Hindawi
International Journal of Forestry Research
Volume 2022, Article ID 7917704, 10 pages
https://doi.org/10.1155/2022/7917704

Research Article

Correlation between Relative Humidity and Forest Seeds Moisture on the Incidence of Fungi

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Received 13 March 2022; Revised 4 July 2022; Accepted 27 July 2022; Published 9 September 2022

Academic Editor: Monika Marković

The objective of the research was to evaluate the effect of relative humidity (RH) and moisture content (MC) on the incidence of pathogenic fungi on the seeds of *Agave lechuguilla*, *Lippia graveolens*, and *Nolina cespitifera*. Seeds were stored 90 days at 60, 75, 80, and 85% RH, and results were processed with a correlation analysis in the R software using the Spearman test. Higher fungi incidence (FI) in seeds was found from 10 to 20% RH; however, correlation between RH and MC of seeds was positive with $r = 0.311$ and $p = 2.2 \times 10^{-16}$. In general, RH is related to MC, but not to fungi incidence, which is related mainly to MC of seeds. Correlation between RH and FI for each seed species was not significant, $r = 0.026$, -0.040, and 0.071 and $p = 0.687$, 0.540, and 0.272 for *A. lechuguilla*, *N. cespitifera*, and *L. graveolens*, respectively. There was a positive correlation between the MC of seeds with fungi incidence; a negative correlation between the RH and the FI; and a positive correlation between the RH and the MC of seeds. In this type of seeds (orthodox), the MC is probably the most important factor in determining its longevity. The seeds under study can be stored in a 60% to 75% RH. Five fungi genera were found, predominating *Aspergillus* with five identified species.

1. Introduction

The seed bank (SB) is an *ex situ* conservation technique used to expand their viability [1] with the aim of supporting the species survival in nature [2] as well as for research and their propagation [3]. Seed storage (SS) is essential for the preservation of physical, physiological, and sanitary qualities, ensuring conservation of genetic diversity of many species for scientific research and agriculture [4]; however, maintaining their viability depends on storage conditions [5].

The period in which seeds remain viable is genetically determined by internal as well as by environmental factors [6, 7], such as environmental conditions during production, pests, diseases, oil content, MC (variable), mechanical damage during processing, storage time, packaging materials, pesticides, air temperature, and RH (variable), as biochemical damage of seed tissue [8–13]. In relation to storage conditions, the MC in seeds and high temperatures are possibly the most important factors which influence seed quality (QS), such as viability and vigor [14–19]; however, the most critical factor is MC [20–22] since small changes in the MC have a great effect on the storage life of seeds [23], that is, seeds stored with low MC or low RH retain their viability for a longer period [24]; however, typical orthodox seeds age and gradually lose their viability when stored [25].

Seed storage can be a challenge, given their inherent nature; however, orthodox seeds can survive desiccation at 3 to 7% MC and temperatures as low as −20°C for indefinite storage period at less than 60% humidity [26–30]. Various mechanisms have been proposed to explain this cytoplasmic resistance to dehydration [31, 32]; one seems to be the role played by water bound to macromolecules, whose presence and disposition prevents irreversible alteration. In orthodox seeds, to increase longevity and limit their deterioration, the MC and storage temperature is reduced [18, 33, 34], and specifically, the long-term conservation of orthodox seeds is generally carried out at low temperature and low RH [35], that is, increase in RH and temperature leads to a progressive decrease in the vigor due the deterioration caused by the loss of the integrity of the membranes [36] and for conservation of the physiological quality (viability, germination and vigor) of seeds for long periods of time. It is necessary to know the physiological behavior (recalcitrant, intermediate or orthodox) of seeds in storage [37].

Experiments have shown that there are thresholds for the beneficial effects of drying, where below a critical level of seeds MC will not improve longevity [38] and may even have a detrimental effect on survival during processing and storage [39, 40]. Reducing MC to certain thresholds increases longevity in a predictable way in approximately 90% of species [41], that is, favorable conditions and inadequate storage systems [42–44] such as high temperature and humidity can not only can affect yield and seed quality but also reduce vigor and SS capacity [45–48], loss of viability [49], and seed deterioration during storage characterized by a progressive loss of viability, vigor, and even death [50] causing considerable losses [42].

Furthermore, storage under inappropriate conditions can lead to microorganism development, loss of dry matter, heating, increased humidity, high levels of free fatty acids in the extracted oil, and loss of seed germination [51]; high RH and temperature can favor fungi and insects activity, thus reducing SQ, [52]. On the other hand, Jyoti [53] mentioned that seeds stored at high MC show an increase in respiration, warming, and fungi invasion. Fungi infected seeds can survive for 5 years if air is dry and stored at 4°C [54], playing an important role in seed degradation during poor storage, being unsuitable for planting and consumption not only for humans but also for domestic animals [55, 56]. Seed-borne pathogens can be the main source of infection and disease transmission [57], for instance: *Alternaria* Nees (Pleosporaceae), *Aspergillus* P. Micheli ex Haller, (Trichocomaceae), *Botrytis* P. Micheli ex Haller (Sclerotiniaceae), *Cladosporium* Link ex Fr. (Cladosporiaceae), *Curvularia* Boedijn (Pleosporaceae), *Doratomyces* (Corda) (Microascaceae), *Fusarium* Link (Nectriaceae), *Helminthosporium* Link (Massarinaceae), *Macrophomina* Petrák (Botryosphaeriaceae), *Nigrospora* Zimm (Trichosphaeriaceae), *Penicillium* Link (Trichocomaceae), *Chaetomium* Kunze (Chaetomiaceae), *Pestalotia* De Not. (Amphisphaeriaceae), and *Rhizopus* Ehrenb. (Rhizopodaceae) represent the most associated fungi with seeds under storage conditions [58, 59].

The most important nontimber forest products from arid and semiarid climates are as follows: lechuguilla, candelilla, oregano, sotol, yucca, agave, jojoba, gobernadora, and cortadillo [60], with social, cultural, or economic importance; in Mexico, there are 42 species of *Agave* to produce mezcal [61], and approximately 26 states of Mexico grow agave [62, 63]; in that matter, knowledge of SS is essential to plan strategies for conservation of plant genetic resources [64]. Due to the lack of necessary information to provide optimal seed storage conditions for orthodox species from arid zones, the objective of the research was to evaluate the effect of RH and MC in seeds of *A. lechuguilla*, *L. graveolens*, and *N. cespitifera* and their relation with pathogenic fungi incidence.

2. Materials and Methods

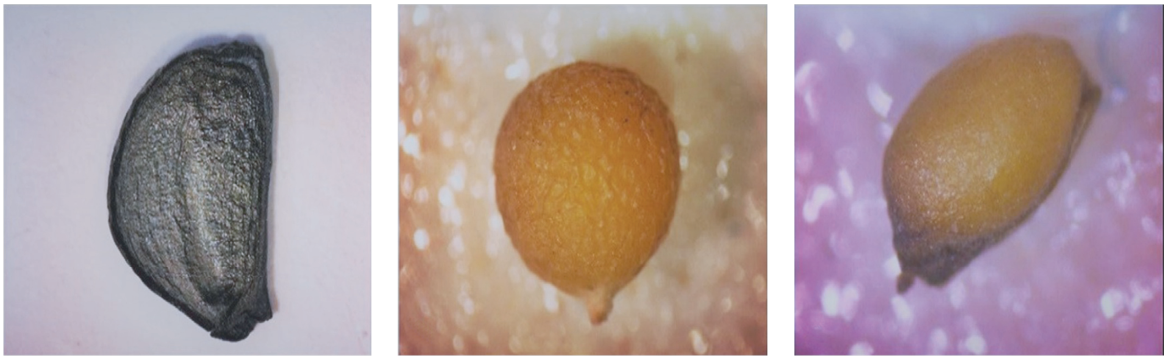
2.1. Experiment Location. Research was carried out at Centro de Capacitación y Desarrollo de Tecnología de Semillas (CCDTS) of Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila. Lechuguilla seeds *Agave lechuguilla* Torr. (Asparagaceae), oregano *Lippia graveolens* Kunth. (Verbenaceae), and cortadillo *Nolina cespitifera* Trel. (*Asparagaceae*) were used (Figure 1), obtained from seed lots of natural populations, from southeast Coahuila, collected in 2016 (Figure 2).

2.2. Time and Storage Conditions. Seeds of *A. lechuguilla*, *N. cespitifera*, and *L. graveolens* were stored for a 90-day period at 60, 75, 80, and 85% RH. To reach 60% RH under storage conditions, a saturated glucose solution ($C_6H_{12}O_6$) was used. For 75% RH, a chloride solution (NaCl) was used, whereas for 80% and 85%, solutions of ammonium sulfate ($(NH_4)_2SO_4$) and potassium chloride (KCl) were used, respectively [65]. A total of 36 treatments were preformed (3 types of seeds; 4 RH percentages, and 3 seed MC) with 20 replicates and 100 seeds per each one. Seeds were placed in a perforated cloth sealed with adhesive tape so that solutions could acted correctly and samples were evaluated during 90 days. Initial evaluation was carried out before storage in order to know the MC of the seeds and the physiological stage, and seeds were later subjected to humidity test in aluminum containers in a drying oven at a temperature of 103°C for 17 h based on the International Seed Testing Association [66]. The MC of seeds was determined with the following formula: MC (%) = fresh weight − dry weight/dry weight × 100. Pathogens were isolated in ADP culture medium and identified by morphological criteria.

2.3. Analysis of Results. Results were expressed in percentage and processed in a correlation analysis in R software by means of Spearman test [67].

3. Results

The RH environments evaluated in the three types of seeds (*A. lechuguilla*, *L. graveolens* and *N. cespitifera*) were from 60 to 85%; in the MC, a numerical variable was obtained, but with continuous values, which ranged from 2.06 to 24.07%. In the correlation between RH and FI in the different seeds,



(a) (b) (c)

Figure 1: Seeds of *A. lechuguilla* (a), *L. graveolens* (b), and *N. cespitifera* (c) used in this research.

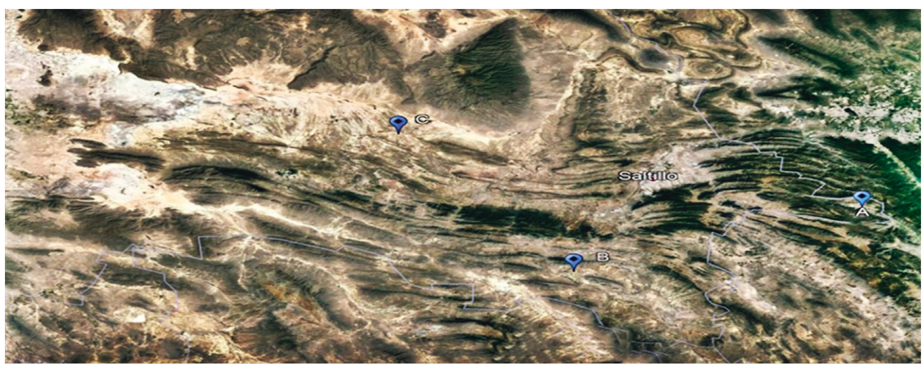


Figure 2: Sampling locations of seeds under study. (a) *N. cespitifera* at Ejido Nuncio, Arteaga, Coahuila; (b) *A. lechuguilla* at Punta Santa Elena, Saltillo, Coahuila; (c) *L. graveolens* at Ejido Piedra Blanca, Parras de la Fuente, Coahuila.

with a correlation coefficient (r) = 0.0205 and p = 0.5835, the correlation was not significant (Figure 3(a), whereas in the MC of the seeds and the FI with $r = 0.2678$ and $p = 2.717 \times 10^{-13}$, the correlation was positive and significant, that is, both variables increase or decrease together, while in the relation of the MC of the seeds and FI, it indicates that, as one variable increases, the other can decrease or increase (Figure 3(b)).

In the correlation between RH and seed MC, a perfect straight line with a positive slope is observed; that is, a positive correlation was obtained with $r = 0.311$ and $p = 2.2 \times 10^{-16}$ (Figure 3(c)), in which the RH is related to MC, but not to FI, which is related to seeds' MC (Figure 4).

Figure 5(a) shows that *N. cespitifera* seeds tend to have higher FI when compared to *A. lechuguilla* seeds and especially with *L. graveolens* seeds. Correlation between RH and FI for each seed species was not significant (Figure 5(a)), with values of r = 0.026, −0.040, and 0.071 and p = 0.687, 0.540, and 0.272 in *A. lechuguilla*, *N. cespitifera*, and *L. graveolens*, respectively. In the correlation of FI and MC, the r = −0.029, −0.039, and 0.110 and p = 0.6520, 0.5520, and 0.0861 were not significant (Figure 5), showing the highest percentages of MC in the *A. lechuguilla* seeds (Figure 6), and the RH correlation with seeds MC was positive (Figure 5(c)), with values of r = 0.53, 0.68, and 0.37 and $p = 2.02^{e-18}, 1.52^{e-33}$, and 3.81^{e-09} in *A. lechuguilla*, *N. cespitifera*, and *L. graveolens*, respectively.

Fusarium sp. and *Alternaria* sp. were observed at 60% RH with values in seeds' MC of 9.11 to 9.15% in *A. lechuguilla* and *N. cespitifera.* In addition to *Fusarium* sp. and *Alternaria* sp., *Cladosporium* sp. and *Aspergillus versicolor* (Vuill.) Tirab. (*Aspergillaceae*) were observed from 4.76 to 12.41% MC, whereas in *L. graveolens*, no pathogens were found. In the range of 60–74% RH, no pathogens were found in seeds. At 75% RH, the same pathogens began to appear in *A. lechuguilla* and *N. cespitifera*, but not in *L. graveolens*; at 80% RH, also *A. glaucus* and *A. niger* appeared in *A. lechuguilla*; in *N. cespitifera*, *A. glaucus, A. ochraceus,* and *Penicillium* sp. also appeared, and for the first time in *L. graveolens*, *A. glaucus, A. niger*, and *Penicillium* sp. were found. Finally, at 85% RH in *A. lechuguilla*, *Cladosporium* sp., *A. terreus*, and *A. glaucus* were detected; in *N. cespitifera*, the same pathogens remained, and in *L. graveolens*, *Penicillium* sp., *A. ochraceus*, and *A. ochraceus*. It should be noted that *A. glaucus* and *Alternaria* sp. were the pathogens with the highest incidence, with over 75% in the seeds (Figure 7).

4. Discussion

Currently, three main categories of the physiological behavior of seeds in storage are recognized: orthodox, intermediate, and recalcitrant [1, 68], and their storage depends on each type [69, 70]; the seeds under this study: *A. lechuguilla*, *L. graveolens*, and *N. cespitifera* are orthodox,

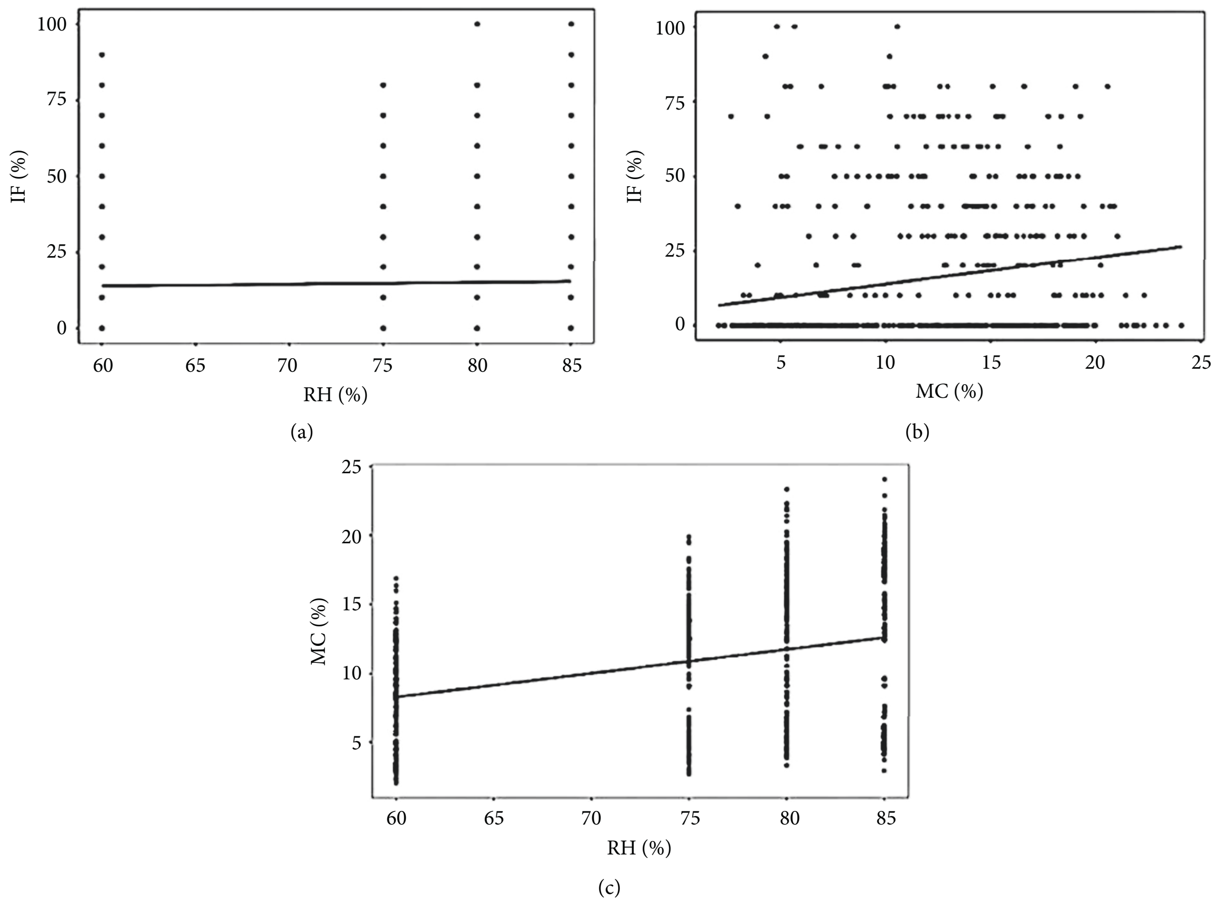


FIGURE 3: General correlations between variables under study. (a) Correlation of HI and RH; (b) correlation of HI and CH of the seeds; (c) correlation of CH of the seeds and RH.

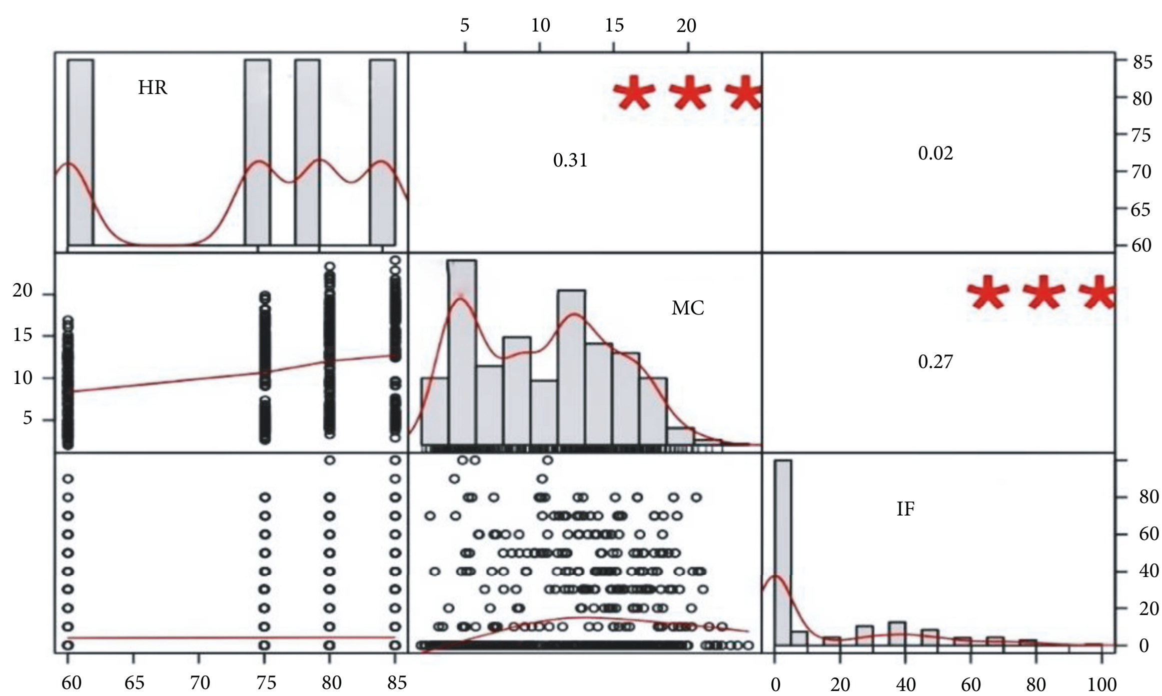


FIGURE 4: RH, MC, and FI correlation matrix. Pearson's correlation coefficient and the significant level indicated by an asterisk are shown in the upper triangular matrix. Significant levels are represented by *** Histograms and scatterplots for RH, MC, and FI are shown in the lower diagonal and triangular matrix, respectively.

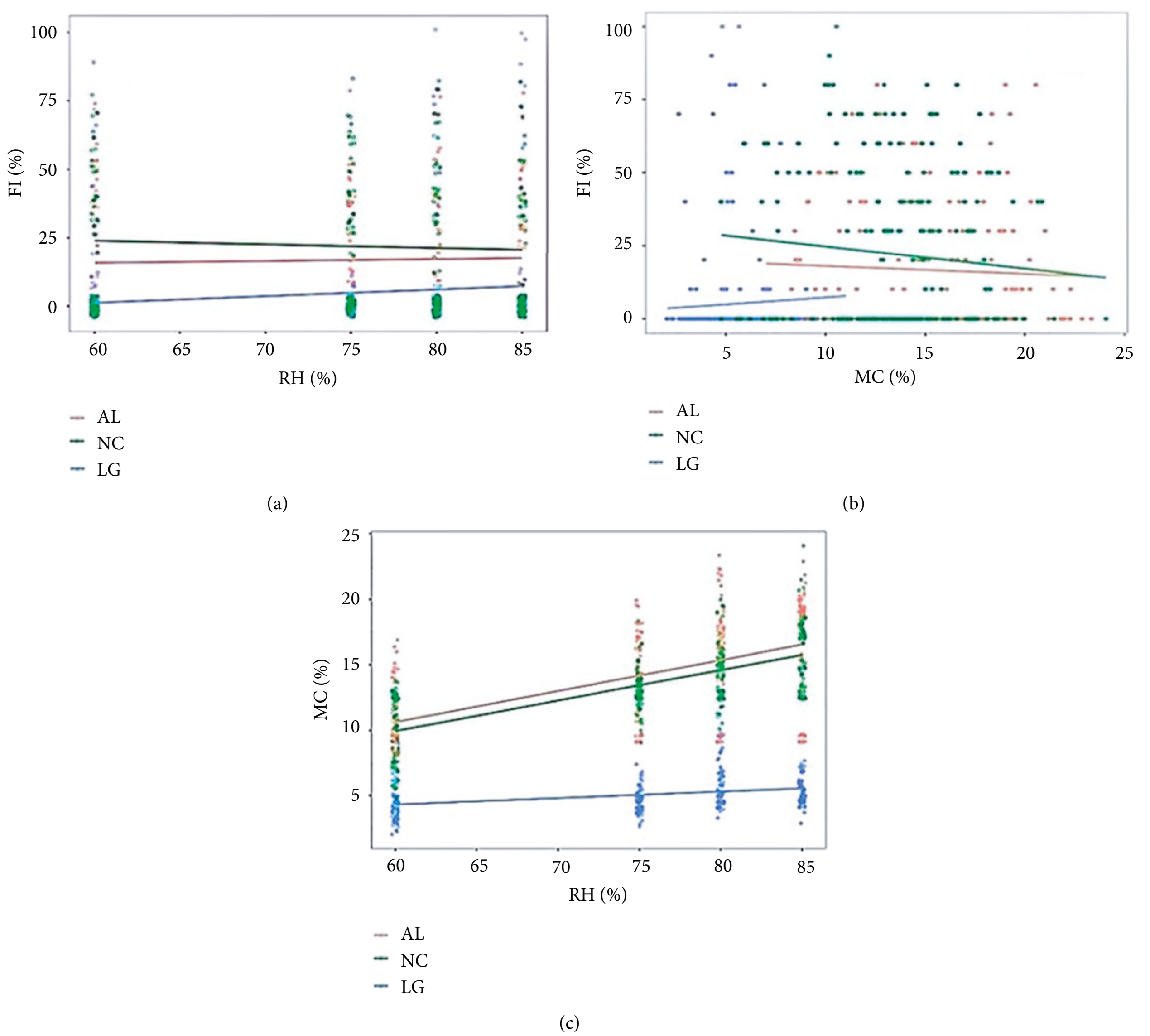


Figure 5: Correlation of the variables and seed types under study. (a) Correlation of FI and RH; (b) correlation of FI and seed MC; (c) correlation of seeds MC and RH; AL = *A. lechuguilla*, LG = *L. graveolens*, and NC = *N. cespitifera*.

and the main characteristic of this type of seeds is that they dried at low MC [71] and conserved without any inconvenience for long time [72]; however, deterioration of the storage seeds depends on the conditions in which it is done, that is, deterioration of the seeds could affect vital functions causing their death [73].

Long-term conservation in SB is based on increasing longevity of the stored seeds, which depends on size, MC, chemical composition, and storage conditions such as temperature, RH, or the type of packaging used (plastic, glass, aluminum, and paper) [74, 75]. *Ex situ* conservation has been the main approach to preserve genetic diversity associated with cultivated plant genetic resources worldwide because it is more likely to be investigated, characterized, and used than if they are conserved *in situ* [76], understanding that seeds will remain viable from the beginning to the end of storage, being alive and able to germinate when removed from storage [77]. In orthodox seeds, longevity increases with the reduction of MC and storage temperature [34], being tolerant to desiccation, they disperse and conserve after reaching a low humidity percentage [78] and can be stored years to decades or centuries, depending on the species [18]. In addition, *ex situ* conservation is a priority activity of the global plan of action to achieve conservation and sustainable use of plant resources of interest for food and agriculture [79].

In addition to the importance of *A. lechuguilla*, *L. graveolens*, and *N. cespitifera*, some endangered species have priority within the phytogenetic resources for their in vitro conservation, and Agave is one of them as well as *Allium* L. (Amaryllidaceae), *Ananas* Mill. (Bromeliaceae), *Canna* L. (Cannaceae), *Colocasia* O. (Araceae), *Ficus*

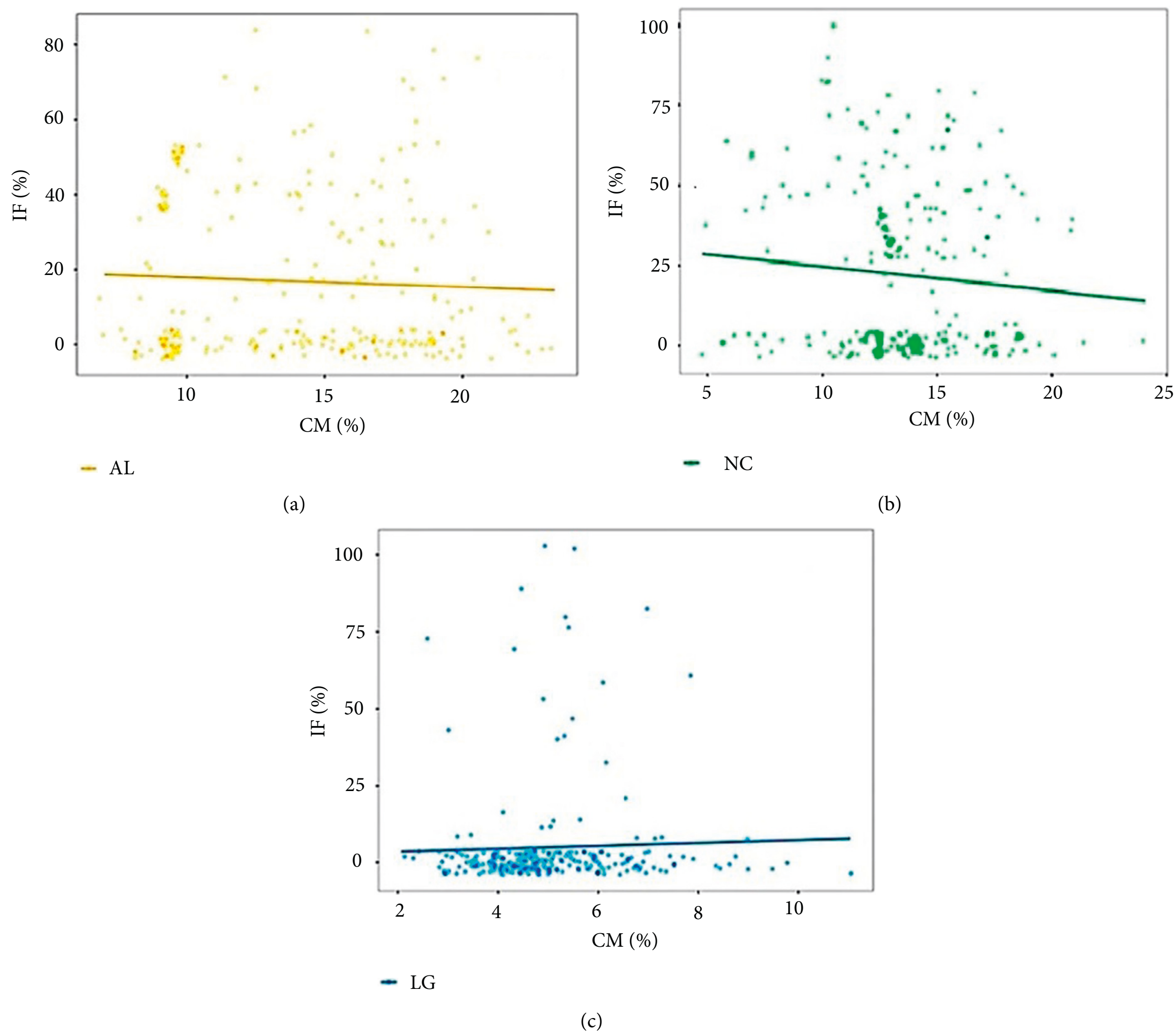


FIGURE 6: FI for each seed species. (a) *A. lechuguilla*; (b) *N. cespitifera*; (c) *L. graveolens*.

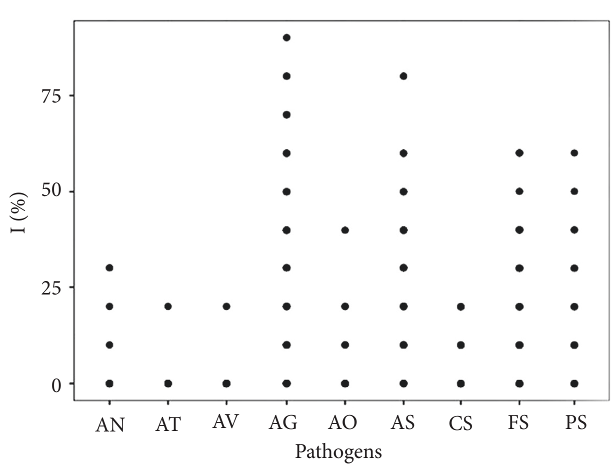


FIGURE 7: FI in seeds. *I* (%) = incidence percentage, AN = *A. niger*, AT = *A. terreus*, AV = *A. versicolor*, AG = *A. glaucus*, AO = *A. ochraceus*, AS = *Alternaria* sp., CS = *Cladosporium* sp., FS = *Fusarium* sp., and PS = *Penicillium* sp.

L. (Moraceae), *Ipomea* L. (Convolvulaceae), *Musa* L. (Musaceae), *Olea* L. (Oleaceae), *Piper* L. (Piperaceae), *Saccharum* L. (Poaceae), *Solanum* L. (Solanaceae), *Vanilla* Plumier ex Miller (Orchidaceae), *Vitis* L. (Vitaceae), *Xanthosoma* Schott (Araceae), and tubers such as fruit trees among others [80–82]. The importance of seeds under study is big; for instance, *A. lechuguilla* is used for the production of aguamiel and pulque, as distilled alcoholic beverages such as tequila and mezcal, and also to obtain fibers, food, and materials for ornaments and construction, among others [83]; *N. cespitifera* is important for a large number of families in rural areas of arid and semiarid zones of northeastern Mexico [84]. It is the main source of economic income, obtaining a high resistance hard fiber used as raw material in the manufacture of brooms, brushes, rustic furniture, and explosive cartridges [85], and *L. graveolens* have industrial and pharmaceutical uses; its essential oil is used as a fragrance in soaps, perfumes, cosmetics, and flavorings, [86]; in addition, it is used as a condiment in the preparation of regional dishes such as sauces, stews, broths, and salads and in international foods as pizzas and the production of sausages [87].

The low disease incidence of *L. graveolens* seeds is probably due to *L. graveolens* has antibacterial, antifungi, antiparasitic, antimicrobial, and antioxidant properties [88], and it has been found that the essential oils of species of the genus *Origanum* L. (Lamiaceae) also have antifungal capacity against *Candida albicans* (Robin) Berkhout (Debaryomycetaceae), *C. tropicalis* (Castellani) Berkhout (Debaryomycetaceae), *Torulopsis glabrata* (Anderson) Meyer & Yarrow (Saccharomycetaceae), *Aspergillus Niger* Tieghem (Aspergillaceae), *Geotrichum* Link (Dipodascaceae), and *Rhodotorula* H. (Sporidiobolaceae) but not against *Pseudomonas aeruginosa* (J. Schröter) Migula (Pseudomonadaceae) [89].

Factors such as time and temperature can influence germination, being optimal conditions low temperatures and low humidity content, probably because it reduces the probability of fungi and insect appearance [90, 91], so, the knowledge of MC of seeds under environmental conditions allows the establishment of safe storage times during a given period. Abdul-Bak and Anderson [92] mentioned that when favorable interactions occur between the genetic component and the environment in which the seed is produced, harvested, processed, and stored, the higher quality level is reached. Bonner et al. [93] on the other hand mentioned that it is better to store orthodox seeds with an MC from 5 to 8% since seeds with less than 5% can have problems due to desiccation, and above 9%, there will be problems with insects and fungi; this type of seeds tolerate dehydration of up to 5% [94], whereas 70% RH is needed for optimal development of insects [95].

Other authors state that storage of seeds below 5% of MC increases seed longevity [95]; Copeland and McDonald [96] mentioned that maximum seed longevity is reached with 5 to 6% RH. Seeds MC in which the highest FI was obtained was from 10 to 20%, and at a MC of 5%, a 100% FI was shown for the first time. Importance of MC in the preservation of seeds lies in the role of water in the physiological processes that determine vigor and longevity of seeds, as well as in the development of insects and fungi during storage [97–99].

The activity of field fungi is delayed during storage with a low MC since they require ≥90% RH in the environment for their growth [100], similar to results found in this research, in which the highest incidences of pathogens in the three seed types were at high RH (85%). Results show that fungi began to appear at 65% RH and at 74%, and no pathogen growth was found in none of the seeds. The importance of having seeds without fungal growth in storage (clean and safe) is because contamination of seeds by these pathogens [101] is the most important factors during storage reducing QS causing a negative effect in appearance and chemical composition. In addition, they can also inhibit germination, disease transmition from seed to seedling, reduce crop yield, and threaten food safety [102, 103].

5. Conclusions

There is a positive correlation between MC of seed species and FI, as well as a negative correlation between RH and FI, with a positive correlation between the RH and seed MC.

In these types of seeds, MC is probably the most important factor determining their longevity.

The seeds under study can be stored in a 60% to 75% RH.

Five fungi genera were found, predominating *Aspergillus sp.* with five identified species.

Data Availability

Data are available on request from the authors. All the data are incorporated in the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Adriana Antonio-Bautista conducted samplings and experiments. Mario Ernesto Vázquez-Badillo designed the research and methodology and conducted samplings and experiments. Armando Rodríguez-García conducted samplings and experiments. Luis Alberto Aguirre-Uribe reviewed and edited the final version of the manuscript. Agustín Hernández-Juárez revised the original draft and prepared the final manuscript. Epifanio Castro-Del Ángel analyzed data. Juan Mayo-Hernández revised the original draft. Jose Luis Arispe-Vazquez analyzed data and revised the original draft and prepared the final manuscript. All authors have read and agreed to the published version of the manuscript.

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