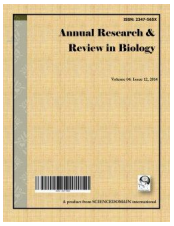

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Biochemical Composition of Seed and Husk of Cowpea (*Vigna unguiculata* (L.) Walp.) Infected by *Colletotrichum destructivum* O'Gara in Storage

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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

Colletotrichum destructivum was isolated from infected seeds and pods of cowpea (Var. IAR-48) with typical symptoms of anthracnose disease. The fungus during the pathogenesis, reduced the protein, fat, ash, crude fibre, carbohydrate, calcium and phosphorus, and increased the amount of iron, sodium, zinc, magnesium and potassium in the infected seed and husk. The carbohydrate, protein and phosphorus contents in the healthy husk reduced from 55.05%, 11.21% and 171.85 mg to 39.94%, 8.92% and 42.92 respectively in the infected sample whereas potassium and sodium contents in the healthy pod increased from 1.03 mg and 78.29 to 2.90 mg and 100.65 mg respectively in the infected husk. The potassium, sodium, magnesium and iron increased from 1292.25 mg, 0.19 mg, 0.09 mg and 11.00 mg in the healthy seeds to 1536.03 mg, 0.28 mg, 0.21 mg and 13.19 mg respectively in the infected seeds. The fungus caused the depletion of phosphorus from 498. 06 mg in the healthy to 430.17 mg in the infected seed, protein from 24.09% to 17.86%, carbohydrate from 57.02% to 34.35%, fat from 1.70% to 1.33% and crude fibre from 3.94% to 2.61%. The average loss of the major nutrient values; protein, carbohydrate and fat were 28.95% and 22.55% for seed and husk respectively after 8 weeks of planting.

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Keywords: Biochemical composition; Colletotrichum destructivum; cowpea; husk; seed.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an annual legume widely grown in Africa, Asia, USA and the Americas [1] whose seeds, pods and leaves are consumed as processed grains and vegetables [2]. As a food crop, it is grown for grain production and its tender leaves are consumed as vegetable in several countries of Asia, the Pacific and Africa [3,4]. The tender leaves are used as spinach substitute, immature pods as snap beans while the green seeds or pulse grains may be boiled or canned [1] and used as supplements to scarce animal protein for millions of people in the tropics and sub-tropics of the world [2].

Colletotichum spp. are worldwide in distribution, causing important diseases in humans, ornamentals and economic crops [5-7] and in humid and sub-humid tropics they occur as saprobes, endophytes or pathogens on leaves, stems, flowers and fruits in susceptible crops [8]. Many economically important diseases including anthracnose have been ascribed to attacks from members of the genus [9,10]. Anthracnose is one of the major important fungal diseases which affect the yield and economic production of cowpea [11], causing up to 50% yield reduction [12]. The disease could also result in loss of quality and market value of the crop through alterations in its anatomy, physiology and biochemical composition and contamination with noxious mycotoxins [13]. The reduction in nutritional values of potato and cassava root by *Rhizoctonia bataticola, Botryodiplodia theobromae,* and *Penicllium expansum* [14,15] suggests that infection of cowpea by *Colletotrichum* could potentially affect its chemical and biochemical composition. The alterations of biochemical composition of seed and husk of cowpea (*Vigna unguiculata* (L.) Walp.) by *Colletotrichum destructivum* O'Gara during storage were investigated and presented in this paper.

2. MATERIALS AND METHODS

2.1 Husk and Seed Sample Preparation

Healthy pods and seeds of cowpea (Var. IAR-48) (50 g) were soaked in spore suspension of *C. destructivum* (1 x 10^5 spores/ml of sterile distilled water) for 5 days, air-dried on filter paper (Whatman No. 1) at room temperature (27^{o}C) and incubated for 3 weeks and then oven-dried at 60^{o}C for 2 days. The control (healthy husk and seeds) were similarly treated but soaked in sterile distilled water alone [16]. The dried husk and seed samples were milled separately into powder using a hand milling machine (Corona Lavesh 250).

2.2 Biochemical Analysis

The procedures adopted by [17] were used in the determination of the proximate composition of the specimens. The elementals were determined using atomic absorption spectrometer (AAS) (Model: AA 7000, Schimadzu, Japan).

2.3 Moisture Content Evaluation

Scanning for the optimum temperature (140^{o}C) and determination of the percentage moisture contents of the samples were carried out using a moisture analyzer (Model: MS-70, A & D Company limited, UK).

2.4 Ash Content

Each of the samples of seed and husk was separately weighed (1 g) into a crucible and charred and then put in a furnace (Model 186A, Fisher Scientific Co.) set at 560^{o}C for 3hours. The percentage ash content was determined using the formula:

$$\text{Percentage ash content (\%)} = \{(W_2 - W_1 / W) \times (100/ 1)\}$$

Where, W_2 is the weight of crucible + ash
W_1 is the weight of dried crucible
W is the weight of sample taken

2.5 Protein

Kjehdahl apparatus was used to digest each sample (5 g). The resulting nitrogen (N) from each digestate was converted to protein using the formula by [18]:

$$\text{Protein content (\%)} = \{(\text{Titre value} \times \text{Normality of acid} \times 1.4007 \times 6.25 / \text{Weight of sample taken}) \times (100/ 1)\}$$

Where: 6.25 is the general conversion factor from nitrogen to protein, and 1.4007 is the factor for nitrogen.

2.6 Fat

Two grams (2 g) of each of the samples were put in the Soxhlet extractor and then extracted with

150 ml of 60% petroleum ether for 3 h at 80°C. The fat content of each extract was calculated based on the formula:

Percentage fat content (%) = {($W_2 – W_1$ / W) x (100/ 1)}

Where: W_2 - weight of flask + extract
W_1 - weight of dried flask
W - weight of sample taken

2.7 Crude Fibre

One gram (1 g) of the samples was separately de-fated and the resulting residues were ashed at 500°C for 3 h. The crude fiber was determined using the formula:

Percentage crude fibre content of sample (%) = {($W_2 – W_1$/ W) x (100/ 1)}

Where: W_2 - weight of crucible + extract
W_1 - weight of dried crucible
W - weight of sample taken

2.8 Determination of Total Carbohydrate Content of Sample

The total carbohydrate content of respective samples was determined based on the proximate composition of other variables as follows:

Percentage carbohydrate content of sample (%) = 100-{∑DC}.

Where: ∑DC is the summation of the determined proximate contents of other variables (crude protein, crude fibre, fat and moisture) in 100 grams of the sample.

2.9 Elemental Determination (Na, K, P, Mg, I, Fe, Ca and Zn)

The various elements in each sample solution made up of its ash and 1N Nitric acid (3 drops of concentrated nitric acid) with 100 ml of distilled water .were analyzed using the AAS (Atomic Absorption Spectrometer) (Model: AA 7000, Schimadzu, Japan). Standard curves were generated and used to determine the respective elements of the samples.

2.10 Data Analysis

The experiments were laid out in a complete randomized design (CRD) with three replicates and the data was analyzed using analysis of Variance (ANOVA). The statistical package used was SPSS. The mean values were separated using least significant difference (LSD) at 5% level of probability.

3. RESULTS

3.1 Effects of *C. destructivum* on Biochemical Composition of Cowpea Husks

The results of proximate analyses of the healthy (uninfected) and infected cowpea pods (husks) (Plates 1A and C respectively) showed that the pathogen caused significant effects on the nutrient contents of the husk. It reduced the carbohydrate, protein and phosphorus contents in the healthy husk from 55.05%, 11.21% and 171.85 mg to 39.94%, 8.92% and 42.92 in the infected husk respectively and increased the potassium and sodium contents in the healthy pod from 1.03 mg and 78.29 to 2.90 mg and 100.65 mg in the infected husk specimens respectively (Table 1).

Infection of seed by *C. destructivum* resulted in an increase of potassium, sodium, magnesium, iron and moisture content from 1292.25 mg, 0.19 mg, 0.09 mg, 11.00 mg and 11.98% in the healthy seeds (Plate 1B) to 1536.03 mg, 0.28 mg, 0.21 mg, 13.19 mg and 13.00% respectively in the infected seeds (Plate 1C). Also, the fungus caused a depletion of the phosphorus from 498. 06 mg in the healthy to 430.17 mg in the infected seed, protein from 24.09% to 17.86%, carbohydrate from 57.02% to 34.35%, fat from 1.70% to 1.33% and crude fibre from 3.94% to 2.61% (Table 1).

3.2 Effect of *Colletotrichum destructivum* on Loss of Nutrient in Cowpea Seed and Husk

Results of the quantitative disruption of major nutrients in seed and husk by the pathogen (Table 2) indicated that husk lost 20.43%, 19.75% and 27.46%, of protein, fat and carbohydrate respectively whereas 25.86%, 21.77% and 39.23% of protein, fat and carbohydrate respectively were lost from cowpea seed due to the activities of *C. destructivum*. The highest average nutrient loss of 28.95% was recorded with the seed whereas the husk had the least percentage mean nutrient loss of 22.55% 8 WAP (Table 2).

Table 1. Effects of *Colletotrichum destructivum* on the biochemical and chemical composition of husk and seed of cowpea 8 WAP

Proximate composition of husk and seed					
Nutrients	**Husk**		**Seed**		**Nutrient value**
	Uninfected	**Infected**	**Uninfected**	**Infected**	**After infection**
Moisture content*	19.09	20.13	11.98	13.00	Increased
Protein*	11.21	8.92	24.09	17.86	Decreased
Fat*	0.81	0.65	1.70	1.33	Decreased
Carbohydrate*	55.06	39.94	57.02	34.65	Decreased
Ash*	11.25	10.94	2.81	2.72	Decreased
Crude Fibre*	22.12	18.61	3.94	2.61	Decreased
Zinc**	2.00	2.85	0.27	0.39	Increased
Calcium**	1.15	0.49	93.10	82.10	Decreased
Sodium **	78.29	100.65	0.19	0.28	Increased
Magnesium**	0.56	0.98	0.09	0.21	Increased
Iron**	1.54	2.68	11.00	13.19	Increased
Potassium**	1.03	2.90	1292.25	1536.03	Increased
Phosphorus	171.85	98.10	498.06	430.19	Decreased
LSD (0.05)	**31.33**	**27.51**	**107.22**	**146.01**	

** Values are in percentages; **values are in mg/g*

Plate 1. Healthy (uninfected) cowpea husks (A) and seeds (B) and *Colletotrichum destructivum* infected cowpea husks (C) and seeds (D)

Table 2. Nutrient loss in cowpea husk and seed after 8 weeks of infection with *Colletotrichum destructivum*

Nutrient	Percentage nutrient loss (%)					
	Husk			Seed		
	Healthy	Infected	% loss	Healthy	Infected	% loss
MC**	19.09	20.13	5.45*	11.98	13.00	7.85*
Pr.**	11.21	8.92	20.43	24.09	17.86	25.86
Fat**	0.81	0.65	19.75	1.70	1.33	21.77
CHO_3**	55.06	39.94	27.46	57.02	34.65	39.23
Ash**	11.25	10.94	2.76	2.81	2.72	3.20
Ca***	1.15	0.49	57.39	93.10	82.10	11.82
CF**	22.12	18.61	15.59	3.94	2.61	33.76
P***	171.85	98.10	42.92	498.06	430.19	13.63
Zn***	2.00	2.85	42.50*	0.27	0.39	44.44*
Na ***	78.29	100.65	28.56*	0.19	0.28	47.37*
Mg***	0.56	0.98	75.00*	0.14	0.21	50.00*
Fe***	1.54	2.68	74.03*	11.00	13.19	19.91*
K***	1.03	1.90	84.47*	1292.25	1536.03	18.86*
LSD (0.05)	**31.33**	**27.51**	**1.79**	**107.22**	**146.01**	**2.54**

***Values are in percentages; ***values are in mg/g, * = Percentage increase*
MC = Moisture content, Pr. = Protein, CHO_3 = carbohydrate, CF = Crude fibre, Ca = Calcium, P = Phossphorus, Zn = Zinc, Na = Sodium, Mg = Magnesium, Fe = Iron, K = Potassium

4. DISCUSSION

The proximate analyses of the healthy and infected cowpea pod and seed in this study indicated that *C. destructivum* reduced the protein, carbohydrate, crude fibre, calcium, phosphorus, and ash contents in the healthy husk and seed and increased the moisture, zinc, sodium, magnesium, iron, and potassium contents in the infected husk and seed (Table 1).

Colletotrichum species as hemibiotrophic pathogens live as biotrophs and necrotrophs inside infected cowpea deriving their food and energy from their hosts [19]. Low levels of protein, carbohydrates, fat and crude fibre were recorded in the infected cowpea seed and husk indicating that *C. destructivum* used the nutrients as a source of metabolizable carbon for its calorific and amino acids needs to build its protoplasm since carbon and nitrogen have been reported as the most important elements for growth and reproduction of the test fungus [20]. The increase in trace elements in the infected cowpea husk and seed may have resulted in cell toxicity and tissue necrosis leading to the anthracnose symptoms which corroborated the report by [21] that high levels of Mg and K in infected tissues of lettuce increased the severity of *Botrytis* infection.

C. destructivum caused significant losses in the major nutrients (protein, carbohydrate and fat) of seed and husk of cowpea (Table 2) which agrees with the reports of [22] and [23] that storage and seed-borne pathogens of legumes and cereals caused qualitative and quantitative losses in the crops. Infection by *C. destructivum* led to loss of major nutrient contents of cowpea pod (22.55%) and seed (28.95%) which corroborated the report of [24] who recorded up to 20.39-38.40% loss in mean protein of seeds of chick pea, green gram, blackgram and pigeon pea due to infection by *A. flavus, A. fumigatus, A. niger, R. stolonifer, F. moniliforme* and *Drechslera tetramera.*

5. CONCLUSION

Anthracnose disease incited by *Colletotrichum destructivum* O'Gara caused significant reduction of major nutrients and an increase in mineral contents of infected cowpea seed and husk. The pathogen reduced the values of calcium, phosphorus, protein, carbohydrate, lipid, crude fibre and ash and increased the accumulation of sodium, potassium, iron, zinc, and magnesium in infected tissues of cowpea seed and husk. The average loss of major nutrients; protein, fat, and carbohydrate in infected seed and husk were 28.95% and 22.55% respectively suggesting the need for adequate and sustainable control of the disease to reduce post harvest losses of stored food products in cowpea due to infection by *C. destructivum.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davies DW, Oelke EA, Oplinger ES, Doll JD, Hanson CV, Putnam DH. Cowpea alternative field crops manual. 2012;1-9. Available:www.hort.pordue.edu/newcrop/afcm/cowpea.html
(Accessed July 13, 2012)
2. SADAFF (South Africa Department of Agriclture, Forestry and Fisheries). Production guildlines for cowpeas. DAIS, Pretoria, South Africa. 2009;1-15.
3. Nielsen SS, Ohler TA, Mitchell CH. Cowpea leaves for human consumption: Production, utilization and nutrient content. In: Singh, R. S., Morgan-Raj, Daswell, K. E. and Jackai, L.E.N (Eds). Advances in Cowpea Research. 1997;326-332.
4. Awurum AN, Enyiukwu DN. Evaluation of the seed-dressing potentials of phytochemicals from *Carica papaya* and *Piper guineense* on the germination of cowpea (*Vigna unguiculata* L. Walp) seeds and incidence of the seed-borne fungi. Continental Journal of Agricultural Science. 2013;7(1):29-35.
5. Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ. Characterization and pathogenecity of *Colletotrichum* species associated with anthracnose disease of chilli (*Capsicum spp.*) in Thailand. Plant Patholology. 2008; 57(53):562-572.
6. Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prishastuti H. *Colletotrichum*: A catalogue of confusion. Fungal Diversity Online. 2009;001-017.
7. Cannon PF, Damm U, Johnson PR, Weir BS. *Colletotrichum* - Current status and future direction. Sudies in Mycology (CBS-KNAW Fungal Biodiversity Centre). 2012; 22-33.
8. Waller JM, Bridge PD. Recent advances in understanding *Colletotrichum* diseases of some tropical perennial crops. In: *Colletotrichum* host specifity, pathology and host-pathogen interaction. Prusky, D. Freeman, S. and Dickman, M. D. (Eds). APS Press, Minesota USA. 2000;337-345.
9. Xie L, Zhang JZ, Wan Y, Hu DW. Identification of *Colletotrichum spp* isolated strawberry in Zhejiang Province and Shangai City, China. Journal of Zhejiang University Science (Biomedicine and Biotechnology). 2010;11(1):61-70.
10. Lubbe CM, Dennman S, Cannon PF, Groenewald JZE, Lamprecht SC, Crous PW. Characterization of *Colletotrichum* species associated with diseases of Proteaceae. Mycologia. 2004;96(6):1268-1279.
11. Begum MM, Saviah M, Puteh AB, Abidim MAZ. Detection of seed-borne fungi and site of infection by *Colletotrichum truncatum* in naturally infected soybean. Journal of Agricultural Research. 2007; 2(9):812-819.
12. Enyiukwu DN, Awurum AN. Fungitoxic principles and antifungal activity of extracts from *Carica papaya* and *Piper guineense* on *Colletotrichum destructivum*. Continental Journal of Biological Sciences. 2013;6(1):29-36.
13. Enyiukwu DN, Awurum AN, Nwaneri JA. Efficacy of plant-derived pesticides in the control of myco-induced postharvest and storage rots of tubers and agricultural products: A review. Net Journal of Agricultural Sciience. 2014;2(2):30-46.
14. Amadioha AC. Interaction of hydrolytic enzymes produced by *Rhizoctonia bataticola* during rot development. Acta Phytopathologica et Entomologica Hungarica. 1997;32(1-2):79-87.
15. Markson AA, Omosun G, Umana EJ, Madonagu RE, Amadioha AC, Udo SE. Differential response of *Solanum tuberosum* L. and *Ipomea batatas* L. to three pathogens. International Journal of Natural Sciences. 2014;2(1):40-51.
16. Amadi JE, Oso BA. Mycoflora of cowpea seeds (*Vigna unguiculata* L.) and their effects on seed nutrient contents and germination. Nigerian Journal of Science. 1996;30:63-69.
17. A.O.A.C. (Association of Organic and Analytical Chemist). Official Methods of Analysis International (17th Ed). Washington DC., USA; 2000.
18. Kayode OF, Okafor JNC, Adeyoju OA, Etoamaihe MA, Ozumba AU. Nutrient composition and sensory evaluation of selected Nigeria traditional soups. Journal of Industrial Research and Technology. 2008;2(1):51-55.
19. Tasiwal V, Banagi VI. Studies on the cultural and nutritional characteristics of *Colletotrichum gloeospoiroides Karmatu.*

Journal of Agricultural Science. 2009; 22(4):787-789.
20. Sangeetha CA, Rawal RD. Nutritional studies of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the incitant of mango anthracnose. World Journal of Agricultural Science. 2008;4(6):71-720.
21. Easterwood GW. Calcium's role in plant nurition. A hydro-Agric. North America Inc. and Fliud Fertilizer Foundation Bulletin 2002.
Available:www.fluidfertilizer.com/calcium/... June 3, 2016.
22. Strivastava S, Strivastava M, Kumar R, Sinha A. Effect of seed-borne mycoflora on the protein and amino acid contents of *Jatropha carcus* L. seeds during storage. International Journal of Plant Research. 2013;26(2):271-279.
23. Naikoo-Abbas WM, Bhat NA, Waheed-U-Zmeena MD, Dar S, Tak MA. Effect of seed-borne mycoflora on the quality of three varieties *of Arachis hypogea* L. International Journal of Agricultural Science Research. 2013;3(1):35-42.
24. Kandhare AS. Effect of common and dominant seed-borne fungi on protein content of pulses. Bioscience Discovery. 2014;6(1):14-17.

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