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Effects of *Phytophthora* Infection on Nutrient Composition of *Theobroma cacao* L.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In this study, the proximate and mineral analyses of non-infected and infected cocoa (*Theobroma cacao* L) pods, nibs and testa were investigated. The ash content (g/100 g) of non-infected pods (10.7) and nibs (8.0) were higher than those of infected cocoa pods (9.3) and nibs (7.8) while that of the testa was 13.1 in non-infected pods, the value was higher in infected pods (15.4). The moisture content (g/100 g) was found to be higher in the non-infected nibs (6.3) and testa (9.8). Crude protein (g/100 g) was found to be higher in infected pods (12.8) and nibs (18.4). However, the testa of non-infected nibs had 19.4. For carbohydrate, the values (g/100 g) in pod (35.7), nibs (40.7) and testa (30.9) were found to be higher in non-infected than those of the infected pods. Zn, Co and Fe of non-infected cocoa pods, nibs and testa were found to be higher than those of the infected pods. The Mn content (mg/100 g) of non-infected pods (5.3) and testa (5.1) were higher than those of infected 4.5 and 3.4 respectively but the nibs of non-infected pods had a value of 4.2. However, the Cu content (mg/100g) of non-infected pod (1.2) was found to be higher than that of infected pod (0.3). Total reducing sugars of infected pod (33.0), nibs (24.1) and testa (32.0) were higher than those of non infected pods (14.6, 20.1 and 18.4 respectively). From this study, it can be concluded that *Phytophthora* infection depleted the nutritional content of *T. cacao* beans.

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1. INTRODUCTION

Cocoa (*Theobroma cacao L*) has become one of the main cash crops in Nigeria grown majorly in the South Western and South Eastern parts of the country. The global annual earnings from cocoa have also led to a major boost in the national income [1]. The cocoa is obtained from pods which grow on cacao trees. The pods are egg-shaped, about eight inches long and contain twenty to thirty beans embedded in a soft white starchy pulp [2].

The yield of the crop for export is affected by many diseases and pests. The global losses of cocoa to pests based on data collected from various cocoa growing countries like Ghana, Nigeria, Sierra Leone, Togo, Trinidad and Tobago and West Cameroon were about 29.4% of its production. Numerous factors are responsible for the losses. Physical damage of the growing trees in the farm, invasion of insect pests but the major players are fungi, viruses and bacteria [3].

The most serious disease of cocoa in West Africa especially Nigeria, Ghana and Cameroon is caused by *Phytophthora palmivora, P. megakarya, P. capsici* and *P. citrophthora* [4-10]. Pod losses have been the bane of cocoa production in Nigeria. The black pod pathogens *Phytophthora palmivora* and *P. megakarya* infect more than 200 species of economic, ornamental and shade trees [11].

P. megakarya is the more aggressive and hence referred to as the most important pathogen of cocoa causing black pod [12]. The primary host of this pathogen is cocoa (*Theobroma cacao*), coconut (*Cocos nucifera*), rubber (*Hevea braziliensis*) and pawpaw (*Carica papaya*) while the secondary hosts include black pepper (*Piper nigrum*), pine apple (*Ananas comosus*), oil palm tree (*Elias guinensis*) and cashew nut (*Anacardium occidentale*) [3].

The cocoa bean contains about 50% fat that has served as a valuable source of vegetable fat -the cocoa butter. The residual cocoa powder is used in producing cakes, biscuits, beverages and in pharmaceuticals [2]. The choice of cocoa beans by cocoa products manufacturers are influenced by flavor, bean size, percentage shell and fat content of the pods [13]. At present there is little information on the chemical composition of cocoa in this part of the world except the work of Folayan [13], Adeyeye and Adejuyo [2] who reported the proximate and mineral composition of nibs and shells of processed un-germinated and germinated cocoa beans and [2] also reported the amino acid profile of cocoa beans. Therefore, this work reports for the first time the effect of *Phytophthora* infection of cocoa pods on the nutrient composition of cocoa husks/pods, nibs and testa.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Fresh healthy mature green cocoa pods were obtained from the cocoa plantation of Ado Grammar School, Ado-Ekiti, Nigeria. The pods were immediately transported to the Department of Microbiology Laboratory of Ekiti State University, Ado Ekiti, Ekiti State and washed with two changes of distilled water. The pods were sundried and later taken into the laboratory and kept in a well ventilated place for 24 hours.

2.2 Cultivation of *Phytophthora palmivora*

Unless otherwise stated, 18 ml of molten Potato Dextrose Agar (P.D.A.) were poured in each Petri-dish for the culture of each fungus. The pure culture of *Phytophthora palmivora* L was collected from Cocoa Research Institute of Nigeria (C.R.I.N.) Headquarters, Idi Ayunre, Ibadan, Oyo State. The fungus was cultivated on Potato Dextrose Agar (P.D.A.) slants, subcultured onto P.D.A. plates and incubated at a temperature of 28℃ for 10 days. Five inoculum plugs each from the advancing edge of 5 day old culture were removed with a sterile cork borer, inoculated in 30 ml of sterile Malt Extract Broth (M.E.B.) and later incubated at 28℃ for 10 days.

Following incubation, the fungal mycelium was harvested by decanting the broth. The mycelium was washed in three changes of distilled water. The contents of five flasks were blended using a warring blender (Sonike Electrical Appliance Co., Ltd, Japan) and adjusted to a standard turbidity by comparison with distilled water as standard reference measured by means of an EEL Absorptiometer **(**Sigma**-**Aldrich Company Ltd, Poole, England**)** using a neutral density filter.

2.3 Infection of *Theobroma cacao* Pods

The dried cocoa pods were surface sterilized by swabbing with cotton wool that has been

moistened with 75% ethanol. A sterile cork borer of 6mm diameter was used to bore hole into the cocoa husk. Mycelial disc containing *P. palmivora* was inoculated into the hole and the husk was replaced to cover the inoculum. Sterile vaseline cream was applied on the burrowed surface to ensure proper sealing of the area. The infected cocoa pods were placed in a sterile polyethylene bag and sprayed with distilled water daily to humidify it and left to stand at 25℃ in th e laboratory for 5-7 days [14]. The experiment was carried out in four replicates.

For the control experiment, cocoa pods were treated as described above but were not inoculated with mycelia disc but with distilled water. The pods were watered with distilled water. The pods were also put in polyethylene bags and similarly incubated at 25℃ in the laboratory for 5 – 7 days.

2.4 Preparation of Samples and Determination of Proximate Composition of Infected and Non-Infected Cocoa Pods, Nibs and Testa

After 120 hours of infection, the infected cocoa pods were broken into pieces and the husk and beans were separated washed in running water tap and finally in two changes of distilled water.

The broken infected husk pieces were further cut into small pieces and the beans and husk pieces oven dried separately. The testa of the dried beans were carefully removed and stored separately from the cocoa beans. The dried cocoa husk, nibs and the testa were grounded separately using a mortar and pestle and stored in different labeled plastic containers and stored at 4^0C in the refrigerator until ready for proximate analysis. Healthy mature green cocoa pods without any traces of blackpod infection and injuries were used as control. The pods were broken and separated into the husks and the beans. These were oven dried and treated /prepared as described for infected samples and kept at 4℃ in the refrigerator [15].

The proximate analyses were determined according to the methods of [16,17].

2.5 Mineral Analyses

The minerals of the samples were analyzed using the solution obtained by dry ashing the sample at 550℃ and dissolving it in 10 % HCl (25 ml) and 5 % lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with deionized water. Mn, Cu, Co, Zn, Fe, Mg, Na, and Ca were determined with a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A). Sodium was measured with a Corning 405 flame photometer (Corning Halstead, Essex, UK, Model 405) [17]. The detection limits had previously been determined using the methods of Varian Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05,Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.04, Na 0.001, ppm (all for aqueous solutions).

The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.40%. phosphovanado-molybdate method using a Spectronic 20 colorimeter (Galenkamp, London, UK) [17]. All chemicals were BDH analytical grade.

2.6 Determination of Total Reducing Sugar of Non-infected and Infected Pods

The total reducing sugars of pods, nibs and testa of infected and non-infected cocoa pods was determined using the non-stochiometric volume method as described by [16] whereby about 10-25 ml of each portion of samples was mixed separately with Fehling's solution into a clean conical flask and 15 ml of sugar solution was added. This was boiled and further quantities of sugar solutions (1ml at a time) was added at 10-15 seconds intervals to the boiling liquid until the blue colour was nearly discharged. About 3-5 drops of aqueous methylene blue solution (1 %) was added and the titration of the boiling liquid until the indicator was completely decolorized.

2.7 Statistical Analysis

Statistical analysis [18] was carried out to determine mean, standard deviation, coefficient of variation in percent. Correlation coefficient was determined using SPSS version 11.

3. RESULTS AND DISCUSSION

The results of the proximate analysis of non infected and infected cocoa pod husks, bean and testa are shown in Table 1. The ash content (g/100 g) of non-infected pods (10.7) and beans (8.0) were higher than those of infected cocoa pods (9.26) and beans (7.8) while that of the testa was 13.1 in non infected pods but higher in infected pods (15.4). The moisture content (%) was found to be higher in the non infected beans (6.3) and testa (9.8) while that of the infected

beans was 5.7 and 8.9 for testa. However, the moisture content of non infected pods (7.9) was lower than that of infected pods (10.2).

The crude protein (%) was found to be higher in infected pods (12.8) and beans (18.4) while that of non-infected pods were 10.0 for pods and 16.5. However, the testa of non infected beans had 19.4 and that of infected beans was 10.3. The fat content (g/100 g) and fibre of pods, beans and testa of non infected cocoa pod were higher than those of infected pods. For carbohydrate (g/100 g) of pod (35.7), beans (40.7) and testa (30.9) were found to be higher in non-infected than those of the infected pods.

The results of the proximate analyses of non infected and infected cocoa pod husks, nibs and testa as observed in the experiments are similar to the observations of Tripathi and Mishra [19] who reported a decrease in the ash content of red pepper powder in a 30 days *in vitro* infection by *Aspergillus niger* from 9.8 to 2.9 g/100 g. However, the result of this work is in contrast to the findings of [20] who reported the ash content of *Cocos nucifera* to increase from 1.19% in healthy sample to 1.7% in infected samples. The probable reason for the reduction in the ash content of the infected cocoa beans might be the infecting fungus must have used part of the mineral content present in the beans for its growth and metabolic activities. The moisture content of non infected pods (7.9) was lower than that of infected pods (10.2). This may be due to the mummifying effect and deterioration of the pod husk by infecting *P. palmivora.* Contrary, the moisture content of the beans (6.3 g/100 g) and testa (9.8 g/100 g) in healthy or non infected pods were found to be reduced in infected beans (5.7 g/100 g) and testa (8.9 g/100 g). This is similar to the findings of [20] who found the moisture content of healthy or non infected *Cocos nucifera* to decrease from 36.4 g/100 g to 10.4 g/100g in infected samples. Similarly [19] also found the moisture content of red pepper powder to increase from 8.0 to 24.1 g/100 g upon infection by *A. niger*. This might be due to the fact that infecting fungus utilizes the moisture content for its survival and growth. The shelf life of any product is influenced by the amount of water present in it [16].

The crude protein (g/100 g) was found to be higher in infected pods (12.8) and beans (18.4) while that of non infected pods were 9.96 for pods and 16.5 for beans. However, the testa of non infected beans had 19.4 and that of infected beans was 10.3. It has been reported by [20-23] that fungi increase the protein content of samples on which they grow. Protein increase could have resulted from protein synthesis by proliferation of microorganisms and a synthesis of enzyme proteins [24] or from a rearrangement of the composition following the degradation of other constituents. However, the protein content of non infected or healthy testa was found to be higher than that of the infected testa. Similarly, [25] 1996 reported a slight difference in the amino acid content of healthy and infected pods from three clones after infection with *Phytophthora megakarya* Bra. and Grif. He however suggested that during host-pathogen interaction, amino acid may act as a substrate for the pathogen or they may have a fungistatic effect through their involvement in metabolic reactions associated with diseases resistance.

The fat content of the pod, bean and testa of healthy or non infected pods was found to be higher than that of the infected pod, bean and testa. The probable reason might be due to the fact that the pathogenic fungi metabolize and deplete the fat content of the healthy pods bean, and testa during infection. The fat content reported for this work differs from the findings of [20] who reported that the fat content of healthy coconut to be 20.3 while that of infected one was 40.8 g/100 g. However, similar result was reported [19]; The fat content of red pepper powder reduced from 13.3 to 2.4 g/100 g after 30 days storage when the pepper powder was infested by *A. niger*.

The crude fibre of the non infected or healthy pod, bean and testa was also found to be higher than that of the infected pod, bean and testa. The result of this work is similar to the findings of [20] who found the crude fibre of coconut (*Cocos nucifera* Linn) to decrease from 13.1 g/100 g in healthy coconut to 10.0 g/100g in infected coconut. Similarly, there was a reduction in the crude fibre of red pepper powder from 19.0 to 7.8 g/100 g after 30 days infection during storage. This may be attributed to the fact that the infecting fungus utilizes the crude fibre for metabolic activities [26].

The carbohydrate content (g/100 g) of pod (35.7), beans (40.7) and testa (30.9) were found to be higher in non-infected than those of the infected pod (29.4), beans (21.6) and testa (13.2). The reason for this is not farfetched because the infecting fungus must have used the carbohydrate in the infected pods for metabolic

Table 1. Result of proximate analysis of non infected and infected cocoa pods (g/100 g)

Parameters	Part used	Non-infected	Infected	Mean	S.D.	C.V. %
Ash	Pod	10.7	9.3	10.0	0.99	9.9
	Nib	8.0	7.8	7.9	0.1	1.3
	Testa	13.1	15.4	14.2	2.3	16.2
Moisture content	Pod	7.9	10.2	9.1	2.1	23.1
	Nib	6.3	5.7	6.0	0.4	7.5
	Testa	9.8	8.9	9.4	1.2	12.8
Crude protein	Pod	10.0	12.8	11.4	1.9	17.4
	Nib	16.5	18.4	17.5	1.3	7.5
	Testa	19.4	10.3	14.9	6.2	41.6
Fat	Pod	8.0	4.9	6.4	6.1	2.5
	Nib	38.1	23.8	31.0	9.8	31.6
	Testa	33.2	28.3	30.8	2.4	7.8
Fibre	Pod	33.5	28.3	30.9	3.7	12.0
	Nib	9.7	3.6	6.7	4.2	62.1
	Testa	10.7	5.3	8.0	3.8	47.7
Carbohydrate	Pod	35.7	29.4	32.6	3.7	11.2
	Nib	40.2	21.6	31.0	12.7	40.9
	Testa	30.9	13.8	22.4	12.0	53.2

S.D. = Standard deviation; C.V. % = % Coefficient of variation

activities. The result is similar to that of [20] who reported the carbohydrate content of *Cocos nucifera* to decrease from 17.8 to 10.62 g/100 g and [25] 1996 also reported a decrease in the carbohydrate content of healthy pods from 91.0 to 13.2 in infected cocoa pods.

The results of the mineral content of non infected cocoa pods, beans and testa and those of infected in (mg/100 g) are shown in Table 2. Zinc, cobalt and iron of non infected cocoa pods, beans and testa were found to be higher than those of the infected pods. The sodium content of the bean (39.1) and testa (44.0) of infected pods were found to be higher than those of non infected pods, but that of non infected pod (25.4) was higher than that of infected pod (24.44).

The magnesium content of infected pod (16.4), beans (31.4) and testa (35.5) were found to be higher than those of non-infected (15.7, 21.1 and 30.5) respectively.

The manganese content of non infected pods (5.3) and testa (5.1) were higher than those of infected 4.5 and 3.4 respectively but the beans of non infected pods had a value of 4.2 while that of infected was 4.6. The copper content of non infected beans (0.1) and testa (0.2) were found to be lower than those of infected pods 0.2 and 0.9 respectively. However, the copper content of non infected pod (1.2) was found to be higher than that of infected pod (0.3).

The phosphorus content of non infected pod (193.24) and testa (203.54) were found to be lower than those of infected pod 206.8 and 219.3 respectively while that of beans (145.0) was higher in non infected beans than infected beans (131.21).

The results of the mineral content of non infected cocoa pods, beans and testa and those of infected in mg/100 g are shown in Table 2. Zinc, cobalt and iron of non infected cocoa pods, beans and testa were found to be higher than those of the infected pods. The reduction in the amount of Zn, Co and Fe of infected pods, beans and testa might be due to the uptake of these metals for metabolic activities by infecting fungus *Phytophthora palmivora*. The result of this work is similar to the findings of [27] 2000 who reported that *A. flavus* depleted the Zn, Cu and Fe from an infected crushed corn. The sodium content of the bean (39.1) and testa (44.0) of infected pods were found to be higher than those of non infected pods, but that of non infected pod (25.4) was higher than that of infected pod (24.4). The magnesium content of infected pod (16.4), beans (31.4) and testa (35.5) were found to be higher than those of non-infected (15.7, 21.1 and 30.5) respectively. The probable reason for this result might be due to the fact that the infecting fungus did not metabolize Na and Mg but there was bioaccumulation and concentration of these two metals in the pods, beans and testa.

The manganese content of non infected pods (5.3) and testa (5.1) were higher than those of infected 4.5 and 3.4 respectively but the beans of non infected pods had a value of 4.21 while that of infected was 4.6. The copper content of non infected beans (0.1) and testa (0.2) were found to be lower than those of infected pods 0.2 and 0.9 respectively. However, the copper content of non infected pod (1.2) was found to be higher than that of infected pod (0.3). The phosphorus content non infected pod (193.2) and testa (203.5) were found to be lower than those of infected pod 206.8 and 219.3 respectively while that of beans (145.0) was higher in non infected beans than that of infected beans (131.2). The infecting fungus might have used up the phosphorous and copper of the beans for its growth and other metabolic activities. These observations are similar to the trends observed by [27] 2000 who reported that *A. flavus* depleted zinc, copper and iron from crushed corns during infection and concluded that there was a direct correlation between metal uptake and mould growth.

The results of the total reducing sugars of non infected and infected cocoa pods are shown in Table 3. The infected pod (33.0), beans (24.1) and testa (32.0) were found to be higher than those of non infected pods (14.7, 20.1 and 18.4) respectively. The infecting fungus might have converted the sugars in non infected/ healthy pods, beans and husk to reduced forms (sugar) during metabolic activities and thus raising the values reported in this work.

Table 2. Result of mineral content of infected and non infected cocoa pods

Parameters	Part used	Non-infected	Infected	Mean	S.D.	C.V%
Sodium	Pod	25.4	24.2	24.8	0.89	3.4
	Nib	30.2	39.1	34.7	6.3	18.2
	Testa	38.0	44.0	41.0	4.2	10.2
Magnessium	Pod	15.7	16.4	16.1	1.7	10.7
	Nib	21.1	31.4	26.3	6.9	26.3
	Testa	30.5	35.5	33.0	3.5	10.7
Manganese	Pod	5.2	4.5	4.9	0.8	17.3
	Nib	4.2	4.6	4.4	0.3	7.2
	Testa	5.1	3.4	4.3	0.8	18.0
Zinc	Pod	14.0	11.5	12.8	0.7	6.1
	Nib	38.0	15.0	26.5	16.3	61.4
	Testa	35.3	19.3	27.3	11.3	41.4
Cobalt	Pod	0.5	0.3	0.4	0.1	35.4
	Nib	0.2	0.1	0.2	0.2	100.0
	Testa	0.3	0.1	0.2	0.1	70.7
Iron	Pod	3.1	2.3	2.7	0.6	20.9
	Nib	22.4	15.0	18.7	5.2	27.9
	Testa	20.5	18.6	19.6	1.5	7.4
Copper	Pod	1.2	0.3	1.0	0.7	68.6
	Nib	0.1	0.2	0.2	0.2	86.6
	Testa	0.2	0.9	0.6	0.3	52.7
Phosphorous	Pod	193.2	206.8	200.0	9.6	4.8
	Nib	145.0	131.2	138.1	9.8	7.1
	Testa	203.5	219.3	211.4	11.2	5.3

S.D. = Standard deviation; C.V. % = % Coefficient of variation

Table 3. Results of the total reducing sugars of non infected and infected pods

Part used	Non- infected	Infected	Mean	S.D.	C.V. %
Pod/husk	14.7	33.0	23.9	12.9	54.3
Nib	20.1	24.0	22.1	2.8	12.5
Testa	18.4	32.0	25.2	9.6	38.2

S.D. = Standard deviation; C.V. % = % Coefficient of variation

4. CONCLUSION

Microbes utilize variety of substrate to synthesize and produce energy for their normal body metabolism. It can however be concluded that *Phytophthora* infection depleted the nutritional values of *T. cacao.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gray A. The world cocoa market outlook. Ghana Conference paper, LMC International Press; 2000. Available:http:/www.treecrops.org/crops/cocoaoutlook
2. Adeyeye EI, Ayejuyo OO. Proximate and mineral composition of nibs and shells of processed un-germinated and germinated cocoa beans. Int. J. Chem Sci. 2010;3(1): 41–50.
3. Opeke LK. Tropical tree crops. Woye and Son Ltd, Ilorin, Nigeria. 1992;109.
4. Nyasse S, Cilas C, Herail C, Blaha G. Leaf inoculation as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytophthora* black pod disease. Crop Protection. 1995;14:657–663.
5. Arowolo JA, Fagbohun ED. Occurrence and distribution of *Phytophthora* canker on cocoa in Ekiti State, Nigeria. Afr. J. of Sci. 2001;1(1):100–106.
6. Omokolo ND, Nankeu DJ, Niemenak N, Djocgoue PF. Analysis of aminoacid and carbohydrate in the Cortex of nine clones of *Theobroma cacao* L. in relation to their susceptibility to *Phytophthora megakarya* Bra. and Grif. Crop Protection 2002;21: 395–402.
7. Omokolo ND, Nankeu DJ, Niemenak N, Boudjeko T. Variation of β– 1,3 –glucanase, chitinase and polyphenol oxidase activities in cacao pods upon *Phytophthora megakarya* inoculation. African Crop Science Journal. 200311:97–106.
8. Boudjeko T, Djocgoue PF, Nankeu JD, Mbouobda HD, Omokolo DN, Hadrami IEL. Luteolin derivatives and heritability of resistance to *Phytophthora megakarya* in *Theobroma cacao* L. Australasian Plant Pathology. 2007;36:56-61.
9. Guest D. Black pod: Diverse pathogens with a global impact on cocoa yield. Phytopathol. 2007;97:1650-1653.
10. Fagbohun DE, Falegan CR, Arowolo JA. *Phytophthora* black pod disease in Ekiti State of Nigeria- Species differentiation and pathogenic variation. Bioscience, Biotechnology Research Asia. 2008; 5(1):157-160.
11. Sansome E, Brasier CM, Griffin MJ. Chromosome size difference in *P. palmivora* a pathogen of cacao. Nature 1975;255:104-107.
12. Hamzat RA, Olaiya AO, Sanusi RA, Adedeji AR. State of cocoa growing, quality and research in Nigeria: Need for Intervention. Distinct Global Concepts Company, Lagos, Nigeria. 2006;3-22.
13. Folayan JA. Effect of quality marketing and acceptability of cocoa in international market. Lecture delivered at the 8th annual symposium and 9th annual general meeting of the Nigeria Institute of Food Science and Technology (Western Chapter), Held at the Polytechnic, Owo, Nigeria; 1993.
14. Saskia CM, Wees V, Song chang H, Zhu T, Glazebrook J. Characterization of early response of Arabidopsis to *Alternaria brasicicola* infection using expression profiling. Plant Physiol. 2003;132;606-617.
15. Fagbohun ED. Antimicrobial activities of cocoa pod (*Theobroma cacao* L) infected with *Phytophthora palmivora*. Ph.D. Thesis, Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria; 2012.
16. Pearson DH. Chemical analysis of foods. Churchhill London. 1976;121-127,335-336.
17. AOAC. Official methods of analysis. Section 968.08, 4.1.28, 12.1.7, 18th Edn. Association of Official Analytical Chemists, Washington DC; 2005.
18. Kothari CR. Research Methodology; Methods and Techniques. New Age International Publishers, India. 2004;256-264.
19. Tripathi S, Mishra HN. Nutritional changes in powdered red pepper upon in vitro infection of *Aspergillus flavus.* Braz. J. Microbiol. 2009;40:139-144.
20. Onifade AK, Jeff-Agboola YA. Effect of fungal infection on proximate nutrient composition of coconut (*Cocos nucifera* Linn) fruit. J. Food Agric and Environ. 2003;1(2):141–142.
21. Raimbault M. General and microbiological aspect of solid substrate fermentation. J. of Biotech. 1998;1(3):101–104.

22. Rodolfo AD, Teresa MA, Valdez SJ, Mariano CM. Feeding value of protein enriched sweet potato for broilers. Research Abstract; 1997–2000.
23. Bilgrami KS, Dube HC. A textbook of modern plant pathology. Vikas Pub. House, PVT Ltd., New Delhi, India; 2001.
24. Popenoe I. Coconut and cashew: North America north trees. WF. Humphrey Press, New York; 1969.
25. Omokolo ND, Tsala NG, Djocgoue PF. Changes in carbohydrate, amino acid and phenol content in cocoa pods from three clones after infection with *Phythphthora megakarya* Bra. and Grif. Annals of Botany. 1996;77:153–158.
26. Burnett JH. Fundamentals of mycology. Edward Arnond Pub. Ltd. 2nd Ed. 1976;673.
27. Aziz NH, Shahin AAM, Abou-Zeid AAM, El-Zeany SA. Correlation of growth and aflatoxin production by *Aspergillus flavus* with some essential metals in gamma irradiated crushed corn. Food. 2000;44(5):354–359.

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