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Association of cowpea (*Vigna unguiculata* L. Walp.) (var. Ife Brown) with *Colletotrichum destructivum* O'Gara: A special reference to nutrients lost by the host

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Abstract. *Enyiukwu DN, Nwaogu GA, Bassey IN. 2020. Association of cowpea (*Vigna unguiculata *L. Walp.) (var. Ife Brown) with Colletotrichum* destructivum *O'Gara: A special reference to nutrients lost by the host. Cell Biol Dev 4: 41-46.* Cowpea or southern pea (*Vigna unguiculata* L. Walp.) is an important African grain and leafy vegetable. Anthracnose caused by *C. destructivum* represents a major biotic drawback to the profitable production of cowpeas in the incontinent. The disease culminates in the loss of grain yield, produce quality, and nutritional values of the crop. This work assessed and quantified the nutrients lost from the aerial organs of cowpea (var. Ife Brown) challenged by anthracnose disease in the field and storage 4 weeks after inoculation (WAI) by classical and spectrometric analyses. The results showed that the disease affected the nutrient contents of the inoculated crop's aerial organs, decreasing the mean quantities of all proximate constituents and major mineral nutrients such as calcium and phosphorus in the test plant materials. Amongst the aerial organs, fat was the most depleted nutrient (36.30%), followed by protein (28.52%), carbohydrate (26.67%), and crude fiber, with 20.04%, was the least. Generally, the highest mean loss of proximate nutrients due to anthracnose disease per organ of the test crop of 27.97% was recorded in the leaf sample, followed by 22.13% in the seeds and 18.03% in the husks was the least. Similarly, the highest mean mineral loss of 42.63 mg 100 g^{-1} occurred in the husks, followed by the stem (26.14 mg 100 g^{-1}), while the seed recording (15.41 mg 100 g^{-1}) was the least. Controlling this important fungal disease of the crop will undoubtedly result in better leaf, grain, and haulm quality, which will rub off as improved farm economy and public health in the continent.

Keywords: Anthracnose, nutrient loss, proximate composition, protein, elemental nutrients, cowpea

INTRODUCTION

Cowpea, or southern pea (*Vigna unguiculata* L. Walp.), is an important leafy vegetable or grain legume eaten in several varieties of food in tropical third worlds (Chikwendu et al. 2014; Igbatim et al. 2014; Enyiukwu et al. 2018). In bean-growing areas of West Africa, anthracnose (*Colletotrichum* spp.) is endemic, destructive, and affects all the aerial organs of the crop. Thus, the disease remains one of the major biotic challenges to economic cowpea production in the sub-region, especially in Nigeria (Adegbite and Amusa 2008; Sabo et al. 2014; Falade et al. 2018). *Colletotrichum destructivum* O'Gara has been identified as the causal agent of the disease in Nigeria (Ogu and Owoeye 2013; Awurum and Enyiukwu 2013). The pathogen is a hemibiotrophic fungus; which survives no-crop seasons as dormant mycelia or spores in bean debris or seed coats, cotyledon, and embryo of infected cowpea seeds (Begum et al. 2007). About 80%, 50%, and 88% of infection of *Colletotrichum* spp. has been reported from surveys on guava fruits, soybean, and cowpea seed lots in Nigeria, Brazil, and India, respectively (França Neto et al. 1989; Emechebe and Florini 1997; Amusa et al. 2006). Similarly, as high as 80-83% seed to the disease's seedling transmission has been reported from studies on common and lima beans in Brazil (Da Mota et al. 2019).

Under warm moist conditions or free moisture on cowpea surfaces; spores of the fungus germinate on susceptible varieties of the crop to initiate anthracnose (Latunde-Dada et al. 1999; Enyiukwu and Awurum 2013b). Affected cowpea develops small, round or irregular tan to brown, depressed lesions with characteristic black acervuli, bearing single-celled setae. Incidence of 83% and mean severity of 6 on a 10-point scale have been recorded on some accessions of cowpea in studies in eastern Nigeria (Enyiukwu and Awurum 2013b; Awurum 2013). Grain yield losses averaging 50% due to the disease have also been reported in Thailand and some parts of the humid rainforest agro-ecological zone of Nigeria; and this may be exacerbated to total crop failure in severe instances of attacks by the disease occasioned by very wet weather (França Neto et al. 1989; Begum et al. 2008, Enyiukwu and Awurum 2013b; Awurum 2014).

Crop pathogenic fungi, including *Colletotrichum* species sap, are susceptible hosts of vital nutrients, energy, and electrolytes to grow, reproduce, build their protoplasm and survive in the ecosystem (Amusa et al. 2006; Amadioha and Enyiukwu 2019a). *Colletotrichum truncatum* has been implicated in sapping electrolytes and a

variety of nutrients in infected seeds of legumes (Begum et al., 2013). Recently Amadioha and Enyiukwu (2019a, b) reported that the interaction of cowpea (var. IAR-48) with *C. destructivum* within 4 weeks resulted in mean protein losses, fat, carbohydrates, calcium, and phosphorus ranging up to 20-30 percent. The Ife Brown is a very popular and commonly grown variety of cowpea in humid southern Nigeria. The variety is known to be seriously affected and constrained by anthracnose. So far, however, there is little or no documented information on the effects of the association of *C. destructivum* on the proximate nutrient composition and basic electrolytes such as K, Na, Mg, and Ca of cowpea (var. Ife Brown),

Therefore, this paper assessed the interaction between anthracnose and cowpea (var. Ife Brown) in field and storage situations; with the aim of quantifying the level of depletion of proximate nutrients and electrolytes due to activities of *C. destructivum* in affected organs of the crop.

MATERIALS AND METHODS

Source of seeds and environmental parameters

The seeds of cowpea (var. Ife Brown) obtained from the Research and Training (R&T) Unit of the University were used for the study. The environmental parameters of the study location were 1,072.10 mm of rainfall over 60 days, temperature range of 29-33°C, and relative humidity of 80.0-87.0% within the study months of August-November, 2018. The soil type was sandy loam with organic carbon recorded at 68000 ppm, pH (water) of 4.17, and altitude of 121.08 meters above sea level (GPS Coordinates 2017).

Isolation and identification of the causal agent

Pods of infected cowpea (*Vigna unguiculata* (L.) Walp.) with typical anthracnose symptoms were collected from the University Research field. The pods were cut in bits using a surgical blade, sterilized in 70% ethanol, and washed in several changes of 200 mL of sterile distilled water. The tissues were plated in Petri dishes containing moistened Whatman No 1. filter paper and incubated for 7 days at 27°C. Then 39.50 g of dehydrated potato dextrose agar (PDA) (Oxoid® ThermoScientific Product, England, UK) was dissolved in 1,000 mL of sterile distilled water in a 2L flask, stirred thoroughly with a glass stirrer, then stoppered, and autoclaved at 15 Psi for 30 minutes. The mycelial growth from the plated tissues was repeatedly sub-cultured to obtain a pure culture of the organism maintained on PDA as prepared above. Finally, the isolate was subjected to pathogenicity tests by re-inoculating it into 2-week-old cowpea seedlings. About 4 days after inoculating the seedlings, typical anthracnose symptoms resembling those observed on the diseased pods were observed on the leaf blades and petioles of the seedlings (Ogu and Owoeye, 2013; Markson et al., 2014; Enyiukwu et al. 2020). Thus, confirming that the organism is pathogenic.

Slides of the organism were then prepared, mounted, and examined under a microscope. The organism's identity was confirmed to be *C. destructivum* with the aid of fungi identification manual by Barnett and Hunter (1995), annotated species of *Colletotrichum* by Damn et al. (2009; 2014), and monographs of the International Mycological Institute IMI (1995).

Preparation of spore suspension

The spores of the pathogen *C. destructivum* were collected from 8 days old culture-agar stock in a Petri dish by lifting a 60 cm^2 piece into a beaker containing 200 mL of sterile distilled water. Next, this spore was sieved through 4-folds of sterile cheesecloth to remove agar and mycelial mesh, and the filtrate was then centrifuged for 10 minutes. After that, the spore suspension was standardized using a hemocytometer counting slide to 10^5 spores mL^{-1} (Awurum and Enyiukwu 2013b; Alberto 2019).

Preparation of test cowpea materials

Four-week-old cowpea seedlings (var. Ife Brown) were grown in pots containing sterilized topsoil (4 kg). At this period, they were spray-inoculated with spore suspension (1 x 10^5 spores/mL of sterile distilled water) of *C. destructivum*. At eight weeks after planting (8 WAP), the percentage (%) incidence of anthracnose on the matured inoculated cowpea plants was calculated using the protocol adopted by Amadioha (2003). The control experiment of the cowpea plants was kept anthracnose disease-free with bi-weekly sprays with benomyl sprays (Awurum 2014). The percentage incidence of anthracnose on the test cowpea cultivar was calculated using the formula adopted by Amadioha (2003):

$$\% \text{ incidence} = \frac{\text{Number of plants affected by anthracnose}}{\text{Total number of plants assessed}} \times \frac{100}{1}$$

Healthy husks and seeds of cowpea (var. Ife Brown) (50 g) were soaked in spore suspension of *C. destructivum* (1 x 10^5 spores mL^{-1} of sterile water) for 1 day and then air-dried on sterile filter papers (Whatman No 1) placed on the laboratory bench for 24 h and incubated in the inoculation chamber for 3 weeks. The control (un-inoculated seeds) were similarly treated but soaked in sterile distilled water alone for the same period; after that, the specimens were separately enveloped and oven-dried at 60°C for 3 days.

One hundred grams of each specimen (leaves, stems, husks, and seeds) from both the infected and healthy specimens were weighed out with a digital balance and ground separately into powder using a hand milling machine (Corona Lavesh 250) (Amadioha and Enyiukwu 2019a, b). Each powder was stored in a dark, air-tight bottle and kept in the laboratory cupboards until required for biochemical analysis.

Biochemical analyses

Samples of healthy and infected cowpea organs (stem, leaf, husks, and seeds) were analyzed separately for protein, carbohydrate, fats, crude fiber, and ash contents after 4 weeks of inoculation. The proximate composition of both the healthy and infected test specimens was determined by the procedure adopted from the AOAC (2000) and Chikwendu et al. (2014); while the elemental nutrients were done by the absorbances of the individual

specimens from the atomic absorption spectrometer (AAS) (Model: AA 7000, Shimadzu, Japan) as described by Enyiukwu et al. (2018).

Determination of the effects of the association of the test fungus on cowpea specimens

The quantitative effects of the pathogenesis of the test fungus on the respective individual and mean nutrient contents of the cowpea materials were assessed and calculated using the formulae adopted by Amadioha (1994) and Amadioha and Enyiukwu (2019a, b) as:

$$\% \text{ loss of each nutrient per organ} = \frac{Ha - Hb}{Ha} \times \frac{100}{1}$$

Whereas the mean% loss of individual nutrients per organ or across organs was calculated as:

$$\text{Mean\% loss of each nutrient per organ or amongst organs} = \frac{\sum [Ha - Hb]}{Ha} \times \frac{100}{1}$$

Where:

Ha = value of a parameter in a healthy cowpea tissue(s)

Hb = value of a parameter in anthracnose-infected cowpea tissue(s)

$\sum$ = Summation of the values of parameters per organ or across organs divided by the number of variables (n) considered in the sum.

Data analysis

Data collected from this study were obtained in three determinations. First, they were analyzed by ANOVA (analysis of variance). The general linear model program (Genstat Release, Windows/PC Vista Version 12.10) at a significant level of 0.05 was used for the analysis. Means were separated and compared using Fisher's LSD at the probability of 5%.

RESULTS AND DISCUSSION

The results of this study showed that a mean percentage incidence of 78.21% of anthracnose disease was recorded on the *C. destructivum* inoculated cowpea (var. Ife Brown). The results of the major fingerprinting of nutrients of cowpea presented in Table 1 also showed that the disease affected the quantities of protein, carbohydrate, fat, crude fiber, ash, calcium, and phosphorus within and amongst the aerial organs of the assayed plant materials. Furthermore, it showed that fats (54.62% and 45.23%) and protein (38.92% and 26.30%) were the highest lost nutrients in the leaf and stem specimens, respectively. Also, carbohydrates recorded at (32.01%) and protein (31.04%) were the most depleted nutrients in the leaf and husk specimens, respectively, due to the activities of the fungus, in terms of loss of macro-elementals, Ca (26.05 mg/ 100 g and 34.69 mg/ 100 g) for the leaf and stem tissues and P recorded at (54.16 mg 100 g^{-1} and 21.04 mg 100 g^{-1}) for the husk and seed represent the most depleted elemental nutrient per organ of the test cultivar.

The results of the nutrient fingerprinting in Table 1 also show the comparative mean depletion of different individual proximate nutrients amongst the aerial organs of cowpea. Overall, it showed that the mean nutrient loss profile amongst the aerial organs of the crop was fat (36.30%) ≥ protein (28.52%) ≥ carbohydrate (26.67%) ≥ crude fiber (20.04%). Similarly, the nutrient profiling on the comparative mean depletion of different individual minerals amongst the aerial organs of the test cultivar revealed that P (27.75 mg 100 g^{-1}) was the most depleted element. However, it was statistically ($P \leq 0.05$) at par with 25.39 mg 100 g^{-1} recorded for Ca in the evaluation.

The mean comparative proximate and elemental nutrient composition per organ of the test crop is presented in Figure 1. The pathogen attack on the test cowpea caused the highest mean loss of proximate nutrients (27.97%) in the leaf specimen, followed by 22.13% and 21.61%, respectively, for the stem and seed samples, while the husk recording 18.05% was the least. Mean losses of 42.63 mg 100 g^{-} followed by 26.14 mg 100 g^{-} recorded in the husks and stem specimens of the inoculated cowpea represented the highest mean losses of minerals in the test organs, while the seed recording a mean loss of 15.41 mg 100 g^{-} was the least (Figure 1).

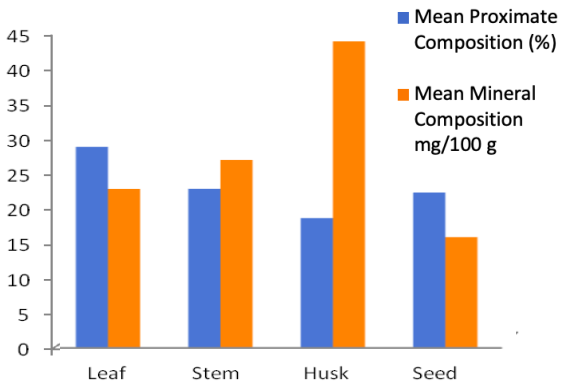

Figure 1. Mean percentage depletion of proximate nutrients (%) and minerals (mg 100 g^{-1}) per organ of cowpea Legend: Y-axis =% composition while X-axis = organs of cowpea

Table 1. Percentage nutrient loss in cowpea aerial organs due to activities of *Colletotrichum destructivum*

Nutrients	Nutrient loss (%) from					
	Leaf	Stem	Husks	Seeds		
		Proximate	Composition	(%)	Means	LSD (0.05)
Moisture content	10.28*	8.61*	10.00*	9.12*	--	0.11
Protein	38.92	26.30	17.83	31.04	28.52	4.41
Fat (lipid)	54.62	45.23	20.16	25.68	36.30	4.80
Carbohydrate	26.08	16.48	32.01	28.49	26.67	3.22
Ash	1.57	1.22	0.99	2.85	1.66	0.21
Crude fiber	18.66	21.43	19.08	20.97.	20.04	3.94
LSD (0.05)	6.28	5.13	4.20	3.81		
		Elemental	Minerals	(mg/100 g)		
Calcium	26.05	34.69	31.09	9.77	25.39	3.06
Phosphorus	18.19	17.58	54.16	21.04	27.75	2.89
LSD (0.05)	0.21	0.17	0.44	0.18	3.45	-

Note: Data are means from 2 separate experiments. *represents% increase

Discussion

The association and infection of cowpea in the field and storage with *Colletotrichum destructivum* have been reported (Enyiukwu and Awurum 2013a, b; Awurum et al. 2014). The results of this study indicated up to 78.21%% incidence of anthracnose in the inoculated cowpea plants. Moreover, this agrees with submissions of other workers who reported a high incidence (80%) of anthracnose (*C. gloeosporioides*) on guava fruit grown in humid Southwest Nigeria (Amusa et al. 2006). It is also consistent with the reports of a high incidence of 83% due to *C. destructivum* induced anthracnose on cowpea in humid southeast Nigeria (Enyiukwu and Awurum 2013b) as well as over 50% incidence due to brown blotch − a closely related disease to anthracnose − caused by *C. truncatum* recorded on soybean in Brazil and Thailand (França Neto 1989). Furthermore, Amusa et al. (2006) observed high humidity and rainfall to have encouraged a high incidence of anthracnose (*C. gloeosporioides*) on guava fruits grown in Ibadan, Southwest Nigeria. Hence, the high ambient temperature, humidity, and rainfall in Umudike, Southeast Nigeria, during the study period, amongst other environmental factors, may partly explain the high incidence of the disease recorded on the crop in this study.

High reductions of all proximate compounds were observed on *Colletotrichum destructivum*-infected aerial organs of the test crop in this study. Furthermore, this is in agreement with reports by Naikoo et al. 2013 who found marked depletions of energy, sugars, and lipids due to storage mold on three varieties of groundnut (Arachis hypogeal L.). The high loss of proximate nutrients (fat, protein, carbohydrate, and crude fiber) from the individual aerial organs and high percentage mean loss of same across the different organs of cowpea uphold that the pathogen is very destructive in deriving energy, amino acids, and other growth factors for its metabolic activities from all parts of the host crop. This finding thus sustained the views of Amusa et al. (2006), Begum et al. (2008; 2013), and Abd-Allah et al. (2018) that *Colletotrichum truncatum*, *C. gloeosporioides*, *Alternaria alternata*, *Fusarium spp. Aspergillus* spp. and some other storage fungi deprived lupine, pea, soybean, common bean seeds, and guava fruits of the same factors during infection in the field and storage. It also agrees with submissions of Amadioha and Enyiukwu (2019a, b), who reported up to 20-30% mean proximate nutrient losses from the interaction of aerial organs of another cultivar of cowpea with *C. destructivum*.

Calcium and phosphorus foster proper cell division and growth, maintain cell wall rigidity and tissue integrity, and resist pathogenic invasion (Better Crops 1999; Easterwood 2002; Imran et al. 2016). The high loss profile of calcium and phosphorus, especially in the husks and stems of the infected host, is in tandem with findings from Begum et al. (2008) and Abbasi et al. (2013). They observed marked reductions of these factors in their studies on the association of *C. truncatum* with soybean and groundnuts, respectively. Therefore, this suggests that the pathogen deprived the host of electrolytes (Amusa et al. 2006) needed for the formation and accumulation of xylans, lignans, and interlocking cross-walls in the host tissues; and thus impaired structural integrity and strength in the matrices of the infected host tissues (Easterwood, 2002; Amadioha and Enyiukwu, 2019a). This depletion of vital mineral factors may explain the girdling, tipping over and breakage of the stems, branches, and pods due to the disease observed in this study.

In all four organs of cowpea tested, the mean percentage losses of both proximate and mineral compositions (Ca and P) were higher in the leaves than in the seeds (Figure 1). This finding agrees with the assertions by Amadi and Oso (1996) and Amadioha and Enyiukwu (2019a, b). They reported higher mean losses of biochemicals in the leaves than in seed samples due to the interaction of *C. cruenta* and *C. destructivum* with cowpea, respectively. The higher loss of nutrients in the leaf, stem, and husk may be due to higher numbers of lenticels and stomata, which provided more and easier entry for the pathogen than the other crop organ (Latunde-Dada et al. 1999). It may also be due to the production of certain exudates such as mannitol, fructose, sucrose, alanine, and xylose-amino acid, which is known to encourage growth, conidiation, and infectivity of the pathogen from these organs than the seeds (Sangeetha and Rawal 2008; Boyette and Hoagland 2012). Or it may suggest the presence of

certain long-chain fatty acids on the cuticle of the severely affected specimens, which has been reported to stimulate the formation of certain adhesive substances, appressoria development, and effective penetration of fungal infection pegs into host tissues than in the seeds (Podilla et al. 1993). Or on the other hand, it may connote the presence of lower concentrations of certain phytochemical inhibitors to the pathogenic fungi such as tannins, polyphenols, flavonoids, and saponins in the husks, leaves, and stem than in the seeds of the test crop (Enyiukwu and Awurum 2013c); since the seed coats of legumes (including cowpea) contain high levels of these fungi-fighting phytochemicals especially flavonoids (Okwu and Orji 2007; Tajoddin et al. 2010).

In conclusion, the challenge of *C. destructivum* causing anthracnose disease of the crop remains a fundamental biotic constraint to profitable cowpea production in sub-Saharan Africa. Besides causing yield losses and crop failure in extreme cases, the disease can lead to serious loss in the quality of cowpea produce. Substantial losses in the proximate and mineral constituents such as protein, carbohydrate, fat, prebiotics, ash, calcium, and phosphorus of the crop were depicted in this study. The mean losses of these 7 nutrients were higher in the leaf, stem, and husk but least in seeds. Therefore adequate control of anthracnose disease will not only improve the farm economy but lead to the production and storage of high-quality, nutritious produce that could contribute to food security in the sub-continent.

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