INFECTION OF YAM (DIQSCQREA SPP) ASSOCIATED WITH CELL WALL DEGRADING ENZYMES BY *ASPERGILLUS NIGER V. TIEGHEM*

ONYENETO,T.C[1]., Nwachukwu*, I.N[2] and Nwogwugwu, N.U[2].

[1] Anambra State University of Science and Technology, Uli, Nigeria.
[2]Department of Microbiology, Federal University of Technology, Owerri, Nigeria.

Abstract: Extract of yam (*Dioscorea* SPP) infected by *Aspergiilus niger*, exhibited cellulase, polygalacturanase and pectin methylesterase activities. These enzymes except for traces of pectin methylesterase were not detected in healthy yam tissues. Maximum activities of the cellulase, polygalacturonase and pectin methylesterase enzymes occurred at PH 4.0, 4.5 and 8,0 respectively. Optimum temperature for cellulase activity was 35^0C and 40^0C for both poly-galacturonase and pectin methylesterase. The enzymes were stimulated by low concentrations of k^+, Na^+,Ca^{2+}, Mg^{2+} and Mn^{2+} but inhibited by iodo-acetic acid, 2,4-dinitrophenol and ethylenediamine tetraacetic acid. The activities of cellulase, polygalacturonase and pectin methylesterase enzymes were affected by the substrate concentrations with maximum activities at 0.3% (w/v), 0.2% (w/v) and 1.5% (w/v) respectively. The Km values for cellulase, polygalacturonase and pectin methylesterase were 1.1mg/ml, 2.5 mg/ml and 9.1mg/ml respectively.
[ONYENETO,T.C., Nwachukwu, I.N and Nwogwugwu, N.U. **INFECTION OF YAM (DIQSCQREA SPP) ASSOCIATED WITH CELL WALL DEGRADING ENZYMES BY *ASPERGILLUS NIGER V. TIEGHEM*.** *Researcher* 2015;7(10):77-82]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 10

Key words: Phytopathogenic, -Deterioration, Pathogenicity.

Introduction:

Yam (*Dioscorea rotundata*), economically the most staple food stuff in tropical Africa, is highly susceptible to a variety of pathogens during storage (Ogundana *et al*, 1971;Amusa,*et al*.,2003). One of the most important micro-organisms associated with this infection is *Aspergillus niger* (Ogundana *et al*, 1970; FAO, 1998;Amusa, 2000). The infection of yam is characterized by softening of the tissues (soft rot) which become watery and often give off an unpleasant flavour and odour. Microbiological attack on stored products usually results in economic loss either by imparting unpleasant flavour, odour and colour or by causing structural changes which render the products unusable for its original value and function {Ogundana *et al*., 1971; Okaka and Okechukwu,1987; Ezeh, 1998). "The genera *Penicillium* and *Aspergillus* embrace all the fungi collectively known as storage fungi (Kenaga, 1970; IITA, 1993). Previous works revealed that rot-causing fungi are strong producers of enzymes that could act jointly to disintegrate host tissues (Ogundana, *et al*., 1971; Onayemi,1983;Morse et al.,2000). Many phytopathogenic fungi produce enzymes in vitro which will degrade cellulose that have been made soluble by chemical treatment and several species have been shown to be capable of degrading more highly ordered forms of cellulose such as insoluble cellulose powder and cotton fibres (Bateman, 1968; Amusa and Baiyewu, 1999). Destruction of native cellulose has been attributed to the ability *of* microorganisms to produce extracellular cellulase enzymes in vitro (Bateman, 1968; Wesheng, *et al*., 2010).

Cellulose is generally regarded as the major structural component of cell wall of plant tissues, comprising the organized phase or micro-fibrillar structures of the cell walls (Bateman, 1964; Dube *et al*, 1976; Wang *et al*., 2011). Many workers have isolated such a complex cellulase enzymes from some *Aspergillus* species (Olutiola & cole, 1976; Olutiola, 1976). Most cellulolytic enzymes haw been grouped as being inductive rather than constitutive (Chapman *et al*., 1975; Ghora *et al*., 1975). Many environmental factors do exert considerable influences on the activity of cellulase enzymes. This has been supported by works of many researchers (Nyuksha *et al*; 1976; Lisker *et al*., 1975). The effect of hydrogen ton concentration (pH) has been revealed to affect the activity of cellulase enzymes by many workers (Salmanora *et al*, 1975). Salmanora *et al*., 1975 showed that pH has a very great effect on the enzymic activity of *Aspergillus niger* and *Trichothecium_roseum*, with maximum activity around 3.5 - 5.5. Other workers, confirmed that most cellulases have optimum activity of pH 4.0 but generally between 3.5 - 5.5 (Fanelli & Cerrone, 1977; Olutiola, 1976). Temperature constitutes another important factor influencing the activity of cellulase enzymes. Inactivation of cellulase of *Rhizoctonia_solani* at 50^0c has been reported (Batenan, 1964). Hurst *et al*, 1977) reported that cellulase enzyme produced by *Aspergillus niger* has maximum activity at PH 4.0 obtained at 45°c It has also been reported that other factors such as substrate concentration and presence of salts and chemical affect the activity of cellulase enzymes (Ikzuk, 1976; Ishii, *et al*, 1975; Russel, 1974).

Pectinases are among the cell-watt degrading enzymes produced by many phytopathogens. They degrade pectic substances which form the middle lamella exclusively (Hagar & Nclntyre, 1972; Dube *et al*, 1976). The two major types of pectic enzymes are (i) Pectin methylesterase (PHE) and Polygalacturonases (PG). However, the actual disintegration of plant cell-wall resulted from the hydrolytic action of polygalacturonase and cellulases (Goel *et al*, 1974, Urbanek *et al* 1975). Alabi & Naqvi (1977) also reported that pathogens causing soft rot of yams and sweet potatoes in storage, produced polygalacturonase and extracellular celuloslytic enzymes. Blagwal *et al*; (1973) reported that *Fusarium* Spp. produced poygalacturonase, pectin methylesterase and cellulase enzymes that play rote in producing tuber rots. Hence, the pathogenicity of those organisms that cause infection is related to their ability to elaborate pectic and cellulosic and enzymes (Ishii *et al*, 1976, Joel *et al*, 1974; Lisker *et al* 1975). Pectic enzymes has been implicated in cell incarceration and cell death (Bateman 1968).

However, pectin methylesterase has been found to occur in health tissues while polygalacturonase and cellulase occur only in infected tissues (Joet *et al,* 1974; Bateman, 1968; Urbanek, 1975). Bateman (1968), revealed that all macerating enzymes when purified, were identified to be pectic enzymes, so cellulase apparently do not contribute to ft. Some environmental and chemical factors also influence the activity of pedic enzymes as in cellulase enzyme (Lister *et al*, 1975; Salmanva *et al.*, 1975; Alabi & Naqvi, 1977; Olutiola &Akintunde, 1979).

The optimum pH is reported to be between 4.0 - 5.0 for polygalaturonase and pectin methylesterase has optimum activity at pH 8.0 (Lister *et al*, 1975; Urbanek *et al.*; 1975; and Olutiola & Akintunde, 1979). Temperatures between 40^{OC} - 50^{0c} predicts maximum activity as shown by many researchers (Hurst *et al*., 1977, Olutiola, 1976; Olutiola & Akintunde, 1979). Some other factors such as concentrations of the substrates, presence of salts or chemicals has been reported to influence the activities of these enzymes (Ishii e*t al.*, 1976, Olutiola & Akintunde, 1979; Ikzuk, 1974). The objective of this research work was therefore to investigate:

(a) Production of cellulolytic and pectic enzymes in yam tissues infected by *Aspergillus niger*.

(b) Some properties of these enzymes.

Material And Method

Inoculation of yam

The isolate (NSPRI. 103) of *Aspergillus niger* employed in this work came from the culture collection of the Nigerian stored products research institute, Ibadan. The stock cultures were maintained on malt yeast extract agar slants prepared by mixing 1% yeats extract, 1% malt extract and 2% agar.

Agar slant cultures (72 hold) were used to prepare inoculums. The inoculums was prepared by washing two agar slants with 40mml of sterile water. The healthy yam was cut into approximately equal thin slices. The yam slices were surface sterilized using 0.1% mercuric chloride ($HgCl_2$) and sterile water. The yam slices were placed in sterile 100ml Erlenmeyer flasks containing 10ml sterile water. Each flask contained 20g of yam plus 10ml of sterile water. Each flask was inoculated with 1ml spore suspension. All the flasks were inoculated except two which served as control. Both the experimental and control flasks were incubated stationary at 30^0C.

Extraction Of Enzymes From Yam Tissue

At 24h interval, the contents of two flasks were analyzed for pectic and cellulolytic enzymes. Each flash was mixed with extractant (0.1m NaCl in 0.05m citrate-phosphate buffer, pH 6.0) and made up to 40ml level in 100ml measuring cylinder. This mixture was poured into the macerating flask, chilled and macerated. The maceration was done using a homogenizer (callenkamp) for two minutes at ten seconds maceration and cooling intervals alternatively. After maceration, the filtrate was allowed to pass through what man No. 1 filter; the clear extract was employed as crude enzyme preparation. The protein content of the extract was analysed by the folin-phenol reagent of Lowry et al. (1951) (Appendix 1.)

Enzyme Assays:

Cellulose

Cellulose activity was assayed viscometrically in an Ostwald viscometer (Bs/u, size D) containing 13ml of 0.7% CM- cellulose in 0.05m citrate- phosphate buffer (ph5.0) and 1ml of crude enzyme solution at 35^oc for 1hour. The activity of the enzyme was expressed as the percentage loss in viscosity of the CM- cellulose by employing the formula,

$P= 100\ (t_1-t_2)t_1-t_w$, where P is the percentage lose in viscosity, t_1 is the initial flow time of, t_2 is the flow time after 1 hour (60 minutes), t_w is the flow time distilled water (Olutiola and Cole, 1976;1977).

The cellulose activity was also determined by measuring reducing sugars released in reaction mixture containing 2.5m1 of 0.2% CM- cellulose in 0.05M citrate- phosphate buffer (pH 5.0) and 0.5m1 of crude enzyme solution. The mixtures were contained in test-tube and incubated at 35^oc for 1 hour. The reducing sugar released were measured by the dinitrosailcylic acid reagent as describe in Appendix 3.

Pectinase

a. <u>Polygalacturonase (PG):</u>

Polygalaruronase activity was determined by measuring the reduction in viscosity of 1% pectin (sigma) in an Ostwald viscometer (BS/u, Size C) containing 1om1 of 1% pectin in 0.05M

citratephosphate buffer (pH5.0) and 1m1 of crude enzyme molution at 35°c foer 1 hour (Appendix 2). Relative activity is expressed as the reciprocal of the time in minues for 50% loss in viscosity multiplied by 10^3 (Bateman, 1963).

Polygalaruronase activity was determined by measuring the reducing sugar released in reaction mixtures. The reaction mixture considered of 2.5m 1 of 0.1% pectin (sigma) in 0.5M citrate-phosphate buffer (pH5.0) and 0.5m1 of enzyme solution at 35°cfor 1 hour. Reducing sugars released were measured by the dinitrosalicyclic acid reagent.

b. **Pectin methylesterase (PME)**

Pectin methylesterase activity was determined by a modification of kertesz method (1951) in which the decrease in Ph of the enzyme-substrate reaction mixture contained 7m1 of 1% pectin (sigma) in 0.05m Tris-HCL buffer (pH 8.0) and 1m1 of enzyme solution. The experimental bottles were covered with their crew caps and placed in water- bath at 35°c for 3 hours. The reaction mixture readjusted to Ph 8.0 by adding 0.02N NaoH.

Effect of Temperature on enzyme activity

Reaction mixture were incubated at various temperatures (30, 35, 40, 45 and 50°c) and analyzed for cellulose, polygalacturonase and pectin methylesterase activities.

Effect of pH on enzyme activity

Carboxymethyl cellulose and pectin were prepared separately at twelve different Ph levels (3.5,4.0,4.5, 5.0, 5.5, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0) and employed as substrate in enzyme assays.

Effect of some chemicals on enzyme activity.

The following chemicals, Nacl, $Cacl_2$, $Mgcl_2$, KCl, $MnCl_2$, Iodo-acetic acid (IAA), 2,4- dinitrophenol (DN2) and ethylenediamine tetra-acetic acid (EDTA) were mixed with pectin or CM- cellulose at final concentrations of 1mm and 10mm. pectin and CM-cellulose containing the various substances were employed as substance in enzyme assays.

Effects of substrate concentration on enzyme activity.

Pectin and carboxymethyl cellulose prepared at different concentrations were employed in enzyme assays. The concentrations used for cellulose assay were 0.05, 0.1, 0.2, 0.3 and 0.4% CM- cellulose. The concentrations used for polygalacturonase assay were 0.01, o.5, 0.1, 0.2 anad0.3% pectin.

Pectin methylesterase was also assayed at concentrations of 0.1, 0.5., 1.0. 1.5, and 2.0% pectin.

Results

Growth of *A. niger* on yam disks and production of enzymes:

There was little growth on the first and second day as of incubation. However, by the third day of incubation, extensive growth occurred and the yam disks were covered with mats of mycelia and conidia. Also during this period, the yam disks became easily teased apart by shaking the flask. As from the fourth day, the yam were completely disintegrated and covered by denser mats of mycelia and conidia.

The extracts of infected yam hydrolysed CM-cellulose and released reducing sugars from this substrates, indicating production of cellulose. Maximum production of cellulose occurred on the fourth day of growth (fig.1).

The extract also caused reduction of the viscosity of buffered pectin and liberated reducing groups, indicating production of polylacturated. Maximum production of polygalacturonase occurred on the third day of growth (fig 1.)

By employing the titration techniques of Kertez (1951), it was show that free acid groups were released from pectin by the infected yam extracts; indicating production of pectin mothylesterase. Maximum production of the enzyme occurred on the fourth day of growth (fig. 1).

Effect of temperature on enzyme activity:

A study of the temperature effect on enzyme activity showed that cellulose has maximum activity at 35°c (fig2), while both polygalacturonase and pectin methylesterase and pectin methlesteranes, there was a gradual increase in activities up to 40°c before a gradual increase was observed. Cellulose also showed increase in activity as the temperature increased up to 35°c beyond which a decrease in activity was observed.

Effect of pH Enzyme activity:

Enzymic activity occurred at each of the pH levels employed. Maximum activities of cellulose, polygalacturonase and pectin methylesteras occurred at pH levels of 4.0, 4.5 and 8.0 respectively (fig. 3) cellulose and polygalcturonase showed poor activities at too alkaline pH levels. Pectin methylesterase on the other hand showed poor activity at too acidic pH levels.

Effect of substrate concentration on enzyme activity:

The activities of pectinase and cellulase were affected by the substrate concentrations. The activity of cellulase increased as concentration of CM-cellulose increased until a CM- cellulose concentration of 0.3% (w/v) was reached beyond which enzyme activity gradually decreased (fig 4.). The activity of Polygalacturonase increased as the concentration of pectin increased and maximum activity occurred at a concentration of 0.2% (w/v) (fig.5). Pectin methylesterase activity also increased achieving a maximum at 1.55 (w/v) pectin (fig,6.)

From the Lineweaver- Burk plots, the km values for the hygrolysis of pectin by polygalaturonase and

pectin methylestonase were 2.5mg/ml and 9.1mg/ml respectively (figs. 8 and 9).

The km for the hydrolysis of CM- cellulose by cellulane was 1.1mg/ml (fig.7).

Effect of some chemical on enzymes activity:

The activities of cellulose, polygalaturonase and pectin methylesteras were inhibited at both 1mM and 10Mm concentrations by ethylenediamine tetra acetic acid (EDTA), Iodo-acetic acid (IAA) and2, 4-dinitrophenol (DNP). The inhibitory effect was higher at 10Mm than at 1Mm concentrations (Table 2).

Table 1: Effect of Cations on enzyme activity.

Enzymes	Cations	1Mm(conc.) Activity (Units/ml)	10Mm(conc.) Activity (Units/ml)
Cellulose	KCL	3.0^{o}	3.6^{o}
	NaCL	3.4^{o}	3.7^{o}
	$MgCL_2$	3.6^{o}	3.8^{o}
	$CaCL_2$	3.8^{o}	4.4^{o}
	$MnCL_2$	4.6^{o}	5.1^{o}
Polygalacturonase	KCL	3.6^{o}	4.4^{o}
	NaCL	4.1^{o}	4.5^{o}
	$MgCL_2$	4.4^{o}	4.7^{o}
	$CaCL_2$	5.0^{o}	5.9^{o}
	$MnCL_2$	5.7^{o}	7.0^{o}
Pectin methylesterase	KCL	56	67
	NaCL	57	72
	$MgCL_2$	58	78
	$CaCL_2$	61	80
	$MnCL_2$	67	83

Each value is the mean of two replicate.

Table 2: Effect of chemicals on enzyme activity.

Enzymes	Chemicals	1Mm(conc.) Activity (Units/ml)	10Mm(conc.) Activity (Units/ml)
Cellulose	EDTA	2.0^{o}	0.5^{o}
	2,4-DNP	1.5^{o}	0.2^{o}
	IAA	1.8^{o}	0.4^{0}
Polygalacturonase	EDTA	1.4^{O}	0.3^{o}
	2,4-DNP	1.5^{o}	0.3^{o}
	IAA	1.7^{o}	0.5^{0}
Pectin methylesterase	EDTA	2.2^{O}	0.4^{o}
	2,4-DNP	2.5^{o}	0.5^{o}
	IAA	2.6^{o}	0.6^{0}

Each value is the mean of two replicates

Discussion

Aspergillus niger, during infection of yam tubers (Dioscorea rotundata) produced enzymes which enabled it to degrade the yam tissue. The enzymes were demonstrated to exhibit cellulose and pectinase activities. Cellulose and pectinase enzymes have been reported to play an important role in the infection of plant tissues (Hunter and Elkan, 1975; Alabi and Naqvi, 1977). Although no traces of cellulose and polygalacturonase activities were detected in extracts from heal thy yam tissues, appreciable quantities occurred in extracts from infected yam tissues. This showed that both enzymes were produces by the pathogen during infection of the yam tissues. On the other hand, traces of pectin methylesterase were detected in extracrs from heal thy yam tissues although comparatively higher amounts of it occurred in infected yam tissues. Presence of traces of pectin methylesterase activity in healthy plant tissues have been reported for potato tubers and cocoa beans (Hagar and McIntyre, 1972; Olutiola and Akintunde, 1979). The greater amount of pectin methylesterase in *Asperillus*-infected tissue extracts could therefore have resulted from a release or perhaps increased synthesis of the yam enzyme rather than production of the enzyme by the pathogen. However, the fact that pectin methylesterase activity was higher in infected yam tissues showed that the pathogen could have secreted the enzyme. Perhaps both host and pathogen could contribute to the overall quantity of pectin methlesterase present in the infected yam tissue extracts. Thus, it is concluded that the three enzymes

must have been produced by *A. niger* during infection of the yam tissue.

The temperature of the environment affected the activity of cellulose and pectinase enzymes in extracts of yam infected by *A. niger*. The optimum activity of the enzymes occurred at a temperature range of 35^0 to 40^0C. in Nigeria, yam tubers are usually stored at room temperature, i.e. approximately 25^0 to 27^0C, and sometimes rising to 30^0C during the dry season. Thus the storage temperature is favourable to the activities of the enzymes which in turn will result in increased infection of the yam by *A. niger*. In genral temperature is believed to be the most important physical factor affecting enzyme activity (Conn and Stumpf, 1972).

The hydrogen ion concentration of the substrates also affected the activity of cellulose and pectinase enzymes in the extracts. The optimum activity of cellulose, polygaleturonse and pectin methylesterase occurred at pH levels of 4.0, 4.5 and 8.0 respectively. Thus, the cellulose and polygalacturonase activities were favoured by acid condition whereas pectin methylesterase activity was favoured by alkaline condition. The pH of the healthy yam tissue extracts was 6.4. Thus, the pH of healthy yam tissues is suitable for the activities of the enzymes.

It was also observed that the activity of pectinase and cellulose enzymes was affected by the concentration of the substrates. The maximum activity of cellulose enzymes occurred at 0.3% (w/v) of CM-cellulose. On the other hand, polygalacturonase and pectin methylesterase activities were maximum at sunstrate concentrations of 0.2% and 1.5% (w/v) pectin respectively. Plant cells contain large quantities of cellulosic and pectic substances in their cell walls (Dube, 1976). Yam tissues are rich in cellulosic and pectic substances (Ogundana, 1971) which contain alpha- 1,4-linkages and therefore can serve as suitable substrate during infection of yam by *A. niger*.

The activities of cellulose and pectinase enzymes were stimulated by various cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}) but inhibited by some chemicals (Iodoacetic acid, 2, 4-dinitrophenol and ethylenediamine tetra-acetic acid (EDTA). Stimulation of hese enzymes by cation and their inhibition by EDTA suggested that the depend on metal ion activation for their activity (Weiseman and Gould, 1971). EDTA is a chelating agent and will inhibit the activities of the enzymes (Leninger, 1975). Also 2,4-dinitrophenol is an inhibitor of aerobic phosphorylation process and therefore inhibition of these enzymes by the agent is suggestive of the importance of metabolic energy in the activity of the enzymes (Leninger, 1975).

Aspergillus niger is an important pathogen associated with rotting of yam tubers in storage (Ogundana *et al*., 1970). As mentioned earlier, yam tubers are rich in cellulosic and pectic substances (Ogundana *et al*., 1971). Thus the ability of *A. niger* to produce cellulose and pectic substances of the yam tissues may be an advantage during infection of yam. The important of cellulose and pectinase enzymes in pathogenicity has been reported by some workers (Hagar and McIntyre, 1972; Hunter and Elkan, 1975).

References

1. Alabi, R. 0. & Naqvi, S. H. Z. (1977) production of cellulolytic and pectic enzyme by *cercospora arachnidicola. Transaction of the British Mycological Society. 68:* 296-298.
2. Amusa, N.A., Adegbite. A.A., Muhammed, S.A. and Baiyewu, R.A.(2003). Yam diseases and its management in Nigeria. *African Journal of Biotechnology,* 2(12): 497-502.
3. Amusa, N.A. (2000). Screening cassava and yam cultivars for resistance to anthracnose using toxic metabolite of *Colletotrichum* species. *Mycopathologia*, 150: 137-142.
4. Amusa, N.A., and Baiyewu, R.A. (1999). Storage and market disease of yam tubers in southwestern Nigeria. *Ogun J. Agric. Res.* (Nig) 11: 211-225.
5. Bateman, D. F. (1963). The macerating enzyme of *Rhizoctina solani. Phytopathology* 53:1178 -1186.
6. Bateman, D. F. (1964). Cellulose and the *Rhizoctonia* disease of bean. *Phytopathology 54:*1372 - 1377.
7. Bateman, D. F. (1968). The enzymic maceration of plant tissue. *Netherland Journal of Plant Pathology* 74*:67*-80.
8. Blagwat, V, Y (1973). Production of Pectinolytic and cellulolytic enzymes by *Fusarium* SPP associated with potato storage rots. Res. Journal 4:113-117.
9. Chapman, E. S.; Evans, E., Jacobelli, M. C. & Logan, A, A. (1975) The cellulolytic and a mytotytic activity of *Papuiospora*_thermophilia. *Mycologia* 67; 608.
10. Dube, H. C & Bil`grami, K. S. (1976). *Text - book of Modern Plant Pathology.* (First Edition), Vikas Publ. House PVT LTD New Dehli, 71pp.
11. Ezeh,N.O. (1998). Economics of production and Post-harvest Technology In: Orkwor, G.C., Asiedu, R. and Ekanayake, I.J.(eds) Food yam; Advances in research IITA and NRCRI, Nigeria, pp. 187-214.
12. Fanelli, C. and Cervone, F. (1977). Polygalacturonase and cellulase production by *Trichoderma Koningii. Transactions* of the *British Myorfogical Society* 68:291-294.
13. FAO (1998). Food and Agriculture Organization Production Year Book, FAO Rome.

14. Goel, S. K. & Mehvotva R. S. (1974). Production of pectilolytic and cellulolytic enzymes by *Rhizoctonia bataticola* Phytopathology 27: 171-177.
15. Hagar, S. S. & McIntyre, g. A. (1972). Pectic enzyme associated with "pink eye" disease of potato tubers. *Canadian J. Botattv.* 50*:* 2479-2488.
16. Hunter, W. J. & Elkan, G. H. (1975). Role of pectic and cellulolytic enzymes in the invasion of the soybean by *Rhizobium japonicum, Canadian* Journal of Microbio L 21: 1254-2258.
17. Hurst, P. L., Nielson, J., Shepherd, M. G. & Sulivan, P. A. (1977). Purification and properties of a cellulolytic and properties of a callulase from *Asperoittos Nicer. Biochemical Journal.* 165*:* 33-41.
18. IITA (1993). Crop Improvement Division/Tuber root Improvement Program Archival Reports (1989 - 1993). Part III yam, *Dioscorea* spp. Ibadan, Nigeria, pp. 20-85.
19. Ikzuk, Z, (1976), Synthesis of pectinases by mutants of *A. Niger* 36 in beet pulp medium. *Acta. Microbiology Pol. Ser. B.* 8: 401-411.
20. Ishii, 3. & Kiho, K. (1976). Evidence for a factor that stimulates tissue maceration by pectinolytic enzymes *Phytopathology* 1077-1081.
21. Kertesz, Z. I. (1951*). The pectic substances.* Interscience Publisher Inc. New-York.
22. Leninger, A, L (1975). *Biochemistry* (2nd ed.) Worth Publishers Inc. New York.
23. Lister, N., Katan, *J. &* Henis, Y, (1975). Sequential production of polygalacturonase (PG), Cellulase (CX) and pectin Lyase by *Rhizoctinia Solani Canadian Journal of Microbiology 21:* 1298-1304.
24. Lowry, O. H., Rosebrough, N.J., Parr, A. L & Randal, R. J. (1951). Protein measurement with the folin-phenol reagent. *Journal of Bid. Chem. 193:* 265-275.
25. Miller, G. L (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry. 31:* 426-428.
26. Morse, S., Acholo, M., McNamara, N. and Oliver, R. (2000). Control of storage insects as a means of limiting yam tuber fungal rots. *J. Stored Product Res.* 36: 37-45.
27. Nysksha, Y. P. & Kossior, L A. (1976). Cellulase activity of fungi as related to the pH of the substrate. *Mikol. Fitopatol.* 10: 477-484.
28. Ogundana, S. K. Naqvi, S. H. Z. & Ekundayo, J. E, (1971). Studies on soft rot of yam in storage. *Transactions of the British Mycological Society* 56: 73-80.
29. Ogundana, S. K. Naqvi, S. H. Z. & Ekundayo, J. E, (1970). Fungi associated with soft rot of yam. *Transactions of the British Mycoioaical Society* 54: 445-451.
30. Okaka, J.C., Okechukwu, P.E., (1987). Yam processing Problems and Prospects In: Quality Evaluation of Sundried yam chips. *Trop. Sci.*30: 265-275.
31. Olutiola, P. O. (1976). Cellulase enzyme systems in culture filtrates of *Aspergillus Flarus. Transactions of the British Mycological Society.* 67*:* 265-268.
32. Olutiola, P. 0. & Akintunde, 0, A (1979). Pectin Lyase and Pectin Methylesterase production by *Penicillium citrinum. Transactions of the British Mycological Society* 72: 49-55.
33. Olutiola, P. 0. & Cole, 0. 0. (1976). Production of cellulase complex in the culture filtrates of *Aspergilius tammarri* associated with moudly cocoa beans in Nigeria. *Physiologia Planterum* 37: 313-282.
34. Olutiola, P. 0. & Cole, 0. 0, (1977), cellulolytic enzymes in culture filtrates of *Aspergillus syndowi, Physiologia Plantarum 39:* 239-242.
35. Onayemi, O. (1983). Observation on the dehydration characteristics of different varieties of yam and cocoyam. Abstract 6th symposium of the Int. Soc. For Trops. Peru February, 1983.
36. Russel, S. (1974). Cellulolytic activity of *Verticillium alboatrum. Acya. Microbiol. Pol. Serv. B.* 6: 97-103.
37. Salmanora, L. S. & Zhdanova, A, A. (1975). Effect of pH on the enzymes activity fungi *Aspergillus Mikrobiol. 11: 214-218.*
38. Urbanek, H. & Zalewska - Sobczak, J. (1975). Polygalacturonase of *Botryi cinera* E-200 Pers. *Biochem. Biophysics Acta.* 377: 402-409.
39. Wang, Z., Tian, S., Fam, Z., Zuo,L., and Wang, J.,(2011).Determination methods of cellulose activity. *African Journal of Biotechnology*, 10(37): 7122-7125.
40. Weiseman, A. & Gould, B. J. (1971). *Enzymes, their nature and role.* Anchor Press. London. 321 pp.
41. Wesheng, Q., Mehdi, D.,Miranda, M.,Kam,T.L., and Canquan, M.(2010). Cellulase activities in biomass conversion: measurement methods and comparison. *Critical Reviews in Biotechnology*, 1-8, Early Online.

10/23/2015