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Effect of the temperature and relative humidity in stored sotol (*Dasyilirion cedrosanum* Trel.) seeds on fungi biodiversity

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ABSTRACT

The objective of the research was to identify the fungi in sotol seeds at different conditions of temperature and relative humidity. Seed were collected at Buñuelos, municipality, and taken to the Laboratory of the Center for Training and Development in Seed Technology (CCDTS) at Universidad Autonoma Agraria Antonio Narro. Seed was stored for a period of 90 days, whit conditions of 60, 75, 80 and 85% of relative humidity kept at 5, 15 and 25 °C. Fungi identifying by morphological criteria. A completely randomized experimental using R software with factorial arrangement whit two replications. Pathogens identified were: *Aspergillus glaucus*, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp., *Aspergillus candidus*, *Cladosporium* sp., *Alternaria* sp. and *Aspergillus ochraceus*. The results showed that the higher the humidity, temperature, storage time and the incidence of fungi tends to be higher. Fungi with higher presence in sotol seeds were: *Aspergillus glaucus* and *Penicillium* sp. Safe storage environments for sotol seeds reported in this work are 5 °C and a relative humidity of 60-75%. Sotol seeds tolerates conditions of 15 °C and a relative humidity up to 75%.

KEYWORDS: Seed, Environment, Conditions, Incidence

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INTRODUCTION

A sotol (*Dasyilirion cedrosanum* Trel.) is an integral part of human history in northern Mexico and southern United States (Valdés *et al.*, 2012). In Mexico the plants can be found in the Sierra Madre Occidental and Sierra Madre Oriental mountain range systems; mainly in the states of Chihuahua, Coahuila and Durango. Sotol is an important ecological component of desertic zones, since it contributes to soil maintenance while being a food source for some of the desertic fauna, particularly rodents and birds (Reyez *et al.*, 2012). Seeds are considered the most important germplasm source for mass production of plants (Poehlman, 2005). The storage conditions of seeds is highly important (Amaral & Lemos, 2009; Oyekale *et al.*, 2012), mainly due to pathogens potentially being transported within the seeds, surviving in it for long periods of time (Mercado, 2018). Species *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Alternaria* were commonly found in post-harvest storage conditions as mold (Chavan, 2011). In order to carry out an adequate storage of seeds, is necessary to minimize viability

loss; factors that influence this during the storage are: humidity content of the seeds, relative humidity and temperature of the environment temperature, as well as biotic factors presented, such as the incidence of fungi and insect pests. Therefore, it is important to identify the species of fungi in stored maize grains with special emphasis on mycotoxigenic species, which pose a potential risk to human and animal health (Castlellarie *et al.*, 2010). Regarding the above mentioned, the aim of this research was to identify the different fungi present in the seeds of sotol (*D. cedrodanum*) stored in twelve different environmental conditions.

MATERIAL AND METHODS

Biological Material Used

Sotol seeds collected in 2016 at Buñuelos, municipality, Saltillo, Coahuila, Mexico, whose coordinates are 25°27'36.1"N 101°01'21.0"W (Figure 1).

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Storage Time and Conditions

Sotol seeds were stored for a 90-day period under relative humidity conditions of 60, 75, 80 and 85% and kept at 5, 15 and 25 °C, respectively. To achieve the 60% of relative humidity in the storage conditions, a saturated solution of glucose was used. Similarly, for the 75% of relative humidity a sodium chloride (NaCl) solution was used; while for the 80% and 85% an ammonium sulfate [(NH₄)₂SO₄] and potassium chloride solutions were used, respectively (Winston & Bates, 1960). Two hundred forty experimental units were placed in perforated cloth meshes and were randomly distributed in the plastic chambers that made up each of the warehouse environments. Sampling was carried out at 0, 15, 30, 60 and 90 days, each with two replications (Table 1).

Isolation, Identification and Incidence of Fungi Pathogens

Ten seeds were placed equidistant by Petri dish with MSA culture medium (two replications) and kept at 25 °C ± 2°C for 192 h. Identification of pathogens was made in a compound microscope following Moreno keys (Moreno, 1988). The results

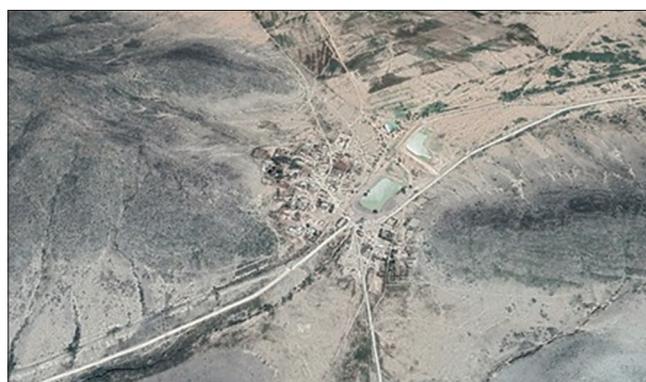


Figure 1: Buñuelos, Saltillo, Coahuila, Mexico

were reported in percentage using the formula (Abdullah & Al-Mousawi, 2010): $\text{Porcentaje incidencia \%} = \frac{\text{number of seeds on which fungi appeared}}{\text{total number of seeds}} \times 100$

Analysis of results

Data collected was evaluated completely randomized in a factorial arrangement of two factors, where: factor A for ambient and factor B for evaluation times. The comparison of means using Tukey test (p=0.05). Results were analyzed through the R software (R Core Team, 2013).

RESULTS

Figure 2 shows the behavior of the incidence of pathogens according the environments (p=0.001), and a higher incidence presented in the environments 11 and 12, with 46 and 36%. The lowest incidences are present in the environments: 7, 2 and 4 with 1.0, 4.0 and 5.0%, respectively.

Figure 3 shows the difference in incidence in the sampling; in first sampling, there was no incidence of pathogens (p=0.001), in second sampling at 15 days with 7%, the third sampling at 30 days with 9.5%, the fourth sampling at 60 days

Table 1: Study environments

Environments	Temperature (°C)	Relative humidity(%)
1	5	60
2	5	75
3	5	80
4	5	85
5	15	60
6	15	75
7	15	80
8	15	85
9	25	60
10	25	75
11	25	80
12	25	85

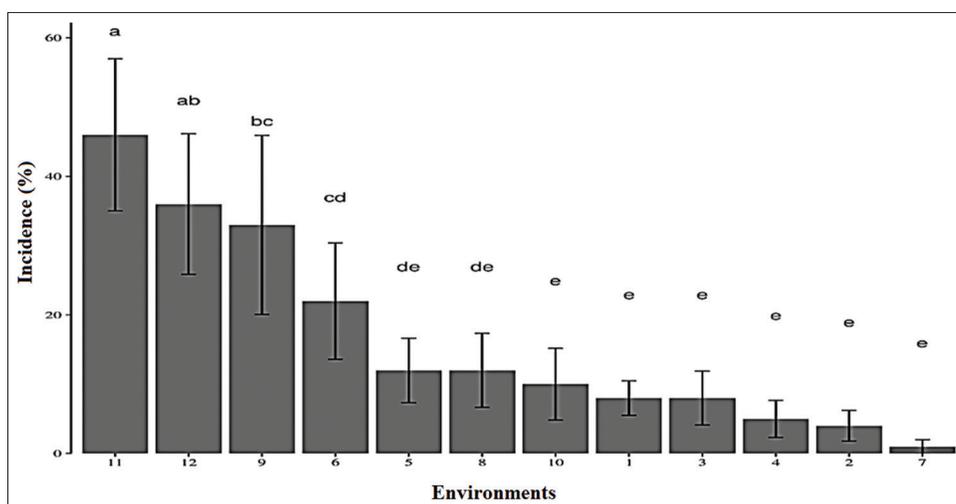


Figure 2: Incidence of fungi in sotol seeds in 12 storage environments, according to Tukey at 0.05, groups with different letters are statistically significant

with 30.8% and finally, in sampling at 90 days with 34.5% (higher incidence).

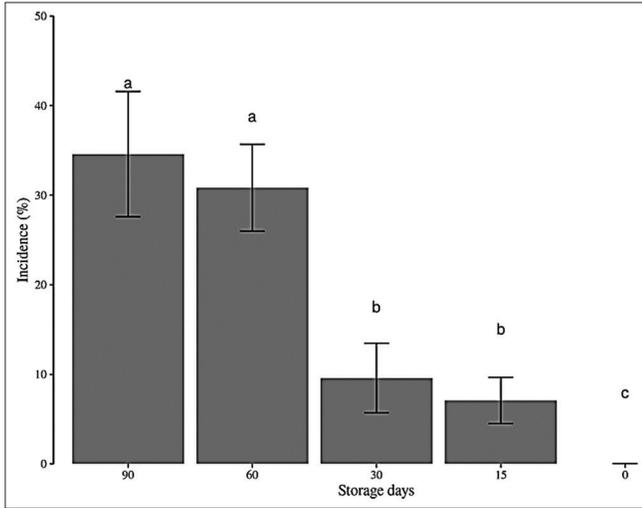


Figure 3: Incidence of fungi in sotol seeds at four storage periods, according to Tukey at 0.05, groups with different letters are statistically significant

Analysis of samples at day 0 presented no detectable pathogens, after a 15-day period of storage fungi was first detected: *A. glaucus*, *A. niger*, *Fusarium* sp. and *Penicillium* sp. with an incidence of 35, 5, 10, 5 and 5% in the environments 3, 6, 10, 11 and 12, respectively. Next, after a 30-day period *A. glaucus*, *Penicillium* sp., and *A. niger* were detected in 5 to 20% of the environments, namely 1, 4, 10 and 12. At day 60, *A. glaucus* was predominant in 30 to 75% of the environments (3, 5, 8, 9, 11 and 12) in addition to *Penicillium* sp., *Aspergillus candidus*, *Cladosporium* sp., *Alternaria* sp. and *Fusarium* sp. (4 to 10%) in the 4, 5, 6 and 9 environments. Finally, after 90 days *A. glaucus* was the predominant pathogen ranging from 5 to 85% of the environments (1, 5, 8, 9, 10, 11 and 12). *A. ochraceus*, *Penicillium* sp. and *Fusarium* sp. were the least detected fungi (5 to 20%) in the environments 3, 4, 5, 9 and 11 (Figure 4). In 2001 isolated nine fungal species from different varieties of *Arachis hypogaea* seeds during a year in storage (Vikas & Mishra, 2010).

DISCUSSION

Environment 12 had the highest presence of fungi (relative humidity of 85% kept at 25 °C), followed by environments 3, 5, 9, 10 and 11 with *Aspergillus* spp. being the predominant

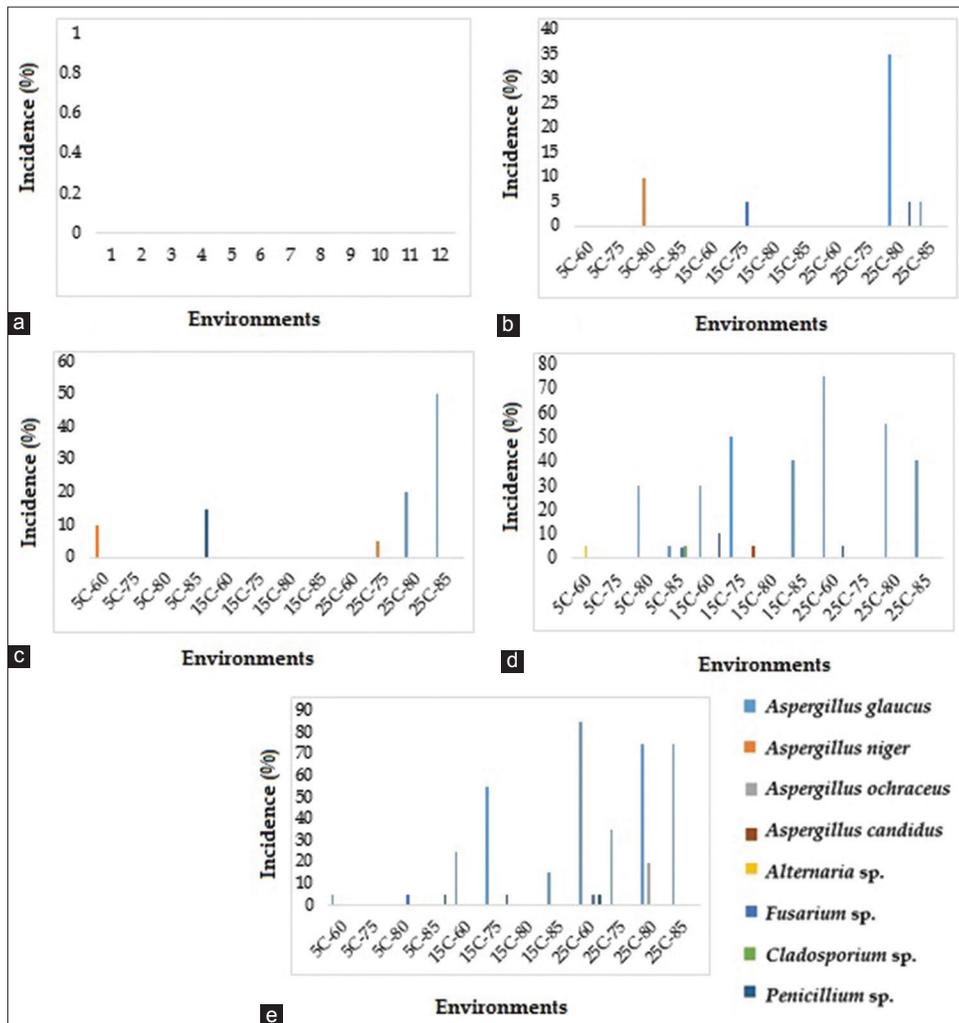


Figure 4: Incidence of fungi at different storage periods. a= 0 day, b= 15 days, c= 30 days, d= 60 days and e= 90 days

genus, followed by *Penicillium* spp., *Aspergillus* and *Penicillium*, are the main pathogens found in stored seeds (Mercado & Precios, 2020), results that agree with various authors (Montes *et al.*, 2009; Pabón-Villalobos & Castaño-Zapata, 2012; Tsedaley & Adugna, 2016; Bhalerao & Chavan, 2017)). Humidity and temperature are the main variables that affect pathogen activity, in storage conditions at higher temperature and humidity microbial proliferation is enhanced (Chulze, 2010; Hell *et al.*, 2010; Martinez *et al.*, 2010), results similar to those obtained in the present research. Best storage conditions are obtained in cool and dry climates, as well these allow grain longevity for a reasonable time (Suleiman *et al.*, 2013).

Other important factor is the control of pest insects; fungi is second after insects as the cause of deterioration and loss of maize (Uzma & Shahida, 2007; Popp *et al.*, 2013), although postharvest losses in maize due to storage insect pests are generally estimated to range between 20 to 30% (Boxall, 2002), for example, the maize weevil (*Sitophilus zeamais*), and larger grain borer (LGB) (*Prostephanus truncatus*) are the major pests in the maize. About 23% losses were observed in maize grains stored for six months, mainly due to infestation of maize weevil and LGB in Benin (Meikle *et al.*, 2002; Kimenju & de Groot, 2010). As the population of insects increases, more heat and humidity will be produced, favoring the development of fungi that affects the grains (Abdullahi *et al.*, 2014).

Blancas (2007) mentioned that fungi *Aspergillus* genus are the more common contaminants in stored seeds, since they are better adapted to environmental conditions in which seeds are generally stored. Warehouse fungi that infects seeds or grains during storage are: *A. restrictum*, *A. glaucus*, *A. candidus*, *Penicillium* spp. needing a relative humidity in the range of 70-90% at 25-35 °C. Similar environmental conditions as the ones in the 10, 11 and 12 environments, that reported higher presence of pathogens. The most clinically important fungi for humans and animals are: *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. In addition, these can develop on a wide range of stored grains and corn seeds (Tola *et al.*, 2016). Said fungi constitute a health hazards that can affect different cereals and their derived products. Fungi like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, cause discoloration, rotting, shrinking, necrosis, loss in germination capacity and toxification in oil seeds (Kakde & Chavan, 2011). Thus the use of certified seeds (pathogen-free) is important (Peralta *et al.*, 2009). Conditions with higher relative humidity and temperature favors the presence of fungi that cause deterioration. Fungi with higher presence in sotol seeds were: *Aspergillus glaucus* and *Penicillium* sp. Safe storage environments for sotol seeds reported in this work are 5°C and a relative humidity of 60-75%. Sotol seeds tolerates conditions of 15 °C and a relative humidity up to 75%.

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