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The Effect of Chromolaena odorata leaf extrct on fungi associated with cassava (Manihot esculenta) (CRANTZ) tuber rot

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Abstract: *Seven fung (Aspergillus niger, Sclerotium rolfsii, Phytophthora palmivora, Rhizopus stolanifer, Fusarium solani, Botryodiplodia theobromae, and Penicillium oxalicum) were isolated from rotten cassava tubers. Pathogenicity test of these fungi implicated them as causal agents of cassava tuber rot. Chromolaena odorata aqueous leaf extracts were tested for controlling cassava tuber rot. In-vitro studies using five different concentrations (2%, 4%, 6%, 8% and 10%) of Chromolaena odorata leaf extract showed increasing inhibitory effects on mycelia growth, sporulation and spore germination with increase in extract concentration. At 2% concentration, all fungi recorded less than 10% inhibition in mycelia growth, while at 10%, growth inhibition ranged between 60.8% for A. niger and 74.9% for R. stolonifer. With respect to sporulation, R. stolonifer recorded the lowest percentage inhibition of 3.1 at 2% concentration while at 10% F. solani had the highest percentage inhibition of 66.2. The least percentage germination inhibition of 10.2was recorded at 2% concentration by R. stolonifer while, at 10%, R. stolonifer recorded the highest inhibition of 70.5%. Phytochemical analysis of the leaf extracts showed high contents of flavonoids, tannins and saponin with moderate amounts of alkaloids, terpenoids and polyphenols. These results indicate that aqueous leaf extracts of C. odorata at high concentrations can be used as agro-pesticide for the control of cassava tuber rot disease. Framers are therefore, encouraged to use this leaf extract instead of the environmentally hazardous Zeneb.*

Key words: *Leaf extract, Cassava rot, Phytochemical analysis, Invitro studies*

I. Introduction

In Nigeria as in most Sub-Saharan African countries, the agricultural sector still accounts for the major share of Gross Domestic Product (GDP), foreign exchange, and employment. Yet, per capita food production has not been able to keep pace with a rapidly growing demand for food. As a result, developing countries have become increasingly dependent on commercial imports and food aid . To reverse this trend, Nigeria and other Sub-Saharan African (SSA) governments have been designing research programs and policy initiatives aimed at achieving national food security. One of the many food crops being considered currently in this effort in SSA is cassava; in terms of its potential to ensure adequate food supply for all and generate rural household income, thereby increasing access to food. While this has led to a major expansion in cassava- based production systems in Nigeria, the desired target is still far fetched (FAO, 2000).

Cassava is an important commodity in many farming systems in Nigeria. It is therefore, not surprising that the Federal government has adopted it as the main crop in its Agricultural transformation agenda. Its relative importance stems from its adaptability to a wide range of agro-ecologies, including marginal lands and erratic rainfall conditions (Okingbo et al.,

2009). This notwithstanding, cassava cultivation in Nigeria is hindered by a number of diseases the most important being, cassava mosaic and tuber rot (IITA 2000; Olufolaji 1999). In order to improve cassava cultivation in Nigeria, a number of control measures have been introduced which include use of agro chemicals, resistant varieties as well as cultural practices.

However, the intensive and indiscriminate use of pesticides in agriculture is highly criticized because it causes serious environmental problems such as water, soil, air, food and animal contamination, poisoning of farmers, elimination of non-target organisms and selection of phytopathogens (Stangarlin et al., 1999). Aiming to minimize these negative effects of pesticides, alternative disease control measures including biological control and the use of natural products are being promoted. Plant extracts have been tested in the control of a number of crop diseases such as cocoa leaf extracts on *Phytophthora* stem canker (Okey et al., 1997); *Azadiracta indica* on *Colletotricum lindemathianum* (Onifade 2000); garlic extract on sweet potato and yam (Udo et al., 2001) and *Ocimun gratissimun* on post harvest yam rot (Okigbo and Ogbonaya, 2006). The present research is aimed at evaluating the potential of crude plant extracts from *Chromolaean*a *odorata* in the control of tuber rot disease of cassava with the view of enhancing its production.

II. Materials And Methods

Materials

Samples of naturally infected cassava tubers were obtained from local markets in Akwa Ibom state, Nigeria. *Chromolaena odorata* is a common weed in Akwa Ibom, leaves were obtained from plants around the Akwa Ibom State University vicinity. Chemicals and equipment were obtained from the Biological Science laboratory, Akwa Ibom State University, Nigeria.

Methods

Isolation and identification of fungi associated with cassava tuber rot

Infected tubers were washed under running tap water and with the aid of a sterile scalpel, 3mm x 3mm sections of the infected tissues were obtained. These sections were surface sterilized with 1% sodium hypochloride solution for three minutes. Then they were blotted dry and three each were placed on plates of potato dextrose agar (PDA) and incubated for six days at ambient temperature of 25 ^{o}C (Baudoni, 1988). The set up was observed daily for possible fungal growth. Pure cultures of the isolates were obtained through sub-culturing. The wet mounts of isolates were examined microscopically using lactophenol in cotton blue and identified based on the colony morphology, mycelia structure, spores and other associated structures (Olds, 1993; Cheesebrough, 1991).

Pathogenicity Test

The procedures of Agrios (2005) were adopted for this test. Healthy tubers were surface sterilized with 70% ethyl alcohol and wound created using a sterile cock borer. A 4mm disc of each isolate was aseptically transferred into separate wounds and the spots covered with vaseline to prevent the entry of other micro-organisms. The set up was incubated at room temperature in the lab. Daily observations were made for rot symptom development and spread on the tubers and final result recorded at 10 days. Disease severity was recorded in each treatment following a modified method of (Latha et al., 2009), where 0=healthy; 1=10%; 2=20%; 3= 30%, 4=40%, 5=50%, 6= 60%, 7=70%, 8=80% and 9≥90% of the infected area. The experiment was conducted in three replicates.

Preparation and analysis of Plant Extract

Fresh leaves of *C. odorata* were collected from nearby bushes around the Akwa Ibom State University Main campus located at Akpaden, Mkpat Enin Local Government, Akwa Ibom State. Materials were washed and 10g of each set were crushed in a mortar with pestle by adding sterile distilled water at the rate of 10ml/g of plant tissue and the homogenates were then centrifuged at 10,000 x g for 15mins at 4 ^{o}C. The supernatant solution was collected and stored in sterile containers. A portion of the extract was used for phyochemical screening following Harbone, (1973) methods. The presence of tannins was tested using ferric chloride, saponin was identified with olive oil, while flavonoids was confirmed using ammonia solution. Dilutions of 2.0, 4.0, 6.0, 8.0 and 10% concentrations (v/v) were prepared and used for bioassays.

Efficacy of extracts on fungal pathogens

The effects of leaf extracts on fungal pathogens was assessed based on three parameters; mycelia growth, sporulation and spore germination. For mycelia growth, Petri dishes of PDA media were amended with 5ml of aqueous extract of 2.0, 4.0, 6.0, 8.0 and 10.0% extract concentrations. The plates were inoculated with mycelia discs (4mm in diameter) taken from the advancing edges of 6 day-old cultures of the different isolates. Negative controls were set up in media without extract while in positive controls, media was amended with an established fungicide (6% Zeneb). The treatments and controls were incubated for 5 days at room temperature. Radial growth was measured at 5 days and then used to calculate percentage inhibition using the formula %inhibition =

$$\frac{P1 - P2}{P1} \quad x \quad \frac{100}{1}$$

P1 = radial distance of pathogens in negative control plates, P2= radial distance of pathogens with treatments.

With respect to sporulation, 10 day old cultures were flooded with 10 ml of sterile distil water. After gentle shaking the contents were filtered through a cheese cloth. Spores were counted using a haemocytometer and percentage inhibition was calculated using the formula:

$$\frac{S1 - S2}{S1} \quad x \quad \frac{100}{1}$$

where S1= sporulation in negative control, S2 = sporulation in in extract amended plates. Spore germination was also assessed by observing their germination status under a microscope. Percentage inhibition was calculated by using the formula % spore inhibition=

$$\frac{A - B}{A} \times \frac{100}{1}$$

where A = spore germination in negative control, B = spore germination in extract treated media.

Statistical Analysis

All experiments were performed trice. Data were analyzed with Analysis of Variance using MSTAT-C program version 2.10 (1991). The least significant difference (LSD) was used to test for significant differences between treatments at $P \leq 0.05$ (Gomez and Gomez, 1984).

III. Results

Isolation and identification of fungi associated with cassava tuber rot

Seven fungi were isolated from naturally infected cassava tubers. These fungi wereidentified as: *Aspergillus niger, Sclerotium rolfsii, Phytophthora palmivora, Rhizopusstolanifer, Fusarium solani, Botryodiplodia theobromae,* and *Penicillium oxalicum.*

Pathogenicity test

All the seven isolated fungi caused tuber rot on cassava but at different degrees (Table 1*).* *Fusarium solani* and *Botryodiplodia theobromae* recorded the highest disease severity of 80% and 81 respectively, followed by *Rhizopus stolonifer* with *Penicillium oxalicum* both causing 70% rots on tubers. *Apergillus niger* had the lowest disease severity of 20%.

Phytochemical Analysis of *C. odorata* leaf extracts

The phytochemical analysis of extracts indicated the presence of different levels of compounds (table 2). Flavonoids, tannin, sapronin were found in high levels while, alkaloids, terpenoids and polyphenols were contained in moderate levels.

Efficacy of extracts on pathogens

Effects of *E. odorata* leaf extracts and Zeneb on mycelia growth

The aqueous leaf extracts from C. *odorata* leaves showed varying degrees of toxicity to the seven pathogens tested, expressed as mean inhibition of mycelia growth (Table 3).

Percentage inhibition increased with increase in extract concentration . At 2% concentration, inhibition levels for all of the fungi were below 10%. However, at 6% concentration, inhibition levels increased for all the pathogens with *P. oxalicum* and *B. theobromae* recording the highest values of 40.6 % and 40.3% respectively. At 10% concentration, all pathogens recorded significantly higher levels of inhibition with the highest being 74.9% for *R. stolonifer*.

Effect of extracts and zeneb on sporultaion

Sporulation of fungi was also inhibited by leaf extracts although, at different levels (tables 4 In all the fungi tested, percentage inhibition of sporulation increased with increase in extract concentration. At 2.0 % extract concentration, sporulation inhibitory levels ranged between 3.1% for *R. stolonifer* and *7.1%* for P. palmivora, while at 6% concentration, percentage inhibition significantly increased to 45.6% for *B. theobromae* and 35.7% for *A. niger.* At 10% concentration, inhibition as high as rose to 66.2% for *F. solani* and 54.8% for *S. rolfsii*.

Effect of extracts and zeneb on spore germination

Leaf extract also inhibited spore germination in all the fungi tested (Fig. 1). The trend was similar to that reported for growth and sporulation on the pathogens. Percentage inhibition increased with increase in extract concentration and also differed among the pathogens.

IV. Discussion

The pathogenicity test in this study indicated that all the seven isolates; *Aspergillus niger, Sclerotium rolfsii, Phytophthora palmivora, Rhizopus stolanifer, Fusarium solani, Botryodiplodia theobromae*, and *Penicillium oxalicum*) can cause rot on cassava tubers with *Fusarium solani* and, *Botryodiplodia theobromae* being most virulent. These fungi have earlier been associated with cassava rots although, (Okigbo et al., 2009) found *A. niger* to be the most virulent. The pathogenicity of these organisms is however, not

restricted to cassava tubers only, but affects a wide range of other plant species such tomato, yams, sweet potato etc. (Stangarlin et al., 1999; IITA, 2000, Okigbo et al., 2009). These pathogens are known to gain entrance into the tubers through natural openings and wounds that are produced at the time of harvest or during transportation.

Different plant extracts have been reported to be effective in the control of a wide range of plant diseases. These extracts are found to be useful in the control of various plant diseases such as Cocoa canker (Okey et al., 1997); rot disease of Amaranthus (Olufolaji, 1999);

Colletotricum lindemathianum (Onifade, 2000); sweet potato and yam (Udo et al., 2001); and cassava rot (Okigbo et al., 2009). In the present investigation, crude extracts from leaves of *C. odorata* were tested for their inhibitory effects on the mycelia growth, sporulation and spore germination of seven pathogens with the view of recommending them for the control of cassava tuber rot. Leaf extracts from *C. odorata* were found to have inhibitory effects on all of the pathogens tested. This inhibition may be attributed to the high levels of fungi toxic substances flavonoids, tannins and sapronin found in the extract. Similar compounds have earlier been reported from *C. odorata* leaves (Akinmoladun et al (2007).

The inhibitory effects on growth, sporulation and spore germination varied with extract concentration as well as pathogenic organism. Increase in concentration had a corresponding increase in percentage inhibition of growth and sporulation of the pathogens. This is not unconnected with the increase in the amount of phytochemical constituents. Since all the pathogens were inhibited with respect to growth, sporulation and spor germination, and these parameters are vital in fungal pathogenic virulence, it is a strong indication that cassava tuber rot can be controlled using *C. odorata* leaf extract. In addition, it can also be reasoned that since only %10 of leaf extract was used and the rate of inhibition increased with increase in concentration, therefore, higher concentrations of extract can be used to match the high inhibition rate recorded for the positive control Zeneb.

V. Conclusion

The significance of this finding lies in its environmental impact. The use of fungicides such as Zeneb in the control of cassava tuber rot has resulted in significant environmental 213. hazards. Therefore, increase cassava yield and reduce the risk of environmental pollution, cassava farmers are advised to employ *C. odorata* leaf extract as agro pesticide instead of the traditional fungicides such as Zeneb.

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Table1: Pathogenicity test of seven isolates on cassava tubers after 8 days of inoculation.

Isolates	Disease Severity (%)
A. niger	20a
S. rolfsii	40b
P. palmivora	63c
R. stolonifer	65c
F. solani	80d
B. theobromae	81d
P. oxalicum	67c

Different letters indicate differences among treatments according to the LSD test ($P \leq 0.05$)

Table 2. Phytochemical constituents of extracts

Chemical Compounds	Level of content
Flavonoids	+++
Tannin	+++
Sapronin	+++
Alkaloids	++
Terpenoids	++
Polyphenols	++

+ = low content, ++ = moderate content, +++ = high content

Table 3: Inhibitory effects of leaf extract and Zeneb on mycelia growth of seven pathogens

Pathogens	Percentage Inhibition					
	2.0%	4.0%	6.0%	8.0%	10.0%	Zeneb 6%
B. niger	8.5a	23.1b	37.8d	49.7e	60.8g	80.5i
S. rolfsii	9.3a	20.5b	36.5d	48.5e	68.0g	82.1i
P. palmivora	8.7a	30.2c	36.9d	47.7e	63.0g	94.0j
R. stolonifer	8.4a	21.0b	37.0d	54.9f	63.4g	95.1j
F. solani	9.1a	30.4c	39.5d	56.1f	74.9h	81.6i
B. theobromae	9.0a	22.5b	40.3d	47.5e	64.0g	81.8i
P. oxalicum	9.4a	20.5a	40.6d	48.1e	69.8h	94.6j

Different letters are significantly different ($P \leq 0.05$)

Table 4: Inhibitory effects of leaf extract and Zeneb on the sporulation of seven pathogens

Pathogens	Percentage Inhibition					
	2.0%	4.0%	6.0%	8.0%	10.0%	Zeneb 6%
C. niger	6.6a	12.8c	35.7f	49.2h	55.6i	80.5k
S. rolfsii	6.5a	11.8c	36.1f	48.5h	54.8i	82.1k
P. palmivora	7.1a	16.5d	35.8f	47.6h	56.1i	94.0m
R. stolonifer	3.1b	19.5e	45.1g	55.9i7.1a	56.7i	95.1m
F. solani	3.5b	20.1e	44.8g	57.1i	66.2j	81.6k
B. theobromae	3.6b	20.3e	45.6g	57.5i	65.2j	81.8k
P. oxalicum	3.4b	21.0e	44.5g	49.1h	64.8j	94.61m

Different letters are significantly different ($P \leq 0.05$)

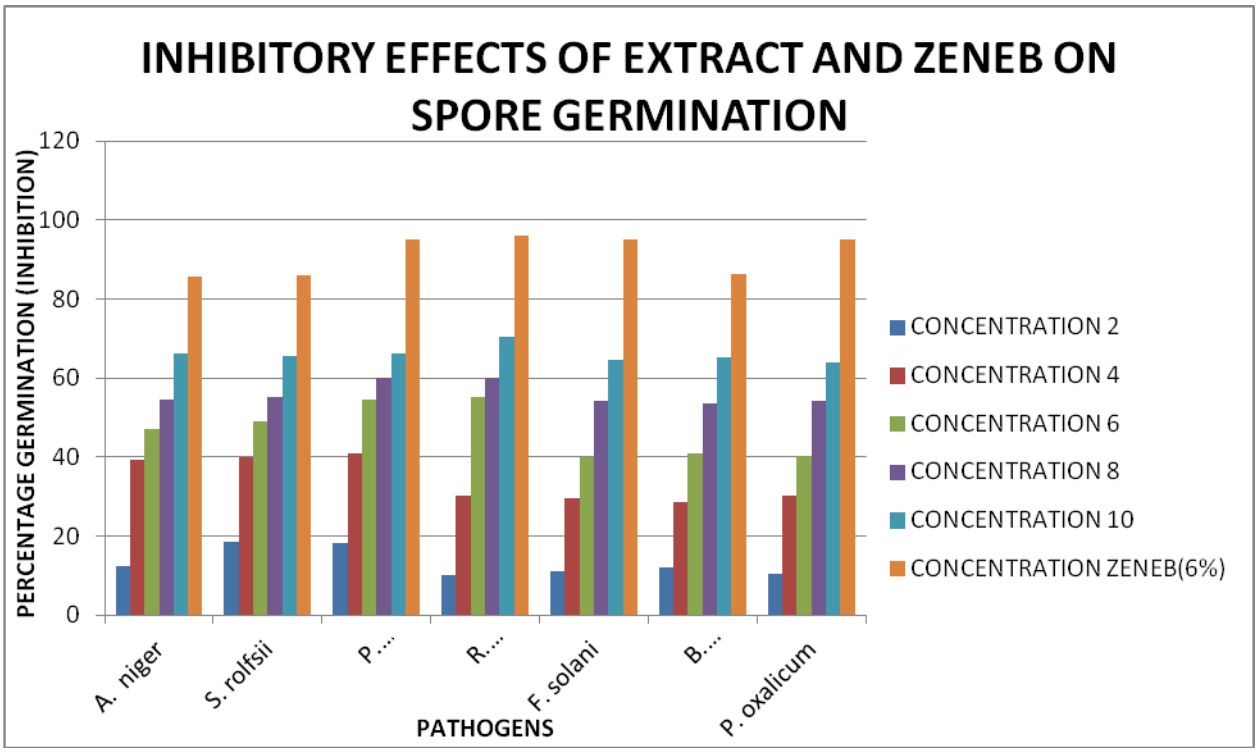

Figure 1: Inhibitory effects of leaf extract and zeneb on spore germination