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Combined effect of chitosan and water activity on growth and fumonisin production by *Fusarium verticillioides* and *Fusarium proliferatum* on maize-based media

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ABSTRACT

The objectives of the present study were to determine the *in vitro* efficacy of chitosan (0.5, 1.0, 2.0 and 3.0 mg/mL) under different water availabilities (0.995, 0.99, 0.98, 0.96 and 0.93) at 25 °C on lag phase, growth rate and fumonisin production by isolates of *Fusarium verticillioides* and *Fusarium proliferatum*. The presence of chitosan affected growth and fumonisin production, and this effect was dependent on the dose and a_W treatment used. The presence of chitosan increased the lag phase, and reduced the growth rate of both *Fusarium* species significantly at all concentrations used, especially at 0.93 a_W. Also, significant reduction of fumonisin production was observed in both *Fusarium* species at all conditions assayed. The present study has shown the combined effects of chitosan and a_W on growth and fumonisin production by the two most important *Fusarium* species present on maize. Low molecular weight (Mw) chitosan with more than 70% of degree of deacetylation (DD) at 0.5 mg/mL was able to significantly reduce growth rate and fumonisin production on maize-based media, with maximum levels of reduction in both parameters obtained at the highest doses used. As fumonisins are unavoidable contaminants in food and feed chains, their presence needs to be reduced to minimize their effects on human and animal health and to diminish the annual market loss through rejected maize. In this scenario post-harvest use of chitosan could be an important alternative treatment.

1. Introduction

Fumonisins are polyketide mycotoxins produced by several *Fusarium* species, especially *Fusarium verticillioides* and *F. proliferatum*, both of which are common pathogens of maize (*Zea mays*) worldwide (Marasas, 2001). The major naturally occurring fumonisin analogues in maize and maize-based products intended for human consumption are fumonisin B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) (Shephard et al., 1996). The contamination of maize with fumonisins is of concern as these mycotoxins cause various animal diseases and occur in maize and maize-based products intended for human consumption (Shephard et al., 1996). Dietary exposure to fumonisins causes adverse effects in farm and laboratory animals. These toxins have been associated with leukoencephalomalacia in horses (Ross et al., 1992), pulmonary edema syndrome in pigs (Harrison et al., 1990), liver and kidney toxicity in rats (Voss et al., 1988) and apoptosis in many types of cells (Jones et al., 2001). Epidemiological studies have shown some evidence between the intake of fumonisins and oesophageal cancer in Africa, Brazil, China and Italy. Also fumonisin B_1 reduces the folate uptake in cell lines, and fumonisin intake has been implicated as a cause of neural tube defects (Marasas et al., 2004). Based on current data, the International Agency for Research on Cancer has classified FB_1 as possibly carcinogenic to humans (group 2B carcinogen) (IARC, 2002).

Previous reports of the mycotoxin situation in South America encompassing the periods 1995–2000 and 1999–2010 showed that fumonisins appeared to be a major problem in maize and maize products (Garrido et al., 2012; Rodriguez Amaya and Sabino, 2002). Since fumonisins are unavoidable contaminants in food and feed chains, their presence needs to be minimized in order to reduce their effects on human and animal health and to diminish the annual market loss through rejected maize. *Fusarium* species are considered as field fungi and fumonisin contamination in maize occurs mainly pre-harvest, although it has been reported that fumonisin production can occur post-harvest under inadequate storage conditions (Chulze, 2010; Marin et al., 2004). Different strategies have been developed to diminish fumonisin contamination. Adequate drying after harvest is the most effective way to reduce fumonisin contamination, but this is not possible in many cases. Post-harvest treatment with antimicrobials agents

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can cause problems such as ambient pollution, chemical residues in food and feed, and the development of chemical resistance fungi species. Due to these problems there is an increased interest on the study and utilization of antifungal compounds obtained from natural sources to replace synthetic fungicides. Chitosan is a possible option, because it is a biodegradable high molecular weight cationic polysaccharide that can be easily obtained by partial alkaline *N*-deacetylation of chitin, which is the most abundant polysaccharide found in nature after cellulose (Chung et al., 2003). Chitosan is a lineal copolymer of β(1–4) 2-acetamido-2deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose. Its biological activities are attributable to several properties including deacetylation degree and molecular mass concentration (Ziani et al., 2009). Due to its unique biological characteristics, including biodegradability and non-toxicity, many applications for chitosan have been found in foods, pharmaceuticals, textiles, water treatment, cosmetic industries and agriculture (Kong et al., 2010).

There are several reports regarding the antifungal activity of chitosan against plant pathogenic fungi, and inhibition of different stages of growth such as mycelia growth, sporulation, germination and spore viability have been observed, as well as inhibition of production of fungal virulence factors (Bautista-Baños et al., 2003, 2004; El Ghaouth et al., 1992; Falcón-Rodriguez et al., 2012; Ziani et al., 2009). Chitosan's antifungal activity depends on its molecular weight, acetylating grade, pH of chitosan solution, and on the target organism (Xu et al., 2007). Recent studies have shown that chitosan is not only effective in stopping the pathogen growth, but it also induces marked morphological changes, structural alterations and molecular disorganization of the fungal cells. The positive charge of the chitosan is due to the protonization of its functional amino group. This group reacts with the negatively charged cell walls of macromolecules, causing a dramatic increase in the permeability of the cell membrane, causing disruptions that lead to cell death (Ziani et al., 2009).

In vitro analysis has demonstrated that chitosan has a fungistatic activity against *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Monilinia fructicola*, *Penicillium digitatum*, *Rhizopus stolonifer* and *Aspergillus niger* (Hernández-Lauzardo et al., 2008; Martínez-Camacho et al., 2010). To date, only a few studies have reported the effect of chitosan on the growth and mycotoxin (aflatoxin) production by *Aspergillus flavus* and *Aspergillus parasiticus* (Cota-Arriola et al., 2011; Cuero et al., 1991). These experiments were carried out in nutrient rich liquid media and grain without any consideration of the effect of key environmental factors, particularly water availability (a_W) and temperature.

Antioxidants or essential oils under different conditions of a_W, and temperature and controlled atmospheres have been evaluated as possible post-harvest strategies for the reduction of growth of *Fusarium* species and fumonisin production in stored maize (Chulze, 2010), but there is no research about the use of chitosan as a possible strategy. Thus, the objectives of the present investigation were to determine the in vitro efficacy of chitosan under different water availabilities at 25 °C on (i) lag phase, (ii) growth rate and (iii) fumonisin production by isolates of *F. verticillioides* and *F. proliferatum* isolated from Argentinean maize.

2. Material and methods

2.1. Chitosan solution

The chitosan used was low-viscous chitosan (Fluka 50494; LVC; viscosity: ≤200 mPa s), obtained from Sigma-Aldrich Co. A stock solution was prepared by dissolving 10 g/L chitosan in 1% acetic acid (AcH) and stirring for 24 h at 28 °C. The pH of the chitosan solution was adjusted to 5.6 using NaOH. This solution was maintained at 4 °C and brought to ambient temperature before use.

2.2. Chitosan characterization

The viscosity–average molecular weight (*Mv*) of chitosan was determined using the intrinsic viscometric method using the Mark–Houwink–Sakurada equation (Knaul et al., 1998).

The percentage of the chitosan amino groups (degree of deacetylation, DD) was determined using an infrared spectroscopy (Bruker Tensor 27) analysis, applying the following equation:

$$DD(\%) = 97.67 - 26.486 \times (A_{1655}/A_{3450})$$

where A_{1655} is the absorbance at 1655/cm of the amide I band and A_{3450} is the absorbance at 3450/cm of the hydroxyl band (Cota-Arriola et al., 2011).

2.3. Fungal strains

F. verticillioides (M7075) and *F. proliferatum* (RC 2080) strains were used. Both strains were isolated from maize in Argentina (Etcheverry et al., 2002; Reynoso et al., 2004). These strains have been characterized using a polyphasic approach: morphologically, amplified fragment length polymorphisms (AFLP) and fumonisin production. The strains are deposited at the Department of Microbiology and Immunology, Universidad Nacional de Rio Cuarto culture collection (RC). Cultures are maintained in 15% glycerol at −80 °C.

2.4. Medium

Maize was finely milled with a Romer mill (Romer Labs GmbH, Tulln, Austria). Mixtures of 2% (*w*/*v*) milled maize in water were prepared and 2% (*w*/*v*) agar (technical agar No. 2, Oxoid) added. The a_W of the basic medium was adjusted to 0.995, 0.99, 0.98, 0.96 and 0.93, by the addition of different amounts of glycerol (Dallyn and Fox, 1980). From the chitosan stock solution different aliquots were taken in order to produce different concentrations (0.5, 1, 2 and 3 mg/mL) and added to the medium before sterilization. The media were autoclaved at 120 °C for 20 min. Flasks of molten media were thoroughly shaken prior to pouring into 9 cm sterile Petri dishes. The final pH of the medium containing chitosan ranged from 5.5 to 6, in order to ensure that all the chitosan amino groups were positively charged (Kong et al., 2010; Liu et al., 2004).The a_W and pH of representative samples of media were checked with an Aqualab Series 3 (Decagon devices, Inc., WA, USA) and a pH meter (Orion 250A, Boston), respectively. Control plates were prepared and measured at the end of the experiment in order to detect any significant deviation of both parameters.

In order to discount fungal inhibition due to acetic acid in the chitosan solution a maize based-medium without chitosan (control AcH) was prepared for each a_W level used. Those AcH control plates were prepared as described above, adding the same volume of 1% AcH used to prepare 3 mg/mL of chitosan amended media.

A preliminary experiment was performed to choose the range of concentration of chitosan (1, 3, 5 and 10 mg/mL) to be added to obtain a dose–response curve. As fungal growth was completely inhibited in 5 and 10 mg/mL chitosan solutions, solutions below 5 mg/mL were used in the following experiments.

2.5. Inoculation, incubation and growth assessment

All media, with and without chitosan, were inoculated centrally with a 4 mm diameter agar disk taken from the margin of a 7-day-old colony of each isolate grown on synthetic nutrient agar (SNA) (Gerlach and Nirenberg, 1982) at 25 °C. The disks were transferred face down onto the center of each plate. Inoculated Petri plates of the same a_W were

sealed in polyethylene bags and incubated at 25 °C for 28 days. A full-factorial design was used where the factors were a_W, chitosan concentration and the response was growth (total number of Petri plates = 5 a_W × 4 chitosan concentrations × 2 isolates × 3 replicates). Experiments were carried out with three replicates per treatment. Assessment of growth was made daily during the incubation period, and two diameters of the growing colonies measured at right angles to each other until the colony reached the edge of the plate. The radii of the colonies were plotted against time, and linear regression applied in order to obtain the growth rate (mm/day) as the slope of the line. The time at which the line intercepted the *x*-axis was used to calculate the lag phase in relation to strain, chitosan concentration and a_W. After the incubation period, controls and treatments were frozen for later extraction and fumonisin determination.

2.6. Fumonisin determination

Toxins were extracted at the end of the incubation period (28 days) for each a_W and chitosan concentration with acetonitrile: water (1:1 *v*/*v*) by shaking the entire contents of the Petri dish (~20 g culture media and mycelia) with the solvent for 30 min on an orbital shaker (150 rpm) and then filtering the extracts through filter paper (No. 4; Whatman International Ltd, Maidstone, Kent, UK). An aliquot of the extracts (1000 μL) was taken and diluted with acetonitrile: water (1:1 *v*/*v*) as necessary for high performance liquid chromatography (HPLC) analysis. An aliquot (50 μL) of this solution was derivatized with 200 μL of an o-phthaldialdehyde (OPA) solution obtained by adding 5 mL 0.1 M sodium tetraborate and 50 μL 2-mercaptoethanol to 1 mL methanol containing 40 mg OPA (Shephard et al.,1990). The fumonisin OPA derivates (50 μL solution) were analyzed using reversed-phase HPLC/fluorescence detection system. The HPLC system consisted of a Hewlett-Packard 1100 pump (Hewlett Packard, Palo Alto, CA, USA) connected to a Hewlett-Packard 1046A programmable fluorescence detector and a data module Hewlett-Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a stainless steel, C_{18} reversed-phase column (150 × 4.6 mm i.d., 5 μm particle size; Luna-Phenomenex, Torrance, CA, USA) connected to Security Guard cartridge (4 × 3 mm i.d., 5 μm particle size; Phenomenex, Torrance, CA, USA) filled with the same phase. Methanol:0.1 M sodium dihydrogen phosphate (75:25, *v*/*v*) solution adjusted to pH 3.35 with orthophosphoric acid was used as the mobile phase, at a flow rate of 1.5 mL/min. Fluorescence of the fumonisin OPA derivatives was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. Fumonisins were measured as peak heights and compared with reference standard solutions of fumonisins B_1 and B_2 (Sigma Chemical Co., St Louis, MO, USA). A mixed acetonitrile: water (1:1, *v*/*v*) stock solution of FB_1, FB_2 and FB_3 containing 50 μg/mL of each toxin was prepared. Four mixed working calibrant solutions (0.25, 0.5, 1.0, and 2.0 μg/mL) were prepared by diluting an aliquot of the stock solution with the appropriate volume of acetonitrile:water (1:1, *v*/*v*). The retention time of FB_1, FB_3 and FB_2 was 7.5, 16.7 and 18.5 min, respectively. Appropriate dilutions of standards and/or sample extracts were made with acetonitrile/water (1:1). The detection limit of the analytical method for the three fumonisins was 1 μg/g based on the signal-to-noise ratio 3:1. Recovery experiment was performed on 2% milled maize agar spiked at levels of 1 to 10 μg/g of each fumonisin (FB_1, FB_2 and FB_3). Mean recovery ranged from 95 to 98% and 94% for FB_1, FB_2 and FB_3, respectively.

2.7. Statistical treatment of results

The growth rates, lag phase and mycotoxin concentration were evaluated by analysis of variance (ANOVA) to determine the effect of chitosan doses, a_W, *Fusarium* species and two- and three-way interactions. When the analysis was statistically significant, the post hoc Tukey's multiple comparison procedure was used for separation of the means. Statistical significance was judged at the level $p \leq 0.01$. All the analyses were done using SigmaStat for Windows Version 2.03 (SPSS Inc.).

Table 1
Effect of chitosan on the lag phase (h) of *Fusarium verticillioides* M7075 and *Fusarium proliferatum* RC2080 on maize-based media at 25 °C.

Fusarium species	Chitosan (mg/mL)	Water activity				
		0.995	0.99	0.98	0.96	0.93
Fusarium verticillioides M7075	0	8^{a}	13^{ab}	13^{a}	15^{a}	17^{a}
	0.5	13^{b}	12^{a}	17^{a}	16^{a}	46^{b}
	1	18^{b}	17^{b}	17^{a}	21^{a}	69^{c}
	2	35^{c}	37^{c}	63^{b}	55^{b}	113^{d}
	3	67^{d}	101^{d}	171^{c}	200^{c}	276^{e}
Fusarium proliferatum RC2080	0	11^{a}	10^{a}	9^{a}	13^{a}	19^{a}
	0.5	12^{a}	11^{a}	13^{a}	7^{b}	27^{a}
	1	19^{b}	19^{b}	10^{a}	22^{c}	53^{b}
	2	32^{c}	45^{c}	46^{b}	56^{d}	124^{c}
	3	99^{d}	142^{d}	197^{c}	149^{e}	209^{d}

Mean values based on triplicate data. Means followed by the same letter within a column are not significantly different according to Tukey test ($p < 0.001$).

3. Results

3.1. Chitosan characterization

Average chitosan molecular weight (Mw) in g/mol determined by Mark–Houwink–Sakurada equation was $3.42 \pm 0.08 \times 10^3$ g/mol. The deacetylation degree (DD) determined by infrared spectroscopy analysis and the equation given above was 77.6%.

3.2. Lag phase

Table 1 compares the effects of treatment on the lag phases (h) prior to growth. In the control treatments there was an increase in the lag phase with decreasing a_W. Interaction with the different chitosan doses resulted in a further increase in the lag phase prior to growth regardless of the a_W and the isolate evaluated. The statistical analysis using ANOVA showed that single factors of a_W and chitosan and their interactions were significant ($p < 0.001$); only the single factor isolate and their interaction with chitosan did not show significant differences (Table 2).

3.3. Growth rates

Fig. 1 shows the effect of chitosan on the growth rate of *F. verticillioides* and *F. proliferatum* isolates on maize based media under all the treatment conditions assayed.

Table 2
Analysis of variance on the effects of water activity (a_W), chitosan concentration (C), and different isolates (i) and their interactions on the lag phase and growth rate of *Fusarium verticillioides* M7075 and *Fusarium proliferatum* RC2080 on maize-based media at 25 °C.

Source of variation	*df*[a]	Lag phase		Growth rate	
		MS[b]	*F*[c]	MS[b]	*F*[c]
I	1	6.050	0.0617	0.337	5.080
C	4	95,208.806	971.684*	48.409	729.716*
a_W	4	10,598.514	108.166*	80.476	1213.085*
i × C	4	302.183	3.084	0.0406	0.612
i × a_W	4	850.036	8.675*	0.767	11.560*
C × a_W	16	6450.164	65.829*	1.934	29.152*
i × C × a_W	16	752.653	7.681*	0.288	4.337*

* $P < 0.001$.
[a] Degrees of freedom.
[b] Mean square.
[c] Snedecor-F.

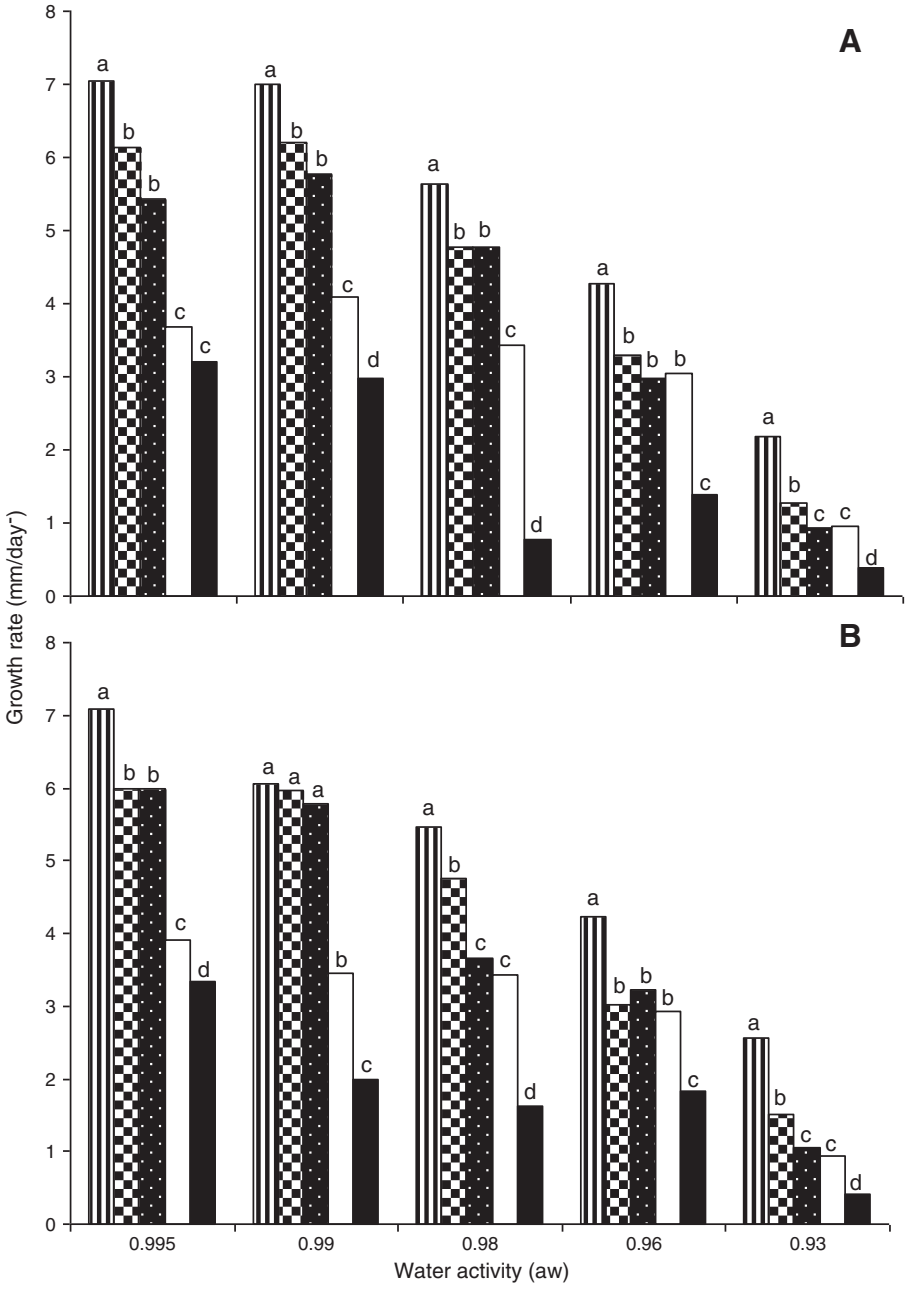

Fig. 1. Effect of different doses of chitosan on growth rate of A) *Fusarium verticillioides* M7075 and B) *Fusarium proliferatum* RC2080 at different a_W levels and 25 °C (▥ Control AcH; ▩ 0.5 mg/mL; ▩ 1.0 mg/mL; □ 2 mg/mL; ■ 3 mg/mL). Mean values based on triplicate data with letters in common for each a_W are not significantly different according to Tukey test ($p < 0.001$).

The results showed that both isolates grew better at high a_W (0.995) with growth decreasing as the water availability of the medium was reduced. In general inhibition of mycelial growth increased as chitosan concentration was increased, regardless of the a_W used. For both isolates, the lowest growth rates were obtained using 3 mg/mL of chitosan and 0.93 a_W.

Statistical analysis showed that growth rate significantly depended on a_W, chitosan concentration and their interaction; only the single factor isolate and their interaction with chitosan did not show significant differences (Table 2).

3.4. Fumonisin accumulation

Table 3 shows the effect of the different chitosan concentration/a_W treatments at 25 °C on fumonisin production by *F. verticillioides*

Table 3
Combined effect of different concentration of chitosan and a_W on fumonisin ($FB_1 + FB_2 + FB_3$ μg/g) accumulation by *Fusarium verticillioides* M7075 and *Fusarium proliferatum* RC2080 on maize-based media at 25 °C.

Fusarium species	Chitosan (mg/mL)	Water activity			
		0.995	0.99	0.98	0.93
F.verticillioides		189[a]		113[a]	
0	175[a]		302[a]		
M7075	0.5	16[b]	9[b]	23[b]	6[b]
	3	20[b]	n.d.[c]	6[c]	n.d.[c]
F. proliferatum	0	930[a]	1140[a]	3653[a]	37.[a]
RC 2080	0.5	776[b]	940[b]	477[b]	16[b]
	3	5[c]	5[c]	13[c]	n.d[c]

Mean values based on triplicate data. Means followed by the same letter within a column are not significantly different according to Tukey test ($p < 0.001$).
n.d..: not detected, below limit of detection.

M7075 and *F. proliferatum* RC2080. Overall, in control treatments *F. proliferatum* produced more fumonisin than *F. verticillioides* under the same conditions. Maximum levels of fumonisin were obtained at 0.98 a_W by both *Fusarium* species. FB_1 was the major fumonisin (approximately in a 70%), in comparison with FB_2 and FB_3.

Overall, production of fumonisins decreased when chitosan dose increased. Fumonisin production was significantly reduced ($p < 0.001$) in almost all a_W conditions used in the presence of chitosan in comparison with the controls (Table 3). For both species, no toxin was detected at the highest doses of chitosan used (3 mg/mL) at 0.93 a_W.

The statistical analysis using ANOVA showed that single factors (isolates, a_W and chitosan) and their interactions were significant ($p < 0.001$) (data not shown).

4. Discussion

The present study has shown the potential use of chitosan for controlling both growth and fumonisin production by *F. verticillioides* and *F. proliferatum* under a range of a_W conditions at 25 °C. Increasing chitosan concentrations associated with a_W reduction increased the lag phase, and diminished the mycelial growth of both *Fusarium* species. As a result the longest lag phase and the lowest growth rate were obtained at the highest chitosan concentration (3 mg/mL) used and 0.93 a_W. At the lowest level of chitosan used we observed reduction in the growth rates and an important effect on fumonisin production on both *Fusarium* species under study, regardless the a_W. There are no other studies in the literature evaluating the effect of both chitosan and a_W on growth and mycotoxin production of *Fusarium* species.

The antifungal activities of chitosan rely on numerous intrinsic and extrinsic factors, such as pH, fungal species, presence or absence of metal cations, pKa, Mw and DD (Kong et al., 2010). For all these reasons some characteristic of the commercial chitosan used during the present work were determined. The results showed that the chitosan had a low Mw and 77.6% DD. For soluble chitosan, pH is a crucial factor relating to solubility, and can further alter antifungal activity. Antifungal activity of chitosan is exhibited only when the pH is below the representative pKa (~6.5), the value at which the soluble molecule can be dissociated as ions in the solution. In the present study the pH of the medium was maintained below the pKa.

Many authors have studied the antifungal effects ""of chitosan. Ziani et al. (2009) studied the potential of chitosan as an antifungal polymer against three fungi, *A. alternata*, *A. niger* and *Rhizopus oryzae*, that cause seed deterioration. They concluded that the antifungal capacity of chitosan is real but difficult to control, as the effectiveness of chitosan depends not only on the chitosan formulation but also on the fungus type and on the type of treatment (using films or solutions). Another survey using chitosan concentrations higher than 1.5% (15 mg/mL) reported a reduction in mycelial growth (greater than 50%) of several phytopathogenic fungi, *Fusarium oxysporum*, *P. digitatum* and *R. stolonifer*, isolated from papaya (Bautista-Baños et al., 2004). Some studies suggest that chitosan may have either a fungicidal or fungistatic effect depending on the concentration used. For example, mycelial growth of *C. gloeosporioides* was totally inhibited using chitosan concentrations of 2.5 and 3% (25 and 30 mg/mL) during 7 days of incubation, whereas at 0.5 and 1.5% (5 and 15 mg/mL) the fungi started to grow at the second and fourth days, respectively (Bautista-Baños et al., 2003). However, at 1.5% a fungicidal effect was reported in *B. cinerea*, *A. alternata*, *C. gloeosporioides* and *R. stolonifer*, the authors of this research attributing the effect to chitosan DD (El Ghaouth et al., 1992). Unfortunately, all these studies were carried out in media (solid and liquid) with freely available water (0.995 a_W), which makes direct comparison with the present work more difficult. Also the previous report took no account of the interactions between the efficacy of chitosan or chitosan derivates and a_W or temperature, which have been demonstrated to be key parameters determining germination, growth and mycotoxin production by different fungal species (Sanchis and Magan, 2004).

A number of possible mechanisms for the antifungal action of chitosan have been proposed, mostly based on the positive charge conferred by protonation of free amino groups at acidic pH, although the exact mechanisms of action are poorly understood. A polycationic chitosan can potentially interact with negatively charged fungal cell membrane components (i.e., proteins, phospholipids, amino acid), thus interfering with the normal growth and metabolism of the fungal cells (Bautista-Baños et al., 2006; Cota-Arriola et al., 2011). Recently, Palma-Guerrero et al. (2009) have demonstrated that chitosan permeabilizes the plasma membrane and kills cells of *Neurospora crassa* in an energy dependant manner.

It is notable that although the chitosan solution used in this study was not chemically modified, it also had an effect in reducing fungal growth and fumonisin production. In the present study, both concentrations of chitosan used (0.5 and 3 mg/mL) at all the a_W tested were able to reduce fumonisin production in *F. verticillioides* and *F. proliferatum* isolates. This is the first report showing the combined effects of different chitosan concentrations and different a_W values, in fumonisin production (in vitro) by both *Fusarium* species.

There are two previous studies that have analyzed the effect of chitosan on mycotoxin production (aflatoxin), but the effect of the a_W was not considered. Cota-Arriola et al. (2011) analyzed the *in vitro* effect of chitosan on growth and aflatoxin B_1 production by *A. parasiticus*. Two different types of chitosan were used; both were obtained from chitin previously extracted from shrimp heads using either a chemical (CS) or biological (CB) process. The experiment was carried out in solid media at 28 °C and final chitosan concentrations were 0.6 to 4.5 mg/mL. Although no fungicidal effect on *A. parasiticus* was shown with either of the chitosans used, for each chitosan, concentrations of 3 and 4.5 g/L were the most effective in retarding fungus growth. These results are similar to those achieved in the present research: the maximum growth rate inhibition was achieved using 3 mg/mL of chitosan. With regard to aflatoxin production Cota-Arriola et al. (2011) reported an increase in toxin levels in maize; the authors attribute this to the stress on the fungus due to the presence of chitosan. Conversely, Cuero et al. (1991) showed that chitosan inhibited the growth of *A. flavus* and aflatoxin production in liquid culture. These authors explain that aflatoxin production was inhibited due to the chelating action of chitosan, selectively binding trace elements such as zinc that is necessary for toxin synthesis. Further studies are needed to elucidate the mechanisms of action of chitosan and the effects on fungal secondary metabolism.

In conclusion, the present study has shown the combined effects of chitosan and a_W on growth and fumonisin production by the two most important *Fusarium* species present on maize. It has also demonstrated that low Mw chitosan with more than 70% of DD at 0.5 mg/mL was able to significantly reduce growth rate and fumonisin production on maize-based media, although maximum levels of reduction in both parameters were obtained at the highest doses used. Since fumonisins are unavoidable contaminants in food and feed chains, their presence needs to be minimized in order to reduce their effects on human and animal health and to diminish the annual market loss through rejected maize. In this scenario, post-harvest use of chitosan could be an important alternative treatment. However it is necessary to continue in depth basic studies that could contribute to explanation of the effect of applying this polymer on maize.

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