SEED-BORNE FUNGI IN CASTOR OIL (*Ricinus cummunis*): EFFECTS OF THEIR METABOLITES AND FUNGICIDES ON SEED GERMINATION AND OTHER SEEDLING PARAMETERS.

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ABSTRACT

An investigation of fungi associated with castor oil seeds collected from four different locations in Nigeria showed Aspergillus niger, A. flavus, Fusarium moniliforme and Penicillium spp. to be the most abundant. Metabolites from these fungi significantly (p=0.05) depressed seed germination and seedling establishment. Four fungicides, metalaxyl + carboxin + furathiocarb [Apron-plus], benomyl [Benlate], mancozeb [Dithan M45] and thiran [Fernasan D] at three different concentrations all significantly controlled seed-borne fungi and increased seed germination. Benomyl proved to be the most effective in controlling mycoflora and increasing seed germination.

Keywords: Fungi, seed-borne, metabolites, fungicides and germination.

Running title: Effects of fungicides and metabolites of seed-borne fungi of castor oil (*Ricinus cummunis*) on seedling parameters.

INTRODUCTION

Castor oil plant is the common name for *Ricinus cummunis,* a half hardy annual herb of the spurge family (Euphorbiaceae) but growing into a tree 40ft high in the tropics. It is indigenous to Africa growing almost everywhere in Nigeria. Its large lobed, long stalked leaves are variable in colour and size and it has green flowers, the sexes being disconnect The male flowers are contained in involucres which open when the pollen is ripe; the female flowers have a three-pointed style and acute-ovate sepals. The fruits are globosely spiny capsule containing three seeds, which are smooth, egg-shaped, shiny brown and spotted. Because of their shape and colouration the seeds are said to resemble beetles.

It is widely grown in Nigeria for ornamental purposes and for the oil extracted from the seeds. There are many varieties of castor oil plant, some of which are very decorative with bronze or red colored leaves. They can be grown successfully in well-drained soil. Castor oil plant is well adapted to the savanna region of west Africa where it is mostly grown by small holder farmers in the home backyards, as border crops around annual crops, sole crop in small holdings or in combination with other crops especially cereals, (21). Seeds play a vital role for the healthy production of a crop as they are known to carry pathogens and may themselves suffer from diseases. Neergaard and Mathus, (23) reported that some of the fungi associated with the seeds may cause *ample* damage to the seeds in various ways. In cases of severe infection, the quality of the seeds deteriorated and sometimes grain may not be suitable for use as seeds, (22), or for consumption due to production of mycotoxic substances in the seeds, (6). Mycotoxins produced by microorganisms such as *Aspergillus flavus* apart from reducing the viability of seeds, is also hazardous to man and livestock, (10). Ekefan and Adie, (11) reported that several fungi including *Aspergillus flavus*, *A. niger*, *Rhizoctonia* spp. and *Botrytis cinerea* were identified on groundnut. The presence of these organisms indicates that groundnut seed is vulnerable to many infections which reduce seed quality.

There is however a dearth of literature on microorganisms borne on seeds of castor oil in Nigeria. This work was conducted to provide information on the most prevalent fungi associated with castor oil seed, establish the effects of these on seeds and to investigate the effects of seed treatment with fungicides.

MATERIALS AND METHODS

Fungi associated with castor oil seed. Seeds for this experiment were collected from four different locations in Nigeria: (i) Kafanchan (94^0N, 82^0E); (ii) Samaru (11^0N, 74^0E); (iii) Zonkwa (94^0N, 82^0E); (iv) Nsukka (7^0N, 75^0E). All samples were stored at 20-25^{o}C until when required.

Seeds were examined for seed-borne fungi using the Potato Dextrose Agar (PDA) method described by (ISTA) International Seed Testing Association (13). Seeds of each location were disinfected by soaking in 1% Sodium hypochlorite solution for five minutes after which they were rinsed several times with sterile distilled water. From these washed seeds, 100 seeds from each location were plated on potato dextrose agar (with streptomycin) medium (PDAS) at the rate of 10 seeds per plate and kept on laboratory benches at room temperature of 25 – 30^o C for 7 – 9 days to allow for fungi growth. Colonies of each fungus that grew were identified, counted and recorded. The fungi were identified with the aid of available manuals (3, 11, 7). Further identification was

achieved by comparing with existing cultures in the plant pathology repository already identified by International Mycological Institute, Egham, U.K.

Effect of metabolites on seed germination and seedling parameters

Pure cultures of three most predominant fungi were obtained through single spore isolation. Each fungus was inoculated separately into Richards medium contained in a 250 ml flask. The liquid medium was prepared as described by CAB (4). They were incubated for 15 days at room temperature of 25–30^{0}C every two days, the flasks were shaken and at the end of incubation, filtrates were obtained by filtering the contents with a two layered cheese cloth. Seeds were soaked in each culture filtrate for 12 hours (2) and divided into two lots. One lot was allowed to germinate in Petri dishes containing sterile filter papers kept on a laboratory bench, while the second lot was sown in plastic pots containing heat-sterilized soil kept in the screen house. Adequate moisture was maintained by adding sterilized distilled water in Petri dishes and tap water for pots in the screen house. One hundred seeds were taken for each assay and percentage germination was calculated seven days after sowing, for seeds sown in Petri dishes and 10 days for potted seeds. Only the potted plants were assessed for plant establishment, plant height, root length, fresh root, shoot weights, dry roots and shoot weights twenty days after sowing.

Effect of fungicides on major seed germination and establishment.

The castor oil seeds used in this study were divided into four lots and each lot dressed with either benomyl (Benlate), mancozed (Dithane M- 45), metalaxy + carbon + furathiocarb (Apron – plus) and thiram (Fernasan D) after surface sterilization as described above.

Each lot of seed was further divided into four sub-plots and dressed with either of the following concentrations: 0 (no fungicide = control), 2.5 g/kg, 3.5g/kg and 5.0 g/kg. One hundred seeds were plated in sterile Petri dishes containing sterile filter paper (13) for each treatment. The trial had three replications and arranged on laboratory benches at room temperatures (25-30^{o}C) in a completely randomized design. Ten days after planting, fungi that grew were counted and recorded.

To study the effect of fungicides on germination and establishment, another set of seed was divided into five lots and four lots dressed with fungicides as described above while the fifth lot was left undressed and served as control. Ten seeds were sown in 25 cm earth ware pots containing field soil replicated three times (each replicate consisted of two pots) and the pots were arranged on the screen house floor in a completely randomized design. Ten days after sowing, seeds that germinated were counted and 21 days after sowing, seedlings were assessed for establishment.

Statistical analysis

Data obtained were statistically analyzed using INSTAT. Angular transformation was carried out where necessary and Duncan's Multiple Range Test (DMRT) was used to establish differences between the means.

Results

Seven fungi, *Aspergillus niger*, *A. flavus*, *Penicillium expansum*, *Fusarium moniliforme*, *Rhizopus stolonifer*, *Phoma* sp. and *Helminthosporium haloides* were isolated from the seeds (Table 1). Phoma sp. was only isolated from seeds collected from Zonkwa and Kafanchan while *R. stolonifer* occurred only on seeds from Kafanchan. *H. haloides* was isolated from seeds of Samaru only. *A.niger* had the highest frequency of occurrence in most of the locations except in Samaru. A higher frequency of fungi was obtained using the blotter technique compared to the PDA method, although *A. flavus* and *A. niger* remained the most dominant species. The effect of fungi metabolites from the four most dominant fungi on seed germination is shown in Table 2. All fungi metabolites significantly (P=0.05) reduced percentage germination compared to the control. The mycotoxins from these pathogens reduced plant heights, root length, fresh root and shoot weights compared to the control (Table 3).

All fungicides proved effective in suppressing the development of one fungi species or the other (Table 4). The fungicides at all concentrations reduced colonies of *R. moniliforme* at 5.0 g/kg, benlate and fernasan D effectively controlled *P. notatum* at 5.0 g/kg. Application of fungicides (except Apron plus) induced a significant (P=0.05) increase in seed germination (Table 5). However, benlate at 5.0 g/kg had the most significant effect in increasing seedling establishment, while seeds treated with Apron plus at 5.0 g/kg depressed germination. Except for seeds treated with Apron plus at 5.0 g/kg other seeds in other treatments that germinated became fully established 10 days after sowing.

DISCUSSION

The fungi found to be associated with castor oil in this study have also been reported to occur on other grain legumes in Nigeria (10, 23) and with castor oil in other parts of the world (2, 16, 22). A few of these fungi are pathogenic to castor oil e.g. *Phoma* spp. (22) while *F. moniliforme* and *H. haloides* could be potentially pathogenic as they are reported to cause disease in other crops (12, 26). Other fungi such as *A. flavus* and *A. niger* are reported to be storage fungi causing severe storage problems, loss of viability (18) and are also toxigenic (12). The isolation of more fungi through the blotter technique support reports by Sinha and Khare (24) and Maduekwe and Umechuruba (17) that this technique was better in detecting seed-borne fungi. However, Gill *et* al. (10) found the PDA technique was better in determining mycoflora associated with seeds of leguminous species.

Table1. Percentage frequency of fungi isolated from castor oil seed (*Ricininus communis*) collected from four locations in Nigeria using the agar plate and blotter paper.

Fungi	% frequency			
	Nsukka	Samaru	Zonkwa	Kafanchan
PDAS medium				
Fusurium moniliforme	0.8d	1.6d	5.7c	1.6d
Aspergillus flavus	9.0b	0.6d	0.8d	1.6d
A. niger	13.9a	3.3c	18.0a	12.3a
Penicillium notatum	5.0c	4.9c	1.6d	3.3c
Rhizopus stolonifer	0	1.0d	0	2.5c
Phoma sp.	0	0	0.8d	12.2a
Heliminthosporium haloides	0	0	5.0c	0
Blotter technique				
F. moniliforme	2.7c	13.4a	3.4c	2.7c
A. flavus	9.7c	11.3a	2.0c	10.7a
N. niger	14.7a	14.0a	19.4a	8.7b
P. notanum	5.4c	5.4c	2.8c	2.7c
R. stolonifer	2.0d	2.0d	2.0d	3.4c
Phoma Sp.	12.7a	0	2.7c	2.1c
Heliminthosporium haloides	0.4d	0	7.5b	0

Figures followed by the same letter do not differ significantly (p=0.05) using Duncan's Multiple Range Test.

Table 2: Effect of fungal metabolites on germination of castor oil seeds collected from four Locations in Nigeria

Source of metabolites	%germination			
	Nsukka	Samaru	Zonkwa	Kafanchan
PDAs medium				
Fusurium moniliforme	3.5b	10.4b	4.6b	5.8b
Penicillum notatum	6.3b	9.3b	8.1b	8.1b
Aspergillus niger	2.3b	3.5b	2.3b	12.8b
A. flavus	3.5b	9.3b	5.8b	3.6b
Control	93.0a	76.0a	86.0a	73.0a

Figures followed by the same letter do not differ significantly (p-0.05) using Duncan's Multiple Range Test.

Table 3: Effect of fungal metabolites on seedling parameters of castor oil.

Source of metabolites					
Fungi	Fresh shoot Weight(g)	Dry shoot Weight(g)	Fresh root Weight(g)	Plant height Weight(g)	Root length Weight(g)
Fusurium moniliform	1.3b	0.5b	0.8b	0.2b	3.2b
Penicillum notatum	1.0b	0.4b	0.8b	0.2b	3.7b
A spergillus niger	1.6b	0.5b	0.7b	0.2b	3.5b
A. flavus	1.8b	0.6b	0.7b	0.2b	3.4b
Control	3.4a	1.4a	1.3a	0.7a	10.7a

Figures followed by the same letter do not differ significantly (p-0.05) using Duncan's Multiple Range Test.

Table 4: Effect of four fungicides on major mycoflora of castor oil.

Fungicide	Treatment (g/kg)	% fungal incidence			
	A. *flavus*	*A. niger*	*F. moniliforme*	*P. notatum*	
Apron-plus	5.0	2.7	0	2.9	0.5
	3.5	2.0	0	1.9	0.7
	2.5	2.7	2.1	3.2	1.7
Benlate	5.0	0	0	0	0
	3.5	0	0	0	0
	2.5	1.7	1.4	2.9	0.7
Dithane M45	5.0	2.1	0	1.5	0.8
	3.5	2.0	0.7	1.5	1.5
	2.5	2.6	1.4	2.6	2.3
Fernasan D	5.0	2.0	0	0	0
	3.5	1.8	0	1.5	0.7
	2.5	2.4	0	1.7	1.3
Control	14.0	12.2	14.6	9.0	3.0

Fungi involved; *Aspergillus flavus, A. niger, Fusarium moniliforme, penicillium notatum.*

Table 5: Effect of fungicides on seed germination in castor oil.

Fungicide	Concentration [g/kg]	% germination	%establishment
Apron-plus	5.0	56.0(7.5)b	80
	3.3	80.0(8.9)a	100
	2.5	80.0(8.9)a	100
Benlate	5.0	86.0(9.3)a	100
	3.3	80.0(8.9)a	100
	2.5	63.1(7.9)b	100
Dithane M45	5.0	69.8(8.4)a	100
	3.3	70.0(8.4)a	100
	2.5	73.0(8.6)a	100
Fernasen	5.0	73.3(8.6)a	100
	3.3	73.3(8.6)a	100
	2.5	62.6(7.9)b	100
Control	0	60.0(7.7)b	95

Figures in bracket are square root transformation of original data.
Figures followed by the same letter are not significantly (p=0.05) different using Duncan's Multiple Range Test.

Seed germination was appreciably affected by metabolites produced from fungi most commonly associated with castor oil seeds in this study. This indicates the probable effects of these metabolites in-situ when they are continuously associated with the seeds. Kulkarni and Deshpande (15), Arya and Mathew (2) also recorded reduced germination in various legume crops studied due to fungal metabolite. The reduced germination observed could have been due to some ultra structural changes in the embryo of the seeds (1) caused by fungal metabolites. These effects would have exerted further suppressive effects on seedling establishment in seeds that germinated as observed. All fungicides, effectively controlled fungi found to be associated with the seeds however; Benlate proved to be the most effective and was reported to be one of the most widespread and successful of the systemic fungicides (24). Zaidi et al. (28) and Kaiser and Hannan (14) also found Benlate to be

most effective in reducing the overall fungal mycoflora in chickpea. Ekpo (8) and Davis (7) also showed that Benlate was effective in preventing seed-borne infections of *Terminalia superb* and *Stylosanthes* sp., respectively. Internally seed-borne fungi appear to play a major role in reducing seed quality as application of fungicides increased seed germination as observed. It therefore becomes apparent that when seeds, especially of low quality, are to be sown, seed treatment with fungicides may be beneficial in increasing seed emergence and stand establishment as reported for soya bean (5). Ellis and paschal (9) reported that the use of fungicides as seed treatment also allows the germination potentials of the seed to be expressed.

REFERENCES

Anderson J.D, E. Baker and W. Washington (2005). Ultrastructural changes of embryos in wheat infected with storage fungi. *Plant physiololgy*. 46:857-859.

Arya, A and D.S. Mathew (2001). Variation in seed germination of castor oil following treatment with fungi metabolites. *Indian Phytopath*. 44: 392-394.

Barnett, U.L. (1999). Illustrated genera of imperfect fungi. Minneapolis. Burgess publ. Co. CAB (2003). Plant pathologists Handbook, 2nd Edition. CAB International, Egham, U.K. 439 pp.

Casela, C.R., M.A. Noguez and A.C.A. Des (2002). Chemical treatment of seed of soya bean (*Glycine max* [L] Merit). Soja Result, Pesq. 1 : 81-90.

Christensen, C.M. and H.H. Kaufmann (2004). Microflora. In "Storage of cereal grains and their products" (ed. Christensen, C. M.) St Paul Minn. 2nd Edition. 549 pp.

Avis, R.D. (1998). Seed borne *Colletotrichum gloeosporiodes* infection and fungicides control in *Stylosanthes* spp. Seed Sc. and Tech. 15: 785-793.

Ekpo, E.N. (1994). Seed borne mycoflora of *Terminalia superb* and control with fungicides. Nig. J. Pl. Protection 15: 74-83.

Ellis, M.A and E. H. Paschall (2000). Effect of fungicide seed treatment on internally seed-borne fungi, germination and filed emergence of pigeon pea (Cajans cajan). Seed Sc. and Tech. 7: 75-81.

El-Wakil, A.A. and Ghonim, M.I. (2000). Survey of seed-borne mycoflora of peanut and their control. Egyptian Journal of Agricultural Research, 78 (1): 47-60.

Ekefan, E.J. and Adie, P. (2003). Occurrence of fungi and fungicidal seed treatment on percentage incidence and viability of groundnut seed in Benue State. Nigeria Journal of Plant Protection, 20 : 93-99.

Gill, L.S., J. U. Obi and S.W.H. Hussaini (2003). Mycoflora of Nigerian leguminious seeds. Legume Research 6: 29-33.

Gillman, J.C. (2001). A manual of soil fungi. Oxford and IBH Publishing Co. New Delhi.

Gupta, R. 1996. Seedborne fungi in sorghum. Plant Genetic Res. News. 106: 39-40.

ISTA (2003). International rules for seed testing Rules 1985. International Seed Testing Association, Zurich.

Kaiser, W.J. and R. N. Hannan. 1998. Seed transmission of *Ashochytarabie* in hickpea and its control by seed treatment fungicides. Seed Sci. and Tech. 16: 625-637.

Kulkarni, G.M. and K.S. Deshpande 1997. Effects of fungi metabolites on seed germination in chickpean. Acta Bot. Indica 15: 181-186.

Lokesh, M.S., R.Y. Hiremath and R.K. Hegde (1998). Seed mycoflora of redgram (*Cajanus caja*). *Plant path News*. 5: 30.

Maduekwe, B.O. and C.I. Umechuruba (1992). Evaluation of classical seed health testing methods for the detection of cowpea seed-borne fungi. *Nig. J. Botany* 5: 119-124.

Marley, P.S. (1996). The role of fungi in cereal grain storage. Noma 12: 13-17.

Marley, P.S. (1997). Dieback disease of pigeon pea (*Cajanus cajan*) caused by *Botryodiplodia theobromeae. Pat. Nig. J. Plant Protect*. 17: 1-9.

Mishra, R.R. and A. Kanajia. (1994). Studies on certain aspects of seed fungi of *Pennisetum tyhoides*. *Comm.. Phytopath. Soc. Hungary* 7: 1-3.

Neergaard, P. and S.K.Mathus (1997). Detection of seedborne fungi: Sorghum and location of *Fusarium moniliforme*. *Seed Sc. and Tech*. 3: 683-690

Nene, Y.L.,V.K. Sheila and S.B. Sharma (1996). A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) pathogens. 5th Edition. Patancheru, 502-324, A.P. India. ICRISAT, 27 PP.

Oyekan, P.O. 1998. Fungicides treatment of seeds. In "University of Ife Institute of Agricultural Research and Training, Annual Report, 1977-78." Ile-Ife, Nigeria. 40 pp.

Pegg, G.F. (1974). Verticillium disease. Plant path. 53(3): 157-176.

Sinha, O.K. and M.N. Khare (1998). Comparative efficiency of four methods for the detection of seed-borne fungi of cowpea. Mysore J. Agric. Sc. 12(3):437-440.

Tarr, S.A.J. (1992). Disease of sorghum, sudan grass and broomcorn. CMI Kew, Surrey U.K. 380 pp.

Toussoun, T.A and P.F. Nelson (1998). A pictorial guide to the identification of *Fusarium* species according to the taxonomic system of Snyder and Hansen the Pennsylvania State University press. University Park, Pennsylvania U.S.A. 51 pp.

Zaidi, S.B.I. M.I Khan and S.K. Saxena (1991). Effects of fungicides on Mycoflora chicpea seeds. *India phytopath*. 44:394-395.

Pourmorad, F. , Hosseinimehr, S, J. ,Shanabi, M, N. (2006). Antioxidant activity, phenolic and flavonoid content of some selected Iranian medicinal plants. *Afr .J .Biotechnol*. 5 (11). 1142-1145.

Price,K,R.,Johnson,I,T.and Fenwic,C,R.(1987).The chemical and biological significance of saponins in food and feeding stuff. *Unpublished Critical Reviews in Food science and Nutrition*.26.27-135.

Robbins,R,J.(2003). Phenolic acids in foods. An overview of analytical methodology *Agricultural and Food Chemistry*.51.2886-2887.

Schippers,R,R.(2000).African Indigenous Vegetables. An overview of the cultivation species. Natural Resources Institute. PCP. EU Technical Centre of Agriculture and Rural Co-operation.Chaton.UK.

Sofowara,R,A.(1984).Medicinal plants and transitional medicine in Africa, *John Wiley Chichester.* a.

Trease, G, E. and Evans, W, C. (1984).*Pharmacognosy*.13th edn.332.

WHO,(1980).Expert committee on diabetes mellitus. Second report. Technical report series 646.World Health Organization,Geneva.12-15.