

Effect of colletotrichum leaf spot disease on growth parameters of *colocynthis citrillus* and *cucurbita pepo*

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ABSTRACT

Colletotrichum lindemuthianum was isolated and identified as the main pathogen causing leaf spot disease of *Colocynthis citrillus* (melon) and *Cucurbita pepo* (pumpkin) in Cross River State, Nigeria. The pathogen's symptoms (observed in May) were known to have impaired growth processes in the hosts. Growth parameters were read at 21 and 45 days post inoculation (21 and 45 Dpi). There was reduction in the Leaf area ratio (LAR) of the diseased plants as infection progressed. The Net assimilation rate (NAR) was higher in infected *C. pepo* at 21 Dpi ($2.3 \times 10^{-3} \text{ gmcm}^2 \text{ day}^{-1}$) and 45 Dpi ($3.2 \times 10^{-4} \text{ gmcm}^2 \text{ day}^{-1}$) than the healthy samples (9.0×10^{-4} and $2.4 \times 10^{-4} \text{ gmcm}^2 \text{ day}^{-1}$ respectively). Reduction in NAR for *C. citrillus* was observed at 45 Dpi (-1.6×10^{-3}). This general situation led to reduction in the relative growth rate (RGR) of the assay cucurbits at 45 Dpi.

INTRODUCTION

Cucurbits belong to the large family cucurbitaceae and has perhaps more species in cultivation than any other family in Nigeria. Hardly a day passes that all or part of the food items prepared in homes in Nigeria is not made up of cucurbits. They constitute very important items in the diet and occupy a special place in the life and culture of the many ethnic groups in Nigeria (Okoli 1984).

Because of their roles in the dietary requirements of Cross Riverians, they are cultivated in nearly all farms in mixed and mono cropping systems. Cucurbits are grown in both temperate and tropical areas. Optimum growth is obtained at temperature within the range of 21 – 28°C, moderate humidity and little diurnal variation (Yaniv *et al*, 2004) Full exposure to sun is beneficial and growth is retarded in humid conditions which encourage leaf diseases, reduce flowering and adversely affect fruit quality. (McGrath, 2004).

C. citrillus has extremely bitter fruits but the seeds are extracted for cooking. They can be removed and roasted as an edible commodity (Soliman *et al*, 1985). The seeds are rich in oils and can be ground into powder and used as a soup thickener or flavoring agent (Badafu and Ogunsua, 1991).

The genus *Cucurbita* contains species with fine-grained flesh and excellent nutritive properties (Wessel-Beaver and Varela, 1991). The spaghetti squash (*C. pepo*), has an internal fleshy texture which resembles strands of spaghetti after cooking. They are all eaten with stew, sauce or soup after cooking (Loy, 1990).

The cultivation of cucurbits in Nigeria is faced with a lot of constraints, among which are the crops susceptibility to diseases and

in, Cross River State, colletotrichum anthracnose is most predominant causing leaf spots which affect growth thus reducing nutritive content and market value generally.

Anthracnose is a very common disease of cucurbits in Cross River State Nigeria. It appears first as a water-soaked spot on the foliage. The spots enlarge, turn brown and shatter Under severe attack, the entire leaf dies and floral abortion is observed. On fruits, sunken areas appear as black, circular spots that do not penetrate deep but provide entry points for ingress of fungi and bacteria. The fungus survives in infected debris (Yamaguchi, 1983).

This study was aimed at assessing the effect of the disease on host growth parameters.

MATERIALS AND METHODS

Cucurbits studied and preservation

Colocynthis citrillus (Melon) and *Cucurbita pepo* (Spaghetti gourds) were the two (2) commonly consumed cucurbits used for this study. Seeds of the cucurbits were collected from the mature healthy pods and preserved in airtight containers sterilized with methylated spirit (Yaniv *et al*, 1994).

Source of inoculum and preservation

Inoculum was obtained from disease host plants across the study area. Preservation of inoculum was in the laboratory on agar slant and in growing hosts (Heist *et al*, 2001).

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In Heist *et al.*, (2001) method, isolates were maintained by scraping fungal spores (with a 0.6cm artist's brush) from culture in Petri dishes into centrifuge tubes containing sterile distilled water. The tubes were swirled gently in the centrifuge for 5 minutes to obtain a spore suspension.

Using a hypodermic syringe equipped with a 20-gauge needle, 1ml of the suspension was sprayed on the abaxial surface of 1-month-old host plant leaves. The leaves were allowed to stay for 2 days before being detached into black polyethylene bags moistened with sterile cotton wool soaked in sterile distilled water. Sporulation was observed after 1-2 weeks post-inoculation (1-2 Wpi). However, periodic transfers to freshly prepared media were also maintained to ensure constant availability.

Isolation of *Colletotrichum lindemuthianum* from host plants.

Test plant leaves with characteristic anthracnose symptoms due to *C. lindemuthianum* (which included dark brown spots) were collected from the field. These leaves were washed under a flowing tap; surface sterilized with ethyl alcohol and finally rinsed with several changes of sterile distilled water. The diseased portions were cut out at the interface between the healthy and the diseased tissues.

These portions were placed on a gelled potato dextrose agar in Petri dishes, incubated at 25±1°C until growth was observed. Through series of sub-culturing, pure cultures were obtained and maintained on agar slant in a refrigerator (Udo *et al.*, 2001)

Pathogenicity test

For the spore inoculation, a spore suspension was produced using the method of Heist *et al.* (2001).

With a sterile hypodermic syringe equipped with a 20 gauge needle, approximately 5×10^5 spores were inoculated on the abaxial surface of wet healthy host leaves by spraying to run-off level.

The leaves were covered with transparent polyethylene bags and allowed to stay for 24 hours. Spores measurement was done with a hemacytometer. The control experiment was carried out with sterile distilled water without spores.

All the experimental set-ups were observed for symptom development. The experiments were replicated five (5) times each.

Effect on leaf area

Data were taken at symptoms development. This was done by ascertaining a correlation between the leaf area and the linear dimensions of the leaf width and length.

The leaf area was calculated using the formula below:-

$$A = KLB \quad (1)$$

where A = leaf area, B = leaf width, L = leaf length and

K = correlation coefficient (constant)

Effect on leaf area ratio (LAR)

This analysis was carried out destructively. It represents the ratio of total leaf area (A) to the whole plant dry weight (W) over a period of time.

The formula used is as follows:

$$\frac{A_1}{W_1} + \frac{A_2}{W_2} + \frac{A_3}{W_3} \dots + \frac{A_n}{W_n} =$$

$$\frac{LAR_1 + LAR_2 + LAR_3 \dots + LAR_n}{N} \quad (2)$$

Effect on net assimilation rate (NAR)

This was analyzed to show the efficiency of the healthy and infected leaves at photosynthesizing materials for the normal growth of the plants.

The formula used is:

$$NAR = \frac{\ln A_2 - \ln A_1}{A_2 - A_1} \cdot \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

Effect on relative growth rate (RGR)

The RGR explains the change in weight (W) of a growing plant over a time interval (t). The formula used for determining RGR is

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad (4)$$

where (t₂-t₁) = time interval,

RESULTS

Effect on leaf area.

At seven days post-inoculation (7Dpi), the infected leaves of *C. citrillus* was seen to increase in area than the healthy leaves. This trend was maintained at 21Dpi up to 45Dpi. In *C. pepo*, increase in leaf area was noted in the infected host at 7Dpi and 21Dpi only. At 45Dpi, there was a marked reduction in leaf of the assay plant (Table 1).

Growth parameters of *colocynthis citrillus* and *cucurbita pepo*

Table 1. Leaf area (cm²) of assay cucurbits recorded within study period.

Dpi	<i>C.citrillus</i>		<i>C. pepo</i>	
	Hi	Di	Hi	Di
7	99.45±0.15	109.99±0.22	244.41±0.88	253.79±0.74
21	124.53±0.50	154.47±0.25	282.82±0.08	308.99±0.61
45	139.44±0.31	158.82±0.03	305.26±0.56	288.49±0.81

NOTE; Data are means of 5 replicates ± S.E., Dpi =Days post inoculation. Hi =Healthy. Di = Diseased.

Effect on leaf dry weight.

The leaf dry weight of the infected test plants were observed to have increased at the initial period of infection. Table 2 shows results of

leaf dry weight for study period. As can be observed, there was an initial increase in most of the test plants dry weight from 7Dpi up to 21Dpi and a drastic drop at 45Dpi for the two (2) test plants.

Table 2. Leaf dry weight (g) of assay cucurbits recorded within study period.

Dpi	<i>C.citrillus</i>		<i>C. pepo</i>	
	H1	D1	H1	D1
7	6.30±0.12	6.48±0.05	7.76±0.22	7.88±0.25
21	6.98±0.17	7.50±0.50	8.48±0.51	9.14±0.31
45	7.22±0.53	6.90±0.23	8.68±0.50	8.36±0.11

NOTE; Data are means of 5 replicates± S.E., Dpi =Days Post inoculation, Hi =Healthy, Di = diseased

Effect on leaf area ratio (LAR)

Leaf area ratios obtained at 21Dpi and 45Dpi showed much variation among the species studied and within ages.

In *C. citrillus*, the LAR for inoculated plants were lower at the two data collection dates (21Dpi and 45Dpi). At 21Dpi, the inoculated

samples had the LAR lower than the healthy by 11.13cm²g⁻¹ while at 45Dpi, it was 3.13 cm²g⁻¹ less. The same trend was observed for *C. pepo* (Table 3).

Table 3. Leaf area ratio (LAR) of healthy and infected test plants at 21 and 45Dpi (Unit=cm²g⁻¹).

SPECIES	DPI			
	21		45	
	Hi	Di	Hi	Di
<i>C.pepo</i>	34.44	21.74	27.92	-31.25
<i>C. citrillus</i>	29.56	18.43	19.82	16.69

NOTE; Data are means of 5 replicates ± S.E., Dpi =Days Post inoculation, Hi =Healthy, Di = diseased

Effect on net assimilation rate (NAR).

The NAR for the inoculated samples were higher in the two (2) test cucurbits than in the healthy specimens. These results are presented on Table 4

Table 4. Net assimilation rate (NAR) of healthy and infected test plants at 21 Dpi and 45Dpi.

SPECIES	DPI			
	21		45	
	Hi	Di	Hi	Di
<i>C.pepo</i>	9.0×10^{-4}	2.3×10^{-3}	2.4×10^{-4}	3.2×10^{-4}
<i>C. citrillus</i>	4.5×10^{-4}	3.2×10^{-3}	6.7×10^{-4}	-1.6×10^{-3}

Effects on relative growth rate (RGR)

The relative growth rate was calculated to obtain the change in weight of the healthy and infected test plants over some time intervals that were taken at 21 and 45 days after inoculation for the infected plants and the same interval for the healthy plants that served as control experiments (Table 5).

In all the species of test plants, there was an increase in biomass at 21 dpi. *C. pepo* had a value of $3.1 \times 10^{-2} \text{ gmcm}^{-2} \text{ day}^{-1}$ for healthy stands and $5.0 \times 10^{-2} \text{ gmcm}^{-2} \text{ day}^{-1}$ for the infected ones at this point of initial infection. For *C. citrillus* $1.33 \times 10^{-2} \text{ gmcm}^{-2} \text{ day}^{-1}$ was obtained for healthy plants (control) while $5.86 \times 10^{-2} \text{ gmcm}^{-2} \text{ day}^{-1}$ was for infected specimens.

Table 5. Relative growth rate (RGR) of healthy and infected test plants at 21 and 45 Dpi

SPECIES	Dpi			
	21		45	
	Hi	Di	Hi	Di
<i>C.pepo</i>	3.1×10^{-2}	5.0×10^{-2}	6.7×10^{-3}	-1.0×10^{-2}
<i>C. citrillus</i>	1.3×10^{-2}	5.9×10^{-2}	1.4×10^{-2}	-2.7×10^{-3}

Unit = $\text{gmcm}^{-2} \text{ day}^{-1}$

DISCUSSION AND CONCLUSION.

The effect of *C. lindemuthianum* attack on growth of the test cucurbits was analyzed from the view point of dry matter production. There was marked decrease in the LAR of the inoculated cucurbits at 21Dpi. This decrease continued at 45Dpi. It will be recalled that there was initial increase in leaf area of the infected cucurbits but even at this, the LAR was observed to drop. This may be because photosynthetic cells at the site of infection were destroyed and it is known that the amount of light intercepted for production of dry matter is always directly proportional to the leaf area. This implies that during infection, photosynthetic efficiency is reduced while respiration is increased. This agrees with the works of Sharma and Sharma, (1990) on excessive respiration in hypertrophied peach leaves infected by *Taphrina deformans*.

The net assimilation rate (NAR), estimates the rate of carbohydrate output from the photosynthesis system minus the loss due to respiration. At 21Dpi, except in *C. sativus*, there was increase in NAR. this may be because at early infection stage, the host cells try to increase in their photosynthetic efficiency to meet up with the high rate of respiration and as the pathogen also increases its efficiency of utilization of the resources, the rate of photosynthesis will reduce as can be seen in the decrease at 45Dpi except in *C. pepo* This agrees with the works of Pawal *et al* (1990) and Lowry *et al* (2002).

The relative growth rate (RGR) is the measure of average efficiency of each unit of dry matter in the rate of production of new dry matter. At 21Dpi, there was increase in the RGR of diseased test plants compared to the healthy plants. At 45Dpi, results for all inoculated plants dropped. According to Ting, (1982), a reduction in the relative

growth rate (RGR) of a particular plant, leads to a concordant reduction in the yield of the plant so affected thus, the yield of cucurbits infected by *C. lindemuthianum* is bound to reduce.

The leaf area ratio (LAR) is an index of a plant's leafiness. A high (LAR) can make for a highly efficient plant. This implies that in a plant infected by a fungus with the main infection site being the leaf, the photosynthetic portion will be reduced compared to the healthy plant and this will in effect reduce the LAR and the whole plant growth in general.

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