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INHIBITION OF MYCOFLORA AND ZEARALENONE ON RICE BY SELECTED ESSENTIAL OILS

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Effect of natural products *viz,* anise, cinnamon, clove, marjoram and peppermint on the inhibition of mycoflora and zearalenone production on rice grains was studied. Population of fungi significantly reduced by spice oils used as 0.1, 0.3, 0.5 and 1.0% and completely inhibited at 1% cinnamon. *Fusarium graminearum* grown in sterilized liquid medium and on cooked and uncooked rice grains showed complete inhibition of zearalenone production where anise, cinnamon and clove as 0.5% were used. Marjoram and peppermint oils as 0.5% on uncooked and as 1% on cooked rice showed inhibitory effects. In rice extract liquid medium, cinnamon and anise oils had most pronounced effect and significantly reduced zearalenone production at 0.02 and 0.05% respectively.

Key words: Mycoflora and zearalenone, Rice, Spice oils.

Introduction

Rice (*Oryza sativa* L.) is one of the basic food of people in Egypt. Fungi are known to cause deterioration of food and feed, and so leading to great economic losses the world over. The seed borne mycoflora of rice (1-4) and their control by pesticides (5-8) have been reported. The spoilage of food by fungi is often accompanied by the production of mycotoxins which are a potential threat to both human and animal health. Of these *Fusarium* spp. represent one of the dominant fungi which has been isolated from rice grains in Egypt [1], where *F. graminearum* is known to produce zearalenone. The secondary metabolites zearalenone has been implicated in causing hyperestrogenism, abnormal estrus, infertility, still birth, small litters and fetal absorption when ingested by swine [9,10]. Dairy cattle show decreased apetite, milk production and fertility when fed with zearalenone [11]. Several pesticides have been used for the control zearalenone-producing molds [12-16]. Spice oils have been reported as effective inhibitors of aflatoxin [17-24], however, their effect on mycoflora and zearalenone production by *F. graminearum* has not been reported.

The present investigation was carried out to show the effect of spice oils on seed-borne fungi of rice and on zearalenone production by toxigenic *Fusarium graminearum* on rice grains and in liquid medium.

Materials and Methods

Spice oils. Spice oils *viz.*, aloe, anise, black cumin, cinnamon, clove, cocoa-nut, fenugreek, horse radish, lettuce, marjoram, peppermint, tamarind and thyme, collected from spice and aromatic shops in Egypt were used at 2.0% concentration for showing their effect on zearalenone production by *F. graminearum.* Anise, cinnamon, clove, marjoram and peppermint, which showed complete inhibition were taken for showing their effect on seed-borne mycoflora, growth and zearalenone production.

Mycoflora of rice grains. Samples of 25g of rice grains in sterile 250 ml flasks were thoroughly mixed with anise, cinnamon, clove, marjoram and peppermint as 0.1, 0.3, 0.5 and 1.0%. Two replicates of each treatment and untreated rice grains were used as control. The flasks were sealed by cotton blugs and incubated for 15 days at 27°C. The dilution plate method described by Christensen [25] were used to show the seed borne mycoflora on 1% Glucose-Czapek's agar medium. Three plates were used per sample, incubated at 27°C for 1-2 weeks and the growing fungi were counted, identified and calculated per g dry rice.

Artificial inoculation of rice. Samples of 25g of rice in 100 ml Erlenmeyer flasks were sterilized with 5% NaOCl solution, washed with sterile distilled water, and sufficient sterile distilled water was added to rice grains to achieve a moisture content of 25%. Another samples of 25 g of moistened grains (25% H_2O) were autoclaved in 100 ml flasks (cooked rice). The cooked (autoclaved) and uncooked rice were treated with the spice oils *viz.* anise, cinnamon, clove, marjoram and peppermint as 0.1, 0.3, 0.5 and 1.0% concentrations. Each flask was inoculated with 0.5 ml of heavy spore suspension containing approx. 10^3 spore of toxigenic *F. graminearum* which was thoroughly mixed and incubated at 27°C for 2 weeks. Three flasks of each treatment and control were used for analysis.

Liquid medium. Rice extract medium (200g of rice ground in a Waring blender for 5 sec. was boiled in 500 ml dist. H_2O for 2 mins, water was decanted and the rice reboiled with an additional 500 ml distilled water. The boiled extracts were combined, filtered through cheesecloth, and the volume in-

creased to 1 L with distilled water (2 g KNO_3, 1 g K_2HPO_4, 0.5g $MgSO_4$-$7H_2O$ and 0.5 g KCl were added per liter). 50 ml of sterilized media was treated with different concentrations of spice oils inoculated with 1 ml of a spore suspensions of *F. graminearum* and incubated at 27°C without shaking for 12 days. Four flasks were used for each concentration and control. The mycelial mat was dried to determine the dry weight of the mycelium.

Extraction and analysis of zearalenone. Zearalenone in the cultures were extracted with chloroform, clean up by silica gel column [26] and quantitatively analyzed according to Mirocha *et al.* [27].

Results and Discussion

Table 1 gives the total count of fungal species associated with rice grains. A total of 7 genera and 13 fungal species *viz.*, *Alternaria alternata* (Fries) Keissler, *Aspergillus flavipes* (Bain. & Sart.) Thom & Church, *A. flavus* Link, *A. fumigatus Fresenius, Emericella nidulans* (Eidam) *Vuillemin, A. niger* Van Tieghem, *A. ochraceus* Wilhelm, *A. sydowi,* (Bain & Sart.) Thom & Church, *A. terreus* Thom, *Cladosporium cladosporioides* (Fres.) de Vries, *Fusarium graminearum* Schwabe, *Penicillium chrysogenum* Thom, *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lindt were isolated and identified. All oils *viz.* anise, cinnamon, clove, marjoram and peppermint significantly reduced the mycoflora of rice grains when used as 0.1% where 43-78% reduction was observed. This depression in fungal count was dose related and reached to 80-100% inhibition at 1% oil concentration. Cinnamon was more effective and followed by clove and anise oils.

The effect of oils on zearalenone production by *F. graminearum* on cooked and uncooked rice is shown in Table 2. In cooked rice, all the oils tested significantly reduced zearalenone production at 0.3% concentration. Anise, cinnamon and clove oils completely inhibited zearalenone production at 0.5% while, marjoram and peppermint completely inhibited zearalenone production at 1% level. When uncooked rice was used, zeara lenone production significantly decreased at 0.1% and completely inhibited at 0.5% of cinnaon oil. Low level of 0.1 % of anise, clove, marjoram and peppermint was ineffective to control zearalenone production whereas at 0.3% level zearalenone production reduced by 73.6, 71.7, 60.4, and 54.7% over the control, respectively. Complete inhibition of zearalenone production was observed of 0.5% level.

In rice extract liquid medium, mycelial growth significantly decreased at 0.02% of anise, clove, peppermint and completely inhibited by cinnamon (Table 3). Production of zearalenone by *F. graminearum in* liquid culture was inhibited by 47% at 0.02% cinnamon and by 44% at 0.05% anise, but, the other tested oils were partially effective.

TABLE 1. EFFECT OF SPICE OILS ON MYCOFLORA OF RICE.

Oil conc. (%)	Spice oils				
	Anise	Cinnamon	Clove	Marjoram	Peppermint
	(Total fungal count/g grain)				
O (control)	164	164	164	164	164
0.1	60*	36*	42*	72*	93*
0.3	57*	12*	28*	67*	71*
0.5	39*	6*	21*	39*	66*
1.0	23*	0*	18*	33*	27*

* Means significant difference compared to the control at 1% level [28].

TABLE 2. EFFECT OF SPICE OILS ON ZEARALENONE PRODUCTION BY *FUSARIUM GRAMINEARUM* ON COOKED AND UNCOOKED RICE.

Rice	Oil conc. (%)	Spice oils				
		Anise	Cinnamon	Clove	Marjoram	Peppermint
		(Zearalenone µg/100 g)				
Cooked	0 (control)	720	720	720	720	720
	0.1	700	640	690	685	670
	0.3	230*	250*	320*	460*	580*
	0.5	0*	0*	0*	144*	290*
	1.0	0*	0*	0*	0*	0*
Uncooked	0 (control)	530	530	530	530	530
	0.1	440	350*	460*	475	455
	0.3	140*	115*	150*	210*	240*
	0.5	0*	0*	0*	0*	0*
	1.0	0*	0*	0*	0*	0*

*Means significant difference compared to the control at 1% level.

TABLE 3. EFFECT OF SPICE OILS ON GROWTH AND ZEARALENONE PRODUCTION BY *F. GRAMINEARUM* IN RICE EXTRACT BROTH AFTER 12 DAYS OF INCUBATION AT 27°C.

Oils	Conc. %	Visual growth after different periods (day)			Mycelial dry weight (mg/50 ml)	Zearalenone production (µg/g dry wt)
		4	6	8		
Control	0	+	+	+	490	320
Anise	0.02	+	+	+	380	210
	0.05	-	-	+	325*	180*
	0.10	-	-	-	0*	0*
Cinnamon	0.02	+	+	+	350	170
	0.05	-	-	-	0*	0*
	0.10	-	-	-	0*	0*
Clove	0.02	+	+	+	340	230
	0.05	-	+	+	270*	215*
	0.10	-	+	+	0*	0*
Marjoram	0.02	+	+	+	430	260
	0.05	-	+	+	360	230
	0.10	-	-	-	0*	0*
Peppermint	0.02	+	+	+	370	280
	0.05	-	+	+	290*	265
	0.10	-	-	-	0*	0*

* Means significant difference compared to the control at 1% level.

Anise, cinnamon clove, marjoram and peppermint oils significatnly decreased the population of fungi on rice grains at 0.1% and completely inhibited at 1.0% cinnamon. Anise, cinnamon and clove oils significantly reduced the mycelial growth and zearlenone production by *F. graminearum* at 0.05% in liquid culture and 0.3% on cooked and uncooked rice, with complete inbihition at 0.5% concentration. Bullerman *et al.* [18] and Tiwari *et al.* [24] found that the oils of anise, cinnamon and clove inhibited aflatoxin production by *Aspergillus parasiticus* at 200-300 ppm in liquid medium. However, in rice powder medium aflatoxin production was inhibited at 0.5% of clove and 10.0% cinnamon [21]. Marjoram and peppermint oils were nearly similar in their activity. They partially retarded the mycelial growth and zearalenone production in liquid medium at 0.02 and 0.05% concentration, but completely inhibited them at 0.1% treatment. On rice, they significantly reduced the production of zearalenone at 0.3% and completely inhibited it at 0.5 and 1.0% on uncooked and cooked rice, respectively. Marjoram and peppermint have previously been classified as antiaflatoxigenic at concentrations of 0.5 to 5% [16,21].

The data obtained during this investigation indicated that the inhibitory activity of these oils offer advantage in the prevention of zearalenone production specially in warm and tropical countries. These results suggest that 0.5% concentration of any of cinnamon, anise or clove was found to control toxin production and also have flavour acceptability of foods.

References

1. O. M. O. El-Maghraby, Ph.D. Thesis, Bot. Dept., Fac. of Sci., Assiut University, Egypt (1984).
2. E. D. Imolehin, Plant Dis., **67**, 1334 (1983).
3. A. S. Ribeiro, Filopatol Bras, **5,** 59 (1980).
4. T.M. Salcado and P.C.T. De Carvalho, Rev. Microbiol., **11,** 60 (1980).
5. A. Garibaldi, Med. Fac. Landbouww-Rijksuniv. Gent, **50** (3b), 1251 (1985).
6. L. P. Kauraw, Indian Phytopathology, **39** (4), 609 (1986).
7. A. J. Kuthubutheen, J. Stored Prod. Res., **20,** 31, (1984).
8. V. K. Tyagi, M.L.Nagar and S. K. Chaudan, Seed Res., (New Delhi) **9,** 35 (1981).
9. K. Chang, H. Kurtz and C. Mirocha, Am. J. Vet. Res., **40,** 1260. (1979)
10. T. Smith, J. Anim. Sci., **50,** 278 (1980).
11. G. G. Long, M. A. Diekman, J. F. Tuite, G. M. Shanon, and R.F. Vesonder, Am. J. Vet. Res., **43,** 1599 (1982).
12. Y. C. Berisford and J. C. Ayres, Entomol., **5** (4), 644 (1976 a).
13. Y. C. Berisford and J.C. Ayres, Agric. Fd. Chem., **24** (5), 973 (1976 b).
14. F. A. Draughon and D. C. Churchville, Phytopathology, **75** (5), 553 (1985).
15. H. A. H. Hasan, Folia Microbiol. **38,** 295 (1993).
16. M. Mostafa, Mycoflora and Mycotoxins of Some Species. M. Sc. Thesis. Botany Dept., Faculty of Sci., Assiut Univ., Egypt., (1990)
17. A. A. Ansari and A. K. Shrivastava, Letters in Applied Microbiol., 13, 75 (1991).
18. L. B. Bullerman, F. Y. Lieu and S. A. Seier, J. Fd. Sci., **42,** 1107,1116, (1977).
19. H. A. H. Hasan and A. L. E. Mahmoud, Zentralbl. Mikroiol, **148,** 543, (1993).
20. H. Hitokoto, S. Morozumi, T. S. Wauke, S. Sakai, and H. Kurata, Appl. Environ, Microbiol., **39,** 818, (1980).
21. S. S. Mabrouk and N. M. A. El-Shayeb, Chem. Mikrobiol. Technol. Lebensm, **6,** 167 (1980).
22. D. L. Marshall and L. B. Bullerman, J. Fd. Prot., **49,** 378, (1986).
23. S. S. Sahay and K. S. Ranjan, Efficacy of Certain Chemicals, Fungicides, Spices and Plant Extracts in the Control of Aflatoxin production by *Aspergillus* in Mustard, Proceedings of National Academy by Science, India, **60** (B), 169-172, (1990).
24. R. Tiwari, R. P. Dikshit, N. C. Chandan, A. Saxena, K. G. Gupta and D. E. Vadehra, J. Fd. Sci. Tech., **20,** 131,(1983).
25. C. M. Christensen, Cereal Chem., **40,** 385, (1963).
26. A. O. A. C,, Official Methods of Analysis, (Association of Official Analytical Chemists, Washington, DC, 1980), 13th ed., pp.429.
27. C. J. Mirocha, B. Schauerhamer and S.V. Pathre, J. Assoc. Off. Anal. Chem., **57,** 1104 (1974).
28. B. Ostle, *Statistics in Research* (The Iowa State University Press Amer. Iowa. U.S.A. 1963).