



Alterations of Biochemical Composition of Leaf and Stem of Cowpea (*Vigna unguiculata* (L.) Walp.) by *Colletotrichum destructivum* O'Gara in Nigeria

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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 during pathogenesis reduced the nutrient values of cowpea leaf and stem. The protein, fat, carbohydrate, fibre contents of the leaf decreased from 34.91%, 5.42%, 43.61% and 19.46% in the uninfected leaf samples to 20.40%, 2.15%, 37.03% and 15.53% in the infected leaf respectively whereas the moisture and ash contents increased by 10.88% and 11.15% in the uninfected leaf sample to 12.51% and 11.24% respectively in the infected leaf samples. The composition of zinc, sodium, magnesium, iron, and potassium in the leaf increased whereas calcium and phosphorous decreased after inoculation with the test fungus. It reduced the protein content from 15.64% in the healthy stem tissue to 12.69% in the infected one, fat from 1.29% to 0.78%, crude fibre from 16.87% to 14.62%, and carbohydrate from 31.11% to 25.39%. Also, the fungus caused a reduction of the calcium and phosphorus contents of the healthy stem tissue from 2.09 mg and 326.50 mg to 1.19 mg and 299.10 mg respectively. Infection of the stem with the pathogen led

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to increasing moisture, potassium, sodium, zinc, iron, and magnesium contents from 11.80%, 230.12 mg, 111.28 mg, 1.66 mg, 0.89 mg and 0.95 mg to 12.65% , 364.21 mg, 203.64 mg, 3.02, 1.52 mg, and 2.18 mg respectively. The average loss of the major nutrients; protein, fat, and carbohydrate was more in the leaf than stem, 34.29% and 18.14% respectively 8 weeks after planting (WAP).

Keywords: Biochemical composition; *Colletotrichum destructivum*; cowpea; leaf; stem.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a food, feed and forage crop which belongs to the family Fabaceae. Cowpea as a food crop is widely grown in the savannas of Africa, Asia, USA and the Americas [1] where the seeds, pods, and leaves can be consumed as processed grains and vegetables [2]. The crop is second most important in Africa and ranked among the top 5 pulse crops in the world [3]. Though cowpea is grown mostly for grain production, the tender leaves of the crop are consumed as vegetable, immature pods as snap beans while the green seeds or pulse grains may be boiled or canned for use in several countries of Asia, the Pacific and Africa [4,1]. This protein-rich grain supplements scarce animal protein for millions of consumers of the crop in the tropics and subtropics of the world [2].

Anthrachnose disease caused by *Colletotrichum destructivum* is one of the major and most important fungal diseases of cowpea [5] which affect its economic production causing up to 50% yield reduction [6]. Besides yield reductions, fungal infections could result in loss of product quality due to alterations in their anatomy, chemical and biochemical composition as well as contamination with noxious mycotoxins [7]. *Colletotrichum* spp. are worldwide in distribution, causing important diseases in humans, ornamentals and economic crops [8,9,10] and in warm moist environment of the humid and sub-humid tropics, they occur as saprobes, endophytes or pathogens on leaves, stems, flowers and sometimes fruits in perennial susceptible crops [11]. Many diseases of economic importance including anthrachnose have been ascribed to attacks from members of the genus [12,13]. The reduction of starch and protein contents of potato and yam tubers by pathogenic organisms [14,15] is an indication that infection of cowpea by *Colletotrichum* could

potentially affect its chemical and biochemical composition.

The present study examines alterations of biochemical composition of leaf and stem of cowpea (*Vigna unguiculata* (L.) Walp.) by *Colletotrichum destructivum* O'Gara in Nigeria.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Samples for Analyses

Four weeks old cowpea seedlings (Var. IAR-48) growing in pots containing sterilized topsoil (4 kg) were spray-inoculated with a spore suspension (1×10^5 spores/ ml of sterile distilled water) of *C. destructivum*. At 8 WAP healthy (uninfected) and infected leaves and stems were harvested. Healthy seedlings (control) were kept anthrachnose free with benomyl sprays. The collected samples were separately enveloped and oven-dried at 60°C for 3 days. Fifty grams each of the infected and healthy specimens (leaves and stems) were separately weighed out with a digital balance and ground into powder using a hand milling machine [16]. Each powder was stored in an air-tight bottle and stored in dark cupboards until required for biochemical analysis.

2.2 Proximate Analysis

The biochemical composition of healthy and infected leaf and stem samples of cowpea were determined to ascertain the effect of the test fungus on the nutrient values and mineral composition of the crop. The procedures by [17] were used in the determination of the proximate composition of the moisture, ash, protein, fat, fibre and carbohydrate (determined by differences) and the elemental composition (Na, K, P, Mg, I, Fe, Ca and Zn) of the specimens.

3. RESULTS

3.1 Effects of *C. destructivum* on Proximate Composition of Cowpea Stem and Leaf

The pathogen infected the crop (Plate 1) and significantly ($P \leq 0.05$) reduced the nutrient contents of the stem and leaf samples of the infected cowpea (Table 1). It reduced the protein content from 15.64% in the healthy stem tissue to 12.69% in the infected one, fat from 1.29% to 0.78%, and carbohydrate from 31.11% to 25.39% giving a total percentage loss of 18.86%, 39.54%, and 18.37% respectively. Also, the fungus caused a reduction of the crude fibre of the healthy stem tissue from 16.87%, to 14.62% whereas the moisture content of the stem increased from 11.80% to 12.65% in the healthy and infected stem respectively, recording a percentage increase of 7.20% (Table 1). The protein, fat, carbohydrate, fibre contents of the leaf decreased from 34.91%, 5.42%, 43.61% and 19.46% in the uninfected leaf samples to 20.40%, 2.15%, 37.03% and 15.53% in the infected leaf respectively whereas the moisture and ash contents increased from 10.88% and 11.15% in the uninfected leaf sample to 12.51% and 11.24% respectively in the infected leaf samples. The percentage increase of moisture and ash contents in the infected leaf tissues were 14.98% and 7.17% respectively whereas 41.56%, 60.33%, 15.09% and 20.19% of protein, fat, carbohydrate and crude fibre respectively of the leaf were lost due to infection by the pathogen.

3.2 Effects of *Colletotrichum destructivum* on the Mineral Compositions of Leaf and Stem

The qualitative trend of effects of *C. destructivum* on the mineral composition of cowpea leaf and stem (Table 2) indicated that the anthracnose disease caused by the test fungus reduced the values of calcium and phosphorus of the healthy stem tissue from 2.09 mg, and 326.50 mg to 1.19 mg and 299.10 mg giving a percentage reduction of 1.89% and 21.13% respectively. Infection of the stem with the pathogen led to increase of potassium, sodium, zinc, iron, and magnesium contents from 230.12 mg, 111.28 mg, 1.66 mg, 0.89 mg and 0.95 mg of the healthy stem to 364.21 mg, 203.64 mg, 3.02, 1.52 mg, and 2.18 mg respectively in the infected stem sample. The composition of zinc, sodium, magnesium, iron and potassium in the leaf recorded an increase

of 15.50%, 81.52%, 2.76%, 37.17%, and 55.50% respectively whereas those stem increased by 81.92%, 83.00%, 11.79%, 70.79%, and 58.27% respectively after inoculation with the test fungus (Table 2).

4. DISCUSSION

The biochemical composition of cowpea leaf and stem (Table 1) showed that the leaves of healthy cowpea contain higher amounts of nutrients than the healthy stems which is in agreement with [18] and [4] who reported higher nutrients and minerals in the leaf than seed specimens of cowpea but at variance with the report of [19] where lower protein value was recorded for leaf than the seed of cowpea. The differences may be due to varietal and age of the crop, processing methods or differences in edaphic and climatic conditions.

C. destructivum obtained nutrients from cowpea, thus distorting their anatomy and physiology which led to lesions on the leaves and stems (Plate 1). The resulting lesions on cowpea leaf has been reported to reduce the photosynthetic area and rate of the crop as well as causing a reduction of the total chlorophyll and nutrient contents of leaves [20, 21] which is in agreement with the findings of this study where the pathogen caused lesions on infected leaves and stems of the crop (Plate 1 A1) and reduced their nutrient composition (Table 1).

Species of *Colletotrichum* derive their food and energy from their hosts while killing the host tissues well in advance of their hyphae [22] which confirms the depletion of the nutrient composition of the test cowpea by *C. destructivum*. Low levels of protein, carbohydrates, calcium and phosphorus were recorded in the infected cowpea indicating that the fungus used the plant as a source of metabolizable carbon for its calorific and amino acids needs to build its protoplasm which could be the reason for the high susceptibility of the test cowpea (Variety IAR-48) to *C. destructivum* in this study. The low level of phosphorus and crude fiber recorded in the infected host tissues suggest that the pathogen dispossessed the cowpea of metabolizable P required for the structural architecture of the crop and compromised the structural integrity of the infected tissues of cowpea [23] that led to reduced disease resistance and growth on the leaf and stem (Plate 1).

Table 1. Effects of *Colletotrichum destructivum* on the proximate compositions of leaf and stem of cowpea 8 WAP

Nutrients	Biochemical composition (%)					
	Leaf			Stem		
	Healthy	Infected	% Loss	Healthy	Infected	% Loss
Moisture	10.88	12.51	14.98*	11.80	12.65	7.20*
Protein	34.91	20.40	41.56	15.64	12.69	18.86
Fat	5.42	2.15	60.33	1.29	0.78	39.54
Carbohydrate	43.61	37.03	15.09	31.11	25.39	18.37
Ash	11.15	11.24	7.17*	12.05	11.98	0.58
Crude Fibre	19.46	15.53	20.19	16.87	14.62	13.34
Mean	20.91	16.48	34.29	14.79	13.02	18.14
LSD (0.05)	5.01	4.29	7.86	4.11	7.20	8.25

Values are means of three replicates in two separate experiments. * = % Increase

Table 2. Effect of *Colletotrichum destructivum* on the mineral compositions of leaf and stem after 8 weeks incubation period

Element	Mineral composition (mg/g)					
	Leaf			Stem		
	Healthy	Infected	% Increase	Healthy	Infected	% Increase
Calcium	1615.2	1584.86	1.89*	2.09	1.19	43.06*
Phosphorous	554.01	436.97	21.13*	326.50	299.10	8.39*
Zinc	121.84	140.73	15.50	1.66	3.02	81.92
Sodium	2216.1	4022.60	81.52	111.28	203.64	83.00
Magnesium	1658.84	1704.58	2.76	1.95	2.18	11.79
Iron	65.21	89.45	37.17	0.89	1.52	70.79
Potassium	13445.25	20907.10	55.50	230.12	364.21	58.27
LSD (0.05)	250.01	414.29	3.23	64.11	87.20	2.88

Values are means of three replicates in two separate experiments. * = % Decrease



Plate 1. Healthy and infected cowpea: A 1-2: Infected cowpea leaf with anthracnose lesions caused by *C. destructivum*; B 1-2: Healthy (uninfected) cowpea leaf without lesions.

In plants, calcium is one of the major nutrients required for its proper growth and functioning since it plays a significant role in strengthening and thickening of plant cell wall culminating in strong structural rigidity occasioned by interlocking cross-walls within the pectin-polysaccharide matrix that holds the cell walls tightly together [24]. The depletion of Ca in this study in the infected cowpea leaf and stem (Table 2) may explain the inability of the plant to develop and maintain structural rigidity against the test fungus and/or inhibit the activities of the cell macerating enzymes of the organism and prevent the initiation, colonization and damage of the crop by *C. destructivum*.

The depletion of nutrients and increase in trace elements like sodium, zinc, magnesium and iron in the infected crop suggest that the fungus interfered with the synthesis of carbohydrate and proteins and utilization of the minerals by the crop which led to accumulation of Zn, Na, Mg, and Fe (Table 2). Excessive amounts of magnesium ions in plants results in reduced rate of photosynthesis indicating that the high magnesium ions detected in this study (Table 2) may have disrupted photosynthetic activity of the crop which agrees with the findings of [21] on common bean where after 4 days post-infection with *C. lindemuthianum*, the organism reduced the photosynthetic rate of the crop by 77% consequently causing a reduction of the total chlorophyll content of its leaves. Potassium (K^+) plays essential role in plant growth and metabolism but excessive amounts of K^+ in a study by [25] on strawberry encouraged infection by *C. gloeosporioides* indicating that the high amount of potassium recorded in this study enhanced infection of cowpea leaf and stem by *C. destructivum*. Also the high amount of sodium ions (Na^+) observed in this study in the infected cowpea affirms the report of [26] that incorporation of high amounts of sodium salts in the growth medium significantly enhanced hyphal growth and enhanced conidial germination of *B. cinerea*.

Infection of cowpea leaf and stem by *C. destructivum* significantly reduced the major nutrient contents and increased the accumulation of minerals in the tissues of infected cowpea (Table 2). This finding is congruent with [16] who reported that infection of cowpea leaves and seeds with *Cercospora cruenta* led to increased severity of the disease, accumulation of elementals and depletion of protein, crude fibre, fat, and calcium in the test organs of cowpea. *C.*

destructivum caused significant losses in the nutrient value of the crop (Table 1). Storage and seed-borne pathogens of legumes and cereals have been reported to cause qualitative and quantitative losses [27,28,29] which are in agreement with the findings in this study where association and colonization of cowpea leaf and stem by *C. destructivum* led to the average loss of 34.29% and 18.14% nutrient values respectively.

5. CONCLUSION

Infection of cowpea leaf and stem by *C. destructivum* significantly reduced the major nutrient contents and increased the accumulation of minerals in the tissues of infected cowpea. The protein, fat, carbohydrate, and fibre contents decreased in the infected leaf and stem whereas the moisture content increased in the infected samples. The composition of zinc, sodium, magnesium, iron, and potassium in the leaf increased whereas calcium and phosphorous decreased after inoculation with the test fungus. Also, the fungus caused a reduction of the calcium and phosphorus contents of the healthy stem tissue whereas infection of the stem with the pathogen led to increase in potassium, sodium, zinc, iron, and magnesium contents. The average loss of the major nutrients; protein, fat, and carbohydrate was more in the leaf (34.29%) than stem (18.14%) after 8 weeks planting.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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