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CONTENTS

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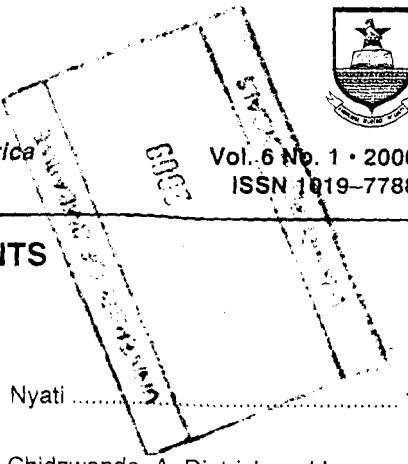

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Natural control of fungi and mycotoxin in grains — a possible means of reducing human and animal contamination

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Essential oils extracted from the edible plants, *Ocimum gratissimum, Thymus vulgaris* and *Cybopogon citratus* were tested from 1997 to 1998 against *Fusarium moniliforme.* This fungus is known worldwide as a producer of mycotoxins that are associated with human and animal oesophageal and liver cancer. The experiments were conducted both at the Biochemistry Laboratory of the University of Yaounde and the Plant Pathology Laboratory of IRAD Yaounde, Cameroon. Poisoning technique was used followed by an incubation of Petri dishes and fungal inoculation for 7 days at 20°C under an alternated cycle of 12 hours of light and darkness. These essential oils inhibited at different levels the radial growth of *F. moniliforme* and expressed a fungistatic and fungicidal property. With *O. gratissimum* no growth of *F. moniliforme* was observed at the concentration of 200 ppm after 7 days of incubation. With *T. vulgaris* and *C. citratus* no growth of *F. moniliforme* was observed with the concentration of 500 ppm after 7 days of incubation. Testing of maize kernels treated with a mixture containing 100 ppm of essential oils extracted from *O. gratissimum* in the proportion 1:10 and Dimethylsulfoxide (9:10) showed a complete elimination of *F. moniliforme* in maize kernels. Other fungi such as *Aspergillus sp.* and *Penicillium sp.* were also killed. This control of *F. moniliforme* which is a producer of carcinogenic *Fumonisin* in maize kernels, using natural substances (essential oils, powders and plant extracts) reduces fungal contamination in food and their bad effects on human and animal health. This can limit the evidence of hepatocarcerogenicity of mycotoxins in humans which has been found impressing and alarming, especially in Africa - where maize is the staple food for many populations.

Keywords: Maize, *Fusarium*, Mycotoxin, Control

Introduction

Fusarium moniliforme Sheldom is among the most prevalent seed-borne fungi of maize in many corn producing countries of the world (Booth, 1971; Hesseltine *et al.*, 1976; Waller and Brayford, 1990, Tagne, 1995, Tagne, 1997). *F. moniliforme* is known as an important toxigenic species in the *Genus Fusarium Section Liseola* (Marasas *et al.*, 1984). Infected grains are toxic to animals (Kellerman *et al.*, 1972; Kriek *et al.*, 1977; Marasas *et al.*, 1976), causing mycotoxicoses such as leucoencephalomalacia in horses and pulmonary oedema in swine (Ross *et al.*, 1990). A number of important mycotoxins have been isolated from *F. monilforme* over the years including Moniliformin (Thrane, 1988); Fusarin C (Bjeldanes and Wei, 1980; Cheng *et al.*, 1985; Gelderblon *et al.*, 1984; Farber and Sanders, 1986); Fumonisins (Gelderblon *et al.*, 1988; Ngoko, 1997, Ross *et al.*, 1990; Thiel *et al.*, 1992); Thricothecenes (Marasas *et al.*, 1984) and Zearalenone (Richardson *et al.*, 1985). Among these mycotoxins, there are some that are mutagenic or cancerogenic to humans and animals; Fusarin C is an example of the former while Fumonisin is a typical example of the latter. Circumstantial evidence of hepatocancerogenicity of mycotoxins in humans has been impressive and alarming, particularly in Africa were many populations remain ignorant of fungal contamination in grains and their bad effects on human and animal health. The antifungal properties of essential oils against food spoilage and mycotoxins have been investigated (Awah, 1989, 1990; Mishra and Dubey, 1994; Amvam Zollo *et al.*, 1997), however little or no attention has been given to *F. moniliforme*.

This study was carried out to investigate the antifungal properties of essential oils extracted from three edible aromatic plants (*Ocimum gratissimum, Thymus vulgaris* and *Cymbopogon citratus)* of the diverse flora of Cameroon. The specific objective was to control *F. moniliforme* in grain and consequently mycotoxin production during storage. The expected result was viewed to help in the prevention of mycotoxins-related diseases and mutagens effect among resource-poor farmers of Cameroon and others who consume maize as their staple food.

Materials and Methods

Materials

The following material were used to carry out the experiment:

Essential oils were extracted from the leaves of *Cymbopogon citratus, Ocimum gratissimum* and the stem of *Thymus vulgaris* by hydro-distillation using the Clavenger apparatus in the Department of Biochemistry, University of Yaounde.

Maize grains of the varieties CMS 8704 and CMS 8501 developed by the cereals program of the Institute of Agricultural Research and largely adopted in Cameroon.

Culture of *Fusarium moniliforme* isolated from maize seeds variety CMS 8704 at the Plant Pathology laboratory of the Institute of Agricultural Research for Development Nkolbisson, Cameroon.

Dimethylsulfoxide (DMSO) for the dilution of essential oils.
Potato Dextrose Agar (PDA) and
Blotter paper of diameter 9 cm.

Methods
Antifungal Properties:
The antifungal property of the essential oil on agar media was determined using the Poisoning technique (Thompson, 1992; Grover and Moore, 1962). The concentrations of essential oil tested are shown in Table 1.

Table 1: Radial growth of *F. moniliforme* after seven days of incubation and inhibition of the radial growth by essential oil.

Concentration/ppm	Essential oils					
	O. grattissimum		T. vulgaris		C. citratus	
	Radial growth** (mm)	Percentage Inhibition	Radial growth** (mm)	Percentage Inhibition	Radial growth** (mm)	Percentage Inhibition
Control DMSO	70	0	70	0	70	0
0	70	0	70	0	70	0
10	23	70	*	*	*	*
50	9	86	67	4	*	*
100	7	90	60	14	58	17
150	6	91	*	*	*	*
200	0	100	22	69	37	48
300	*	*	7	90	21	70
400	*	*	6	91	6	91
500	*	*	0	100	0	100

* not tested.
** Mean diameter of the 3 plates and the 4 replicates prepared for each concentration.

The essential oil was diluted (1:10) final solution with Dimethylsulfoxide (DMSO) (9:10). This mixture was further dilute into 20 ml of PDA at a given quantity to obtain each of the concentration in parts per million (ppm). This media was then dispensed into a sterile glass Petri plates of diameter 9 cm. These plates were inoculated with a mycelial disk of *F. moniliforme* grown on PDA for 5 to 7 days, cut with a cork borer of 0.6 cm. The inoculated plates were incubated for 7 days at 20°C under an alternated cycle of 12 hours of light and darkness. The radial growth of the culture of *F. moniliforme* was evaluated daily up to 7 days after inoculation. The lowest concentration of the essential oil where no growth of *F. moniliforme* occurred on the PDA was recorded as the Minimum Inhibitory Concentration (MIC).

Twelve Petri plates in 4 replicates of 3 were prepared for each concentration and the test was repeated; two controls were prepared, one with DMSO and the other with PDA only. The inhibition was calculated as percentage of the difference between the control and the radial growth obtained for a given concentration.

Treatment of maize kernels with essential oil

Four hundred grains of each maize variety were treated to a mixture of the essential oil extracted from *O. gratissimum* (1:10) and DMSO (9:10) prepared as a solution containing 100 ppm of essential oil. The treatment was performed by soaking the grain for about 5 minutes.

Testing of treated kernels for fungal infection

After the treatment, the treated kernels were removed and directly plated 10 in each 9 cm-diameter Petri dish. The Petri dishes was prepared by placing 3 pieces of wetted blotter paper in the dish. Four hundred kernels of each variety were plated and handled as 4 replicates of 100 kernels. The Petri dishes and the plated kernels were incubated for 7 days following the deep freezing blotter method described by Singh *et al.*, (1974). Full details of the method are: 20°C under an alternated cycle of light and darkness for 24hours, followed by -20°C in darkness (deep freezer) for 24hours and then 20°C under an alternated cycle of light and darkness for 5 days.

After incubation the kernels were observed one by one under a stereo microscope to identify mycelial growth and the chain arrangement of *F. moniliforme* microconidia. Preparations were made from fungal growth on the kernel using deionised water and observed under a leitz compound microscope. The identification was made following the keys of Ram Nath *et al.*, (1970) and Burgess *et al.*, (1988).

Results

Antifungal Properties

The 3 essential oils tested inhibited at different levels the radial growth of *F. moniliforme* on PDA after 7 days of incubation (Table 1).

The essential oil from *O. gratissimum* inhibited the growth of *F. moniliforme* by 70 percent at the concentration of 10 ppm; with 200 ppm no growth was found indicating that the Minimum Inhibitory Concentration (MIC) of *O. gratissimum* against *F. moniliforme* is less than or equal to 200 ppm (MIC ≤200 ppm).

The essential oil from *T. vulgaris* inhibited the growth of *F. moniliforme* only 4 percent at 50 ppm and completely inhibited the growth at 500 ppm. This indicates that the Minimum Inhibitory Concentration of this essential oil against *F. moniliforme* is less than or equal to 500 ppm (MIC ≤500 ppm).

The essential oil from *C. citratus* produced only 17 percent inhibition at 100 ppm and a complete inhibition at 500 ppm. This proved that the Minimum Inhibitory Concentration of this essential oil against *F. moniliforme* is less than or equal to 500 ppm (MIC ≤500 ppm).

No inhibition of *F. moniliforme* was noted on the 2 controls after 7 days of incubation.

Table 2: Impact of the essential oil from *O. gratisimum* on *F. moniliforme* infection in corn.

Treatment	Maize varieties	
	CMS 8501	CMS 8704
No treament (control)	97%*	100%
Dimethylsulfoxide	96%	98%
Essential oil from O. gratissimum	0%	0%

* Percent infection found in the 400 tested kernels.

Control of Fusarium moniliforme in grains

The treatment with the essential oil from *O. gratissimum* completely eliminated the infection of *F. moniliforme* in maize grains (Table 2). In the kernels of the two maize varieties, CMS 8501 and CMS 8704 treated with this essential oil, no infection of *F. moniliforme* was found. In the untreated control, 97 percent and 100 percent infection of *F. moniliforme* was recorded respectively for CMS 8501 and CMS 8704. Treated kernels with DMSO yielded 96 percent and 98 percent infection of *F. moniliforme* respectively for the CMS 8501 and CMS 8704, showing a non significant effect of this product on the infection of *F. moniliforme* in the kernels of the two varieties.

Discussion

The essential oils extracted from *T. vulgaris* and *C. citratus* inhibited the radial growth of *F. moniliforme* with MIC's of less than or equal to 500 ppm. The essential oil from *O. gratissimum* inhibited the radial growth of *F. moniliforme* with a MIC of less than or equal to 200 ppm. No inhibition was found on PDA and with DMSO, meaning that the inhibition of the radial of the fungus is the effect of the essential oil. These observations demonstrated the antifungal activity of the three essential oils studied against *F. moniliforme*.

No growth of *F. moniliforme* and also of other fungi was found on corn kernels treated with a solution of essential oil and DMSO containing 100 ppm of the essential oil, *O. gratissimum*, after 7 days of incubation. This proved that the essential oil extracted from *O. gratissimum* completely eliminated the infection of *F. moniliforme* in corn grains as well as any other common fungal contaminant such as *Aspergillus sp.* and *Penicillium sp.* that may have been present in the samples. This result is similar to that of Awah (1989, 1995) and Mishra and Dubey (1994) who found the toxicity of some essential oils against fungi causing deterioration of stored food and grains. This essential oil can be potentially used as a natural

protectant of grains against important seed-borne fungi such as *F. moniliforme*, and also fungal contaminants such as *Aspergillus sp.* and *Penicillium sp.* The advantage of using essential oils, powder or extracts of edible plants such as *O. gratissimum*, *T. vulgaris* and *C. citratus* to protect grain before and during storage, was that it limited its contamination by *F. moniliforme* and other mycotoxigenic fungi such as *Aspergillus sp.* and *Penicillium sp.* This will reduce the consumption of contaminated foodstuff and mycotoxins, especially among the resource-poor population to whom maize is a staple food. Consequently, this will be of tremendous help in limiting the evidence of hepatocarcerogenicity of mycotoxins in humans, a trend which of late has been reported to be alarming in Africa, particularly in Cameroon (Cardwell, 1995).

This study presents, for the first time, evidence of the control effect of essential oil of *O. gratissimum* on *Fusarium moniliforme* and the possibility of it being used to protect maize grain against fungal contamination and probably mycotoxin. The need of further in-depth studies to formulate the essential oils or the aromatic plant material used into efficient, cost effective and ecologically friendly bioprotectant to improve the storage of maize grain against fungal contamination and mycotoxin is highly illustrated by this work.

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REFERENCES

Amvam Zollo, P. H., Biyiti, L. Tchoumbougnang, F., Menut, C. and Bouchet P. H. 1998 Aromatic plants of Central Africa. Part XXXII, Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour and Fragrance 13.

Awah, R. T. 1995 Possible utilization of plant products in grain storage. Proceedings of the workshop on mycotoxins in food in Africa; November 6–10, 1995. Cotonou Benin. DANIDA-IITA-IDRC, 32.

Awah, R. T. 1989 Fungitoxic effects of extracts from some west African plants. *Annals of Applied Biology* **115**: 451–453.

Bjeldanes, L. F. and Wei, L. A. 1980 Mutagenic mycotoxins from *Fusarium moniliforme*. *Environmental Mutagenesis* **2**: 240–241.

Booth, C. 1971 The genus *Fusarium*. Commonwealth Mycological Institute Kew, Surey England. 237p.

Burgess, L. W., Lidell, C. M. and Summerell, B. A. 1988 Laboratory Manual for *Fusarium* Research. University of Sydney 156p.

Cardwell, K. F. 1995 Proceedings of the workshop on mycotoxins in food in Africa; November 6–10, 1995. *Cotonou Benin*. DANIDA-IITA-IDRC, 5.

Cheng, S. J., Jiang, Y. Z., Li M. H., Lo, H. Z. 1985 A mutagenic metabolite produced by *Fusarium moniliforme* isolated from Linxian county , China. *Carcinogenesis* **6**: 903–905.

FARBER, J. M. AND SANDERS, G. W. 1986 Fusarin C production by North America isolates of *Fusarium moniliforme. Applied Environmental Microbiology* **51**: 381–384.

GELDERBLOM, W. C. A., MARASAS, W. F. O. AND VAN DER MERWE, K. J. 1984 Natural occurrence of fusarin C a mutagen produced by *Fusarium moniliforme* in corn. *Journal of Agricultural Food Chemistry* **2**: 1064–1067.

GELDERBLOM, W. C. A., JASKIEWICZ, K., MARASAS, W.F.O., THIEL, P. G., HORAK, R. M., VLEGAAR, R. AND KRIEK, N. P. J. 1988 Fumonisins a novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme. Applied Environmental Microbiology* **54**: 1806–1811.

GROVER, R. K. AND MOORE, J. D. 1962 Toximetric studies of fungicides against brown rot organism *sclerotinia fruticola* and *S. laxa Phytopathology* **52**: 876–880.

HESSELTINE, C. W., SHOTWELL, O. L., KWOLEK W. F., LILLEHOJ, E. B., JACKSON, W. K. AND BOOTHAST R. J. 1976 Aflatoxin occurance in 1973 corn at harvest. II Mycological studies. *Mycologia* **68**: 341–353.

KELLERMAN, T. S., MARASAS, W. F. O., PIENAAR, J. G. AND NAUDÉ T. W. 1972 A mycotoxicosis of Equidae caused by *Fusarium moniliforme* Sheldon Ondersteport. *Journal of Veterinary Research* **39**: 205–208.

KRIEK, N. P. J., MARASAS, W. F. O., STEYN, P.S., VAN RENGSBURG, S.J. AND STEYN M. 1977 Toxicity of a moniliformin-producing strain of a *Fusarium moniliforme* var. subglutinans isolated from maize. *Food and Cosmetics Toxicology.* **15**: 579–587.

MARASAS, W. F. O., KELLERMAN, T. S., PIENAAR, J. P. AND NAUDÉ,T.W. 1976 Leucoencephalomalacia: A mycotoxicosis of Equidae caused by *Fusarium moniliforme* Sheldon. Onderstepoort. *Journal of Veterinary Research* **43**: 113–122.

MARASAS, W. F. O., NELSON, P. E., TOUSSON, T . A. 1984 Toxigenic *Fusarium* Species: Identity and Mycotoxicology, The Pennsylvania State University Press 310p.

MISHRA, A. K. AND DUBEY, N. K. 1994 Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied Environmental Microbiology* **60(4)**: 1101–1105.

NGOKO, Z. 1997 Maize diseases — a threat to our survival. Paper Presented during the 5th Annual Biosciences Conference 17-19 December 1997. The Cameroon Biosciences Society (Abstract).

RAM NATH , NEEGAARD, P. AND MATHUR, S. B. 1970 Identification of *Fusarium* species as they occurred in the blotter test. *Proceedings of the International Seed Testing Association* **35**: 1.

RICHARDSON, K. E., HAGLER, J. R., CAMPBELL, W. M. AND HAMILTON, P. B. 1985 Production of zearalenone, T-2 toxin and dexoynivalenol by *Fusarium sp.* Isolated from plant material grown in North Carolina. *Mycopathologia* **90**: 155–160.

ROSS, P. F., NELSON, P. E., RICHARD, J. L., OSWEILLER, G. D., RICE, L. G., PLATTNER, R. D. AND WILSON, T. M. 1990 Production of Fumonisins by *Fusarium moniliforme* and *Fusarium proliferantum* isolates associated with equine leukoencephalomalacia and a pulmonary oedema syndrome in swine. *Applied Environmental Microbiology* **56**: 3225–3226.

SINGH, D. V., MATHUR, S. B. AND NEERGAARD, P. 1974 Seed Health testing of maize. Evaluation of testing technique with special reference to *Dreschlera maydis. Seed Science and Technology*: 349–365.

TAGNE, A., THÉ . C., HANSEN, H. J. AND MATHUR, S. B. 1997 Les espèces *Fusarium* isolées des graines de maïs du Cameroon: Pertes après recoltes et Mycotoxines. (Fusarium species isolated from maize grains of Cameroon: Post-harvest losses and mycotoxins) Paper presented during the 5th Annual Biosciences Conference 17–19 December 1997. The Cameroon Biosciences Society (Abstact).

TAGNE, A. 1995 *Acremonium strictum* and *Fusarium moniliforme* in maize seed samples of Cameroon: Infection Biology Seed Transmission and Treatment. M Sc.Thesis The Royal Veterinary and Agricultural University, Denmark, 125p.

THIEL, P. G., MARASAS, W. F. O., SYDENHAM E. W., SHERPHARD, G. S. AND GELDERBLOM, W. C. A. 1992 The implications of naturally occuring levels of fumonosins in corn for humans and animals health. *Mycopathology and Aplied Mycology* **117**: 3–9.

THOMPSON, D. P. 1992 Inhibition of Mycelial growth of mycotoxigenic fungi by phenolic oxidants. *Mycologia* **84(5)**: 791–793.

THRANE, U. 1988 Geographic distribution of toxigenic Fusarium species. Proccedings of the Japanese Association of Mycotoxicology Special issue N°1: 226–227.

WALLER, J. W. AND BRAYFORD D. 1990 *Fusarium* diseases in the tropics. *Tropical Pest Management* **36**: 181–194.