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Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B_1 production by *Fusarium proliferatum* in maize grain

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Abstract

The effect of cinnamon, clove, oregano, palmarose and lemongrass oils on growth and FB_1 production by three different isolates of *F. proliferatum* in irradiated maize grain at 0.995 and 0.950 a_w and at 20 and 30 °C was evaluated. The five essential oils inhibited growth of *F. proliferatum* isolates at 0.995 a_w at both temperatures, while at 0.950 a_w only cinnamon, clove and oregano oils were effective in inhibiting growth of *F. proliferatum* at 20 °C and none of them at 30 °C. Cinnamon, oregano and palmarose oils had significant inhibitory effect on FB_1 production by the three strains of *F. proliferatum* at 0.995 a_w and both temperatures, while clove and lemongrass oils had only significant inhibitory effect at 30 °C. No differences were found using 500 or 1000 μg essential oil g^{-1}. At 0.950 a_w, none of the essential oils had any significant effect on FB_1 production. The results suggest that mainly cinnamon and oregano oils could be effective in controlling growth and FB_1 production by *F. proliferatum* in maize under preharvest conditions.

Keywords: *Fusarium*; Fumonisins; Essential oils; Maize; Water activity

1. Introduction

More than 1340 plants are known to be potential sources of antimicrobial compounds but few have been studied scientifically (Wilkins and Board, 1989). Several other studies have examined the effect of compounds isolated from essential oils extracted from plants on fungi to search for natural fungicides and a number of these oil constituents have shown to be inhibitory (Chao and Young, 2000). It appears that there is a relationship between the chemical structures of the most abundant compounds in the essential oils and their antifungal effect. Generally, the extent of the inhibition of the oils could be attributed to the presence of an aromatic nucleus containing a polar functional group (Farag et al., 1989). Over 30,000 different components isolated from plant oils, compounds containing phenol groups are those most used in the food industry (Meeker and Linke, 1988).

Many publications have documented the antimicrobial activity of lemongrass, cinnamon, clove, palmarose and oregano oils against different microbial species (Farag et al., 1989; Chao and Young, 2000; Hammer et al., 1999; Inouye et al., 1998; Mishra and

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Table 1
Essential oils tested: the main components and their relative contents (%)

Cinnamon oil		Clove oil		Lemongrass oil		Palmarose oil		Oregano oil	
α-curbebene	< 1.0	humulene	1.5	limonene	5.4	linalool	3.8	γ-terpinene	1.8
linalool	1.8	cariophyllene	8.2	methyl heptenone	1.4	cariophyllene	2.5	*p*-cimeme	16.7
cariophyllene	4.6	*eugenol*	*88.2*	*neral*	*28.0*	*geraniol*	*87.6*	linalool	2.1
eugenol	*82.3*	cariophyllene oxide	< 1.0	*geranial*	*52.0*	geranil acetate	1.1	cariophyllene	2.1
cinnamaldehyde	1.0			geraniol	2.0	3-carenene	1.5	thymol	0.2
2-propionyl benzodioxol	1.0			geranil acetate	3.6			*cravacrol*	*70.0*
eugenil acetate	2.1							phenyl-methyl-benzoate	1.3
cinnamil alcohol acetate	1.0								

Dubey, 1994; Paster et al., 1995; Patkar et al., 1993; Pattnaik et al., 1996; Salmeron and Pozo, 1991; Sinha et al., 1993). Lemongrass oil is an effective postharvest fungitoxicant of higher-order plant origin potentially suitable for protection of foodstuffs against storage fungi (Mishra and Dubey, 1994). The inhibitory effect of clove and cinnamon oils on growth and aflatoxin production by *Aspergillus flavus* has been reported (Bullerman et al., 1977; Montes-Belmont and Carvajal, 1998; Sinha et al., 1993). In maize grain, cinnamon and clove oils were effective against aflatoxin formation by *A. flavus* after 10 days under favourable conditions for mycotoxin production (Sinha et al., 1993). The ability of oregano oil to inhibit growth of *A. flavus*, *A. ochraceus* and *A. niger* has been evaluated previously (Paster et al., 1990, 1995). In vivo studies showed that oregano oil was highly effective in controlling internal wheat fungi (Paster et al., 1995). Pattnaik et al. (1996) reported that palmarose oil had some inhibitory activity against 12 fungi; the response ranged from various degrees of susceptibility to total inhibition. It was shown that the response to this oil is dependent on the species of fungi.

Fusarium verticillioides and *F. proliferatum* are probably the most important producers of fumonisin B_1 (FB_1) as they are common contaminants of corn in many areas of the world (Shephard et al., 2000). They are usually described as field fungi but they occasionally develop in storage when a_w is high and temperature is low (Lacey and Magan, 1991). Thirty-seven essential

Table 2
Analysis of variance of the effect of different concentrations (*c*) of essential oils on growth of *F. proliferatum* isolates (*i*) at different a_w and temperature (*t*) levels

	DF	Cinnamon oil		Clove oil		Lemongrass oil		Palmarose oil		Oregano oil	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
c	2	1093.3	19.7**	3539.3	68.8**	815.9	18.9**	773.18	16.4**	3112.8	67.8**
t	1	12,250.8	220.2**	8002.6	155.5**	3722.7	86.2**	5209.3	110.5**	6188.6	134.9**
cxt	2	1216.1	21.9**	663.1	12.9**	146.1	3.4*	353.9	7.5**	343.0	7.5**
i	2	153.1	2.8	331.9	6.5**	14.6	0.3	26.9	0.6	135.3	3.0
cxi	4	294.8	5.3**	50.7	1.0	217.6	5.0**	166.6	3.5**	81.2	1.8**
txi	2	318.8	5.7**	140.7	2.7	486.0	11.3**	149.6	3.2*	220.9	4.8
cxtxi	4	200.0	3.6**	104.8	2.0	11.8	0.3	41.9	0.9	60.4	1.3
a_w	1	75,681.3	1360.2**	70,368.8	1366.9**	68,170.7	1578.8**	66,807.6	1416.5**	85,216.3	1856.8**
cxa_w	2	59.4	1.1	1399.9	27.2**	600.5	13.9**	878.1	18.6**	102.0	2.2
txa_w	1	4283.7	77.0**	2822.1	54.8**	1100.3	25.5**	810.2	17.2**	1571.5	34.2**
$cxtxa_w$	2	653.6	11.8**	292.8	5.7**	84.2	2.0	189.4	4.0*	83.5	1.8
$a_w xi$	2	301.5	5.4**	420.8	8.2**	148.9	3.5*	114.8	2.4	343.2	7.5**
$cxa_w xi$	4	276.8	5.0**	101.5	2.0	178.6	4.1**	239.4	5.1**	147.5	3.2
$txa_w xi$	2	134.2	2.4	104.2	2.0	152.5	3.5*	5.9	0.1	33.0	0.7
$cxtxa_w xi$	4	54.9	1.0	38.7	0.8	37.1	0.9	50.7	1.1	47.9	1.0

* $P < 0.05$.
** $P < 0.01$.

oils have been screened for inhibition of mycelial growth of *F. verticilloides*, *F. proliferatum* and *F. graminearum* in maize meal extract agar (Velluti et al., submitted for publication(a)). It was found that cinnamon, clove, oregano, palmarose and lemongrass oils were the best oils tested. In irradiated maize grain, these five essential oils have shown to have inhibitory effect on growth of *F. verticillioides* under different temperature (20 and 30 °C) and water activity (a_w) (0.950 and 0.995) conditions and to inhibit FB_1 production at 30 °C and 0.995 a_w (Velluti et al., submitted for publication(b)). The objective of the present work was to study the effect of cinnamon, clove, oregano, palmarose and lemongrass oils on growth and FB_1 production by *F. proliferatum* in irradiated maize grain at different a_w and temperatures conditions.

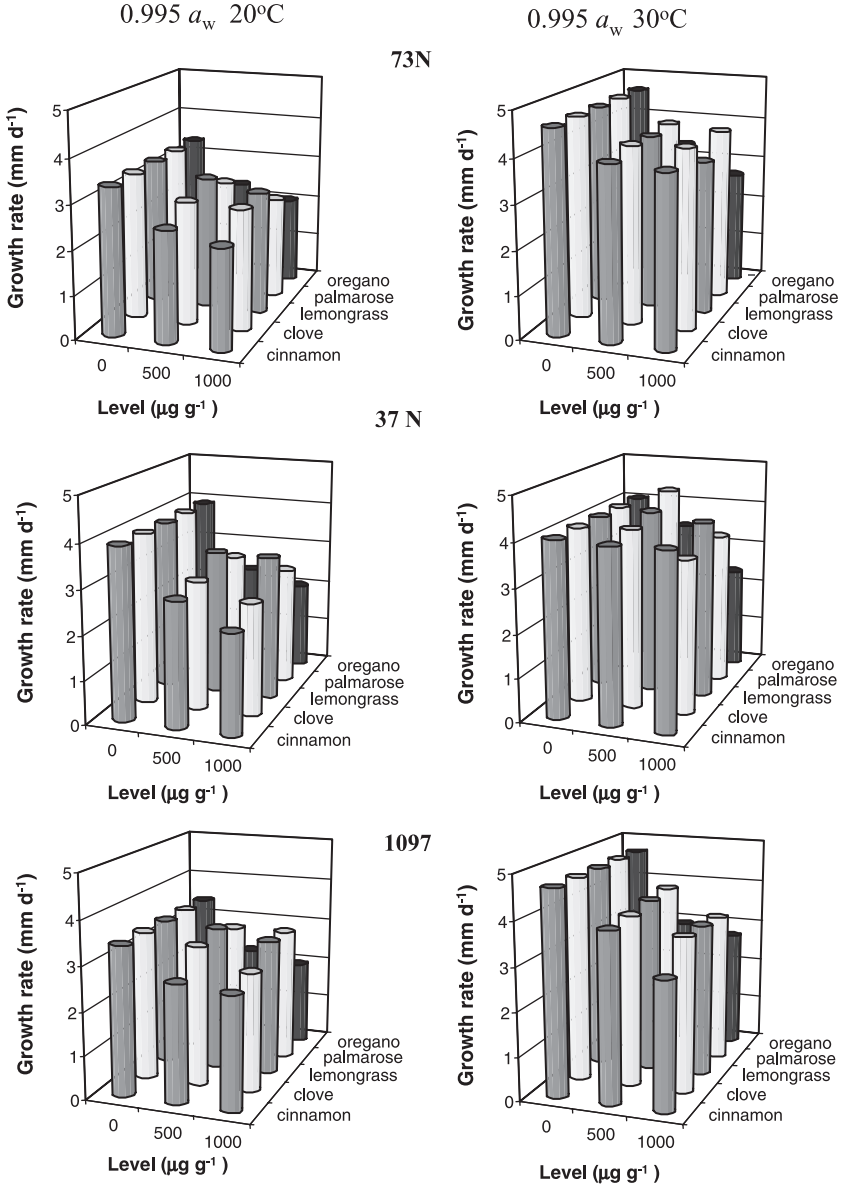

Fig. 1. Effect of essential oils on growth rates of *F. proliferatum* isolates at 0.995 a_w.

2. Materials and methods

2.1. Culture material

Three different isolates of *F. proliferatum* were used for this study: isolate 1097 provided by the Department of Biology, University of Roma "La Sapienza", Rome, Italy, and isolates 73 N and 37 N, which belong to the Food Technology Department fungi collection of the University of Lleida, Spain. All strains used in the study were maintained as Potato dextrose agar (PDA) plugs suspended in sterile distilled water at 7 °C in the Food Technology Department fungi collection of the

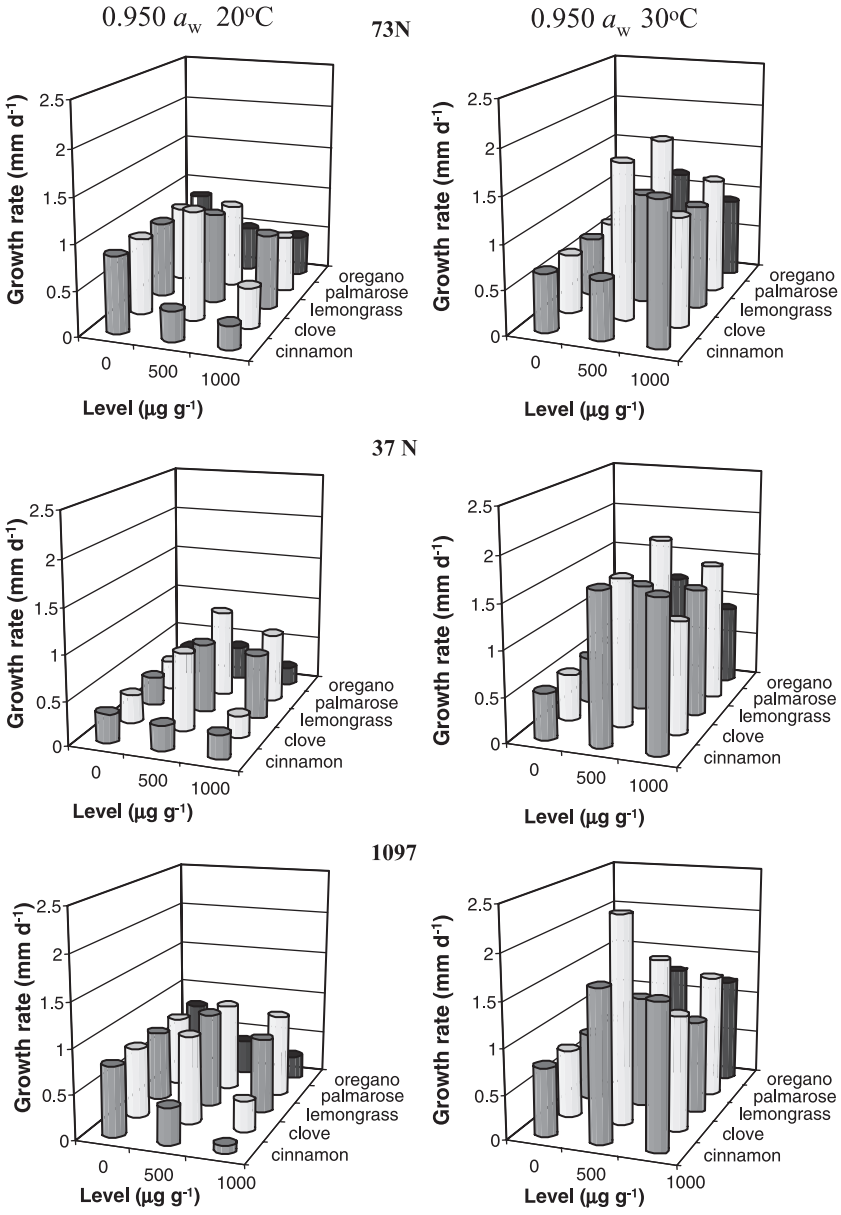

Fig. 2. Effect of essential oils on growth rates of *F. proliferatum* isolates at 0.950 a_w.

University of Lleida, Spain. For this study, strains were grown on maize meal agar (MMEA) at 25 °C for 5 days.

2.2. Essential oils

The essential oils used were cinnamon, clove, lemongrass, oregano and palmarose (Ravetllat Aromatics, Barcelona, Spain). The analyses of the main components of each essential oil (Table 1) were run on a Hewlett Packard GC-MS system (GC 6890; MSD 5973, Hewlett Packard, Vienna, Austria) by the Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Química Orgánica General, Madrid, Spain.

Stock solutions of each essential oil were prepared at 12 and 24 mg essential oil ml^{-1} using Tween 80 (10%) as an emulsifying agent.

2.3. Grain preparation at different water activities (a_w) and addition of essential oils

Spanish dent maize grain was irradiated with 12 k Grays of gamma irradiation and stored aseptically at 4 °C. In this way, the grain contained no fungal infection but had retained the ability to germinate. The initial water activity of the grain was 0.71 a_w, equivalent to a moisture content of 139 g kg^{-1}. For all experiments, irradiated maize was weighed into sterile flasks (240 g) and rehydrated to the desired a_w levels (0.950 and 0.995) by addition of sterile distilled water. Essential oil solutions (10 ml) were added to the irradiated maize to give final concentrations of 500 and 1000 μg g^{-1} of maize; 10 ml of Tween 80 (10%) was added to the control treatments. Flasks were vigorously shaken to homogeneously distribute water and essential oils in the grain. The amount of water and essential oils added was calculated from the moisture adsorption curve of the grain. The treated grains were allowed to equilibrate at 7 °C for 48 h, with periodic shaking. Finally, the a_w values were confirmed by using a water activity meter (AquaLab, Pullman, WA, USA).

2.4. Inoculation, incubation and growth assessment

Rehydrated maize was placed in sterile Petri plates (20 g per plate, approximately) forming a single layer of grains covering the whole plate. A 5-mm diameter agar disk was taken from the margin of a 5-day-old growing colony on MMEA at 25 °C of each isolate and transferred to one grain placed in the centre of each plate. Plates containing grain at the same a_w level and the same essential oil were placed in containers along with beakers containing

Table 3
Analysis of variance of the effect of different concentrations (c) of essential oils on FB_1 production by *F. proliferatum* isolates (i) at different a_w and temperature (t) levels

	DF	Cinnamon oil		Clove oil		Lemongrass oil		Palmarose oil		Oregano oil	
		MS	F	MS	F	MS	F	MS	F	MS	F
c	2	18,532.1	11.9**	1758.8	0.3	8871.9	3.7*	27,510.5	18.4**	5652.6	24.5**
i	2	5654.5	3.6*	35,739.1	6.1**	18,522.6	7.7**	6871.9	4.6*	10,401.7	45.0**
cxi	4	5015.6	3.2*	1304.4	0.2	2282.4	1.0	4977.7	3.3*	1075.3	4.7**
a_w	1	133,838.6	85.6**	368,379.3	63.3**	212,172.5	88.2**	88,947.5	59.4**	62,553.0	270.8**
cxa_w	2	17,637.6	11.3**	1772.2	0.3	8415.0	3.5*	27,505.4	18.4**	5880.4	25.5**
ixa_w	2	5744.1	3.7*	36,306.2	6.2**	19,087.3	7.9**	7154.2	4.8*	10,809.9	46.8**
$cxixa_w$	4	5205.5	3.3*	1506.4	0.3	2384.4	1.0	5108.5	3.4*	1191.6	5.2**
t	1	70,319.1	45.0**	263,544.6	45.3**	113,928.0	47.4**	39,897.1	26.7**	41,567.9	180.0**
cxt	2	4494.0	2.9	7781.4	1.3	1531.6	0.6	7802.3	5.2*	10,289.2	44.6**
ixt	2	1634.2	1.0	26,237.7	4.5*	6805.3	2.8	2327.2	1.6	9056.3	39.2**
$cxixt$	4	2291.7	1.5	573.6	0.1	1333.2	0.6	2211.9	1.5	1246.6	5.4**
a_wxt	1	69,012.3	44.1**	260,698.5	44.8**	113,518.4	47.2**	42,754.1	28.6**	38,233.0	165.5**
cxa_wxt	2	4611.8	3.0	7464.5	1.3	1490.5	0.6	7258.7	4.9*	10,713.9	46.4**
ixa_wxt	2	1653.5	1.1	26,906.0	4.6*	7101.2	3.0	2373.7	1.6	9237.5	40.0**
$cxixa_wxt$	4	2426.9	1.6	612.5	0.1	1322.8	0.6	2373.8	1.6	1392.8	6.0**

* $P<0.05$.
** $P<0.01$.

glycerol water solutions of the same a_w as the grains in order to create an atmosphere with the same equilibrium relative humidity. Containers were incubated at 20 and 30 °C. All treatments were repeated twice. Diameters of growing colonies were measured every day with the aid of a binocular magnifier. Two diameters were obtained from each colony and growth rates expressed as mm day^{-1} were calculated by linear regression of colony radius against time for each strain at each set of conditions tested. After 28 days, the grains were frozen at −20 °C for later FB_1 analysis.

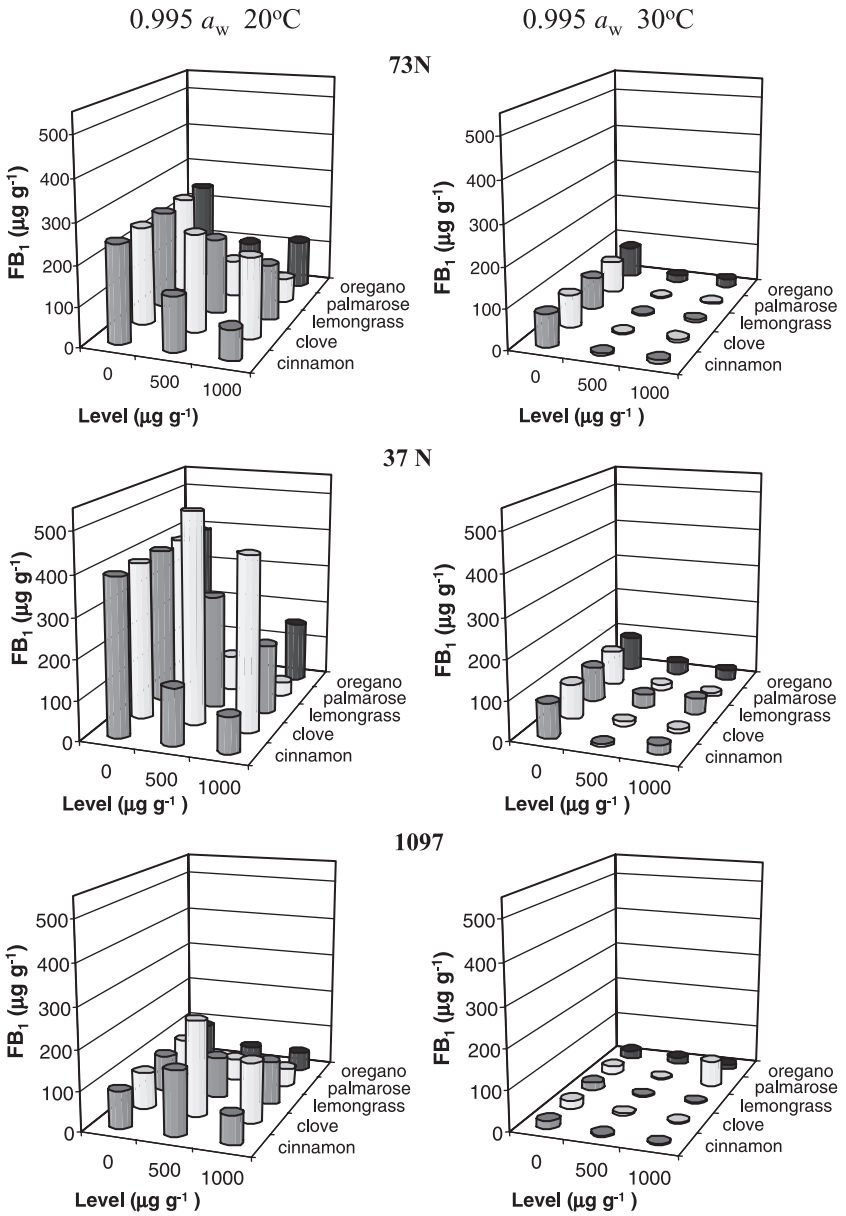

Fig. 3. Effect of essential oils on fumonisin B_1 production at 0.995 a_w by *F. proliferatum* isolates.

2.5. Fumonisin B_1 analyses

European Committee for Standardisation (CEN, 2000) method for determination of fumonisin B_1 by HPLC was followed. Subsamples (10 g) were ground and extracted by blending in 20 ml methanol–water (3 + 1). Extracts were filtered. Filtrate (8 ml) was loaded on a preconditioned SAX (strong anion exchange) column and eluted with 0.5% acetic acid in methanol. The eluate was evaporated to dryness in a rotavapour, redissolved in methanol, and finally evaporated under a gentle stream of nitrogen and

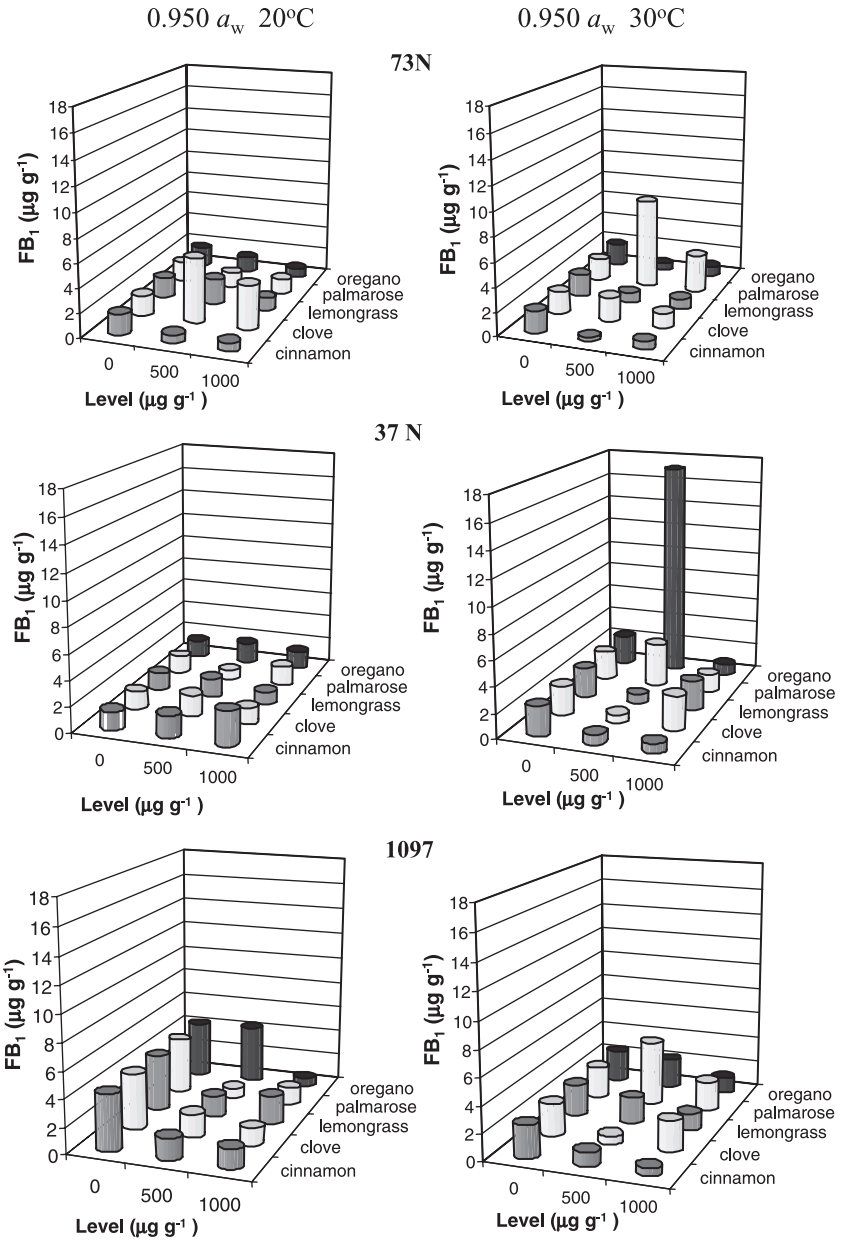

Fig. 4. Effect of essential oils on fumonisin B_1 production at 0.950 a_w by *F. proliferatum* isolates.

dissolved in methanol for HPLC. Sample was coupled to OPA (phthaldialdehyde) reagent and assayed by HPLC, by comparison with external standards, using methanol: 0.1 M dihydrogen sodium phosphate (3 + 1) (pH = 3.35) as mobile phase at a 1-ml min^{-1} flow rate. The reference standard of FB_1 was purchased from Sigma, St. Louis, MO, USA.

2.6. Dry matter determination

The dry matter content of each sample was determined by drying subsamples of approximately 10 g at 105 °C for 17 h (ISTA, 1976). Thus, all results are presented on a dry weight basis.

2.7. Statistical analyses of the data

A full factorial design was used. The factors were a_w, temperature, isolates and concentration of essential oils and the responses were radii of growing colonies and FB_1 concentration. Analysis of variance was performed for colony radii and FB_1 concentrations using SAS version 8.02 (SAS Institute, Cary, NC, USA). Statistical significance was judged at the 5% level.

3. Results

3.1. Effect of the addition of the essential oils on growth rates of F. proliferatum

a_w, temperature and concentrations of essential oils as well as some two- and three-way interactions had a significant effect on the growth of *F. proliferatum* (Table 2). No significant differences between the three isolates were shown when cinnamon, oregano, palmarose and lemongrass oils were used. The different isolates, however, had different responses to a_w, temperature and essential oil concentration. The five essential oils had a significant inhibitory effect on growth of *F. proliferatum* at 0.995 a_w at both temperatures (Fig. 1). At 0.950 a_w, the effect of essential oils on growth rates was dependent on the temperature (Fig. 2). At 20 °C, cinnamon, clove and oregano oils (1000 μg essential oil g^{-1}) had significant inhibitory effect, while at 500 μg g^{-1} only cinnamon and oregano were effective. Lemongrass and palmarose oils enhanced growth of the three strains tested. At 30 °C, none of the essential oils analysed had any inhibitory effect on growth rates of the three strains of *F. proliferatum*. Moreover, the growth rate was enhanced when the essential oils were added, however, none of the enhance effects were significant.

3.2. Effect of the addition of the essential oils on fumonisin B_1 production by F. proliferatum

Almost all single factors a_w, temperature, concentrations, and isolates as well as some of their interactions had a significant effect on FB_1 production by *F. proliferatum* (Table 3).

FB_1 production was very dependent on a_w levels. In the absence of essential oils, higher levels of FB_1 were found at 0.995 a_w, more at 20 °C than at 30 °C (Fig. 3). At 0.950 a_w, less FB_1 was produced (< 4.5 μg g^{-1}), sometimes more at 20 and 30 °C, depending on the isolates (Fig. 4).

When statistical analysis were made separately for a_w level, it showed that at 0.995 a_w and both temperatures, cinnamon, oregano and palmarose oils had significant inhibitory effect on FB_1 production by the three strains of *F. proliferatum* tested, while clove and lemongrass oils had only significant inhibitory effect at 30 °C. No differences were found using 500 or 1000 μg oil g^{-1}. At 0.950 a_w, none of the essential oils tested had any significant effect on FB_1 production.

4. Discussion

The present study showed that the five essential oils tested had significant inhibitory effect on growth and FB_1 production by *F. proliferatum* isolates at 0.995 a_w and 30 °C. At 0.995 a_w and 20 °C, the five essential oils also inhibited growth of *F. proliferatum* and cinnamon, oregano and palmarose oils inhibited FB_1 production. At 0.950 a_w, none of the essential oils tested had effect on FB_1 production and at 20 °C only cinnamon and oregano oils were effective in inhibiting growth of *F. proliferatum.*

Inhibitory effect of cinnamon, clove, palmarose, lemongrass and oregano oils on the growth of different isolates of *F. verticillioides* on irradiated maize was demonstrated at 20 °C at 0.995 and 0.950 a_w. At 30 °C, cinnamon, lemongrass and oregano oils also inhibited growth of *F. verticillioides* at 0.995 a_w.

However, FB_1 production was only inhibited at 0.995 a_w and 30 °C (Velluti et al., submitted for publication(b)). Consequently, the efficacy of the five essential oils studied is similar for both *Fusarium* species tested; the higher the a_w of the grain, the better the inhibitory effect of the essential oils. It might be assumed that the penetration of the oils into the internal parts of the grain is improved in the presence of water, and therefore pathogens could be more easily controlled in the inner parts of the moist grain (Paster et al., 1995).

Infection of maize by *Fusarium* spp. is most likely just after silk emergence and its prevalence is considerably increased with wet weather later in the season; at this time moisture of the kernel is approximately 30% ($\cong 0.98-0.99$ a_w) (Bilgrami and Choudhary, 1998). The essential oils tested, mainly cinnamon, palmarose and oregano oils, could then be effective in controlling growth and FB_1 production by *F. proliferatum* in maize under preharvest conditions.

There was no correlation between growth inhibition and FB_1 inhibition. This means that the reduction of FB_1 was not mainly due to the decreased growth of *F. proliferatum*. Similarly, no correlation has been found between inhibition of growth and FB_1 production by *F. verticillioides* (Velluti et al., submitted for publication(b)). Other authors have reported that, sometimes, toxin production may be inhibited without fungal growth being affected (Bullerman, 1974).

It has been suggested that antimicrobial activity of essential oils depends on chemical structure of their components (Guenther, 1961). Three out of the five essential oils tested, oregano, clove and cinnamon, have aromatic compounds among their major components. Eugenol (the main component of clove and cinnamon oils) and carvacrol (from oregano oil) are phenols. The antimicrobial activity of these oils can be attributed to the presence of an aromatic nucleus and a phenolic OH group that is known to be reactive and to form hydrogen bonds with active sites of target enzymes (Farag et al., 1989). Although clove and cinnamon oils have eugenol as a major component, the abiotic conditions under which they had inhibitory effect on FB_1 production by *F. proliferatum* were not exactly the same. These results suggest that minor compounds may play an important role. Milos et al. (2000) has suggested that the effectiveness of complete essential oil is higher than the activity of each separated compound.

The main component of palmarose oil is geraniol, an aliphatic alcohol, and geranial and neral, aliphatic aldehydes, the main components of lemongrass oil. It has been reported that, geraniol, nerol and citronellol (aliphatic alcohol) completely suppressed the growth of *A. flavus* and consequently prevented the formation of aflatoxin (Mahmound, 1994). Essential oils containing aliphatic alcohols and phenols exhibited significant action against *A. aegyptiaceus*, *Penicillium cyclopium* and *Trichoderma viride* (Megalla et al., 1980). These results together with ours suggest that aliphatic alcohols could have antifungal action against a broad spectrum of fungi.

Further studies with other pathogenic and mycotoxigenic maize fungi on this topic should be performed to establish the overall effectiveness of the essential oils and therefore to optimise the abiotic parameters that encourage their antifungal power. Both under field and storage conditions the moulds occur and compete with other species (Magan and Lacey, 1984). Therefore, it is important to find essential oils with antimycotic effect against a broad range of maize grain fungi. Moreover, as no differences were found in general between both concentrations tested, further studies in order to reduce essential oils concentrations should be performed.

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