidulans*
$WIIT_{C11}$	Brown	Spherical	Smooth walled	Smooth slightly rough	*A. nidulans*
$WIIT_{C21}$	Brown	Spherical	Smooth walled	Smooth slightly rough	*A. nidulans*
$WIIT_{C22}$	Brown	Spherical	Smooth walled	Smooth slightly rough	*A.nidulans*

TABLE 3: FUNGAL ISOLATES FROM SOAKED AND WET BAMBARA NUTS FROM DIFFERENT LOCATION

Soaked Bambara nuts					Wet Bambara nuts		
Sample Location	**Sample**	**Colour**	**Identification**	**Sample Location**	**Sample**	**Colour**	**Identification**
Bodija Market Ibadan	SBJ_{A11}	Brown	*A.tamarii*	Bodija Market Ibadan	WBJ_{A11}	Brown	*A.tamarii*
	SBJ_{B11}	Brown	*A.tamarii*		WBJ_{B12}	Greenish yellow	*A.flavus*
	SBJ_{B11}	Green	*A.parasiticus*		WBJ_{C11}	Brown	*A.tamarii*
	SBJ_{A21}	Black	*A.niger*		WBJ_{C21}	Greenish yellow	*A.flavus*
	SBJ_{B21}	Greenish yellow	*A.flavus*	Ota Market			
Ota Market	SOT_{B11}	Black	*A.niger*		WOT_{A11}	Greenish yellow	*A. flavus*
	SOT_{B12}	Green	*A.parasiticus*		WOT_{A12}	Green	*A.parasiticus*
					WOT_{B12}	Brown	*A.tamarii*
				IITA Ibadan	$WIIT_{A11}$	Brown	*A.nidulans*
					$WIIT_{C11}$	Brown	*A.nidulans*
					$WIIT_{C21}$	Brown	*A.nidulans*
					$WIIT_{C22}$	Brown	*A.nidulans*

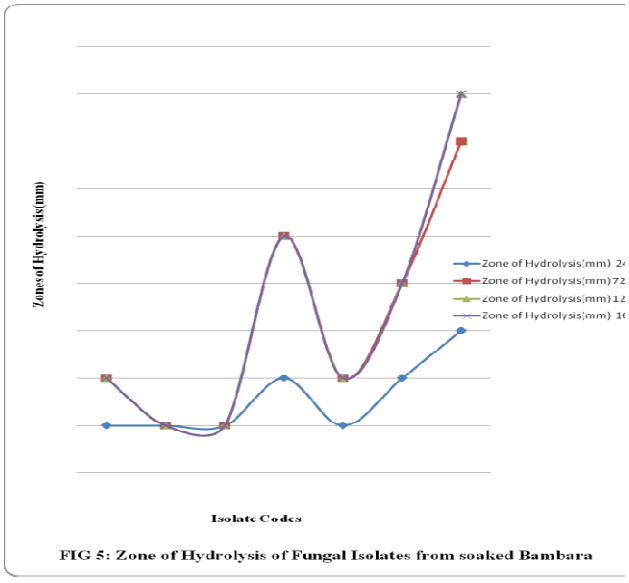

FIG 5: Zone of Hydrolysis of Fungal Isolates from soaked Bambara

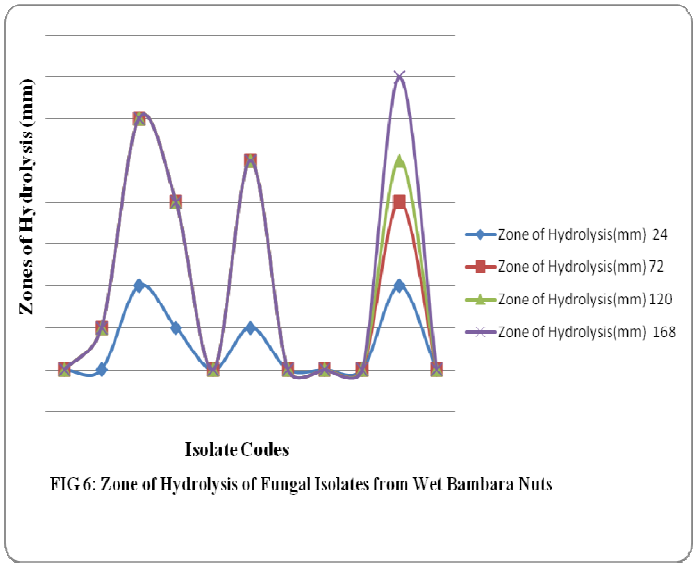

FIG 6: Zone of Hydrolysis of Fungal Isolates from Wet Bambara Nuts

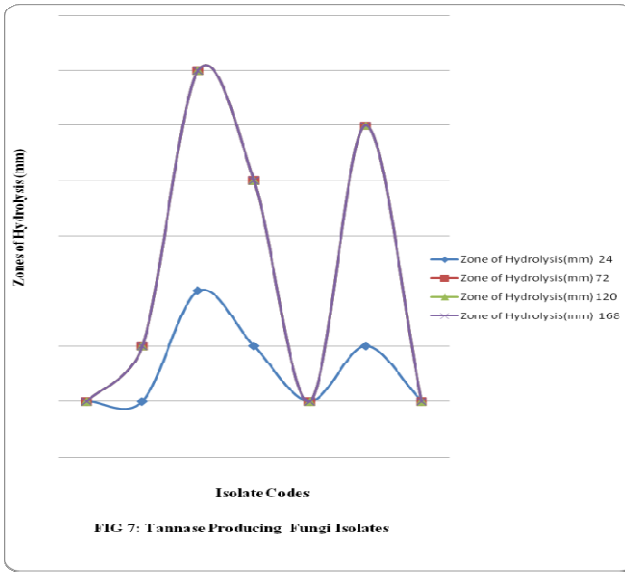

FIG 7: Tannase Producing Fungi Isolates

DISCUSSION

This study revealed the isolation and screening of fungal isolates from bambara nuts for tannase production. Most of the fungi isolated were of the genus *Aspergillus* (14) had earlier isolated different species of *Aspergillus* from various legumes sampled. This correlates with the findings of (15) and (16) where fungi especially of the genus *Aspergillus* were employed successfully as sources of producing different enzymes. *A. niger* isolated from the soaked bambara nuts showed high tannase production. *Aspergillus niger* strains was isolated from bambara nuts of three different colour seed coats and the strain of *A.niger* from the red colour seed coat showed the highest potential for tannase enzyme (17). However, this is in contrast to the wet method whereby *A. flavus* showed the highest tannase production. Tannase production by *A. flavus* had been reported (18). Filamentous fungi of the *Aspergillus* and *Penicillium* genus have been widely used for tannase production (19). Tannase as an extracellular inducible enzyme produced in the presence of tannic acid by fungi, bacteria and yeast was reported (20).

Conclusion

Despite the long history and numerous publications on tannase, it is still considered as one of the costly industrial enzymes. This study adds to existing reports on isolating high productive strains of tannase in view of the growing demand for tannase.

Acknowledgement

The Authors are grateful to the Covenant University Centre for Research Innovation and Discovery (CUCRID) for publication cost.

REFERENCES

1. Abou-Bakr, H. A., El-Sahn, M. A. and El-Banna, A.A., 2013. Screening of Tannase-Producing Fungi Isolated from Tannin-Rich Sources. *Int. J. of Agri and Food Res.* 2(3): 1-12.

2. Costa, A. M., Ribeiro, X. W., Kato, E., Monteiro, A. R. G. and Peralta, R.M. 2008. Production of tannase by *Aspergillus tamarii* in submerged cultures. Braz. *Archives of Bio. and Tech.* 51(2): 399-404

3. Natarajan, K. and A. Rajendran, 2009. Effect of Fermentation Parameters on Extracellular Tannase Production by *Lactobacillus plantarum* MTCC 1407. *J. of Chem.* 6(4): 979-984.

4. Yaoa J., Guob, G. S. Renb, G.H. Liub, Y. H. 2014 Production, Characterization and Applications of Tannase. *J. of Mol. Cataly B: Enzymatic* 101: 137–147.

5. Girdhari, S.N. and Peshwe, S.A. 2015. Isoaltion and Screening of Tannase producing Fungi. *Int J. of Curr. Microbio. App. Sci.* 4(7): 765-774.

6. Atolagbe, O.M., Ajayi, A. A. and O. Edegbo, O. 2016. Characterization of alpha–amylase from soursop *(Annona muricata* Linn.) fruits degraded by *Rhizopus stolonifer*, *Pakistan J. of Biol Sci.* 19: 77-81 doi:10.3923/pjbs.2016.77.81

7. Lokeswari, N. and Jayaraju, K. 2007. Tannase Production By *Aspergillus niger*. *J. of Chem* 4(2): 192-198.

8. Paranthaman, R. S., Vidyalakshmi, Murugesh, S. and Singaravadivel, K, 2008. Optimisation of Fermentation Conditions for Production of Tannase Enzyme by *Aspergillus oryzae* using Sugarcane Baggasse and Rice Straw. *Global J. of Biotechnol. Biochem.* 3(2): 105-110.

9. Ajayi, A. A., Osunlalu, E.O., Peter-Albert, C.F. and Adejuwon, A. O., 2014. Studies on Pectinolytic and Proteolytic Enzymes from Deteriorated grapes. *Covenant Journal of Physical and Life Sciences* 1(2): 1-15.

10. Rodríguez-Durán, L. V., Valdivia-Urdiales,B., Contreras-Esquivel, J.C., Rodríguez-Herrera, R. and Aguilar, C.N. 2011. Novel Strategies for Upstream

and Downstream Processing of Tannin Acyl Hydrolase. *Enz Res*., pp 20 doi:10.4061/2011/823619

11. Gautam, A.K. and Bhadauria, R., 2011. Diversity of Fungi and Mycotoxins associated with stored triphala churn and its ingredients. *J. of Biol Sci*., 11: 226-235.

12.Belmares, R., Conttreras-Esquivel, J.C., Rodriguez-Herrera, R., Coronel, A.R. and Aguilar, C.N. 2004. Microbial production of tannase: An enzyme with potential use in food industry. *Lebensm Wiss. Technology*.37: 857-864.

13.Pinto, G. A. S.., Leite, S.G.F. Terzi, S. C. and Couri, S., 2001. Selection of Tannase-producing *Aspergillus niger* strains. *Braz J. of Microb*. 32: 24-26.

14. Salem, A. and Ebraim, M. K. H., 2014. Production of amylase by fungi isolated from legume seeds collected in Almadinah Almunawarah, Saudi Arabia. *J. of Taibah Uni. of Sci*., 8: 90-97.

15.Mrudula, S. and R. Murugammal, 2011.Production of Cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. *Braz J. of Microb*. 42(3): 1119-1127.

16.Max, B., J. M. Salgado, N. Rodríguez, S. Cortés, A. Converti, and J. M. Domínguez, 2010. Biotechnological Production of Citric Acid. *Braz J. of Microb*. 41(4): 862-875

17. Difo, H.V., E. Onyike, E., Ameh, D. A. Njoku, G.C. and Metwally, S. M. A., 2015. Optimization of Tannase Production by *Aspergillus flavus,* Egypt J. of Expt. Bio. 11(2): 121-127.

18. El-Shora H.M., O.A. Awadalla and S.M.A. Metwally, 2015. Optimization of tannase production by *Aspergillus flavus*. *Egypt J. of Expt. Bio*. 11(2): 121-127.

19. Belur, P.D. and Mugeraya, G. 2011. Microbial Production of Tannase: State of the art, *Res. J. Microbiol*. 20(6): 25-40. DOI:10.3923/jm.2011.25.40.

20. Battestin, V. and Mecedo, G.A. 2007. Tannase production by *Paecilomyces* variation. *Biores Tech.* 98: 1832-1837.