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ANTIFUNGAL ACTIVITIES OF *Jatropha curcas* AND *Ricinus cumunis* SEEDS ON *Fusarium verticilliodes* AND *Aspergillus flavus* IN YAM

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ABSTRACT

Antifungal properties of *Jatropha curcas* and *Ricinus cumunis* seed extracts in the control of mycelia growth and rot development of yam caused by *Fusarium verticilliodes* and *Aspergillus flavus* was investigated *in-vitro* and *in-vivo*. Water extracts of deoiled, crude extract and extracted oil of *Jatropha curcas* and *Ricinus cumunis* (castor oil) were tested against *A. flavus* and *F. verticilliodes*. The effect of these treatments were compared with Super homai® and distilled water. Data on mycelia growth were recorded by day 4th, 5th, 6th, and 7th days, respectively. The result showed that *in-vitro* and *in-vivo*, castor oil seed crude extract lowers mycelia growth of *F. verticilliodes* significantly at ($P \leq 0.05$) compared to other treatments, it also lowered the rot index in yam. Similarly, castor seed oil (crude extracts) had the lowest mycelial growth on *A. flavus* in-vitro while in-vivo, *J. curcas* crude extract and deoiled castor seed oil (crude extract) significantly ($P \leq 0.05$) reduced rot depth of yam compared with other treatments. The findings indicate promising potentials of *J. curcas* and *R. cumunis* seeds in management of plant fungal diseases.

Keywords: yam, rot development, plant fungal disease, *Aspergillus flavus, Fusarium verticilliodes, Jatropha curcas, Ricinus cumunis*.

INTRODUCTION

Yam (*Discorea spp.*) is a preferred stable tuber crop in West Africa with has a high nutritive value. It is a major source of carbohydrate, calcium, iron and vitamins such as riboflavin, thiamine, and ascorbic acid (Normal *et al*., 1995). This food crop is subject to several diseases among which is storage disease caused by different genera of fungi (Okigbo and Ikediugwu, 2000). Dry rot which is a storage disease of yam usually start from the soil and progressed to storage period, and infected tubers do not normally show any sign of exeternal symptoms. The disease is therefore seen during harvest in Nigeria between June and September (Amusa, 2000).

The major micro-organisms that cause diseases in yam tubers include *Aspergillus niger var*) *tiegh*, *Botryodiploidia theobronmae pat, Fusarium oxysorum Schlecht ex fr,* (*Fusarium Solani Mart.) Sacc, Penicillum, Chrysogenum Thom, Rhizoctenia sp., Oxalicum currie and Thom, Triichzoctenia viride per* and *Rhizopus nodosus N'amyslowski* (Okigbo and Ikediugwu, 2002, Okigbo, 2004). The predisposing factors include insect attack especially storage beetles (*Coleptera)* mealy bug (*Plannococcuscitri*) and scale inscect (*Aspidiella hartii)* during storage (Morse *et. al*, 2000).

The symptoms of these diseases vary depending on the invading pathogen, and the infected tissue becomes hard and dry. *Fusarium spp.* shows yellowish boarder on the infected tissue. Yam infected with *Aspergillus spp.* turn brown with yellowish margin while *Rosellinia bunods* and *Botryodiplodia the obramae* has been reported to cause dry black rot (IITA, 1993).

Aspergillus flavus is pathogenic in both plant and animal species, and is favoured by dry hot condition with an optimal growth temperature between 25 - 42°C which contribute to its pathogenicity in human. It infects vegetative reproductive tissues without any symptoms developing. Insect, herbivoury pressure, and physiological stress facilitate disease development (Miller and Trenholm, 2000).

Jatropha curcas (physic plant) is a small tree shrub which is leafless and its mature seed changes from green to yellow. The active ingredient in jatropha seed is curcin which is very toxic due to diterprene ester. The seed has been used as antihelminthes, arbortifacient, used in treatment of gout, paralysis and skin disease (Weding *et al*., 1986).

Castor oil seed is found in the tropics, and the oil contains a triglyceride ricinoleic acid. (Weding *et al*., 1986). The seeds, leaves and stem are highly toxic to animals. It has immunotoxigenic effect, which gives it anti-tumor activity.

The objective of this work is to access in-vitro the mycelia growth of *F. verticilloides* and *A. flavus* pretreated with *J. curcas* and *R. cuminis* extracts and also to investigate the in-vivo inoculum capacity. Furthermore to determine the rot index of yam set applied on treated *F. verticilloides* and *A. flavus* with *J. curcas* and *R. cuminis* seed extracts.

MATERIALS AND METHODS

Collection of samples

Jatrophas curcas seed and castor oil seed were obtained from Gwagwalada, Abuja. The samples were kept in a polythene bag prior to when the oil and cake

were being extracted two yam tubers were purchase from Bosso market, Minna, Niger state, Nigeria.

Culture medium

Potatoes dextrose agar was prepared. 200g of peeled Irish potatoes was accurately weighed into a beaker using electronic weighing balance and placed on a bunsen burner. This was heated for 45mins with distilled water until thick solution was obtained. The mixture was filtered using cheese cloth and made it up to 500ml with distilled water. Each of the dextrose and agar (20g) were added to the filterate and also 0.5g of choramphenicol. It was placed on the bunsel burner and stirred thoroughly with a stirrer. The medium was sterilized using autoclave at $121^{O}C$ for 15 minutes. It was further stirred thoroughly and allowed to cool. The bench top was sterilized with disinfectant using detol to minimize contamination. The medium was allowed for cool to about 30 minutes to an hour before dispensing into sterile petri-dishes and allowed to gel.

Isolation of fungi

Three petri-dishes were washed and lined with filter paper; the paper was moistened with distilled water. A piece of infected yam was sliced into smaller bits and put into the lined petri-dishes. The petri-dishes were covered and kept in the inoculating hood. After 6 days, colony of fungi was observed.

Identification of *Fusarium verticilliodes* and *Aspergillus flavus* sub-culturing

This involved the preparation of stains using clean microscopic slide, each of the fungi culture was aseptically placed on the sterile slide using a forceps, and the fungi were then stained with dye (lactophenol blue) on the centre of the slide and covered with cover-slip and viewed under 40 objective lens of the microscope. The identification was done using a fungi catalogue in the Microbiology Department of the Federal University of Technology, Minna. The pure culture of the identified fungi *Fusarium verticilliodes* and was aseptically sub-cultured and were kept in the inoculating hood for 72 hours. The same procedure is used in identification of *Aspergillus flavus.*

Preparation of *Jatropha curcas* seed and castor seed extracts

Some seeds were sun dried for 7 days after which they were broken to remove the hull with the aid of a pestle and mortal. They were further dried to be able to grind easily using mortar and pestle and sieved with cheese cloth to get a fine powdery substance. 20g each of the castor oil seed and Jatropha seed powder were used for crude extract preparation while 102.9g of castor powder and 134.1g of Jatropha were used for oil extraction.

Oil extraction

The oil from the physic plant and castor oil plant were extracted using N-hexane 600 ml as the choice of the solvent. Weighed samples of *J. curcas* powder was introduced into a thimble and inserted into the soxhlet extractor. The solvent is poured into the round bottom flask and fixed to the extractor. The condenser is also fixed to the extractor and clamped with a retort stand. The extractor set at $60^{o}C$. The inlet of the condenser is fixed to a tap that supply water which is used to cool the vapor of the solvent as it refluxes and condenses back to the extractor, thereby washing the oil from the sample and going back to the flask containing the solvent. The extraction was carried out for 8 hours, and the end of the extraction process the heater was switch off, the thimbles removed from the extractor and the apparatus allowed to cool. The solvent is completely removed from the oil using a steam bath. The oil obtained was stored in a dry test tube and kept in the refrigerator for further studies. The non-soluble portion which is the cake (deoiled) is kept in a sealed container. This was also repeated for the castor oil kept in a dry test tube.

Preparation of the treatment used

1 ml of distilled water was poured into each petri-dish containing:

- Crude castor seed
- Crude Jatropha seed
- Deoiled castor seed
- Deoiled Jatropha seed

These were mixed throughout using a stirring rod and left overnight. Each of the these treatment was filtered using cheese cloth and each filtrate was poured into four different petri dishes and labeled as follows:

- Castor seed crude extract
- Physic nut crude extract
- Deoiled castor seed extract
- Deoiled physic nut extract

A loop of the *Fusarium verticilliodes* already grown on the PDA plate was transferred into each of these petri-dishes including those of the synthetic fungicide, physic nut oil, castor oil and labeled accordingly: The procedure is repeated for *Aspergillus flavus.*

- Deoiled castor seed extract
- Deoiled physic nut extract
- Physic nut oil
- Castor seed oil
- Castor seed crude extract
- Physic nut crude extract
- Synthetic fungicides
- Distilled waters

Inoculation of PDA and yam tubers

Twenty four petri dishes of PDA were also prepared and 3petri dishes containing PDA were inoculated each with the same treatment and were kept in inoculating hood. Data on mycelium growth were collected at 4, 5, 6 and 7 days. Two tubers of yam were cut into twenty four pieces with 3 pieces of the yam

inoculated each with the same treatment and were kept in inoculating hood. Data on mycelia growth were collected at 11 days after inoculation (DAI).

Data collection and analysis

Measurement of mycelium growth was carried out on the 3, 5, 7, 9 days, respectively. All results obtained from the measurement of mycelium growth on (PDA) on the yam, including the rot index were subjected to analysis (ANOVA) using mathematical packaged for social sciences scientist (SPSS). The (DMRT) Duncan multiple range test at 5% level of probability was used to ascertain the significance between the different treatments used.

RESULTS

The results showed the effect of *Jatropha curcas* and castor seed extract on the mycelium growth of *F. verticilloides* on PDA and yam, and also on the indices of yam tuber inoculated with *F. verticulliodes.*

Table-1. Effect of Jatropha and castor oil seed extracts on mycelium growth of *F. verticilloides.*

S. No.	Treatment	4DAI	5DAI	6DAI	7DAI
1	Deoiled jatropha seed extract	0.85^{b}*	1.25^{ab}	1.25^{ab}	1.50^{a}
2	Deoiled castor oil seed extract	1.25^{a}	1.40^{a}	1.50^{ab}	1.50^{a}
3	Jatropha seed oil	0.55^{d}	0.95^{bc}	0.95^{bc}	1.10^{b}
4	Castor oil seed extract	0.75^{bc}	1.23^{ab}	1.35^{ab}	1.50^{a}
5	Jatropha seed crude extract	0.50^{d}	0.50^{d}	0.75^{cd}	0.85^{b}
6	Castor oil seed crude extract	0.45^{d}	0.40^{e}	0.50^{d}	0.50^{d}
7	Synthetic fungicides	0.00^{e}	0.00^{f}	0.00^{e}	0.00^{e}
8	Control (distiled water)	0.50^{d}	0.50^{de}	0.50^{d}	0.75^{c}

*Means on the same column with same superscripts are not significantly different (P>0.05)

Table-2. Effect of *Jatropha curcas* and castor oil seed extracts on mycelium growth of *F. Verticilliodes* on yam.

S. No.	Treatment	3DAI	5DAI	7DAI	9DAI
1	Deoiled *Jatropha* seed extract	0.27^{cd*}	0.63^{cd}	0.63^{cd}	0.67^{dc}
2	Deoiled castor oil seed extract	0.67^{c}	0.83^{cd}	0.90^{cd}	1.17^{cd}
3	*Jatropha* seed oil	1.33^{b}	1.67^{b}	1.83^{b}	1.83^{b}
4	Castor oil seed extract	0.33^{b}	1.33^{bc}	1.27^{bc}	1.67^{bc}
5	*Jatropha* seed crude extract	0.43^{cd}	0.57^{cd}	0.87^{dc}	1.30^{bc}
6	Castor oil seed crude extract	0.00^{d}	0.43^{cd}	0.50^{cd}	0.83^{de}
7	Synthetic fungicides	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{e}
8	Control (distilled water)	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}

*Means on the same column with same superscripts are not significantly different (P>0.05)

Table-2 shows that at 3DAI, the highest mycelium growth on yam (3.0) was recorded in the control and was significant different (p<0.05). At 5DAI, 7DAI and 9DAI, application of synthetic fungicides on *F. Verticilliodes* completely hampered the mycelia growth and is significantly lower than all other treatments. Deoiled *Jatropha* and deoiled castor seed extract also have lower mycelia growth apart from synthetic fungicides.

Table-3 shows that application of super homai® on inoculated yam with *F. Verticilliodes* has the lowest rot index and its significantly lower(p<0.05) than treatments and the control recorded the highest rot index, followed by castor oil and Jatropha seed oil extracts. However, castor oil seed extract also is recorded the lowest rot index apart from synthetic fungicides.

Table-3. Effect of *Jatropha curcas* and castor oil seed extracts on yam rot (cm) inoculated with *F. vercilliodes.*

S. No.	Treatment	11DAI
1	Deoiled *Jatropha* seed extract	1.00^{bc}
2	Deoiled castor oil seed extract	0.63^{bc}
3	*Jatropha* seed oil	1.46^{bc}
4	Castor oil seed extract	1.90^{b}
5	*Jatropha* seed crude extracts	0.93^{bc}
6	Castor oil seed crude extracts	0.096^{bc}
7	Synthetic fungicides	0.00^{c}
8	Control (distilled water)	3.90^{a}

*Means on the same column with same superscripts are not significantly different (P>0.05)

Table-4. Effect of physic nut and castor oil seed products on mycelium growth (cm) of *A. flavus* on yam tuber.

S. No.	Treatment	3DAI	5DAI	7DAI	9DAI
1	Physic nut crude extract	0.30^{c*}	0.33^{b}	0.47^{bc}	0.50^{c}
2	Castor seed crude extract	0.27^{c}	0.43^{b}	0.57^{bc}	0.70^{bc}
3	Physic nut oil	0^{d}	0.57^{b}	1.83^{a}	1.83^{a}
4	Castor oil	0^{d}	0.60^{b}	0.63^{bc}	0.73^{bc}
5	Deoiled physic nut extract	1.43^{ab}	1.57^{b}	1.83^{a}	1.83^{a}
6	Deoiled castor extract	0.00^{d}	0.33^{b}	0.67^{bc}	0.83^{bc}
7	Superhomai®(synthetic)	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}
8	Distilled water (control)	1.00^{b}	1.77^{a}	2.00^{a}	2.00^{a}

*Means on the same column with same superscripts are not significantly different (P>0.05)

Table-4 Shows that at 3DAI there was no mycelia growth of the fungi on yam applied with physic nut oil, castor oil, deoiled castor seed extract and with superhomai®. Mycelia growth on yam applied with deoiled physic nut extract was significant higher (P<0.05) than their any treatment (2.00cm). Closely next to this was the growth on the yam applied with physic nut oil and deoiled physic nut.

Table-5. Effects of physic nut and castor seed extracts on rot indices (rot depth) of yam tuber inoculated with *A. flavus.*

S. No.	Treatment	11DAI
1	Physic nut crude extract	1.47^{ab*}
2	Castor seed crude extract	0.29^{ab}
3	Physic nut oil	1.41^{ab}
4	Castor oil	0.52^{ab}
5	Deoiled physic nut extract	1.82^{ab}
6	Deoiled castor extract	0.22^{ab}
7	Superhomai®(synthetic)	0.00^{b}
8	Distilled water (control)	2.48^{c}

*Means on the same column with same superscripts are not significantly different (P>0.05) 98 iu

Tables 3 to 6 show that 11DAI yam tubers applied with superhomai® had zero rot index and was significantly different (P>0.05) from the control. This result showed the effect of physic nut and castor seed extracts on mycelia growth of *Aspergillus flavus* on PDA yam tuber and rot indices of yam tubers.

Table-6. Effect of physic nut and castor seed product seed products on mycelium growth (cm) of *A. Flavus* on PDA.

S. No.	Treatment	4DAI	5DAI	6DAI	7DAI
1	Physic nut crude extract	0.60^{b*}	0.67^{ab}	1.00^{a}	1.37^{bc}
2	Castor seed crude extract	0.37^{b}	0.40^{bc}	0.40^{c}	0.40^{cd}
3	Physic nut oil	0.63^{bc}	0.77^{b}	0.77^{b}	1.50^{b}
4	Castor oil	0.50^{b}	0.50^{ab}	0.87^{ab}	1.27^{bc}
5	Deoiled physic nut extract	0.10^{b}	$0,10^{cd}$	0.37^{c}	1.27^{bc}
6	Deoiled castor extract	$0.87b^{cd}$	0.87^{a}	0.87^{ab}	2.97^{a}
7	Superhomai® (synthetic)	0.00^{b}	0.00^{d}	0.00^{d}	0.00^{d}
8	Distilled water (control)	0.50^{b}	0.50^{ab}	0.50^{c}	2.60^{a}

*Means on the same column with same superscripts are not significantly different (P>0.05)

DISCUSSIONS

Fungi are known to be one of the major causes of rot in yam tubers. This results in colossal losses in the crop and market value of these crops (Onifade, 2000). The antifungal activities of some plants extracts in controlling different pathogens have been reported by several workers (Tewarri and Nayak, 1991; Amadioha, 2000; Okigbo and Ajalie, 2005) It is noteworthy that the active principles present in plants were influenced by many factors which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials (Amadioha and Obi, 1991; Okigbo and Ajalie, 2005; Okigbo *et al*., 2005).

In this research, it was observed that *Aspergillus flavus* and *Fusarium verticilliodes* is among the pathogenic fungi that affects yam. This confirms (Okigbo, 2005) that yam rot is caused by *Aspergillus spp., Fusarium spp.* and other fungi.

Also, the fungitoxic effect *of Jatropha curcas* was due to the presence of the active principle curcin. *J. curcas* crude extracts and deoiled castor seed extracts significantly (P<0.05) reduces the rot index of yam in comparism to other plant extracts. The inhibition is due to the fungitoxic activities of the plant extracts which agreed with the report of other workers (Qasem and Abu-Blan, 1996; Amadioha, 2000; Okigbo and Nmeka, 2005).

From this work, it was observed that in-vitro and in-vivo, castor oil seed crude extracts is highly fungi-toxic, this is due to the presence of ricin which is the active ingredient that inhibits mycelia growth. It lowered mycelia growth of Fusarium *verticilliodes* and *Aspergillus flavus in vitro*, compared to other treatments.

RECOMMENDATIONS

From the finding of this research, *J. curcas* and *R. cumunis* water extracts could be used as protective fungicide. This approach to plant disease management is economical, easy to apply and poses little environmental risk and the plants are available in Nigeria. It is thus recommended that further studies under field condition should be done on these plants extracts to determine if their effect is fungicidal or fungistatic.

REFERENCES

Amadioha A.C., Obi V.I. 1999. Control of Anthracnose disease of cowpea by *Cymbopogon citrates* and *Ocimum gratissimum*. Acta phytopathology Entomology Hungerica. 34(1-2): 85-89.

Amadioha A.C. 2000. Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch phytopathology Pflan. 4: 1-9.

Amusa N.A. 2000. Screening cassava and yam cultivars for resistance to anthracnose using toxic metabolites of *Colletotrichum glocosporioidies* infected yam leaves. International Journal of Tropical plant Diseases. (India). 128: 161-162.

IITA. 1993. Crop Improvement Division /Tuber root Improvement. Program Archival Reports (1989-1993). Part 111 yam (*Discorea spp).* Ibadan, Nigeria. pp. 20-85.

Miller J.D. and Trenholm H.L. 1994. Mycotoxin in grains compound other than aflatoxin. Eagan press, USA. pp. 3-541.

Morse S., Acholo M., McNamara N., Oliver R. 2000. Control of storage insects as a means of limiting yam tuber fungal rots. Journal of store product research. 36L37-45.

Norman M.J.T., Peerson C.J., Searle P.G.E. 1995. Tropical food crops in their enviroment. 2nd Ed. Cambridge University Press, U.K. pp. 305-316.

Odebayi O.O. 1993. Plant medication. 48: 44.

Onifade A.K. 2000. Antifungal effect of Azadirachta indica. A juss extract on *Collectorium lindemuthianum*. Global journal of Pure and applied Sciences. 6: 425-428.

Okigbo and Ikedigwu FEO. 2000. Studies on biological control of poor harvest rot of yams. (*Discorea spp)* with *Tricodermal viride.* Journal of phytopathology. 148: 351-355.

Okigbo R.N., Ikediugwu F.E.O. 2002. Evaluation of water losses in different regions of yam (*Discorea spp*) tuber in storage. Nigeria Journal of expository and applied Biology. 3: 317-321.

Okigbo R.N., Ajalie A.N. 2005. Inhibition of some human pathogens with tropical plants extracts *Chomolinaena odorata* and *citrus aurantifolia* and some antibiotics. International Journal Molecular of Medical Advanced Sciences (Pakistan). 1(1): 34-40.

Okigbo R.N., Emoghene A.O. 2004. Antifungal activity of leaf extracts of some plants species on *Mycosparaella fijiensis morellet*, the causal organism of black sigitoka disease of banana (*Musa acuminate)*. KMITL Science Journal. 4: 20-31.

Okigbo R.N. 2004. A review of biological control methods for post harvest yam (*Discorea spp.*) in storage in south Eastern Nigeria. KMITL Science Journal. 4(1): 207-215.

Tewarri S.N., Nayak N. 1991. Activity of four plants leaf extracts against three fungal pathogens of rice. Tropical Agriculture. (Trinidad). 68: 373-375.

Qasem J.R., Abu-Blan H.A. 1996. Fungicidal activity of some common weeds extracts against different pathogenic fungi. Journal of Phytopathology. 144: 157-161.

Wedin G.P., Neal J.S., Everson G.W. and Krenzelok E.P. 1986. Castor beans poisoning. America Journal of Emergency Medication. 4(3): 259-261.