©2018 Scienceweb Publishing

International Journal of Biotechnology and Food Science
Vol. 6(2), pp. 33-41, July 2018
ISSN: 2384-7344
Research Paper

Effect of some physico-chemical parameters on the production of hydrolytic cell wall degrading enzymes by 2 strains *Fusarium oxysporum* f. sp. *elaeidis*

Ekhorutomwen O. E.[1,2*] • Samuel T. O.[2] • Chidi N. I.[1,2] • Udoh M. E.[1] • Ogbebor C. O.[1] • Omoregie K. O.[1]

[1]Nigerian Institute for Oil Palm Research (NIFOR), Plant Pathology Division, PMB 1030 Benin City, Edo State, Nigeria.
[2]Department of Botany, University of Lagos, Yaba, Lagos State, Nigeria.

E-mail: martinsdonsteve@yahoo.com, endurance.ekhorutomwen@nifor.org.ng.

Accepted 1st June, 2018

Abstract. Extracellular endoglucanase, exoglucanase, β-glucosidase, and endo-polymethylgalacturonase enzyme activities were detected in culture filtrates of two pathogenic strains of *Fusarium oxysporum* f. sp. *elaeidis* of oil palm. The effect of carbon source, incubation period, pH and temperature were used to detect the production of these enzymes. Although there were no significant differences in the means of enzymes between the two carbon sources used, pectin medium enhanced higher production of endoglucanase and exoglucanase, while β-glucosidase and pectinase show higher production in carboxymethylcellulose medium. On the effect of incubation period on enzymes production, the optimum production of these enzymes was achieved on both 4th and 8th day. Optimum production of enzymes occurred at pH 5.5 except for β-glucosidase which optimum production was pH 7.5. Lastly, optimum temperature for the production of these enzymes was 30°C. Result from this study shows that physico-chemical parameters such as carbon sources, incubation period, pH and temperature influenced the production of these enzymes.

Keywords: Endoglucanase, exoglucanase, β-glucosidase, endo-polymethylgalacturonase, *Fusarium oxysporum* f. sp. *elaeidis.*

INTRODUCTION

The oil palm, *Elaeis guineensis* Jacq., of the family *Arecaceae* with chromosome number 2n = 32 is a perennial monocot of great importance both in small-scale and large-scale farming in most countries in the world. The oil palm is classified into 3 separate groups based on the shell thickness of its fruit: Dura (thick shell), Tenera (relatively thin shell) and Pisifera (absence of shell). Tenera with higher oil extraction efficiency is a genetically-formed hybrid between Dura and Pisifera (Hartley, 1988; Harminder *et al*., 2010). Constraints limiting the extension and profitability of oil palm cultivation are ecological (destruction of natural forests), high labor demand, poor agronomic practice, pests and diseases.

Some of the major diseases of the oil palm include; vascular wilt diseases, bud rot disease, basal stem rot, etc. The oil palm is susceptible to these diseases on each of the three continents (Africa, Asia and South America) where it is mostly cultivated. Strategies are being developed against the main diseases of the oil palm such as *Fusarium oxysporum* f. sp. *elaeidis* (Foe) and *Ganoderma*, by using sustainable genetic resistance. A genetic hence, eco-friendly approach has been taken to successfully produce vascular wilt-tolerant planting material (Cochard *et al*., 2005).

F. oxysporum f. sp. *elaeidis* is a soil-borne fungus (Fraselle, 1951; Aderungboye, 1981) and it has shown to be a pathogen responsible for vascular wilt disease of the oil palm. The disease attacks oil palm seedlings in the nursery, as well as young and adult palms in plantations

and in the groves. The external symptoms of the disease in the adult palms include stunted growth, yellowing of the leaves, desiccation and fracture of the fronds, followed by death of the palms within 6 months (acute type) or a few years (chronic type). The internal symptoms include blackening and necrosis of the cortex and plugging of the vascular system. In the nursery seedlings, the symptoms commence with stunted growth, followed by yellowing of leaves, desiccation and death. The mechanism by which this disorder is brought about in the oil palm is yet to be fully studied. However, studies conducted on vascular wilt diseases of plants have reported involvement of cell wall degrading enzymes in the disease (Hancock *et al.,* 1964; Bateman, 1968; Cooper, 1983; Goel and Mehrotra, 1974).

Studies have shown that *F. oxysporum* f. sp. *elaeidis* among other plant pathogens has the ability to produce cell wall degrading enzymes (Osagie et al., 1999, 2013); but studies have not been tailored towards understanding the concept of host-pathogen interaction pattern with the cell wall degrading enzymes and as such limiting proper characterization of these enzymes. This study is therefore necessary in order to investigate the production rate of various cell wall degrading enzymes produced by *F. oxysporum* f. sp. *elaeidis* for proper characterization and documentation.

MATERIALS AND METHODS

Fungal isolates

Stock culture of two strains of *F. oxysporum* f. sp. *elaeidis* isolated from Nigeria and Ghana with accession number: Abak 508.1746; OPC 2 (will be designated as strain N and G during the course of this study) were collected from Plant Pathology Division, N.I.F.O.R., and maintained on potato dextrose agar slant.

Media formulation

Czapeck Dox broth (sodium nitrate 2 g, potassium nitrate 1 g, potassium chloride 0.5 g, magnesium sulphate 0.5 g, ferrous sulphate 0.01 g, sucrose 30 g) was formulated such that its sucrose was substituted with equivalent amount (30 g/L of distilled water) of the appropriate carbon source (Okunowo et al., 2010).

Production of enzymes

The extracellular enzymes were produced through submerged fermentation. Each fungus was inoculated at 1% (v/v) into the defined enzymes production media containing 30 g/L for each carbon source in the modified Czapeck Dox broth. The initial pH was adjusted and sterilized under pressure at 121°C for 15 min. The medium inoculated in duplicates were optimized for enzymes production (Singh and Hayashi, 1995).

Effect of carbon source on enzymes production

Effect of two carbon source viz., carboxymethylcellulose (CMC) and pectin were used for this study. The broth was distributed into different flasks and containing 1.0% of each carbon sources in the modified Czapeck Dox broth, each flask was inoculated with each isolates and incubated for 7 days at 30°C in an orbital shaker at 150 rpm for enzyme assay (Hussain *et al*., 2012).

Effect of incubation period on enzymes production

Effect of incubation period is an important parameter for enzymes production (Hussain et al., 2012). In this study, incubation was carried out up to 8 days and production rate measured at 48 h interval at 30°C using pectin broth.

Effect of pH on enzymes production

The most suitable pH of the culture medium (pectin broth) for enzymes production was determined by adjusting the pH of the culture medium at different levels in the range of pH 4.5 to 7.5 using 1.5 M NaOH and 1.0 M HCl for pH adjustment (Hussain *et al*., 2012). Incubation was done at 30°C for 4 days.

Effect of temperature on enzymes production

In order to determine the effective temperature for enzymes production, incubation was carried out for 4 days at 5°C intervals (Hussain *et al*., 2012) in the range of 25 to 40°C also using pectin broth and the pH of the medium was adjusted to 5.5.

Enzymes extraction

Crude enzymes extract were obtained by filtering each enzyme medium through nylon cloth; then, the filtrates were centrifuged for 10 min at 12000 rpm to remove fungi and substrate residues (Shamala and Sreekantiah, 1986). The supernatants were used to check for endoglucanase, exoglucanase, β-glucosidase and pectinase activity.

Enzyme assay

β-1,4-Endoglucanase activity

The β-1,4-endoglucanase activity was determined

according to Zaldivar *et al*. (2001); using carboxymethylcellulose (CMC) as substrate and the formation of reducing sugars was measured by reaction with dinitrosalicylic acid (DNS). The reaction mixtures containing 10 mg CMC in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml culture supernatant (enzyme extract) were incubated at 50°C for 30 min. The reducing sugar formed was measured with dinitrosalicyclic acid (DNS). One milliliter (1 ml) of DNS reagent was added to 2 ml of the test sample. The colour was developed by boiling the mixture in water bath for 5 min and diluted appropriately with distilled water. Absorbance was read at 540 nm using spectrophotometer (SG8 072218, Spectronic GENESYS 8, England). Reducing sugar concentration was obtained from a standard glucose concentration curve (the net reducing sugar formation was determined by subtracting the value for the reaction with no enzyme addition). One unit of CMCase activity was defined as the amount of enzyme releasing 1 μmol of reducing sugar per minute at 50°C.

β-1,4-Exoglucanase activity

The β-1,4-exoglucanase activity was assayed as above using microcrystalline cellulose (Avicel) as substrate (Okunowo et al., 2010).

β-Glucosidase activity

The β-glucosidase activity was assayed using nonchromogenic substrate by incubating 0.1 ml of the culture filtrate (enzyme extract) with 0.5 ml of 0.05 M sodium acetate buffer (pH 5.0) containing 2.5 mg cellobiose at 50°C for 10 min (Zaldivar *et al*., 2001). 0.6 ml of the glucose released in above reaction mixture was added to 1.4 ml of 5% trichloroacetic acid (TCA), thoroughly mixed, and allowed to stand for 10 min. 2 ml of O-toluidine was then added to 2 ml of the reaction mixture, thoroughly mixed. The mixture was kept in a boiling water bath for 10 min, cooled, and then the optical density read at 620 nm. The concentration of the glucose released (μg /ml) was measured as one unit of activity was defined as the amount of enzyme required to liberate 1 μmol of glucose per minute under the assay conditions.
(Note: when the substrate is a polysaccharide, reducing sugars liberated will be measured by the DNS method).

Endo-polymethylgalacturonase activity

Endo-polymethylgalacturonase assay was done according to the method of Mandels (1985). Half milliliter of 1% pectin in 0.1 M citrate buffer (pH 5.8) was placed in a test tube and 0.5 ml of culture filtrate (enzyme extract) was added. The reaction mixture was incubated at 50°C for 30 min and the reaction terminated by adding 1.5 ml 3,5-dinitrosalisylic acid (DNS) reagent. The tubes were heated at 100°C in a boiling water bath for 15 min and diluted appropriately with distilled water. The absorbance was read at 540 nm. Enzyme activity is expressed as micromole glucose released per min-1 ml of culture filtrate as enzyme solution. One unit (U) of pectinolytic activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol galacturonic acid under the assay conditions (Minjares-Carranco et al., 1997).

Total extracellular protein

The total extracellular protein was determined by Lowry's method using bovine serum albumin (BSA) as standard (Lowry et al., 1951). Five milliliter (5 ml) of alkaline solution was added to the protein sample solution. This was mixed thoroughly and allowed to stand at room temperature for 10 min. Folin-Ciocalteau reagent (0.5 ml) was added and mixed. After 30 min, the absorbance was read against reagent blank at 750 nm. The protein concentration in the test sample was estimated from the standard protein concentration plot.
Note: Enzymes and protein concentrations were determined from the mathematical equation below:

$$\text{E.A (IU/ml)} = \frac{\text{Absorbance of enzyme soln.} \times \text{Conc. of standard (μM/ml)} \times \text{Dilution factor}}{\text{Absorbance of standard}}$$

Statistical analysis

All the data obtained were analyzed by ANOVA (Analysis of variance), DMRCT (Duncan's multiple range comparison test) and LSD (Least significant difference) using GenStat Version 8.1.0 software.

RESULTS

Effect of carbon source on enzymes production

In carboxymethylcellulose (CMC) medium; strain N has the highest enzyme activity in endoglucanase (3.625 μg/ml), exoglucanase (6.2678 μg/ml) and endo-polymethylgalacturonase (3.803μg/ml) while strain G has the highest enzyme activity of β-glucosidase (0.241 μg/ml) (Table 1a).

In pectin medium; a similar trend in enzymes production was also observed. Strain N has the highest enzyme activity in endoglucanase (4.271 μg/ml), exoglucanase (7.057 μg/ml) and β-glucosidase (0.194 μg/ml) while strain G has the highest enzyme activity of endo-polymethylgalacturonase (2.803 μg/ml) (Table 1b).

Effect of incubation period on enzymes production

As shown in Figure 2, on day 2, strain N has the highest

Table 1a. Effect of carboxymethylcellulose on enzymes production.

Parameter		Isolates	
		F. oxysporum (N)	*F. oxysporum* (G)
Total protein (µg/ml)		323.077	401.920
Enzyme activity (IU/ml)	Endoglucanase	3.625	2.910
	Exoglucanase	6.267	2.183
	β-glucosidase	0.225	0.241
	Endo-polymethylgalacturonase	3.803	2.810

Table 1b. Effect of pectin on enzymes production.

Parameter		Isolates	
		F. oxysporum (N)	*F. oxysporum* (G)
Total protein (µg/ml)		480.769	409.615
Enzyme activity (IU/ml)	Endoglucanase	4.271	3.667
	Exoglucanase	7.057	3.108
	β-glucosidase	0.194	0.190
	Endo-polymethylgalacturonase	1.983	2.803

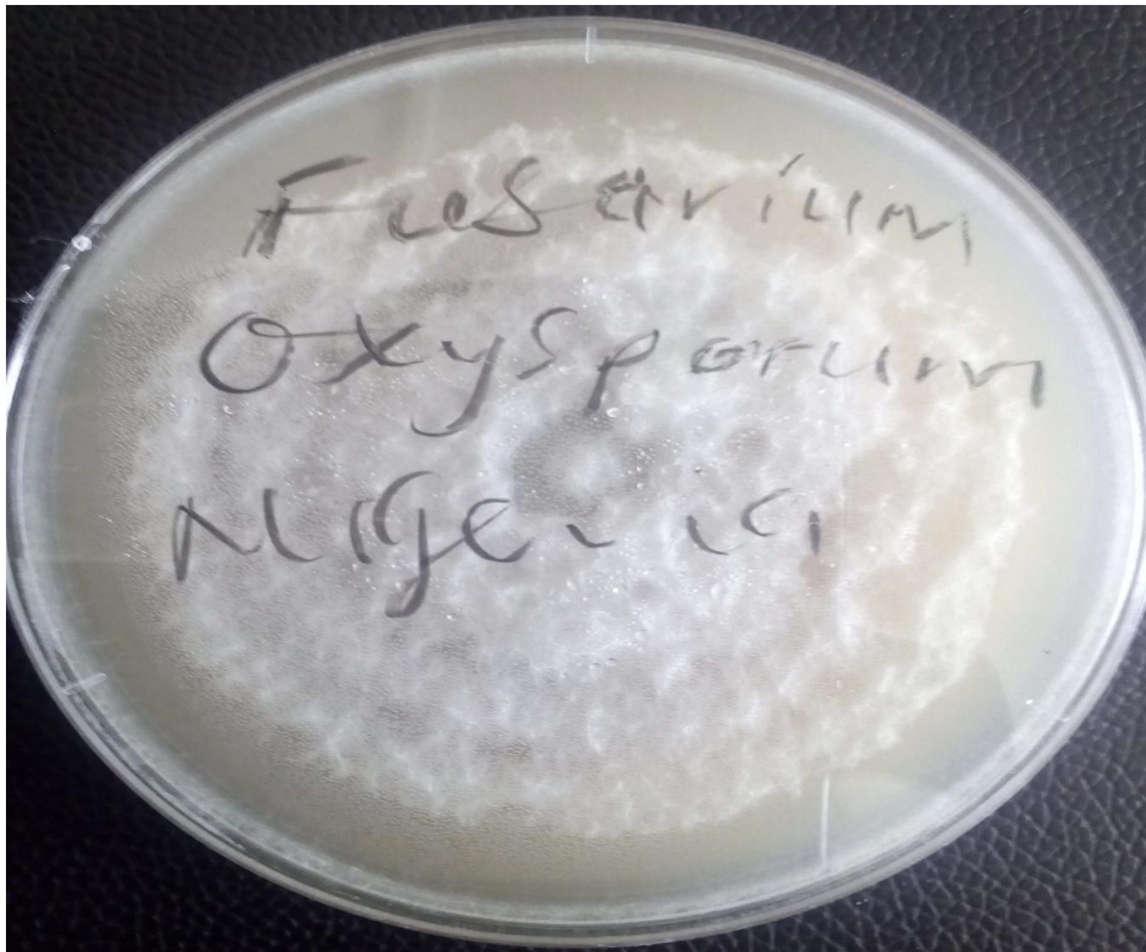

Figure 1a. A 7 day old fungus N on PDA.

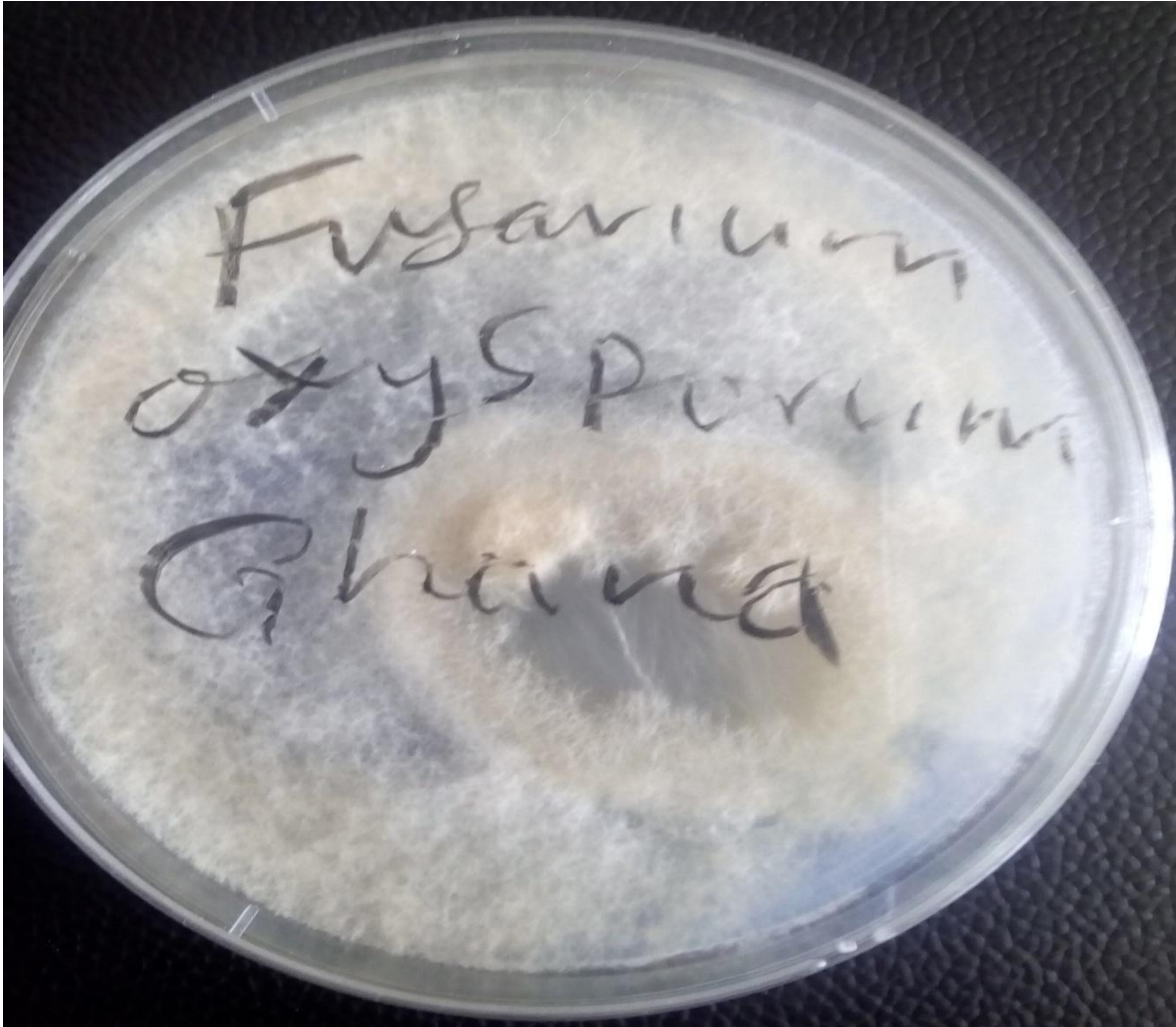

Figure 1b. A 7 day old fungus G on PDA.

enzyme activity of endoglucanase (5.86 µg/ml), β-glucosidase (0.115 µg/ml) and endo-polymethylgalacturonase (7.815 µg/ml) while strain G has the highest enzyme activity of exoglucanase (7.574 µg/ml).

On day 4, strain G has the highest enzyme activity of endoglucanase (8.125 µg/ml) and exoglucanase (8.276 µg/ml) while strain N also has the highest enzyme activity of β-glucosidase (0.248 µg/ml) and endo-polymethylgalacturonase (8.042 µg/ml).

On day 6, strain G has the highest enzyme activity of endoglucanase (6.215 µg/ml), exoglucanase (5.678 µg/ml) and endo-polymethylgalacturonase (1.895 µg/ml) while strain N has the highest enzyme activity of β-glucosidase (0.072 µg/ml).

On day 8, strain G has the highest enzyme activity of endoglucanase (8.888 µg/ml), exoglucanase (8.593 µg/ml) and endo-polymethylgalacturonase (8.684 µg/ml) while strain N has the highest enzyme activity of β-glucosidase (0.211 µg/ml).

Effect of pH on enzymes production

Figure 3 shows the effect of pH on enzymes production in the range of pH 4.5 to 7.5.

At pH 4.5, strain N has the highest enzyme activity both of endoglucanase (6.592 µg/ml) and endo-polymethylgalacturonase (9.650 µg/ml) while strain G has the highest enzyme activity of exoglucanase (8.344 µg/ml) and β-glucosidase (0.263 µg/ml).

At pH 5.5, a similar trend was noticed as strain N has the highest enzyme activity of endoglucanase (9.250µg/ml) and endo-polymethylgalacturonase (8.842 µg/ml) while strain G has the highest enzyme activity of exoglucanase (11.749 µg/ml) and β-glucosidase (0.262 µg/ml).

At pH 6.5, a similar trend was also noticed as strain N has the highest enzyme activity of endoglucanase (4.787 µg/ml) and endo-polymethylgalacturonase (9.741 µg/ml) while strain G has the highest enzyme activity of exoglucanase (5.641 µg/ml) and β-glucosidase (0.206 µg/ml).

At pH 7.5; strain N has the highest enzyme activity both

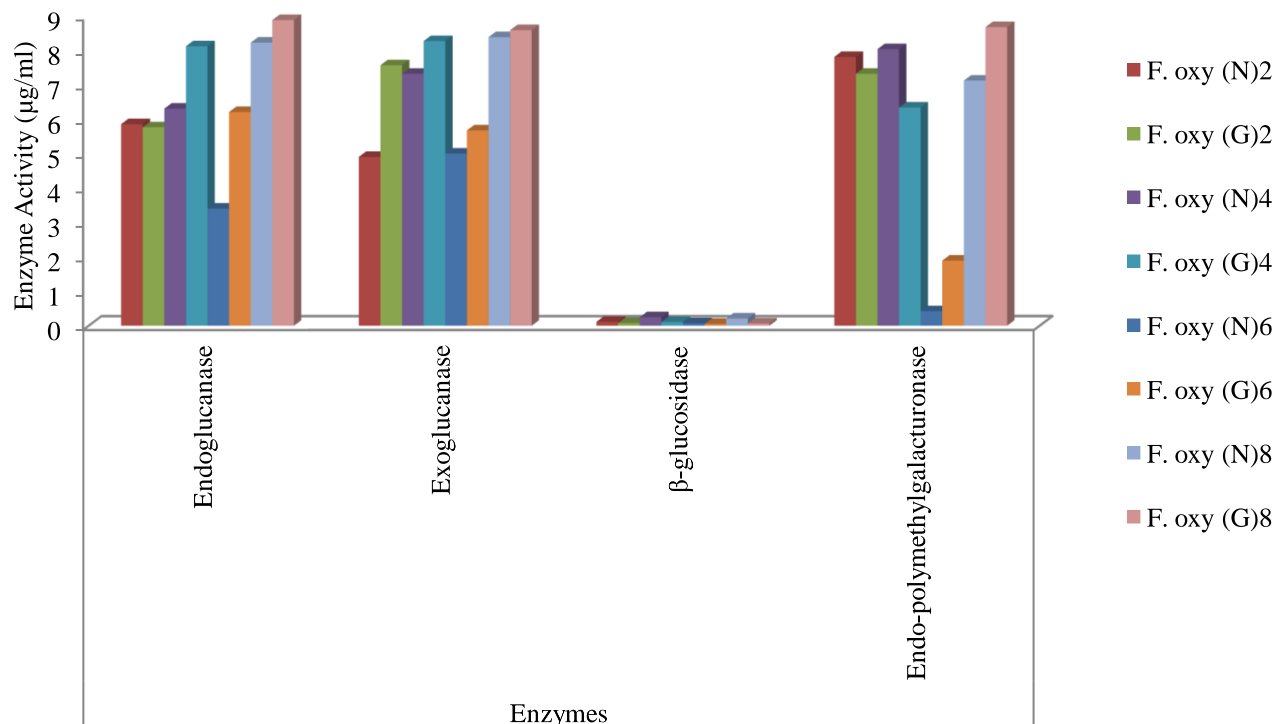

Figure 2. Effect of incubation period on enzymes production for 8 days in 48 hours (2 days) interval by the two strains of *F. oxysporum*.

of endoglucanase (7.695µg/ml) and exoglucanase (7.053 µg/ml) while strain G has the highest enzyme activity of β-glucosidase (0.402 µg/ml) and endo-polymethylgalacturonase (7.739 µg/ml).

Effect of temperature on enzymes production

Figure 4 shows the effect of temperature on enzymes production in the range of 25 to 40°C.

At 25°C, strain N has the highest enzyme activity of endoglucanase (6.314 µg/ml), exoglucanase (5.083 µg/ml) and endo-polymethylgalacturonase (5.890 µg/ml) while strain G has the highest enzyme activity of β-glucosidase (0.107 µg/ml).

At 30°C, a similar trend was noticed as strain N has the highest enzyme activity of endoglucanase (7.740 µg/ml), exoglucanase (9.239 µg/ml) and endo-polymethylgalacturonase (7.332 µg/ml) while strain G has the highest enzyme activity of β-glucosidase (0.183 µg/ml).

At 35°C, strain N has the highest enzyme activity of endoglucanase (2.895 µg/ml), exoglucanase (5.083µg/ml) and endo-polymethylgalacturonase (5.890 µg/ml) while strain G has the highest enzyme activity of β-glucosidase (0.107 µg/ml).

At 40°C, strain G has the highest enzyme activity of endoglucanase (1.865 µg/ml), β-glucosidase (0.070 µg/ml) and endo-polymethylgalacturonase (2.786 µg/ml) while strain N has the highest enzyme activity of exoglucanase (2.658 µg/ml).

DISCUSSION

There is now evidence that cell wall degrading enzymes play an important role when lesions are formed in plants by bacteria and fungi (Bateman and Millar, 1966). The need by researchers to investigate host-pathogen interaction of *F. oxysporum* f. sp. *elaeidis* on oil palm would assist in raising reliable planting materials against this deadly pathogen. Earlier work done by Osagie *et al.* (2013) suggested that further studies would be required to elucidate this factor that could be responsible for relatively low levels of enzyme activity found in the wilt-tolerant seedlings, which could justify host-pathogen interaction. This suggestion initiated the basis for this study.

In the current study, the enzymes production capacities of two strains of *F. oxysporum* f. sp. *elaeidis* under the same physico-chemical conditions were comparatively examined. In the study, each fungus was able to grow in the two carbon sources employed. This is an indication that cellulolytic and pectinolytic enzymes that can degrade cell wall were secreted by the isolates to depolymerize the carbon sources to simple sugars for growth (Bateman and Basham, 1976). The high protein released in both cellulosic and pectinolytic materials

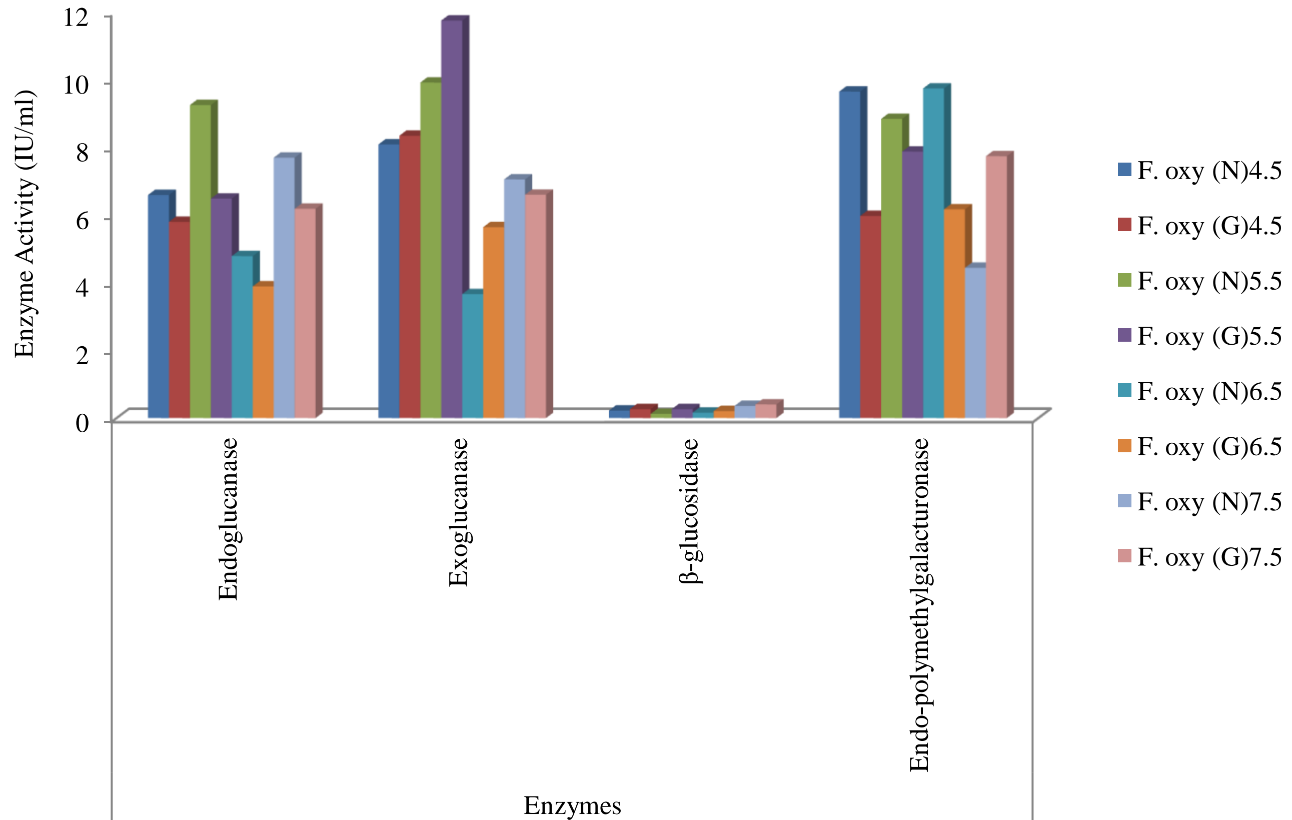

Figure 3. Effect of pH on enzymes production in the range of pH 4.5 - 7.5 by the two strains of *F. oxysporum.*

suggest the presence of other proteins (beside the cellulose and pectic enzymes) which may include other cell wall hydrolyzing enzymes (Chinedu *et al*. 2011). The result obtained showed that each *F. oxysporum* strain produced endoglucanase, exoglucanase, β-glucosidase and endo-polymethylgalacturonase during fermentation period in submerged cultures (Table 1a, b). Pectin medium enhanced higher production of endoglucanase and exoglucanase, while β-glucosidase and endo-polymethylgalacturonase show higher production in carboxymethylcellulose medium. The differences in the production of these enzymes on carboxymethylcellulose and pectin by the different strains could be assessed to various factors such as heterogeneity structure carboxymethylcellulose and pectin, and the ability of the different strains to degrade the substrates at different degree (Bateman and Basham, 1976). Duncan's multiple range comparison test (DMRCT) shows that there is a significant difference between the two strains in enzymes production in pectin medium but there is no significant difference in enzymes production in carboxymethylcellulose medium.

On the effect of incubation period on enzymes production, the maximum production of these enzymes varies between the 4th and 8th day, but statistical analysis revealed that the 4th day was significant. This might be due to the fact that during this phase the fungi were in stationary phase (Hussain et al., 2012) or possibly transcending into the log phase during the growth cycle. Also, the higher level of endo-polymethylgalacturonase detected on both the 2nd and 4th day might indicate that during infection by the pathogen pectinase enzymes are produced first in other to kill the host cells thereby increasing the chance of further invasion by the plant pathogen (Carpita and Gibeaut, 1993; Annis and Goodwin, 1997). The result obtained is in accordance with those of earlier work conducted on cellulolytic fungi by Aurangzeb et al. (1997). Although, there was a slight increase on the 8th day but 4th day is most significant in enzymes production. There is also no significant difference between the two strains in enzymes production in each of the days.

Cell wall degrading enzymes appear to depend on pH value. pH 5.5 influenced the highest enzymes production except for β-glucosidase whose production was highest at pH 7.5. This study somehow agrees with the work of Juhasz *et al.* (2004), who reported that the maximum cellulase production was obtained at pH ranging from 3.0 to 5.0. Like pH, temperature is also an important factor that influences the production of cell wall degrading enzymes. Most work concerning the effect of incubation temperature on growth of filamentous fungi supports the finding that is within limits, increased incubation temperature results in increased growth rate. However, in

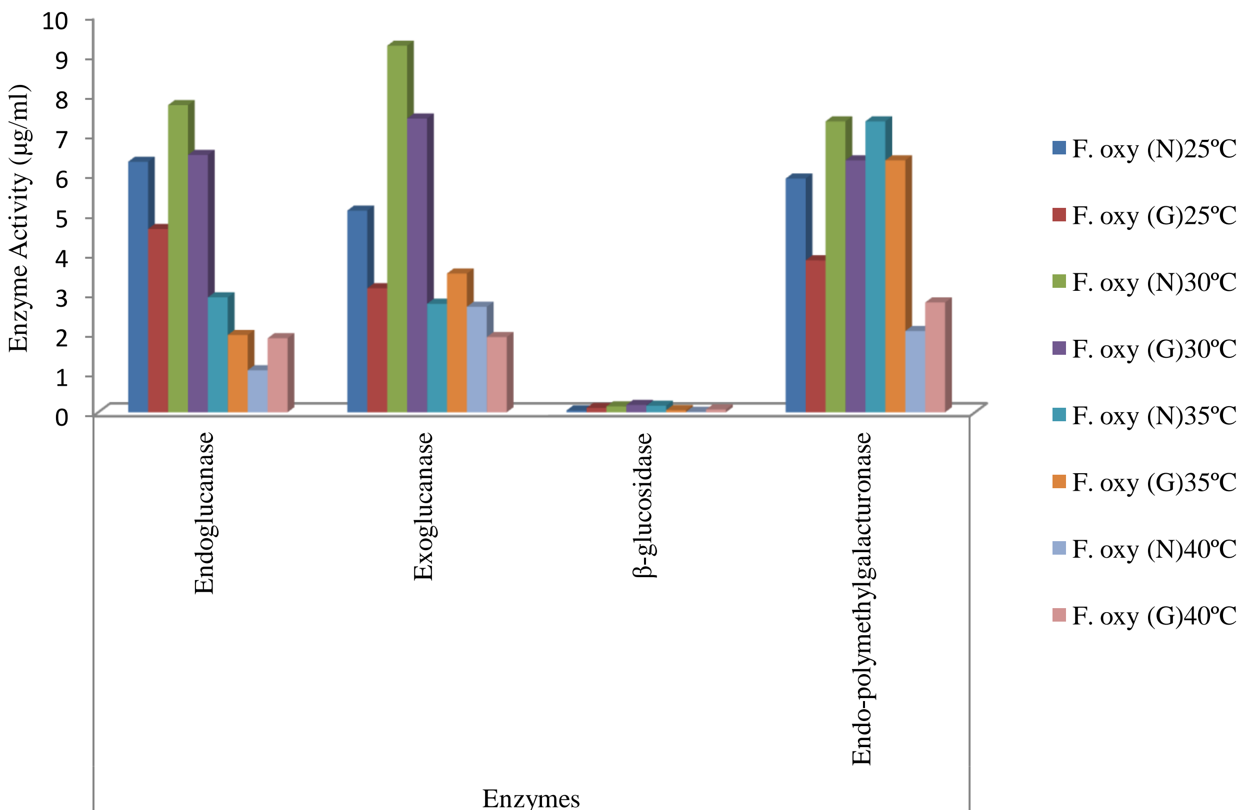

Figure 4. Effect of temperature on enzymes production in the range of 25 to 40°C by the two strains of *F. oxysporum*.

most published works the effect of incubation temperature on cellulolytic and pectinolytic materials is not fully discussed. In this study, the optimum temperature for enzyme production was 30°C. This temperature is in line with the optimum temperature for cellulose production by *T. Reesei* was 28 to 30°C (Ahmed *et al*., 2009), while the optimum temperature for cellulose production by *T. harzianum* Rut-C 8230 was 28°C (Kocher et al., 2007). This study also agrees with the work of Hussain *et al*., (2012). In the study, increase in temperature above 30°C reduced the production of endoglucanase, exoglucanase, β-glucosidase, and endo-polymethylgalacturonase which reveals that temperature might have directly influence the production of these enzymes by these 2 strains of *F. oxysporum* f. sp. *elaeidis*. There is also no significant difference between the two strains in enzymes production in incubation temperature.

CONCLUSION

This study has revealed the production of endoglucanase, exoglucanase, β-glucosidase and endo-polymethylgalacturonase by 2 strains *F. oxysporum* f. sp. *elaeidis.* Physico-chemical parameters such as carbon source, incubation period, pH and temperature influenced the production of these cell wall degrading enzymes. These parameters should serve as a guide in further studies for proper characterization and factors limiting these enzymes in wilt-tolerant oil palm.

REFERENCES

Aderungboye FO (1981). Significance of vascular wilt in oil palm plantations in Nigeria. In: Pushparajah E. and Chew Pop Sew (eds). *The Oil Palm in Agriculture in the Eighties*. Kuala Lumpar, Malaysia: Incorporate Society of Planters. pp. 1-11.

Ahmed S, Bashir A, Saleem H, Saadia M, Jamil A (2009). Production and purification of cellulose degrading enzymes from a filamentous fungus *Trichoderma harzianum.* Pak. J. Bot. 41:1411–1419.

Annis SL, Goodwin PH (1997). Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. Eur. J. Plant Pathol. 103: 1-14.

Aurangzeb M, Jalal N, Kauser T (1997). Isolation of cellulolytic fungi from indigenous sources and shake flask studies on their nutritional requirements for enzyme production. Biologia, 43(1): 113-119.

Bateman DF (1968). Degradation of Plant Cell Walls and Membranes by Microbial Enzymes. Encycl. Plant Physiol. 43(8):16-355.

Bateman DF, Basham HG (1976). Degradation of Plant Cell Walls and Membranes by Microbial Enzymes. Encycl. Plant Physiol. 4**3**(8):16-355.

Bateman DF, Millar RL (1966). Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. ***4:*** 119-146.

Carpita NC, Gibeaut DM (1993). Structural models of primary cell walls in flowering plant: consistency of molecular structure with the physical properties of the walls during growth. Plant J. 3:1-30.

Chinedu SN, Okochi VI, Omidiji O (2011). Cellulase production by wild strains of *Aspergillus niger, Penicillium chrysogenum* and *Trichoderma harzianum* grown on waste cellulosic materials. Ife J. Sci. 13(1):57-62.

Cochard B, Amblard P, Durand-Gasselin T (2005). Oil palm genetic improvement and sustainable development. Oilseeds and Fats Crops and Lipids. 12(2):141-147.

Cooper RM (1983). 'The mechanisms and significance of enzymic degradation on host cell walls'. In: Callow, J. A. (ed.). Biochem. Plant Pathol. Wiley, Chichester, New York. pp. 101-137.

Fraselle JV (1951). Experimental evidence of the pathogenicity of *Fusarium oxysporum* to the oil palm (*Elaeis guineensis* Jacq.). Nature. 167:447.

Goel SK, Mehrotra RS (1974). Pectin methylesterase production by *Rhizoctonia bataticola* (Taub.) Buttler in culture and infected *Abelmuschus exculentus* Moench roots. J. Indian Bot. Soc. 53:110-114.

Hancock JG, Miller RL, Lorbeer JW (1964). Pectolytic and cellulolytic enzymes produced by *Botrytis allii, B. cinerea, and B. squamosa in vivo*. Phytopathol. 54:928-931.

Harminder S, Rajinder S, Abduh R, Rahimah O, Cheng LLE, Ti E (2010). Method for identification of a molecular marker linked to the shell gene of oil palm. PCT/MY2009/000192, International application published under the Patent Cooperation Treaty (PCT), World Intellectual Property Organization International Bureau. International publication, 33 pp. Retrieved from the Internet at: http://www.sumobrain.com/patents/wipo/Method-identification-molecular-marker-linked/WO 2010056107A2.pdf (November 3, 2010).

Hartley CWS (1988). The oil palm (*Elaeis guineensis* Jacq.), 3rd ed. Longman scientific and technical Essex, UK. pp. 761.

Hussain A, Shrivastav A, Jain SK, Baghel RK, Rani S, Agrawal MK (2012). Cellulolytic enzymatic activity of soft rot filamentous fungi *Paecilomyces variotii.* Adv. Biores. 3(3)10-17.

Juhasz T, Szengyel Z, Szijarto N, Reczey K (2004). The effect of pH on the cellulase production of *Trichoderma reesei* RUT C-30. Appl. Biochem. Biotechnol. 113–116(1): 201–211.

Kocher G, Kalra K, Banta G (2007). Optimization of cellulase production by submerged fermentation of rice straw by *Trichoderma harzianum* Rut-C 8230. Internet J. Microbiol. 5(2):1-5.

Mandels M (1985). Applications of cellulases. Biochemical Society Transactions. 13: 414-416.

Okunowo WO, Gbenle GO, Osuntoki AA, Adekunle AA, Ojokuku SA (2010). Production of cellulolytic and xylanolytic enzymes by a phytopathogenic *Myrothecium roridum* and some virulent fungal isolates from water hyacinth. Afr. J. Biotechnol. 9(7):1074-1078.

Osagie IJ, Obuekwe CO (1991). Extracellular hydrolytic enzyme production by pathogenic strains of *Fusarium oxysporum f. sp. elaedis.* Mycol. Res. 95(1):116-122.

Osagie IJ, Ojomo EE, Obuekwe CO, Maduewesi AE (2013). Cell wall degrading enzymes in vascular wilt of oil palm. Niger. J. Agric. Food Environ. 9(1):28-32.

Shamala TR, Sreekantiah KR (1986). Production of cellulases and D-xylanase by some selected fungi isolates. Enzyme Microbial Technol. 8:178-182.

Singh A, Hayashi K (1995). Microbial cellulase, protein architecture, molecular properties and biosynthesis. Adv. Appl. Microbiol. 40:1-44.

Zaldivar M, Velasquez JC, Contreras I, Perez LM (2001). *Trichoderma aueviride* 7-121, a mutant with enhanced production of lytic enzymes; its potential use in waste cellulose degradation and/or biocontrol. Electron. J. Biotechnol. pp. 1-7.