Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.8, 2015

Effect of Some Plant Leaf Extracts on Mycelia Growth and Spore Germination of Botryodiplodia Theobromae Causal Organism of Yam Tuber Rot

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Abstract
Water and ethanol extracts of leaves of *Cassia alata* L., *Azadirachta indica* A. Juss., *Citrus aurantifolia* (Christm.) swingle and *Anacardium occidentale* L. were studied for in vitro activity against mycelia growth and spore germination of *Botryodiplodia theobromae* Pat, causal organism of yam tuber rot. The phytochemical screening of the extracts of the plant species revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phytobatannins and terpenes. The water and ethanol extracts showed varying degrees of fungitoxicity with the ethanol extracts being more effective. Mycelial growth of *B. theobromae* was significantly ($P < 0.05$) reduced by ethanol extracts of *C. aurantifolia* (60.37 – 73.83%), *A. indica* (51.44 – 60.46%) and *C. alata* (50 – 58.51%) during the period of incubation. Spore germination was also significantly ($P < 0.05$) reduced by ethanol extract of *C. aurantifolia* (59.68%), *A. indica* (48.69%) and *C. alata* (47.19%). The present study showed the potentials of extracts of some plant species to control *B. theobromae* in yam.
Keywords: Botryodiplodia theobromae, yam tuber rot, Cassia alata, Azadirachta indica, Citrus aurantifolia, Anacardium occidentale

1. Introduction

Nigeria is the largest producer of yam in the world (CBN, 2003; FAO, 2008). Yam tubers are of very high value as food where it is a major source of carbohydrate, minerals such as calcium, phosphorus, iron and vitamins (riboflavin, thiamine and Vitamins B and C) (Okigbo and Ogbonnaya, 2006). In addition to its nutritional value yam has considerable social and cultural significance especially among the people of South Eastern Nigeria (Okigbo *et al.,* 2013). With the importance of yam, it is plagued by an array of diseases one of which is tuber rot. Rotting is a major factor limiting the postharvest life of yams (Osagie, 1992).

Studies have shown that microbial rot is the greatest cause of tuber loss in storage (IITA, 1993, Emehute, *et al*., 1998). Olurinola *et al.,* (1992) estimated microbial postharvest losses in yam at 40% while Okigbo and Ikediugwu (2000) indicated that between 20 and 39.5% of stored tubers may be lost to decay. Microbial agents causing rot of yam are mainly fungi (IITA, 1985, Cornelius and Oduro, 1999) but some bacteria, notably *Corynebacterium, Serratia* and *Erwina* species have been implicated as causal agents of yam tuber rot (IITA, 1993; Emehute *et al.*, 1998; Amusa and Baiyewu, 1999). The principal species of microorganisms associated with yam rot in Nigeria are *Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium oxysporum, Penicillium oxalicum* and *Aspergillus niger* (Amusa and Baiyewu, 1999; Okigbo and Ikediugwu, 2000; Okigbo, 2003). The disease causing agents not only reduce the quantity of yam produced, but also reduce the quality by making them unappealing to the consumers (Amusa *et al.,* 2003).

The control of these pathogens relies heavily upon the use of synthetic chemicals. However, the obvious pollution problems in the environment and the toxic effects of synthetic chemicals on non target organisms have prompted investigations on exploiting pesticides of plant origin (Amadioha, 2000). Pesticides of plant origin are specific, biodegradable, cheap, readily available and environmentally safe (Okigbo and Nmeka, 2005).

Although several workers have reported on the toxic effects of plant extracts on plant pathogenic organisms (Onifade, 2000; Amadioha, 2002; Okigbo and Emoghene, 2003; Chiejina, 2005; Nduagu *et al.,* 2008; Ijato *et al.,* 2011), there is paucity of information on the control of *Botryodiplodia theobromae* by the use of natural plant products. The present study evaluated *in vitro*, the effect of water and ethanol leaf extracts of some plant species on mycelia growth and spore germination of *B. theobromae* which is a major pathogen of tuber rot disease of yam.

MATERIALS AND METHODS

Source of pathogen

Botryodiplodia theobromae was isolated from rotted yam tubers obtained from Cable Point and Hausa markets in Asaba, Delta State, Nigeria. Tissue segments (1 – 2mm diameter) were cut from the periphery of infected portions of the tuber using sterilized scalpel. The cut tissues were surface-sterilized in 10% sodium hypochlorite (NaOCL) solution for 2 minutes to remove surface contaminations and rinsed twice in sterile distilled water (Okigbo *et al.,* 2009). Four sections of the sterilized tissue pieces were plated out in Potato Dextrose Agar (PDA).

The plates were incubated at 28ºC for 7 days. Five subcultures were made on PDA to obtain pure culture of the fungus. Morphological identification was done to ascertain its identity with the aid of compound microscope and identification guide (Barnett and Hunter, 1999). *Botryodiplodia theobromae* was observed under the microscope to possess simple short conidiophores with dark and ovoid 2-celled conidia. However, on the PDA, it produced white to dirty white fluffy mycelia, black underneath radiating uniformly from the centre of the plate.

Source of plant materials

Fresh leaves of *Cassia alata* L. (ringworm plant) *Azadirachta indica* A. Juss (neem), *Citrus aurantifolia* (Christm.) *Swingle* (lime) and *Anacardium occidentale* L. (cashew) were collected from Asaba, Delta State, Nigeria in May, 2011 and identified by a taxonomist in the Department of Forestry and Wildlife, Delta State University, Asaba Campus, Asaba, Nigeria.

Preparation of leaf extracts

The collected leaves were surface-sterilized (10% NaOCL for 2 min) rinsed in five changes of sterile distilled water and air dried at 28ºC for 7 days. The air dried leaves were ground with the aid of a Tower blender (Model BL-NC-6802D) to obtain 500g powder of each plant species. Water extract was obtained by adding each powder (100g) to 500 ml of sterile distilled water in 1000ml conical flasks. Each suspension was hand shaken for two minutes and allowed to stand for 12 hours before being filtered into fresh flask using four fold cheese cloth.

For ethanol extract, 100g of each powdered plant material was extracted with 500 ml of 95% ethanol. The extract was evaporated to dryness on water-bath. Ten grammes of the extract were reconstructed in 50 ml sterile distilled water and used as the test solution.

Phytochemical analysis

Phytochemical analysis was carried out on part of the pulverized plant materials to reveal the presence of secondary metabolites in them using the method of Poongothai *et al.,* (2011).

Effect of plant extract on radial growth and of Spore germination

One milliliter of each plant extract was pipetted separately and aseptically into 9 ml of cool molten PDA medium in each of the Petri dishes. Each plate was gently swirled on the laboratory bench to ensure even dispersion of extracts. The PDA-extract medium was allowed to solidify and 2 mm diameter mycelia disc obtained from 7 day-old culture of the fungus was inoculated into the center of each Petri dish. The plates were incubated at 28ºC and radial growth was measured for 6 days at two days interval. Colony diameter was taken as the means along two preset diametral lines on the reverse side of the plates.

To determine toxicity of extracts against spore germination plates with each extract were inoculated with one drop (0.1 ml) of conidial suspension (5.0×10^4 conidial ml^{-1}) of the fungus. Spore suspension was obtained from 10 day-old cultures of the fungus following the method of Osai and Ikotun (1996) and adjusted to a final concentration of 5.0×10^4 conidial ml^{-1}. Spore concentration was measured using a haemocyometer. Plates were inoculated at the centre of each of four sectors. Counts of germinating spores were made under the low power (x100) of the microscope after 24 hours incubation (Osai and Ikotun, 1996). Germination was based on the mean of 100 conidia counted per sector in each plate. Plant extract free PDA plates similarly inoculated with mycelia discs or spores served as controls. Three replicates were maintained for each experiment. Percentage inhibition of mycelia growth or spore germination was calculated by a modification of the formula of Pandey *et al.,* (1982) as follows:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where:

dc = average diameter of fungal colony/average number of spore germination in control plates

dt = average diameter of fungal colony/average number of spore germination in treated plates

Statistical design analysis

The experimental design used was Completely Randomized Design (CRD) with three replicates. Data were subjected to analysis of variance (ANOVA) and significant means were separated with the Duncan's Multiple Range Tests (DMRT) using SAS (2002).

RESULTS AND DISCUSSION

The results of the phytochemical analysis showed that the leaves of the four plants had flavonoids and tannis. In addition, *C. aurantifolia* contained glycosides and phylobatannins. *Azadirachta indica* contained alkaloids, glycosides, saponins and terpenes. *Anacardium occidentale* contained alkaloids, saponins and terpenes. The component, anthraquinones was not detected in the plant materials tested (Table 1). These metabolites present in

the plant materials are in various amounts. Other investigators (Gill, 1992; Ahmad *et al.*, 1998; Shariff, 2001; Okwu *et al.*, 2006) have reported the presence of these components in members of the families,

Table 1: Phytochemical analysis of the plant extracts

	Citrus aurantifolia		*Azadiratcha indica*		*Anacardium occidentale*		*Cassia alata*	
Phytochemical	**Water**	**ethanol**	**Water**	**ethanol**	**Water**	**ethanol**	**Water**	**ethanol**
Anthraquinones	-	-	-	-	-	-	-	-
Alkaloids	-	+	+	+	-	+	+	+
Flavonoids	+	++	+	+	+	-	++	+++
Glycosides	+	+	+	+	-	-	+	+
Saponins	-	-	++	++	+	-	+	+
Tannins	+	+	+	+	+	+	+	+
Phytobatannins	+	++	-	-	-	-	-	-
Terpenes	-	-	+	+	-	+	-	-

Keys: - = Not detected
\+ = Low concentration
++ = Moderate concentration
+++ = High concentration

Rutaceae, Maliaceae, Anacardiaceae and Caesalpiniaceae, one to which the plants used in the current investigation belong. The inhibitory effects of these plants on the pathogen may be due to the presence of the above phytochemicals. Scientists have shown that these metabolites play defence roles in the plants producing them. For example, Haralampidis *et al.,* 2001) reported that secondary metabolites have been implicated as chemical defence against attack by soil fungi. In the same paper, they further reported that many plants synthesize secondary metabolites as part of their normal programme of growth and development, often sequestering them in tissues to protect them against microbial attack.

The water and ethanol extracts of the four plant species screened, *in vitro* showed varying degrees of toxicity to *B. theobromae*, expressed as percentage inhibitions to mycelia growth and spore germination. Results in Table 2 showed that ethanol extract of *C. aurantifolia* recorded the highest growth inhibition of *B. theobromae* (60.37 – 73.83%) during the period of incubation. This was significantly ($P < 0.05$) higher than other extracts. Treatments containing ethanol extracts of *A. indica* and *C. alata* were similar, giving percentage inhibitions of 51.44 – 60.46% and 50 – 58.51% respectively. Water extract of *A. occidentale* was the least inhibitory (5.93 – 15.35%) on mycelial growth of *B. theobromae*. Ethanol extracts were more effective in reducing the growth of the rot-causing pathogen than water extracts of all the plants tested. The highest growth reduction of the pathogen by the plant species was recorded after 2 days of culture and the efficacy of the plant products generally decreased thereafter.

On the effect of extracts on spore germination of *B. theobromae* (Table 3), ethanol extract of *C. aurantifolia* gave the highest inhibition (59.68%) in spore germination of the fungus. This way significantly ($P < 0.05$) higher than other extracts. Ethanol extracts of *A. indica* and *C. alata* had similar inhibitory effects on spore germination, with percentage inhibitions of 47.19 and 48.69% respectively. The least percentage inhibition of spore germination was recorded with water extract of *A .occidentale* and control. Ethanol extracts were relatively more effective than water extracts of all the plant species.

Results from this study indicated the presence of fungitoxic compounds in the different plant extracts tested. This is in agreement with the reports of some earlier workers (Amadioha, 2000; Okigbo and Nmeka, 2005, Pawar and Thaker, 2006; Bediakao *et al.,* 2007). The fungicidal effects of plant extracts on different pathogens of crops have been widely reported (Amadioha and Obi, 1999; Olufolaji, 1999; Onifade, 2000; Okigbo and Ogbonnaya, 2006) Among the four

Table 2: Reduction of mycelial growth of *Botryodiplodia theobromae* by leaf extracts at different dates

	Growth reduction (%) at different dates (days)					
	Water extract			**Ethanol extract**		
Plant species	**2**	**4**	**6**	**2**	**4**	**6**
Citrus aurantifolia	54.75^{d}	45.67^{d}	44.44^{d}	73.83^{d}	65.73^{d}	60.37^{d}
Azadiratcha indica	33.73^{c}	27.28^{c}	22.22^{c}	60.46^{a}	53.24^{c}	51.44^{c}
Cassia alata	31.07^{c}	24.83^{c}	23.78^{c}	58.51^{c}	53.07^{c}	50.00^{c}
Anarcardium occidentale	15.35^{b}	6.17^{b}	5.93^{b}	40.77^{b}	40.50^{b}	35.22^{b}
Control	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Means in the same column with different superscripts are significantly different ($P < 0.05$)

Table 3: Percentage inhibition of spore germination of *Botryodiplodia theobromae* by four plant leaf extracts

	Inhibition (%)	
Plant species	**Water extract**	**Ethanol extract**
Citrus aurantifolia	42.33[c]	59.68[d]
Azadiratcha indica	29.10[b]	48.69[c]
Cassia alata	26.72[b]	47.19[c]
Anarcardium occidentale	0[a]	39.33[b]
Control	0[a]	0[a]

Means in the same column with different superscripts are significantly different ($P < 0.05$)

plant materials screened, *C. aurantifolia* leaf gave the best result especially the ethanol extract which recorded between 60.37 and 73.83% reduction on the mycelial growth of the pathogen. The inhibitory activity of *C. aurantifolia* has been reported against *Fusarium oxysporum* (Okwu *et al*., 2006) and *Phaeoramularia angloensis* (Dongmo *et al.,* 2009). Ethanol extracts exhibited relatively stronger fungitoxicity than water extracts on the test fungus. The differences in fungitoxicity between the extraction medium can be attributed to the difference in the nature of their active ingredients (Onifade, 2000; Okigbo and Odurukwe, 2009). The impressive fungitoxic activity of the *C. aurantifolia* extract suggests that the chemical contained in the plant material are highly soluble in the extracting solvents. Extracts of the test plants were most effective in reducing the radial growth of the pathogens after 2 days in culture, which decreased as incubation period increased indicating that the efficacy of the active compounds of the plant species were not persistent in the culture medium or they depreciated in toxicity after two days of culture.

5. Conclusion

The study reveals the potential of natural plant products in the management of yam tuber rot caused by *Botryodiplodia theobromae*. The study also found out that the best plant extract that could arrest the growth and germination of this pathogen is *C. aurantifolia,* followed by *A. indica* and *C. alata.*

References

Ahmad, I. Mehomood, Z. and Mohammed, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology* 62(2): 183 – 193.

Amadioha, A. C. (2000). Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Protection 19: 287 – 290.

Amadioha, A. C. (2002). Fungitoxic effects of extracts of *Azadirachta indica* against *Cochliobohus miyabeanus* causing brown spot disease of rice. Arch. Phytopath. Pflanz, 35:37-42.

Amadioha, A. C. and Obi, V. I. (1999). Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimu. Acta Phytopathol Entomol. Hungerica* 34(2): 85 – 89.

Amusa, N. A. Adegite, A. A. Mohammed, S. and Baiyewu, R. A. (2003). Yam disease and its management in Nigeria. *African Journal of Biotechnology* 2(12): 497 – 502.

Amusa, N. A. and Baiyewu, R. A. (1999). Storage and market disease of yam tubers in South Western Nigeria. *Ogun Journal of Agric. Res.* 11:211.

Barnett, H. L. and Hunter, B.R. (1999). Illustrated genera of imperfect fungi. Fourth edition. Academic Press San Diego, California USA 218p.

Bediakao, A., Showemimo, F.A., Asiamo, Y.O. and Amewowor, D. H. A. K (2007). *In vitro* analysis of growth media and the control of yam minisett-rot. *Biotechnology* 6: 1- 4.

Central Bank of Nigeria (CBN) (2003). Annual Reports and Statement of Accounts.

Chiejina, M. V. (2005). Antifungal properties of leaf extracts of *Carica papaya* Linn. On three fungal pathogens of tomato (*Lycopersicon esculentum* Mild). *Nigerian Journal of Plant Protection* 22: 172 – 179.

Cornelius, E. and Oduro, K. A. (1999). Storage disease of white yam (*Dioscorea rotundata*) causes, varietal susceptibility and control. *Journal of the Ghana Science Association* 3:45 – 52.

Dongmo, P. M. J., Tatsadjieu. L. N., Sonwa, E. T., Kuate, J., Zollo, P. H. A. and Menut, C. (2009). Essential oils of *Citrus aurantifolia* from Cameroon and their antifungal activity against *Phaeoramimaria angolensis. Afri. J. Agric. Res.* 4(4): 354 – 358.

Emehute, J. K. U., Ikotun, T. Nwanzor, E. C. and Nwokocha, H. N. (1998). Crop In: Orkwor, G. C., Asiedu, R. and Ekanayeke, I. J. (eds.) Food Yams: Advances in Research. IITA/NRCRI. Pp 141-186.

Food and Agriculture Organization (FAO). (2008). Production Year Book, FAO, Rome.

Gill, L. S. (1992). Ethnomedical uses of plants in Nigeria. Uniben Press, University of Benin, Benin City 275pp.

Haralampidis, K., Bryan, G. Qi, X., Papdopoulou, K., Bakht, S., Melton, R. and Osbourn, A. (2001). A new class of oxidosqualene cyclases direct synthesis of antimicrobial phytoprotectants in monocots. *Proceedings of the national academy of science of the United States of America* 98(23): 13431 – 13436.

Ijato, J. Y., Adebiyi, A.O. and Ijadunola, J. A. (2011). Antifungal effects of four tropical aqueous and ethanol extracts on postharvest rot of tomato (*Lycopersicum esculentum*) in Ado-Ekiti, *Nigeria: New York Science Journal* 4(1): 64-68.

International Institute of Tropical Agriculture (IITA) (1985). Yam disease in common African Pests and diseases of cassava, yam, sweet potato and cocoyam. IITA Ibadan, Nigeria. 108pp.

International Institute of Tropical Agriculture (IITA) (1993). Crop Improvement Division/Tuber root improvement program archival reports (1989 – 1993). Part III yam (*Dioscorea* spp.) Ibadan, Nigeria pp. 20 – 85.

Nduogu, C., Eketan, E. J. and Nwankiti, A. O. (2008). Effect of some crude plant extracts on growth of *Colletotrichum capsici* (Synd) and Bisby, causal agent of pepper anthracnose. *Journal of Applied Bioscience* 2:184 – 190.

Okigbo, R. N. (2003). Fungi associated with peels of postharvest yams in storage. *Global J. Pure Applied Sci*. 9(1): 19-23.

Okigbo, R. N. and Emoghene, A. O. (2003). Effects of leaf extracts of three plant species on *Mycosphaerella fijiensis* Morelet the causal organism of black sigatoka disease of banana (*Musa acuminata*). *Nigerian Journal of Plant Protection* 20:101-110.

Okigbo, R. N. and Ikediugwu, F. E. O. (2000). Studies on biological control of postharvest rot of yams (*Dioscorea* spp.) with *Trichoderma viride. J. Phytopathol*. 148:351 – 355.

Okigbo, R. N. and Nmeka, I. A. (2005). Control of yam tuber rot with leaf extract of *Xylopia aethiopica* and *Zingiber officinale. African Journal of Biotechnology* 4(8): 804 – 807.

Okigbo, R. N. and Odurukwe, C. N. (2009). Occurrence and control of fungal rot pathogens of yam (*Dioscorea rotundata* Poir) with leaf extract of *Chromolena odorata, Carica papaya* and *Aspilia Africana. Nigerian Journal of Mycology* 2(1): 154 – 165.

Okigbo, R. N., and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant leaf extracts *Ocimum gratissimum* and *Aframomum melegueta* on postharvest yam (*Dioscorea* spp.) rot. *African Journal of Biotechnology* 5(9):727 – 731.

Okigbo, R. N., Anukwuorji, C. A. and Ezeabara, C. A. (2013). Effects of leaf extracts of *Chromolaena odorata* (L.) and *Terminalia catappa* (L.) on mycelia growth of yam tuber pathogens. *Niger. J. Plt. Prot*. 27(1): 74 – 84.

Okigbo, R. N., Eme, U. E., Asiedu, R. and Ramesh, P. (2009). Effect of crude extracts of *Allium sativuum* Lin., *Cymbopogon citratus* CD. Stapf and *Terminalia catappa* on rot causing fungi at *Dioscorea* species. *Nigeria Journal of Botany* 22(2): 359 – 369.

Okwu, D. E., Awurum, A. N. and Okoronkwo, J. I. (2006). Phytochemical composition and *in vitro* antifungal activity screening of extracts from citrus plants against *Fusarium orxysporum* of Okra plant (*Hibiscus esculentus*) *African Crop Science Conference Proceedings* Vol. 8 pp. 1755 – 1758.

Olufolaji, D. B. (1999). Control of wet rot disease of *Amaranthus* spp. Caused by *Chonehora curcubitaricum* with extracts of *Azadirachta indica. J. Sust. Agric. Environ.* 1(22): 183 -190.

Olurinola, P. F., Ehimmidu, J.O. and Bonire, J. J. (1992). Antifungal activity of n-tributyltin acetate against some common yam rot fungi. *Applied and Environmental Microbiology* 58(3): 758 – 760.

Onifade, A. K. (2000). Antifungal effect of *Azadirachta indica* A. Juss extracts on *Colletotrichum lindemuthianum*. *Global Journal of Pure and Applied Sciences*. 6(3): 423 – 428.

Osagie, A. U. (1992). The yam tuber in storage. Postharvest research Unit, Department of Biochemistry, University of Benin, Benin-City, Nigeria. Pp 146 – 149.

Pawar, V. C. and Thaker, V. S. (2006). *In vitro* efficacy of 75 essential oil against *Aspergillus niger*. *Myloses* 9(4): 326 – 333.

Shariff, Z. U. (2001) Modern Herbal therapy for common ailments. Nature Pharmacy series (Vol. 1). Spectrum Books Ltd, Ibadan, Nigeria in Association with Safari Books (Export) Ltd, United Kingdom, pp. 9 -84.

Statistical Analytical System (SAS) (2002). User's guide statistics version 91, SAS Institute Inc. Raleigh, N. Y. USA.

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