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INFECTION RELATED CHANGES IN NUTRITIONAL CONTENTS OF FLUTED PUMPKIN (*Telfairia occidentalis)* INFECTED BY *Diplocossum spicatum* AND CONTROL USING PLANT EXTRACTS

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ABSTRACT

Tailairia occidentalis is an important vegetable crop in the south-eastern part of Nigeria. It is consumed in various ways by the different communities. Most farmers grow it for sales hence; it is a high income earner. In the 2007 and 2008 cropping seasons, a high incidence of leaf spot disease was observed. A Pathological study based on Completely Randomized Block Design (CRBD) was carried out and the result implicated *Diplocossum spicatum*, as the fungal pathogen causing leaf spot disease of the crop in Cross River State, Nigeria.

Infection was mainly through spores and symptoms normally develop in the early rains in April,. Infection by this fungus significantly reduced the carbohydrate and vitamins contents in the host plant. However, there was increase in the protein (+ 4.64%) and moisture (+ 6.16%) contents of the infected leaf samples ($P< 0.05$). For fat content, there was only a negligible decrease in the infected samples. Fungal sporulation was effectively controlled with aqueous extract of *Sacocephalus latifolium* (0.8×10^{1}) and ethanolic extract of *Danielli olivera* (0) all, at 30% concentration. Spore germination was as well reduced to 0% by ethanolic extract of *Danielli olivera.*

KEYWORDS: *DiplocossIum spicatum, Danielli olivera,* Sporulation, *Sacocephalus latifolium,* Moisture Content

INTRODUCTION

Telfairia occidentalis (Fluted pumpkin) belongs to the family Cucurbitaceae. The leaves are an important food vegetable for many people especially in the mid-western and eastern parts of Nigeria. It is a climber with long coiled tendrils and the stem can be as long as 10 metres (Osagie and Eka, 1993).

According to Tindall (1983), approximately 0.5kg of leaves and shoot can be obtained from one plant per harvest and up to 15 harvests can be obtained between 3-4months. Apart from the leaves and young shoots being used for soup, the seeds are also cooked and eaten by humans and can as well be pounded after cooking and used as soup thickener in some parts of West Africa.

Due to its various uses, this crop seems to be more in vegetable cultivation than any other species in places where it is consumed. However, the production of this vegetable is faced with a lot of constraints which include, attack by insects, nematodes and susceptibility to diseases. However, infections can be avoided by knowing the disease that threatens the crop, their causative agents, method of spread and immediate control processes.

This work therefore was aimed at isolating the fungal pathogen causing leaf spot disease of *T. occidentalis* in Cross River State, Nigeria, its effect on host nutritive contents and control using plants extracts.

METHODS

Isolation of Fungal Pathogens

Isolation of the pathogen was done using the method of Amadioha, (1998). In this, diseased test plants were collected from four farms across the State in May 2005. The infected leaves were washed in different changes of sterile distilled water and then surface sterilized with 70% ethanol. Using a sterile scalpel, the infected spots were cut into sections and plated on potato dextrose agar (PDA). The plates were incubated at 27^0C until visible growth was observed, and pure cultures of the pathogens were maintained as stock culture at 4^oC. Identification of the fungi was done by matching and comparing features of the isolated fungi with features of those in a compendium of fungi by Barnett and Hunter (1972).

Pathogenicity Test

To test pathogenicity of the isolated fungi, some pieces of mycelia and spores were re-inoculated into potted healthy host plants that were grown in sterilized garden soil in a green house.

On a wet healthy host leaf, an infection court was created by scraping with a sterile needle. A piece of mycelium of each fungus was inoculated on wound created and thereafter covered with a transparent polyethylene bag for 24 hours. In the control, wound was created and only a piece of gelled PDA was introduced.

For the spore inoculation, a spore suspension was produced using the method of Madunagu *et al* (2005). With a sterile hypodermic syringe, 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 haemocytometer. The control experiment was carried out with sterile distilled water without spores. The experimental set-up was replicated 5 times and observed daily for symptoms development.

Determination of Nutritive Contents

One hundred (100) grammes of fresh leaves of the test plant were collected from the green house after which both the healthy and diseased leaves were selected and thoroughly washed under a flowing tap. The samples were wrapped in aluminium foil and dried in a hot air oven (70^oC for 24hrs), ground into powder and stored in a refrigerator in airtight bottles. The samples used for moisture content were shredded fresh with a sterile kitchen knife. Fresh samples were also used for vitamin determination.

The samples were each analysed for the various nutrient constituents by the methods of Association of Official Analytical Chemists (A.O.A.C 1975). The percentage (%) increase (+) or decrease (-) in the nutritive contents of the samples was analysed using the method of Udo, *et. al.* (2007) as shown below;

$$Dc = (Di - Hi)$$

$$Dc\ (\%) = \frac{Dc}{Hi} \times \frac{100}{1}$$

Where Dc = Difference in contents

Dc (%) = % difference in contents

Hi = Healthy

Di = Diseased

Preparation of Extracts

Medicinal plants used for the experiment were *Danielli olivera* Mill. (Fabaceae), *Sacocephalus latifolium* Schum (Sapindaceae), *Smilax krausiana* L. (Liliaceae) *and Salacia pyriformis* Chev. (Fabaceae). Plant nomenclature was confirmed from the Forestry/Wildlife and Botany Herbaria of Cross River University of Technology and University of Calabar, respectively. Collection was done according to Singh and Jain (2004). The leaves were collected in the morning and taken in a vasculum to the laboratory for preparation for screening. Only leaves of these plants were used. Choice of the plants was based on availability in the farming areas, proximity to the farmers and accessibility to growth area. Only healthy and mature leaves were collected for use.

Aqueous Extraction

Five (5) grams of shade-dried ($28\pm1^{o}C$) and pulverized test plants leaves was added to 25ml of distilled water in a test tube. The mixture was covered with aluminium foil and allowed to stand for two (2) days. The sample was filtered and the filtrate stored in a refrigerator.

To prepare ethanolic extract, 25ml of 80% ethanol was mixed with five (5) grams of each of the pulverized plant samples in test tubes. The mixture was allowed to stand for two (2) days after which it was filtered and the filtrate stored in a refrigerator as stock solution.

Phytochemical Screening of Plants Extracts

The methods of Culei (1982) and Sofowora (1984) were used to test for presence of the various active components found in the medicinal plants.

Antifungal Test

Effect of the extracts was determined by measuring fungal sporulation, radial growth and spore germination.

Effect on Sporulation

This was done according to Mao and Newman.(1998). In doing this, 25ml of sterile distilled water was poured on a 5-day old culture growing in plates containing different percentages (0%, 10%, 20%, and 30%) of the extracts. The plates were covered and carefully swirled for the spores to drop from the sporophores. The water and, spore mixture was decanted into centrifuge tubes and centrifuged at 4,000 x g for 5 min. The supernatant was decanted and, 3 ml of sterile distilled water added. Spore concentration was then estimated for every 1ml of spore mixture with a haemocytometer under a binocular microscope.

Effect of Plant Extracts on Spore Germination

A 5ml spore suspension of a 5-day old culture of test fungus was filtered through sterile cheesecloth, mixed with 1ml of each extract and, dropped on a film of molten medium on a glass slide. This was incubated in a micro-humidity chamber at room temperature ($28\pm1^{0}C$) for 24 hours. In the control experiment, no extract was added. The number of spores germinated was counted under the light microscope (X10 magnifications) after 6 hours (Onifade, 2000).

The percentage inhibition of spore germination was calculated using the formula below:

$$\% \text{ inhibition} = \frac{NC - NT}{NC} \times \frac{100}{1}$$

Where:

NC = number of spores germinated in the control

NT = number of spores germinated in the treatment

RESULTS

Isolation of Causal Agent

The fungus that was isolated from the test plant was observed and identified as *Diplocossum spicatum.* Growth of the pathogen on the artificial media was observed three (3) days after inoculation.

Pathogenecity Test

Symptoms development was observed 6 days post inoculation (6dpi) for mycelial inoculation and 5 dpi for spore inoculation. It was also observed that leaf spot only developed at the point of inoculation for mycelial inoculation and at several points for spore inoculation. Symptoms were seen to have developed from abaxial surface of the leaves.

Effect on Nutritional Content

Results of analysis for nutritional contents of the healthy and infected leaves are as shown on Table 1. The results showed that, there was an increase in protein content of the infected leaves (45.10mg/100gm) over the healthy leaves (43.10mg/100gm) at 14dpi. A reverse trend was observed for carbohydrate content of the test leaves. The infected samples had a lower carbohydrate content (1.60mg/100g) compared to the healthy one (2.30mg/100g). There was no significant difference in the amount of fat in the infected (66.mg/100g) and healthy 66.5mg/100g) samples. Moisture content was more in infected leaves (91.40mg/100g) than in non-infected (86.10mg/100g). The healthy leaves had more vitamin content than the infected samples.

Phytochemical Contents of Medicinal Plants

The results of phytochemical screening of equeous and ethanolic contents of the medicinal plants are presented on Table 2.

Antifungal Test

Effect on Sporulation

Table 3 shows the result of sensitivity test of the aqueous extract of medicinal plants on *D. spicatum.* The results show that *S. latifolium* could reduce sporulation of the fungus at 30% concentration to about 10 spores per ml of spore suspension. The least effective (0.9×10^2/ml of spore suspension) was aqueous extract of *D. olivera.*

The result of ethanolic extracts' potency on the test fungus is presented on Table 4. Highest potency was shown by *D. olivera at 30% concentration.* This was followed by *S. latifolium* (0.9×10^1) and the least potent (0.6×10^2) was *S. pyriformis.*

Effect of Plant Extracts on Spore Germination of *D. spicatum*

The result of effect of aqueous and ethanol extracts of the test plants on spore germination of *D. spicatum* is shown in Tables 5 and 6 respectively. All the plant extracts (except the control), had varying degrees of potency on spore germination of the test fungus.

Aqueous extract of *S. krausiana* recorded the least percentage spore germination (16.5%) at 30% conc. and was not significantly different ($P>0.05$) from that of *S. latifolium*. The highest percentage germination was shown by *D. olivera* (83.4%).

For ethanolic extract, the highest percentage of spore germination suppression was shown by *D. olivera* at 30% concentration. No spore (0%) was observed to have germinated at this concentration.This was followed by extracts of *D. olivera* (16.4% at 20% conc.) and *S latifolium* (16.6% at 30% conc). which were not significantly different in performance. The least effective was *S. pyriformis* which recorded 82.7% as permissible spore germination (PSG) rate at 10% concentration. The reduction in spore germination increased with increase in the concentration of the extracts.

DISCUSSIONS

The result of the experiment implicated *Diplocossus spicatum* as the pathogen that caused the leaf spot disease of *Telfairia occidentalis* in Cross River State, Nigeria. It was found out that the main inocula were the spores and infection was by June during the early rains in the cropping season.

When analysis was carried out on the effect of attack by the fungus on the host plant, results showed that there was an increase in protein content of the diseased leaves. This agrees with the work of Mehrotra and Aggarwal (2004) who explained that during infection, there is always an increase in the quantity of protein in the host due to, the production of more protein by the pathogen and the production of more enzymes to strengthen the host immune system. There was reduced carbohydrate content in the infected leaves compared to the healthy ones. This may be due to the increase in respiration of the host plant and the utilization of carbon in carbohydrate by the pathogen for its physiological processes (Uritani, 1971).

There was no difference in fat content. Moisture content in the diseased test plant increased because of increase in growth of the pathogen (Mishra and Rath 1986). There was reduction in vitamin content of the infected plants. This may be because during pathogenesis, the vitamins are broken down and their contents serve as energy source for the pathogen and may also be because, photosynthesis is impaired so, there is reduction in the production of vitamin by the infected host.

The antifungal test revealed that production of spores (which were the main inocula) can be checked with some plants with medicinal potentials. Aqueous extract of *S. latifolium* showed high potency for Sporulation at 30% concentration and aqueous extract of *S. krausiana* recorded the least percentage spore germination (16.5%) at 30% conc.. This implies that at a higher concentration, spore production can be inhibited and spore germination by *S. krausiana* especially at a higher concentration. Ethanolic extract of *D.olivera* inhibited spore production totally at the same concentration of 30%. The potency must have been conferred by the presence of flavonolds (Malik and Singh, 2002). This shows that aqueous extract of *S. latifolium* and ethanol extract of *D. olivera* can be used for control of the leaf spot disease in this area. The reason for their potency will be due to the content of active phytochemicals in the extracts (Enokpa, 1995, Udo, *et al* 2001, Markson, *et al* 2005, Madunagu *et al* 2005).

Table 1: Effect of *D. spicatum* Infection on Nutritional Composition (mg/100g) of *T. occidentalis*

S/N	Nutrient	Di	Hi	Dc	Dc (%)
1.	Protein	45.10	43.10	+ 2.00	+ 4.64
2.	Carbohydrate	1.60	2.30	- 0.70	- 30.43
3.	Fat	66.40	66.50	- 0.10	- 1.50
4.	Moisture	91.40	86.10	+ 5.30	+ 6.16
5.	**Vitamins:**				
	A (RE)	1532	1550	- 18.00	- 1.16
	B_1	0.66	0.68	- 0.02	- 2.94
	B_2	1.15	1.17	- 0.02	- 1.71
	C	0.089	0.091	- 0.002	- 2.20

Note: RE = Retinol Equivalent
Di = Diseased (infected)
Hi = Healthy (Non-infected)

Table 2: Phytochemial Contents of Aqueous and Ethanolic Extracts of Medicinal Plants

Phytochemical	Plants Extract							
	D. olivera		*S. latifolium*		*S. krausiana*		*S. pyriformis*	
	A	E	A	E	A	E	A	E
Tannins	+	-	+	-	+	-	+	-
Anthraquinons	-	-	-	-	-	+	-	+
Phlobatannins	+	+	-	+	-	-	-	-
Cardiac glycoside	-	-	+	-	+	+	+	+
Alkaloids	+	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-
Flavonoids	-	+	-	-	-	-	-	-
Polyphenols	-	-	-	+	+	+	+	+

Note: + = Present; - = Absent; A = Aqueous; E = Ethanolic

Table 3: Spore Count in Aqueous Extracts of Medicinal Plants

Conc.(%)	Spore Count (Approx./ml of Suspension) / Plant Extract			
	D. olivera	*S. latifolium*	*S. krausiana*	*S. pyriformis*
0	2.0×10^3	2.0×10^3	2.0×10^3	2.0×10^3
10	1.2×10^3	1.5×10^2	2.6×10^2	1.4×10^2
20	1.8×10^2	0.9×10^2	1.3×10^2	1.1×10^2
30	0.9×10^1	1.0×10^1	0.5×10^2	0.6×10^2

Table 4: Spore Count in Ethanolic Extracts of Medicinal Plants

Conc.(%)	Spore Count (Approx./ml of Suspension) / Plant Extract			
	D. olivera	*S. latifolium*	*S. krausiana*	*S. pyriformis*
0	2.0×10^3	2.0×10^3	2.0×10^3	2.0×10^3
10	1.8×10^2	1.3×10^2	1.6×10^2	1.1×10^3
20	0.4×10^1	1.0×10^2	0.4×10^2	2.4×10^2
30	0	0.8×10^1	1.2×10^1	1.1×10^2

Table 5: Percentage Spore Germination in Aqueous Extract of Medicinal Plants

Conc.(%)	Permissible Spore Germination Rate (%) / Plant Extract			
	D. olivera	*S. latifolium*	*S. krausiana*	*S. pyriformis*
0	100^{a}	100^{a}	100^{a}	100^{a}
10	83.4^{b}	58.5^{d}	54.2^{d}	64.5^{c}
20	60.6^{c}	26.1^{f}	22.8^{g}	35.9^{e}
30	26.8^{f}	18.3^{h}	16.5^{h}	21.5^{g}

Values in the same column with same superscript are not significantly different (P< 0.05)

Table 6: Percentage (%) Spore Germination in Ethanolic Extract of Medicinal Plants

Conc.(%)	Permissible Spore Germination Rate (%) / Plant Extract			
	D. olivera	*S. latifolium*	*S. krausiana*	*S. pyriformis*
0	100^{a}	100^{a}	100^{a}	100^{a}
10	51.8^{d}	56.3^{c}	45.2^{e}	82.7^{b}
20	16.4^{i}	21.4^{h}	29.8^{g}	53.4^{d}
30	0^{j}	16.6^{i}	21.4^{h}	37.4^{f}

Values in the same column with same superscript are not significantly different (P< 0.05)

CONCLUSIONS

Due to residual effect of ethanol and its cost which may be higher than the financial ability of the poor farmers who produce this crop, aqueous extract of *S. latifolium* and *S. krausiana* are recommended at a higher concentration for control of spore formation and germination respectively.

Concentration of the extracts and solvent of extraction exerted significant influence on the performance of the extracts. Effect of the extracts (water and ethanol) was concentration-dependent with, higher concentrations recording

lower spore germination. It was also observed that there were differences between the aqueous and ethanolic extracts in the type of phytochemical found. This suggests that, plant extracts differed in the amount and type of phytochemical compounds contained in them based on extraction solvent.

REFERENCES

1. Amadioha, A. C. (1998). Control of powdery mildew in pepper (*Capsicum annum* L.) by leaf extract of papaya (*Carica papaya* L). *Journal of Herbs, Spices and Medicinal Plants 6* (2):41-47.
2. A. O. A. C. (1975). Official methods of analysis. 11th edition. Washington DC.
3. Barnett, H. L. and Hunter, B. B. (1972). Illustrated genera of imperfect fungi, (3rd edition). Bungess Publishing Company. Minnesota.
4. Culei, T. (1982). Methodology for analysis of vegetable drugs. Practical manual on the industrial utilization of medicinal and aromatic plants.Centre Blvde, Romania.
5. Enokpa, E. N. (1995). Studies on the effects of lichen extracts on soybean pathogenic fungi. Ph.D. Thesis. University of Calabar, Nigeria.
6. Madunagu, B .E, Udo, S. E., Umana, E. J., and Markson, A.A. (2005). Exploitation of phanerogamic parasites and epiphytic plants for their medicinal values. *Nigerian Journal of Plant Protection. 22*: 17-23
7. Malik, V. K. and Singh, S. (2002). Effect of leaf extract of different botanicals on spore germination of *Ustilago hordei. Indian Phytopathology.* 55(3) : 395.
8. Mao, M. and Newman, I. (1998). Antimicrobial Effects Of Aqueous Extracts On The Fungi *Microsporium Canis* and *Trichophyton Rubrum* and on Three Bacterial Species; *Letter of applied Microbiology*.
9. Markson,A. A., Madunagu,B. E., Umana,E.J. and Udo, S. E.(2005).A survey and biocontrol of post harvest fruit rotting fungi of tomato. *Nigerian Journal of Plant Protection.22*: 122-131.
10. Mehrotra, R. S. and Aggarwal, A. (2004). Alteration in plants physiological functions due to plant-pathogen interaction. *Plant Pathology*. .McGraw-Hill.
11. Mishra,D. and Rath,G.C. (1986).Changes in dry matter and mineral contents of bingal fruits due to fusarial rots. *Indian Phytopathology. 38*: 584-585.
12. Onifade, A. K. (2000). Antifungal effect of *Azadirachta indica* A Juss extracts on *Colletotrichum lindemuthianum*. *Global J. of Pure and Applied Sciences*. 6 (3): 425-428.
13. Osagie, A. U. and Eka, O. U. (1998), Nutritional quality of plant foods. AMBIK press, Benin City. Nigeria. Pp. 120-133.
14. Sofowora, E. A. (I984). Medicinal plants and tranditioal medicine in Africa. John wiley and Sons Inc. Chichester London.
15. Tindall, H. D. 1983. Vegetables in the tropics. Mcmillan press, London.
16. Udo, S. E., Madunnagu, B.E. and Isemin, C. D. (2001). Inhibition of growth and sporulation of fungal pathogens on sweet potato and yam by garlic extracts. *Nigerian Journal of Botany.14*:35-39
17. Uritani, I. (1971). Protein changes in diseased plants. *Annual Review of Phytopathology. 9*: 211-234.