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MYCOFLORA ASSOCIATED WITH PUMPKIN SEEDS (*Cucurbita moschata* DUCHESNE) SUBMITTED TO THE METHOD: *BLOTTER TEST*

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Abstract: The seed health test seeks to analyze seeds in order to identify lots with high sanitary quality for good crop production. The objective of this work was to point out the incidence of fungi in pumpkin seeds (Cucurbita moschata) var. Brazilian Girl, as well as the use of osmotic potential restriction, through the Blotter test method. Test I used two different batches: marketed dried seeds (SC) and fresh fruit seeds (SF), all of which were incubated for seven days for analysis of fungal incidence. Test II used only SC submitted to six treatments: NaCl (-0.6 Mpa); NaCl (-0.7 MPa); KCl (-0.6 MPa); KCl (-0.7 Mpa) and freezing and incubated for seven days. Both assays were subjected to sanity tests using the Blotter test method. In the two seed lots tested SC and SF, there was 96% germination and incidence of four and five, respectively, different fungal genera, also listed for test II. The use of Mpa did not significantly prevent seed germination, however, there was an incidence of fungi of the genus Rhizopus sp., Fusarium sp., Aspergillus sp., Trichoderma sp., Colletotrichum sp., Cladosporium sp., Penicillium sp., Curvularia sp., and Bipolaris sp. These, find in the storage, conditions of humidity and temperature favorable to its development, therefore, the treatment of seeds can be an adequate measure to inhibit pathogens in the phases of production still in field and of storage. The use of water restriction appears to be a promising methodology for seed health analysis.
Keywords: Incidence, Fungal diversity. Water restriction.

INTRODUCTION

The process of obtaining vegetable seeds consisted of different processing steps: pre-cleaning, cleaning and seed classification (Nascimento, 2005). The subsequent step is seed storage, which must remain until sowing (Labbé, 2003). All these stages take into account plant health, as fungi present in the seeds, mainly due to favorable climate conditions, can trigger diseases and these reach the plantations in the field (Bruno et al., 2000). Thus, the emergence of these seed contaminating microorganisms is also strongly influenced by storage conditions(Parrella, 2012).

Seed health analyzes aim to identify the presence or absence of pathogenic agents such as bacteria, fungi, nematodes and viruses, in addition to stored grain pests. In order to prove the health of the seeds, the seed health test revealed existing pests and pathogens. Among the phytopathogens associated with seeds, fungi stand out because they are easily transmitted by seeds (Brasil, 2009). Therefore, obtaining healthy seeds depends on good management in the harvesting, drying, processing and storage phases (Parrella et al., 2012).

The sanitary, physiological and physical quality of stored seeds can be obtained through treatments (Parisi, 2012). Seed treatments concern carrying out processes or introducing substances to improve their execution or even preserve them, these treatments can be by chemical, physical, biochemical and biological methods (Lucca Filho, 2003; Machado 2006, Menten, 2010).

Among the analyzes of sanity if minds is water restriction. This method is related to the submission of the seed in substrate with a certain water potential, allowing the absorption of water by the seed, only to carry out processes prior to germination, but without allowing the emission of the radicle (Menezes et al., 2009). This way, water restriction plays a fundamental role in the sanitary analysis of seeds, since, when fungi are submitted to a culture medium to which they have undergone osmotic changes, seed germination is delayed, but does not interfere with the development of the fungus (Coutinho et al., 2001). In view of this, this study aimed to point out the incidence of fungi in pumpkin seeds (*Cucurbita moschata*

Duchesne) submitted to the *Blotter test method* and use of water restriction.

MATERIALS AND METHODS

OBTAINING THE SEEDS

The seeds were obtained from pumpkin fruits (*Cucurbita moschata* Duchesne) from the municipality of Tianguá, Ceará and seeds sold in the municipal market of Parnaíba, Piauí.

TEST WITH COMMERCIAL SEEDS AND FRESH SEEDS

Fresh seeds were first extracted from pumpkin fruits, washed and stored until the beginning of the test. The *Blotter test method by Neergaard* (1979) (Goulart, 2004) was used. A total of 400 seeds were used (Brasil, 2009). Then the seeds were previously disinfected in a 3% sodium hypochlorite solution for three minutes and then washed 3x in distilled and sterilized water for one minute and placed in plastic Petri dishes measuring 90x15mm containing two sheets/layers of paper sterilized filter and soaked in distilled and sterilized water, with nine seeds distributed in plates. Subsequently, the plates containing the seeds were incubated at a temperature of 25ºC±2 for seven days. After incubation, the seeds were analyzed under a stereomicroscope and an optical microscope to identify the incident fungi.

TEST WITH COMMERCIAL SEEDS

The seeds were disinfested in a 3% sodium hypochlorite solution for three minutes, once and then washed in distilled and sterilized water (A.D.E) for 3x followed by one minute for each wash. The trial was conducted in a Completely Randomized Design (D.I.C) with six treatments and 24 replications. The treatments (solutions) were: NaCl (-0.6 MPa), NaCl (-0.7 MPa), KCl (-0.6 MPa), KCl (-0.7 MPa), Freezing (24 h) and Witness. The method *Blotter test* Neergaard (1979) was used. The seeds were then distributed in Petri dishes (90x15 mm) containing three layers of previously sterilized filter paper and dipped in the solutions (treatments), for the freezing and witness treatments the papers were soaked in A.D.E.

For each treatment, six Petri dishes were used with nine equidistant seeds distributed. The plates with the seeds were incubated in a B.O.D. (*Biochemistry Oxygen Demand*), with a photoperiod of (12 h light / 12 h dark) and temperature of 25°C+ 2. For this test, the parameters of number of germinated seeds and radicle length were evaluated.

ANALYSIS OF THE INCIDENCE OF FUNGI

After the incubation period, the seeds were analyzed under a stereoscopic microscope and, when necessary, microscopic preparations were performed to identify the fungi. The results were obtained in percentage of fungi associated with the seeds.

STATISTICAL ANALYZES

Root length data were submitted to Snedcor's ANOVA F test and the means compared by the Scott-Knott test at a 5% probability level, using the Assistat program, version 7.7 beta (Silva, 2009). In the freezing treatment, the mean root length values were adjusted using the x+1 formula.

RESULTS AND DISCUSSIONS

TEST WITH COMMERCIAL SEEDS AND FRESH SEEDS

The seeds used in this test showed 96% of germination. Fresh seeds contained a greater diversity of fungi associated with them, with five genera and their respective percentages of incidence: Fusarium sp. (30%), Aspergillus sp. (30%), Rhizopus sp. (20%), Cladosporium sp. (10%) and Colletotrichum sp. (10%). The commercialized seeds, in turn, presented the following genera and incidences: Rhizopus sp.

(78.04%), Trichoderma sp. (13%), Aspergillus sp. (4.03%) and Fusarium sp. (4.03%). Therefore, the commercialized seeds had a lower level of fungus infestation. The presence of phytopathogens in seeds, as reported here, can damage germinal development, interfering with their emergence, vigor, yield and storage time (Pedroso, 2009). For example, Aspergillus sp., a genus that contains species that are suitable for storage, may be responsible for causing seed rot (Tonel et al., 2009).

TEST WITH COMMERCIAL SEEDS

Seeds subjected to this test showed significant differences between treatments in terms of radicle length (Table 1). The greatest length was observed in the control treatment (4.2 cm), followed by the NaCl -0.6 Mpa (3.6 cm) and CKl -0.6 Mpa (3.7 cm) treatments. The smallest lengths occurred in the NaCl -0.7 Mpa (2.8 cm), KCl -0.7 Mpa (1.8 cm) and freezing (1.5 cm) treatments (Table 1). The germination percentages show that the control treatment presented the highest percentage (87.04%), followed by NaCl -0.6 Mpa (81.01%), KCl -0.6 Mpa (71.30%), NaCl -0.7 Mpa (58.08%), KCl -0.7 Mpa (53.24%) and freezing (04.63%) (Table 1).

Treatments	Radicle length (cm)	Germination percentage (%)
NaCl (-0,6 Mpa)	3,6 b	81,01
NaCl (-0,7 Mpa)	2,8 c	58,08
KCl (-0,6 Mpa)	3,7 b	71,30
KCl (-0,7 Mpa)	1,8 d	53,24
freezing	1,5 e	04,63
Witness	4,2 a	87,04%

Means followed by the same letter do not differ statistically according to the Scott-Knott test at the 5% probability level.

Table 1. Root length and germination percentage of pumpkin seeds as a function of water restriction.

Root growth as a function of osmotic potentials showed an inversely proportional relationship, that is, the higher the applied osmotic potential, the lower the radicle growth. These results also corroborate those in the literature, where Torres (2007), when evaluating papaya seeds under different osmotic potentials, observed the same behavior. Góis (2010), when working with gherkin seeds (Cucumis anguria L.), using sodium chloride (NaCl) at -0.20 Mpa, obtained the lowest average germination percentage with 41.5%, while the highest percentage was found in the 0.0 Mpa treatment, reaching 94%, that is, the greater the osmotic potential applied, the greater the delay in seed germination. The results found in our study also corroborate this proportional relationship, as the greater the applied osmotic potential, the lower the germination percentage of the seeds (Table 1). Probably, this occurs as a result of the high amount of soluble salt causing a reduction in the water potential of the substrate, restricting the water absorption of the seeds.

The diversity and incidence of fungal genera in relation to treatment are shown in Table 2. A total of six genera were identified (*Aspergillus* sp., *Penicillium* sp., *Colletotrichum* sp., *Fusarium* sp., *Rhizopus* sp., *Cladosporium* sp., *Curvularia* sp., and *Bipolaris* sp), entre eles o que apresentou maior incidência foi *Colletotrichum* sp., up to 60.81% incidence was reached with NaCl treatment (-0.7 Mpa) and 90% with freezing treatment (Table 2).

Fungal genera	Treatments					
	NaCl (-0,6 MPa)	NaCl (-0,7 MPa)	KCl (-0,7 MPa)	KCl (-0,6 MPa)	Freezing	Witness
	Incidences (%)					
Aspergillus sp.	04,60	17,57	10,00	04,08	02,50	04,65
Penicillium sp.	03,45	01,35	02,50	04,08	02,50	09,30
Colletotrichum sp.	49,42	60,81	52,50	55,1	90,00	41,86
Fusarium sp.	00,00	00,00	1,25	02,04	00,00	08,14
*Rhizopus*sp.	39,08	17,57	31,25	34,70	05,00	34,89
Bipolaris sp.	00,00	02,70	02,50	00,00	00,00	00,00
Cladosporium sp.	02,30	00,00	00,00	00,00	00,00	01,16
Curvularia sp.	01,15	00,00	00,00	00,00	00,00	00,00

Table 2. Diversity of fugitive genera and incidence in pumpkin seeds submitted to treatments with water restriction by NaCl and KCl solutes, freezing and control.

The presence of *Aspergillus* sp. and *Penicillium* sp. they may result from deteriorated seeds and grains and cause losses such as nutritional changes, damage to the germ, loss of color (Cardoso Filho, 2011). However, the incidence of these genres showed low rates in all treatments.

The presence and high incidence of Colletotrichum sp. associated with the seeds demonstrated here has great epidemiological relevance since fungi belonging to the genus *Colletotrichum* sp. can reach the aerial part of the plant, causing defoliation and unusability of the fruits. The fungus can also cause post-harvest disease, also causing damage to fruits and making them unfit for consumption Galli (1980).

The genus *Fusarium* sp. causes rot in the roots and in the collar of Cucurbitaceae such as pumpkin, watermelon and melon (GALLI, 1980). In addition, they cause damage to the aerial part, such as flower abortion and even seed rot in storage, with species of this genus capable of releasing mycotoxins (SILVA, 2012).

CONCLUSION

There is a relative contamination in pumpkin seeds, however the diversity of genera that occur is small, the low diversity may be due to the short storage period. The use of water restriction appears to be a promising methodology for seed health analysis, as it allows the delay of radicle emission, thus enabling the health analysis of these propagation organs.

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