CYMBOPOGON PROXIMUS (HALFA BARR) EXTRACTS TO CONTROL MAIZE EAR ROTS AND MINIMIZE MYCOTOXINS ACCUMULATION

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Abstract

A field trial was carried out to control maize ear rots and reduce the production of fumonisin FB1 produced *by Fusarium verticillioides* and aflatoxin B1& B2 produced by *Aspergillus flavus* by using extracts of *Cymbopogon proximus* Stapf. These extracts were superior to extracts of *Thymus vulgaris* and *Salvadora persica* in reducing infection with the target fungi. *C.proximus*-hexane extract gave the best effect in this respect. Using *C.proximus*-hexane extract as a protective application significantly affected the disease comparable to application after fungal inoculation. All treatments successfully reduced production of aflatoxins B1 compared with the untreated control, whether in rotted or adjacent kernels. Aflatoxin B2, however, was completely prevented to accumulate due to these treatments. *C.proximus*-hexane extract caused more reduction in aflatoxin production compared with other extracts. Fumonisin FB1 production was reduced in the discoloured as well as the adjacent kernels. The treatment was more effective when used before than after the fungal inoculation in minimizing FB1 accumulation.

INTRODUCTION

From ripening through harvest and storage, maize grains are vulnerable to fungal attack by process commonly referred to as weathering (Halloin, 1981) and storage deterioration (Christensen & Kaufmann, 1969). Fungi which infect grains in the field can develop during storage causing deterioration. The Specialized National Council (Anonymous, 2002) estimates the post harvest annual losses of the national food crops being 500 millions L.E. for principal crops including maize. No doubt that rejection of food with visible fungal growth and/or its probable contamination with mycotoxins represents a part of the losses in the crop. Studies done by some investigators threw the light on some aspects concerning the behavior and seriousness of grain and ear rotting fungi of maize in Egypt (El-Sayed, 1996, El-Shabrawi, 2001). Aflatoxins produced mainly by *Aspergillus flavus* & *A.parasiticus* and fumonisins produced by *Fusarium verticilliodes & F.proliferatum* are considered the most serious and dangerous mycotoxins contaminating maize commodities in significant amounts (Mohamed, 1999). It was emphasized by El-Assiuty *et al.* (2006b) that *Cymbopogon*

proximus extracts have the potential to inhibit the growth of *F.verticillioides & A.flavus in vitro.* Fumonisin (FB1) and aflatoxins (B1& B2) production have been drastically decreased due to the effect of these fractions in growing media. Eight compounds were isolated by El-Assiuty *et al.* (2006a) from *C.proximus*-hexane extract and identified as sesquiterpenes. They were potent inhibitors to the growth of *F.verticillioides & A.flavus* in growing media, indicating the potential of this fraction in disease control.

The present investigation was carried out to reduce infection with ear rots and mycotoxin production in maize kernels by applying *C.proximus* extracts rather than using fungicides as an environment- friendly approach and safe to public health.

MATERIALS AND METHODS

Plant material:

Thyme (*Thymus vulgaris*), tooth brush tree (*Salvadora persica*) and halfa barr (*Cymbopogon proximus*) were obtained from the local market and kindly verified through The Herbarium of Faculty of Science, Cairo University, Giza.

Extraction:

Materials were air dried, ground to fine powder and subjected to fractionation by soaking into solvents differing in polarity, *i.e.*N-hexane, di-ethyl ether, methanol, ethanol & chloroform + methanol. Solvents were added to each of plant material (50 g) in a sufficient amount (about 100ml) in glass bottles. Bottles were sealed with tape and kept in the dark. After two weeks, extracts were filtered through Whatman no.1 filter paper and filtrates were dried at room temp. in the dark. The residues were weighed and kept in a refrigerator.

Pathogens:

Fusarium verticillioides (Sacc.) Nirenberg (syn. *F.moniliforme* Sheldon), st 77 and *Aspergillus flavus* L.K., st 15. were used in the present study. They were isolated from infected maize kernels and kept in the Type Culture Collection of the Lab. of Maize Diseases, Plant Pathology Research Institute, ARC, Giza.

Fungal inoculation:

Conidial suspensions of each of the two pathogens, *i.e.F.verticillioides & A.flavus* were used. Conidia from culture of each pathogen were harvested from 7 day-old Czapek agar slant incubated at 25° C, after adding 5ml of 0.01 % tween 20 to each slant. Cultures were then agitated, and the spore suspension was transferred to sterile screw-capped tubes and adjusted to 10^6 spores/ml with sterile distilled water. Maize plants, c.v. single cross Nagah (Egaseed Comp.) were grown in single-row plots 5.0 m long and 1.0 m apart with 20 plants per row and arranged in a randomized

block design. The work was done at the Farm of Egaseed Company (Beni- Hedare, Giza) and treatments were quadruplicated. Ten plants of each replicate, 15 days after midsilk, were inoculated following the cob-tip inoculation technique (Chungu *et al.*,1996). Aqueous spore suspension (2-3 ml) were inserted into the cob-tip by the aid of 50 mm long, hypodermic needle, and inoculated ears were covered by craft bags for 24 hrs.

Evaluation of material extracts and mold assessment :

N-hexane, di-ethyl ether, and methanol-fractions of *C.proximus,* ethanol and chloroform + methanol (2:1) of *T.vulgaris,* and ethanol, & chloroform + methanol (2:1) of *S.persica* were chosen in the present study. They were found in a previous investigation (El-Assiuty *et al.,* 2006a) to be the more potent inhibitors to the growth of the target fungi *in vitro*

Each dried fraction was dissolved in 5 ml of the same solvent and an aqueous solution was prepared to give the concentration 5000 ppm and few drops of tween 20 as a dispersing agent were added to each solution. Plant extracts (2-3 ml) were inoculated to maize ears following the method of cob-tip technique as described above, one day before or after the fungal inoculation. Plant ears inoculated with sterilized water acted as control. Five replicates, ten plants each were used.

One month after application, percentage of infection was calculated for each treatment and infected area of the inoculated corn ears were recorded.

Mycotoxin analysis:

Fumonisin FB1 produced by *F.verticillioides* and aflatoxin B1& B2 produced by *A.flavus* were determined in discolored and adjacent maize kernels. Kernels were ground to fine powder and analyses were carried out as follows:

-Determination of fumonisin FB1:

Fumonisin FB1 was determined in the powdered samples at the Central Lab. of Biotechnology, Plant Pathology Research Institute, ARC, Giza. The VICAM method, 1998 (Fluorometer Series 4, Watertown, USA) for fumonisin measurement was used in the analysis.

- Determination of aflatoxin B1&B2:

To determine the aflatoxin B1& B2 in the powdered maize kernels, the method of BF recommended by Williams (1984) was followed.

Statistical analysis:

Duncan`s multiple range test (DMRT) and averages were compared to the least significant differences (LSD) using Irristat Michigan State Univ., USA,1993, based on Duncan Test (1955).

RESULTS AND DISCUSSION

Cymbopogon proximus-extracts were applied to control ear rot of maize compared with other effective plant extracts shown in Table (1) under artificial inoculation with *Aspergillus flavus* and *Fusarium verticillioides* as described under Material & Methods.

Data, in Table (1), show that all the plant extracts used significantly reduced ear rot caused by *A.flavus* and *F.verticillioides*. These extracts were highly effective in controlling the disease when used before than after the fungal inoculation, in general. Extracts used in this study along with other plant extracts were recently reported by El-Assiuty *et al* (2006 b) as potent inhibitors which completely suppressed the linear growth of these pathogens, when introduced into the growing medium *in vitro*. As regards to *A.flavus,* data in Table (1) show that *C.proximus*-hexane extract gave the best effects in reducing the disease followed by *C.proximus*-methanol, *C.proximus*-hexane+methanol (1:1) and *S.persica*-ethanol extracts where infection percent ranged between 5.2 and 12.3 % compared with the non-treated control (33%). *F.verticillioides,* however, was significantly affected by *C.proximus*-hexane and *T.vulgaris*-chloroform+methanol extracts (9.3 & 10.4 % infection, respectively). El-Assiuty *et al.* (2006 a) isolated and identified the chemical compounds in the *C.proximus*-hexane extract and found eight sesquiterpene compounds which posses antifungal properties against *A.flavus* and *F.verticillioides*. It is known that terpenes are widespread, mainly in plants as constituents of essential oils and some of them are antibiotics that may affect microorganisms and plant pathogens as reported by Krol-Bogomilski, (2006) and Mevy *et al.* (2007).

Table 1. Effect of some plant extracts on average percentage of infected kernels of maize ears inoculated with *A.flavus & F.verticillioides,* Beni-Hedare, 2004.

Treatment (extract)	% infection			
	A.flavus		*F.verticillioides*	
	Before[I]	After[II]	Before	After
1-*C.proximus* (hexane)	5.2 ^{a}	11.0 ^{b}	9.3a	18.4 ^{b}
2-*C.proximus* (methanol)	8.9 b	21.6 ^{d}	--	--
3-*C.proximus**	11.1b	17.9 ^{c}	--	--
4-*C.proximus* (di-ethyl ether)	--	--	18.9 ^{bc}	20.0 ^{bc}
5-*C.proximus***	--	--	31.7 ^{cd}	22.3 ^{c}
6-*T.vulgaris* (chloroform+methanol)****	22.0 ^{d}	31.5 ^{e}	10.4 ^{a}	16.3 ^{b}
7-*S.persica* (ethanol)	12.3 ^{b}	13.9 ^{bc}	--	--
8-*S.persica* (chloroform+methanol)*****	--	--	31.7 cd	21.3 ^{c}
Control	33.0 ^{e}	33.0 ^{e}	46.5 ^{e}	46.5 e

Means with the same letters don`t differ significantly according to Duncan`s Multiple Range Test at5% level.

I,Treatment done 24 h before fungal inoculation

II,Treatment done 24 h after fungal inoculation

* Treating with a mixture of *C.proximus*-hexane & *C.proximus*-methanol extracts (1:1)

** Treating with a mixture of *C.proximus*-hexane & *C.proximus*-di-ethyl ether extracts (1:1)

*** Treating with *T.vulgaris*-chloroform+methanol (2:1) extract

**** Treating with *S.persica*-chloroform+methanol (2:1) extract

Mycotoxin produced in kernels of inoculated maize ears and the effect of plant extracts in minimizing their accumulation are shown in Tables (2&3). As regards to aflatoxins B1& B2, data in Table (2) indicate that infection with *A.flavus* resulted in higher accumulation of aflatoxins B1 in the discolored kernels of untreated control plants (166.6 ug/g kernels), whereas it was detected in lower concentrations in the symptomless kernels adjacent to the rotted ones (15.0 ug/g kernels) in the controls. Aflatoxin B2, on the other hand, accumulated in both discolored and adjacent kernels in amounts of 35 & 4.3 ug/g, respectively.

However, treatment with extracts of the tested plant species shown in Table (2) caused drastic decrease in the production of aflatoxin B1 and complete prevention of aflatoxin B2 production comparable to the controls, whether in the discolored or adjacent kernels. Applying plant extracts before inoculation of maize ears with *A.flavus* was more efficient in reducing the toxin accumulation than applying after the fungal inoculation, as the extracts interfered with the infection process and subsequent colonization.

Table 2. Aflatoxins (B1&B2) production (ug/g) in kernels of maize ears inoculated with *A.flavus*

Treatment (plant extract)	Discolored kernels		Adjacent kernels	
	B1	B2	B1	B2
1-*C.proximus* (hexane):				
Before fungal inoculation	0.0	0.0	0.0	0.0
After fungal inoculation	0.0	0.0	1.6	0.0
2-*C.proximus* (methanol):				
Before fungal inoculation	3.0	0.0	0.0	0.0
After fungal inoculation	16.9	0.0	4.0	0.0
3-*C.proximus* (1+2) *				
Before fungal inoculation	0.0	0.0	0.0	0.0
After fungal inoculation	23.5	0.0	0.0	0.0
4-*T.vulgaris* (chloroform+methanol) **				
Before fungal inoculation	6.2	0.0	1.0	0.0
After fungal inoculation	16.0	0.0	3.0	0.0
4-*S.persica* (chloroform+methanol)***				
Before fungal inoculation	12.0	0.0	4	0.0
After fungal inoculation	0.0	0.0	6.3	0.0
Control (inoculated untreated)	166.6	35	15.0	4.3

*Treating with a mixture of *C.proximus*-hexane + *C.proximus*-methanol extracts (1:1)

** Treating with *T.vulgaris*-chloroform + methanol (2:1) extract

*** Treating with *S.persica*-chloroform + methanol (2:1) extract

It is concluded from this result that these extracts could be used as protective treatments to reduce infection with the pathogen and decrease, in turn the aflatoxin production. It is also obvious from data in Table (2) that *C.proximus*-extracts were superior in reducing the production of aflatoxins compared with the other extracts used in this study, where hexane extract completely prevented both types of aflatoxins to accumulate when used before the fungal inoculation. This is true in the discolored and adjacent kernels. After the fungal inoculation, traces (1.6 ug/g) of B1 were detected in the adjacent kernels. These results are supported by the findings obtained by El-Assiuty *et al.* (2006b), who found that *C.proximus*- hexane extracts reduced the production of aflatoxins produced by *A.flavus in vitro.* Some compounds isolated from the hexane-fraction of *C.proximus* were found to prevent conidial production of *A.falvus,* but partially affected the growth of this pathogen (El-Assiuty *et al.* 2006a). As spores being the source of fungal infection, prevention of sporiolation by these extracts is a success in controlling ear rot.

As regards to fumonisin (FB1) production, results (Table 3) indicate that infection with *F.verticillioides* led to the accumulation of this mycotoxin in the molded (discolored) and the adjacent (symptomless) kernels of non-treated ears (29.0 & 14.0 ppm respectively). These results are consistent with the findings obtained by Munkvold and Hellmich (1997) who reported that several *Fusarium* species can infect maize ears without causing visible symptoms, but affect grain quality and produce mycotoxin. Also, Desjardins *et al.* (2002) reported that maize inoculated with a high fumonisin-producing *Fusarium* isolate had a high frequency of kernel infection without visible symptoms of disease, but fumonisin levels in symptomless kernels were generally low.

Table 3. Fumonisin (FB1) production (ppm) in kernels of maize ears inoculated with *F.verticillioides*

Treatment (plant extract)	Discolored kernels	Adjacent kernels
1)*C.proximus* (hexane):		
Before fungal inoculation	0.0	0.0
After fungal inoculation	6.0	0.0
2)*C.proximus* (di-ethyl ether):		
Before fungal inoculation	3.0	0.0
After fungal inoculation	12.0	6.0
3)*C.proximus* (1+2) *		
Before fungal inoculation	29.0	10.0
After fungal inoculation	29.0	13.5
4)*T.vulgaris* (chloroform+methanol)**		
Before fungal inoculation	27.0	12.7
After fungal inoculation	26.0	14.0
5)*S.persica* (ethanol):		
Before fungal inoculation	18.0	15.0
After fungal inoculation	20.5	14.5
Control (inoculated untreated)	29.0	14.0

* Treating with a mixture of *C.proximus*-hexane +*C.proximus*-di-ethyl ether extracts (1:1)

** Treating with *T.vulgaris*-chloroform + methanol (2:1) extract

It was found (Table 3) that *C.proximus*-hexane extract followed by di-ethyl ether extract gave the best effect in reducing the production of FB1 when applied to maize ears whether before or after the fungal inoculation. Applying these extracts before the fungal inoculation of ears was more effective than applying after fungal inoculation. Treatments with the other extracts used in this trial were ineffective in reducing FB1 contamination compared with the control. These results confirm those previously obtained by El-Assiuty *et al.* (2006b), who reported that these extracts were highly efficient in reducing the capability of *F.verticillioides* to produce fumonisin FB1 in the laboratory.

As hexane extract obtained from *C.proximus* was found in a previous investigation (El-Assiuty *et al.*,2006b) to have the potentiality to inhibit the growth of several fungal pathogens *in vitro*, chemical analysis was made to identify the active principles in this extract (El-Assiuty *et al*,2006b). Eight sesquiterpenes inhibitive to the growth of these two target fungi were isolated and identified by mass spectrophotometry. Terpenes are widespread mainly in plants as natural antibiotics (Krol-Bogomilski, 2006). However, the antifungal effect of *n*-hexane extract may be attributed to the poisonous effect of the antibiotic nature of compounds in this fraction.

As recommended nowadays of using natural products rather than the pesticides that pollute environment and seriously affect public health, findings obtained through the present investigation threw the light on the possibility of using *C.proximusn*-hexane extract to control maize ear rot. Further study to develop practical method for application of such extract is needed.

REFERENCES

1. Anonymous. 2002. Annual Report of the Specialized National Council, 2002.
2. Christensen, C.M., and H.H. Kaufmann. 1969. *Grain Stored-The Role of Fungi in Quqlity loss.* Univ.of Minnesota Press, Mineaplis.
3. Chungu, C., D.E. Mather, I.M. Rid and R.I. Hamilton. 1996. Comparison of techniques for inoculation of maize silk, kernel, and cob tissues with *Fusarium graminarum.* Plant Disease, 80: 81-84.
4. Desjardins, A.E., G.P. Munkvold, R.D. Plattner and R.H. Proctor. 2002. *FUM1*-a gene required for fumonisin biosynthesis, but not for maize ear rot and ear infection by *Gibberella moniliformis* in field tests. Mol.Plant-Microbe Interact., 15: 1157-1164.
5. Duncan, D. B. 1955. Multiple range and multiple F-test. Biometrics, 11:1-42.

6. El-Assiuty, E.M., Fawziya M. Bekheet, Zeinab M. Fahmy, A.M. Ismael and T.S.M. El-Alfy. 2006 a. Potentiality of some isolated compounds from *Cymbopogon proximus* Stapf. against the toxigenic fungi, *Fusarium verticillioides & Aspergillus flavus.* Egypt.J.Phytopathol., 34,(2): 75-84.
7. El-Assiuty, E.M., Zeinab M. Fahmy, Fawziya M. Bekheet, A.M. Ismael and E.M. Hob-Allah. 2006b. Effect of some medicinal and indigenous plant extracts on some plant pathogens and mycotoxin production *in vitro.* Egypt.J.Agric.Res., 84 (5): 1345-1358.
8. El-Sayed, Fawziya M.B. 1996. Mycotoxin pollution in stored corn grains. Ph.D. Thesis, Instit.Environ.Stud. & Res.,Ain Shams Univ.pp 121.
9. El-Shabrawi, S.M. 2001. Studies on ear and kernel rot of maize caused By *Aspergillus* and *Fusarium* spp. M.Sc.Thesis, Fac.Agric., Tanta Univ.pp80.
10. Halloin, J.M.1981.Weathering: changes in planting seed quality between Ripening and harvest. P.286-289.in Proc.Beltwide cotton Prod.Res.Conf., New Orleans, LA.
11. Krol-Bogomilski, J. 2006. Natural antibiotics, sesquiterpenes from Lactarius fungi. http://www.geocities.com/jerzor/sesquiterpenes / antibiot.html
12. Mevy,J.P., J.M. Bessiere, M. Dherbomez, J. Millogo and J. Viano. 2007. Chernical composition and some biological activities of the volatile oils of a chemotype of *Lippia chevalieri* Moldeke. Food Chemistry, 101: 682-685.
13. Mohamed, M.I. 1999. Biochemical Studies on Fumonisin Mycotoxins. Ph.D. Thesis. Fac.Agric.Cairo Univ., Egypt, pp136.
14. Munkvold, J.P., R.L. Hellmich and W.B. Showers. 1997. Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology, 87 (10): 1071-1077.
15. Williams, S.1984.Official methods of analysis (14^{th} ed.) Association of Official Analytical Chemists. Inc., Arlington. USA.1141pp.

مستخلصات نبات الحلفا بر فى مكافحة عفن كيزان الأذرة الشامية وخفض السموم الفطرية

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أجريت تجربة حقلية لمحاولة تقليل الإصابة بعفن الكيزان فى الذرة الشامية وكذا إنتاج وتراكم السموم الفطرية وهى الأفلاتوكسينات والفيومونيسين والتى تفرز بواسطة فطرى الأسبرجيلوس فلافس والفيوزاريوم فيرتيسيللويدز وهما أهم المسببات لعفن الكيزان والحبوب فى الذرة الشامية. فقد تمت معاملة الكيزان ببعض المستخلصات النباتية والتى أثبتت سابقا قدرتها على تثبيط نمو الفطرين المذكورين فى المعمل وذلك بحقنها بهذه المستخلصات قبيل وبعد حقنها صناعيا بهذين الفطرين. وكانت أكثر المعاملات تأثيرا هى المعاملة بمستخلص الهكسان لنبات الحلفا بر "سيمبوبوجون بروكسيموس" حيث تم خفض الإصابة بالمرض الناتج عن الحقن الصناعى بأى من الفطرين المذكورين. هذا وقد كان لهذه المعاملات تأثير فعال فى تقليل إنتاج السموم الفطرية وهى الأفلاتوكسينات ب١،ب٢ والفيومونيسين ب١ بدرجة ملحوظة وذلك فى الحبوب المصابة وكذلك فى الحبوب المجاورة للإصابة. وكانت المعاملات قبل الحقن بالفطريات اكثر فاعلية من المعاملات بعد الحقن بصفة عامة.