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Evaluation of fungicidal action of four products on external fungal biota of grains

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Abstract

Corn, the main crop in Argentina, is normally harvested and stored in silo bags above the safe storage moisture content for 3-6 months, and faces high risk of quality losses. The evaluation of fungicidal/fungistatic products that could potentially be used to protect wet grain stored in silo bags, has not been widely studied. Thus, the objective of this study was to make a preliminary in-vitro comparative evaluation of four different fungicidal treatments in corn: 1) propionic acid (3 L/t); 2) a quaternary ammonium compound (3 L/t, diluted 1:25 with water); 3) sulfur dioxide (initial dose: 15% v/v); 4) phosphine (initial dose: 6.6 g/m^3) along with their respective controls (non-treated). In treatments 1 and 2 (liquids) corn was sprayed with the product and then stored in a sterilized glass jar. In treatment 3, sulfur dioxide was injected into a previously sealed glass jar. In treatment 4, glass jars were placed inside a plastic drum of 60 L capacity with two aluminum phosphide tablets (0.2 g phosphine each) and closed. Both liquids and the phosphine treatments had an exposure time of 15 d (at 25 ± 1°C), while in the sulfur dioxide treatment, three exposure times were evaluated (5 min, 5 h and 24 h, all at the same temperature). After the various treatments, the fungal biota (molds and yeasts) was evaluated. The results showed that the propionic acid treatment achieved a total control of fungal biota. Treatment with sulfur dioxide showed a reduction of 2-3 log10 (CFU/g DM) compared to non-treated samples. Finally, the quaternary ammonium compound and phosphine treatments only achieved a reduction in the number of colonies of 1 log10 (CFU/g DM). Based on the results of this study, propionic acid seems to be a good solution for improving the storability of wet corn in silo bags. Nevertheless, more information should be generated at laboratory and full scales.

Keywords: Propionic acid, Quaternary ammonium compound, Sulfur dioxide, Phosphine, Fungal colonies

Introduction

Molds have been designated as one of main causes of quality losses during grain storage (CAST, 1989). Fungal activity can cause rapid deterioration of grain, leading to dry matter loss, deterioration of nutritive value, seed viability reduction and production of mycotoxins. Storage molds are able to grow at relatively low water activities (a_w, 0.70-0.75) initiating grain spoilage (Magan and Lacey, 1988).

In the past few years, corn has become the most important crop in Argentina (50 Mt by 2019/20; Melo, 2021). Due to weather conditions in the fall, corn is usually harvested at 15-18% moisture content (m.c.) corresponding to a_w from 0.77 to 0.89, which is above the safe storage m.c. (<14%; a_w <0.7) and therefore requiring drying. However, grain drying is not always feasible at the farm level because most farms do not have grain dryers, and the price of fuel at the farms is too expensive. As a result, many farmers have to store wet corn in silo bags for 3-6 months and face a high risk of quality losses. The silo bag is a temporary storage system, is potentially hermetic, has an average holding capacity of 180-200 t of corn (2.74 m diameter, 60 m long, 235 µm polyethylene).

When wet grain is stored inside silo bags, the favourable modification of the atmosphere can assist in delaying the grain deterioration process. However, the airtightness of silo bags in the field is highly variable (depending on the sealing system implemented, perforations caused by animals, or misuse of equipment and tools around the bag) and normally decreases during storage (Cardoso et al., 2012). When stored in non-airtight silo bags, grain quality deteriorates after only a few months (Bartosik et al., 2008).

Several compounds had been proposed to reduce mold activity in corn and others grains, including organic acids (Chulze, 2010), phosphine (PH_3) (De Castro et al., 2001), and sulfur dioxide (SO_2) (Magan, 1992). There are also quaternary ammonium compounds (QAC) which are non-toxic products and have fungistatic or antifungal properties (normally used for disinfection of surfaces in the food industry) which were not previously evaluated in grain (Chauret, 2014).

Evaluation of fungicidal/fungistatic products that could potentially be used to protect wet grain stored in silo bags has not been widely studied. Liquids and gaseous candidate products must be applied under the particular conditions of storage in silo bags at the farm level. Liquid products can only be sprayed during the filling (or bagging) operation, usually at harvest time, while gaseous products can be applied during storage in silo bags with variable levels of airtightness. Thus, the objective of this study was to make a preliminary in-vitro comparative evaluation of four different fungicidal treatments in corn.

Materials and methods

The study was carried out at INTA Balcarce Research Station, Argentina. Four different products (two liquids and two gaseous) were evaluated: Test 1) Propionic acid (Lupro-Grain®, 3 L/t), Test 2) Quaternary ammonium compound/QAC (Virukill® = 3 L/t, diluted 1:25 with water), Test 3) Sulfur dioxide/SO_2 (initial dose: 15% v/v), Test 4) Phosphine/PH_3 (initial dose: 6.6 g/m^3), and their respective controls (non-treated).

A full-randomized design was proposed for each test. A factorial analysis was considered where each treatment was a combination of two factors: dose and storage time (Table 1).

The experimental unit was a glass jar with 1.8 kg of corn at 15% m.c., and three replicates were conducted for each treatment. In Tests 1 and 2 (liquids), half of the corn was sprayed with the product (and the grain mixed by hand), while the other half was used as a control. Both halves were divided into 4 portions of 1.8 kg each and stored in sterilized glass jars (non-hermetic). Test 3 consisted of different exposure times at 15% SO_2. The glass jar filled with corn was hermetically sealed and the SO_2 mixture (15%) was injected for 1 min through a rubber septum inserted in the metal lid of the jar (while purging was made through another needle inserted in the septum). The SO_2 concentration was measured with colorimetric tubes. In Test 4, the glass jars were placed inside a plastic container of 60 L capacity with two aluminum phosphide tablets (0.2 g phosphine each) and then closed with a partial sealing. Phosphine concentration was measured at different times employing colorimetric tubes inserted through a valve. All treatments were stored in a controlled temperature chamber (at 25 ± 1°C) during the exposure times indicated in Table 1.

Table 1. Dose and exposure time of different tests

Test number	Product	Dose	Exposure time
Test 1	Propionic acid	Non-treated	15 d
		3 L/t	
Test 2	Quaternary ammonium compound/QAC	Non-treated	15 d
		3 L/t (diluted 1:25)	
Test 3	Sulphur dioxide/SO_2	Non-treated	24 h
		15% v/v	5 min
			5 h
			24 h
Test 4	Phosphine/PH_3	Non-treated	15 d
		6.6 g/m^3	

After the prescribed exposure time, the jars were opened and the fungal biota (molds and yeasts) was evaluated using the method of counting in Petri dishes in potato dextrose agar (Britania®), with the addition of chloramphenicol (0.01% Anedra®). Plates were incubated in an oven at 28°C in darkness for 5 d (Pitt and Hocking, 2009). Counts were expressed as colony forming units per gram of dry matter (CFU/g DM).

Comparisons of treatments were performed with ANOVA, employing R software (version 3.6.3). Tukey's HSD (0.05) post hoc test was also used for mean comparison. Figures were created with Excel (Microsoft Office Professional Plus 2016).

Results and discussion

Table 2 shows that CFU counts in the non-treated samples had certain variability, from 3.42 log_{10}/g DM ($2.6x10^3$ CFU g/DM) for propionic acid to 4.12 log_{10}/g DM ($1.35x10^4$ CFU g/ DM) for SO_2. Despite the fact that the grain came from the same batch, this variability was expected because the tests were carried out at different times.

The four evaluated treatments had different results (Table 2). Test 1 with propionic acid completely eliminated the fungal load after 15 d (0 CFU/g DM). Test 2 with QAC only had a reduction of 1 log_{10} CFU/g DM in the fungal biota after 15 d of treatment ($p<0.05$). In Test 3 with SO_2 all of the exposure times resulted in a high reduction in the fungal biota ($p< 0.001$) of between 2-3 log_{10} CFU/g DM compared to the control. However, there were no significant differences among the various exposure times ($p>0.05$). The average final CFU count across all exposure times was 1.12 log_{10} CFU/g DM (13 CFU/g DM). In Test 4 with PH_3 there was a reduction of 1 log_{10} CFU/g DM, with the final count being 2.94 log_{10} CFU/g DM ($8.7x10^2$ CFU/g DM) ($p<0.05$), similar to that of Test 2.

Table 2. Average CFU counts (± SD) for different tests in wet corn. Different letters mean statistical differences within same product (p-value: 0.05)

Product	Dose	Exposure time	Log_{10} CFU/ g DM
Propionic Acid	Non-treated	15 d	3.42 ± 0.52 A
	3 L/t		0.00 ± 0.00 B
Quaternary ammonium compound/QAC	Non-treated	15 d	3.92 ± 0.62 a
	3 l/t (Diluted1:25)		2.94 ± 0.11 b
Sulfur dioxide/SO_2	Non-treated	24 h	4.12 ± 0.78 A
	15% v/v	5 min	1.80 ± 0.56 B
		5 h	1.08 ± 0.99 B
		24 h	0.50 ± 0.92 B
Phosphine/PH_3	Non-treated	15 d	3.93 ± 0.36 a
	6.6 g/m^3		2.94 ± 0.06 b

Gimeno and Martins (2011) proposed a threshold of $4x10^4$ CFU/g DM for a grain sample to be considered of good mycological quality. The CFU counts of all the control samples (non-treated) were below that threshold, indicating the grain used for this study was in good microbiological condition. Higher CFU counts (i.e., 10^5 CFU/g DM) would have indicated an early spoilage process (Magan, 1993).

Liquid products can only be applied during the bagging operation at the beginning of storage. In this sense, the desirable characteristics of the liquid products include an immediate reduction in fungal load and the persistence of this effect over time (residuality).

Propionic acid had the best performance of all the evaluated products as it completely eliminated the fungal load after 15 d. These results were in agreement with those reported by Fernandez Zambón (2018), who evaluated a dose of 4 L/t in wet corn (17% m.c.) stored for 6 months at pilot-scale (15 kg bags). He reported an immediate and significant reduction in CFU counts after the application, and that the CFU counts remained stable during storage (residual effect). Raeker et al. (1992) reported that lower doses than that evaluated in our study (0.5 and 1.0% (v/v)) allowed the preservation of wet corn (≥ 17.6% m.c.) for more than one year in laboratory tests. However, the application of low doses at real-scale represents a challenge in terms of achieving a proper distribution of the product throughout the entire grain mass (Chulze, 2010). As a negative effect of the use of propionic acid, a certain degree of grain discoloration was reported even in treatments with lower doses (Fernandez Zambón, 2018; Raeker et al., 1992), which could affect the commercial quality of the grain.
Quaternary ammonium compounds had certain fungicidal properties and had no negative effects on the grain (i.e., discoloration). However, a higher dose than what was explored in this study should be evaluated for achieving a suitable control. In addition, although this product showed a good residual effect on disinfected surfaces (Chauret, 2014), a more comprehensive study should be carried out for establishing its residual behavior in a grain matrix.

Gaseous treatments could be implemented at any point during storage, but they do not have residual effect. Additionally, a minimum airtightness is required to achieve the effective concentration-time in order to produce the expected fungicidal/fungistatic effect.

The theoretical concentration in the PH_3 treatment was 5000 ppm. However, after 120 h, the mean PH_3 concentration dropped to 3500 ppm, and to 800 ppm after 15 d, implying that the container was not hermetic. This PH_3 loss was about 270 ppm/day, equivalent to that achieved in a semi-hermetic system with a pressure decay test lower than 1 min (250-125 Pa) (Navarro and Zettler, 2000). This level of airtightness was similar to that reported by Carpaneto et al. (2016) in fumigated silo bags, implying that the results of the present study could be extrapolated to a real scale silo bag. The concentration-loss condition evaluated in the present study would reduce, but not eliminate, the fungal biota. A higher airtightness could yield higher fungicidal effect. De Castro et al. (2001) evaluated different PH_3 doses (0-4 g/m^3) and exposure times (1 to 15 d) in wet corn (0.85 and 0.98 a_w). They concluded that in the lower a_w condition, exposure time was more relevant than PH_3 concentration. Similarly, Lacey (1992) noted that PH_3 at 3.0-3.5 g/L over 90 d was fungicidal to 89% of storage fungi and fungistatic to another 7%. Lower PH_3 doses (i.e., 0.1 g/m^3) during longer exposure times could produce a slight decrease in fungal population or retard fungal growth (Hocking and Banks, 1993).

The evaluated dose of SO_2 during a short exposure time (1-24 h) produced a better result than PH_3 and QAC treatments. Magan and Aldred (2007) conducted a review about the fungicidal effect of SO_2, but their dosage recommendation was not perfectly elucidated. On the one hand they stated that 1.5% SO_2 (v/v) was required to reach long-term (5 m) grain conservation. However, studies suggested that much higher concentrations might be required because of the adsorption and binding of the SO_2 in the grain matrix. Furthermore, for wetter grain (0.92-0.96 a_w), 2 g/L (76% v/v) of SO_2 was suggested in order to reduce the fungal biota population from 1 to 2 log.

The shorter exposure time of SO_2 in comparison with the PH_3 treatment (1-24 h) implied that moderate/lower airtightness could be required and that a faster treatment could be made. However, at full-scale, a significantly higher volume of gas should be injected in the silo bag at the field, implying a greater operational complexity than the PH_3 treatment.

An additional challenge that must be considered for a full-scale application of SO_2 and propionic acid is their corrosive effect on metallic structures. This could disqualify the use of these products in metal bins, but not in silo bags since the polyethylene is not affected. Note, however, the application of propionic acid during the loading operation could also cause corrosion in the bagging machine. Based on the result of this study, propionic acid seems to be a good solution for improving the storability of wet corn in silo bags. Nevertheless, more information should be generated at laboratory and full-scale levels in order to make practical recommendations.

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