

Detection, Transmission and Pathogenic Fungi in Chia Seeds

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Abstract

This template is designed using the previous setting; kindly copy and paste your text to this template. The study related to seed pathology is essential to know the sanitary quality, since the presence of pathogens in favor of seed can spread disease, and physiological influence the quality of seeds, resulting in low germination. Thus, this study aimed to identify the detection, transmission, and pathogenicity of fungi associated with the seeds of Chia. The work was conducted in the Phytopathology Laboratory and experimental area of the Federal University of Tocantins, Chia seeds produced being evaluated in Gurupi (Brazil) and Katueté (Paraguay).

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To check the sanitary quality of Chia seed was used the method of the filter paper to the present lifting mycobiota which was subsequently isolated and cultured in a potato dextrose agar culture (PDA). For seed fungi, the transmission test to the plant was sowed 100 seeds of Chia divided into four replicates of 25 seeds each. The seeds were sown in pots of 4 dm³ (four seeds per pot) evaluations were made to ten, twenty and thirty days after emergence by observing the characteristic symptoms. The pathogenicity of fungi transported by the seed was evaluated by inoculation in the aerial part of seedlings. The fungi found associated with Chia seeds were: *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *Rhizoctonia* spp, e *Rhizopus* spp. the fungus *Colletotrichum* spp. and *Fusarium* spp. They are transmitted to seedlings via seed. Genres *Colletotrichum* spp. and *Fusarium* spp. Are pathogenic to plants of Chia.

Keywords: *Salvia hispanica* L; Sanitary quality; Seed pathology.

1. Introduction

The Chia (*Salvia hispanica* L.) is a plant of family Lamiaceae, originally from the Midwest Mexico up until northern Guatemala. In the Pre-Columbian period was an important staple food used by civilizations that inhabited Central America, behind only corn and beans, but with greater emphasis than other crops like amaranth [1]. The grain consumption of Chia has increased, mainly in Mexico, the southwestern United States, and also in America Southern. Although Chia is not widely known in Europe, it has have been introduced, particularly in the Products Market dietetics. The seeds have been subject to investigation, and they recognized beneficial effects on health due to its high protein content, antioxidants, and dietetic fiber [2]. The occurrence records in Brazil of fungi causing diseases in the Lamiaceae family of plants are few about the number of cultivated species. There are pathogenic fungi reports transmitted by seeds, hindering the development of seedlings, causing leaf spots or wither [3]. The most common fungal diseases in medicinal plants, aromatic, and condiments are anthracnose, rust, coal, rottenness, mildew, oidium, mildew e wilt [4]. However, the presence of fungi in seeds, in addition to enabling the introduction of pathogens in growing areas and cause diseases of crops, may also cause changes in plant metabolism affecting its therapeutic properties and flavor. In storage, some fungi cause physical and chemical changes in the tissues of the seeds, causing loss of lipids, carbohydrates, proteins, and increased fatty acid, besides influencing the seed's germination [5]. They are also capable of producing mycotoxins, being the genres *Aspergillus*, *Penicillium*, and *Fusarium* responsible for the production of most substances known [6]. The main mycotoxins found in food are aflatoxins (B₁, B₂, G₁, G₂, and M₁), fusaric acid, fumonisins (B₁ and B₂), fumonisins, ochratoxins (A, B and C), patulin, citrinin, zearalenone and trichothecenes [7]. The identification of pathogenic fungi in the seeds can facilitate the choice of disease control measures, being of great importance for the success of agricultural production. According to reference [8], an extremely important factor in determining the quality of seeds is the sanitary attributes. Thus, the sanitary quality of the seeds should be analyzed, once the association of pathogens with seeds it is not limited to direct losses of the population in the field, but it can also harm the nutritional properties of seeds. The objective of this study was to evaluate the sanity by the detection method, transmission, and pathogenicity of fungi associated with Chia Seeds.

2. Materials and Methods

The work was conducted in the Phytopathology Laboratory and experimental area of the Federal University of Tocantins, the Campus University of Gurupi, characterized by the geographical coordinates 11° 44' 48" of south latitude and 49° 03' 12" of west longitude, at an altitude of 285 m. The study was conducted from August 2015 to June 2016, Chia seeds produced were evaluated in Gurupi (Brazil) and Katueté (Paraguay). Chia seeds collected from Gurupi were obtained in the harvest 2014/2015, the experimental area in the Federal University of Tocantins, Campus Gurupi, while the seeds coming from Katueté in Paraguay were provided by a farmer from the country 2013/2014. The seeds produced in Gurupi were wrapped placed in plastic bottle and stored in Germplasm Bank (temperature 5 °C), and the seeds from the Katueté were stored in plastic bottle in natural. To check the sanitary quality of Chia seeds was carried out the detection test, transmission test, and pathogenicity test.

2.1 Detection Test

The seeds were disinfected with alcohol 50% for 30 seconds and then in sodium hypochlorite 1% for 30 seconds, then washed in sterilized distilled water and left to dry on sterilized filter paper. Subsequently, the seeds were placed on plates, containing two paper layers GERMITEST® sterilized and moistened with 3 ml and sterilized distilled water. Used 200 seeds distributed in four repetitions, 50 seeds per petri dish for each treatment (Figure 1). Then, the seeds were kept for 4 hours in the laboratory, under controlled temperature and humidity conditions, and then brought to the refrigerator for another 4 hours, to stop their germination process, to facilitate the identification of pathogens. Finally, the seeds were incubated for five days at a temperature around 25° C and a 12-hour photoperiod. After the incubation period, the seeds were individually examined with the aid of a stereoscope, registering the presence or absence of fungal elements, thus making the identification of pathogens based on their morphological characteristics. When necessary, slides were prepared for display in the optical microscope for microscopic characteristics, with the aid of specialized literature to facilitate the identification of pathogens [9]. The present fungi were isolated and cultured in potato dextrose agar culture (PDA) for confirmation of identification. In sequence, each fungus was identified was and transferred to other Petri dishes, in sequence, each fungus identified was peel and transferred to other Petri dishes containing culture medium PDA, for later use in pathogenicity testing. Data obtained from incidence from fungi in the seeds evaluated in they were expressed in percentage (%).



Figure 1: Detection test seeds of Chia.

2.2 Fungi Transmission Test Via Seed

For seed fungal transmission tests for the plant, they have sowed 100 seeds of Chia divided into four replicates of 25 seeds each. The seeds were sown in plastic pots of 4 kg (four seeds per pot) in the proportion 3:1 (soil: sand), soil type being used Latosol red yellow dystrophic, medium texture [10], properly disinfected, making use of the methodology proposed by [11]. The seedlings were kept in the field with daily irrigation, once a day. Evaluations were made at ten, twenty, and thirty days after emergence by observation of symptoms characteristic. They have evaluated the following parameters: a) normal plants (emergence and formation of complete structures of the plant); b) abnormal plants (characterized by incomplete formation of plant structures) and c) ungerminated seeds. The parts of the plant that showed symptoms they were isolated in means PDA for confirmation of transmission of pathogenic.

2.3 Pathogenicity Test

Isolates of *Fusarium spp.* and *Colletotrichum spp.* they were obtained in the detection test by isolation on the PDA medium. The Chia seeds used belonged to provenance to origin Gurupi (Brazil), it contemplated for both the occurrence of the test fungus detection, as the seed of the same transmission to the plant. The sand was used and soil type Dystrophic red yellow latosol medium texture [10], properly disinfected, making use of the methodology proposed by [11]. The sowing of seeds from Chia proceeded three plastic pots of 4 kg (four seeds per pot) in the proportion 3:1, one pot of plants corresponding to the genre *Fusarium*, the second to genre *Colletotrichum* and the third pot used a witness, not inoculated with the fungus. The plants were kept in the field with daily irrigation until thirty-five days. Later, taken to the for inoculation of the spore solution. This was prepared from the Petri dishes containing the inoculated fungi. It was added 10 ml of sterile distilled water and with the help of a detection, transmission and pathogenic fungi in Chia seeds brush soft bristle made the loosen the spores, then filtering carried out with sterile gas. Plants were inoculated spraying solution of the spores, manual sprayer for using such a procedure. They were then placed in a dark and moist chamber for 48 hours. After this period, it was observed the development of the disease. The assessment (symptoms of view) was performed at four and seven days after, inoculation of the fungus, and this was evaluated pathogenic fungi, observing the presence or absence of symptoms in inoculated plants and witness (Uninoculated). The fungi that caused symptoms in seedlings were reisolated amid PDA, thus fulfilling the steps of the postulates of Koch. The identification of the level of gender according to the descriptions of [9] and with the use of an optical microscope.

3. Results and Discussion

The detection test conducted on Chia seeds were found a total of nine genera of fungi (Table 1). In seed form Gurupi (Brazil) it was observed the presence of genres: *Alternaria spp.*, *Aspergillus spp.*, *Bipolaris spp.*, *Colletotrichum spp.*, *Curvularia spp.*, *Fusarium spp.*, *Penicillium spp.*, *Rhizoctonia spp* e *Rhizopus spp* (Table 1 and Figure 2).

Table 1: Percentage of occurrence of fungi (%), identified by the detection method, associated with seeds of *Salvia hispanica* L., two provenances of Gurupi (Brazil) and Katueté (Paraguay).

Pathogens	Gurupi (Brazil)	Katueté (Paraguay)
<i>Fusarium spp.</i>	31.0%	0%
<i>Colletotrichum spp.</i>	7.5%	0%
<i>Penicillium spp.</i>	4.5%	3%
<i>Aspergillus spp.</i>	3.5%	7%
<i>Alternaria spp.</i>	1.5%	0%
<i>Rhizopus spp.</i>	1.5%	0%
<i>Curvularia spp.</i>	1.0%	0%
<i>Bipolaris spp.</i>	0.5%	0%
<i>Rizoctonia spp.</i>	0.5%	0%

The genre with the highest incidence in the seeds analyzed was the *Fusarium* spp. (31%) is one of the main fungi with wide host range high expression economic, as well as producing toxins [12]. According to reference [13], the *Fusarium* is regarded as pathogenic fungus, by cause problems in germination and plant diseases. The second genre of higher frequency in seeds was *Colletotrichum* spp. (7,5%), is frequently reported in different species of plants cultivated worldwide, with the potential to cause disease in plants [14]. The fungus *Colletotrichum* can be transmitted via seed, causing deterioration of seed, seedling death, and systemic infection in adult plants, also, to be common the appearance of symptoms cotyledons, characterized by necrosis thereof, after seedling emergence [13]. Studies with species of the same family of Chia also found foliar diseases caused by *Colletotrichum* in *Mentha piperita* [15, 16].

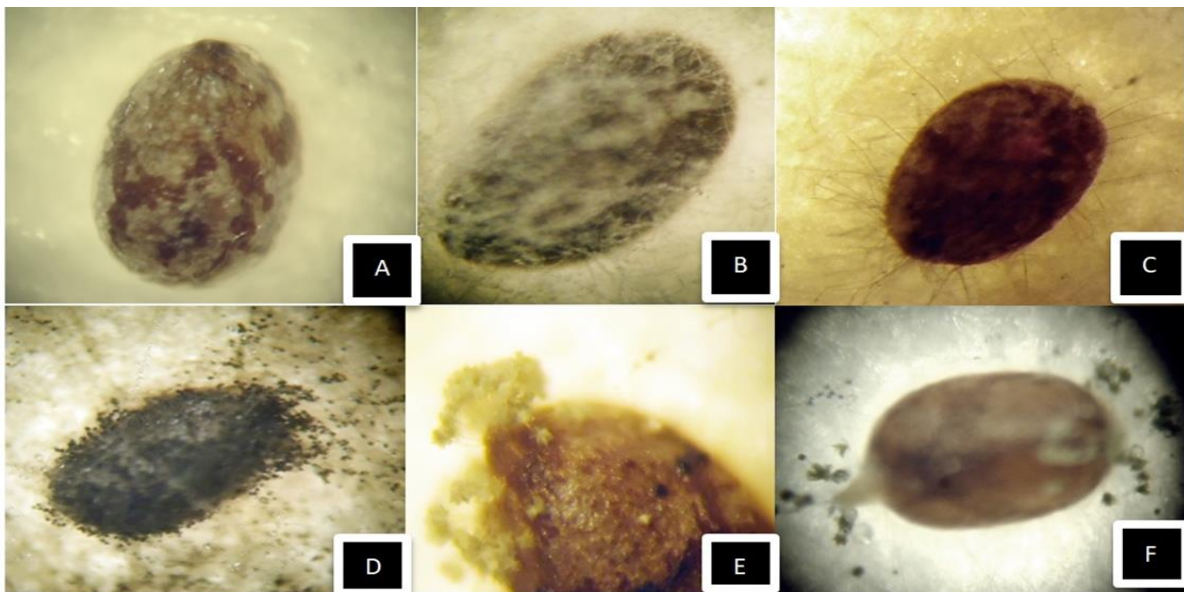


Figure 2: Pathogen detection test associated with seeds. (A) healthy seeds, (B) *Fusarium* spp., (C) *Colletotrichum* spp., (D) *Rhizopus* spp., (E) *Aspergillus* spp. and (F) *Penicillium* spp.

Other important genera were found on how *Aspergillus* spp. (3, 5%) and *Penicillium* spp. (4, 5%), considered storage fungi and their presence usually indicate inadequate drying conditions and storage of seeds, causing the reduction of grain quality [5]. According to [17], the genres *Penicillium* and *Aspergillus* have the ability to reduce of germinative power of the seed and cause the death of the embryo. The results obtained in this study were also similar to those found by reference [18] that found the presence of the genres *Penicillium*, *Aspergillus*, and *Rhizopus* associated the Chia seed. The genres *Alternaria* spp. and *Rhizopus* spp. presented 1.5% occurrence in the seeds, being pathogens commonly found in sanitary analyzes of seeds, considered saprophytic fungi and or pathogenic (nourish yourself with organic matter of waste plants and animals) and contaminants. The genres *Alternaria* is very common in seeds, being, most of the time, associated only as saprophytic and depending on the incidence may cause a loss in seed quality [13]. According to reference [19] found the interference on the viability and vigor of coriander seeds. The pathogens *Bipolaris* spp., *Curvularia* spp. and *Rhizoctonia* ssp. were those who had a lower incidence or equal to 1% in the seeds analyzed. When having a high incidence, it can cause damage to crops, in addition to being transmitted via seed to seedling. According [13] the *Rhizoctonia* is a natural inhabitant of the soil, causes significant losses in production, and, once installed, remains in the soil for several years, in the form of sclerotia and mycelium in crop residues. The seeds produced in Katueté (Paraguay) presented only storage fungi as well as *Aspergillus* and *Penicillium* with an incidence of 7 and 3%, respectively. According to reference [17] are fungi that its incidence has increased by improper storage conditions. These results corroborate with reference [20], who found in Chia seed fungi *Penicillium* sp., *Aspergillus* sp. and *Curvularia* sp., The reference [21] assessing the sanitary quality of Chia seeds, they revealed the presence of genres *Fusarium*, *Aspergillus* and *Penicillium*. The reference [22] assessing the culture of Chia, found field diseases caused by *Fusarium* sp. and *Rhizoctonia* sp. studies with species from the same family as from *Salvia hispanica* also noted the existence of *Fusarium* [23, 24]. It is observed that there were differences in the fungal genera found in lots of two locations, only genres *Aspergillus* and *Penicillium* were common in the samples analyzed. Thus, it is evident that the seeds originating in Paraguay have better sanitary quality. This may be related to the driving of the crop in the field, in addition to the different climatic conditions of the two locations. Are observed in table 2, information about the transferability test via Chia seed, only the Gurupi seeds showed transmission of fungi *Fusarium* spp. and *Colletotrichum* spp. In the sample Gurupi seed germination percentage was 75%, there were still 7% of abnormal seedlings and 18% of seeds did not germinate (Table 2). It was also verified the existence of symptomatic plants with fungi structures *Fusarium* spp. and *Colletotrichum* spp, as well as some non-germinated seeds.

Table 2: Percentage of normal, abnormal seedlings, on-germinated seeds and fungi found in plants in transmission test via seed in *Salvia hispanica* L., of two origins in Gurupi (Brazil) and Katueté (Paraguay).

Origin	Normal seedlings	abnormal seedlings	Dead seeds / not germinated	Fungi
Gurupi	75%	7%	18%	<i>Fusarium</i> spp./ <i>Colletotrichum</i> spp.
Katueté	11%	----	89%	-----

According to [25], the species of *Fusarium* are commonly associated with the seeds of various crops, causing loss of germination and vigor, also assisting in complex pathogens that cause topples in plants. Symptoms caused by the fungus *Colletotrichum* spp. are irregular lesions with light brown to dark brown color accompanied by necrosis of the vein of the leaf. Seeds from the Katueté were subjected to inappropriate storage conditions, thus showing low germinating power seed (11%) can be viewed in Figure 4. Although 89% of the seeds did not germinate, no symptoms of any pathogen in seedlings that germinated and nor was there evidence that the seeds did not germinate. Comparing the two origins of the seeds as the physiological and sanitary quality, it was found that the seeds of Gurupi have better physiological quality and those of Katueté best sanitary quality.



Figure 3: Transmission test on seeds of *Salvia hispanica* L. from two locations (Gurupi and Katueté).

Therefore, it is important that the quality of the seeds cannot be improved during storage, but under favorable storage conditions, it can be preserved, thus demonstrating to be fundamental conditions during storage. According to reference [26], seed lots with percentages similar germination, though with different levels of vigor, may exhibit different behaviors concerning deterioration, depending on storage conditions. According to reference [27] decreasing the vigor of Chia seed during storage may be observed by the reduction in germination rate. In pathogenicity test can confirm or not the fungi identified in the seeds and transmitted seeds to seedlings are even pathogenic Chia (Table 3).

Table 3: Pathogenicity of fungi in Chia plants (*Salvia hispanica* L.)

Fungi	Pathogenicity
<i>Fusarium</i> spp.	+
<i>Colletotrichum</i> spp.	+
Witness	-

(-) Non-pathogenic; (+) pathogenic.

In this study, fungal genera *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. we're not used for pathogenicity tests because, often, these genres are related in the majority to inadequate storage conditions, or

are contaminants in the seed and the pathogens *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp. and *Rhizoctonia* spp., per present low occurring in seeds (Table 1). It was found that *Fusarium* spp. and *Colletotrichum* spp. were pathogenic the seedlings of *Salvia hispanica* L. (Table 3). After his inoculation, observed symptoms, wilt followed by molting death inoculated with *Fusarium* spp. and leaf spot in light brown color the dark brown, characteristics of the fungal from gender *Colletotrichum*. There is the difference about the witness, therefore, demonstrating that the symptoms occurred as a result of inoculation confirming the pathogenicity of the fungi to Chia plants much information about Chia culture is still lacking, demonstrating the need for in-depth studies on sage seed pathology *Salvia hispanica* L. The fungi found in Chia seeds were: *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *Rhizoctonia* spp, and *Rhizopus* spp. The pathogens *Colletotrichum* spp. and *Fusarium* spp. are transmitted to seedlings via seeds and pathogens to Chia plants.

References

- [1]. Ayerza R, et al. (2004). Composition of Chia (*Salvia hispanica*) grown in six tropical and subtropical ecosystems of South America. *Tropical Science*, v. 44, n. 3, p. 131–135.
- [2]. Ixtaina VY, et al. (2011). Characterization of Chia seed oils obtained by pressing and solvent extraction. *Journal of Food Composition and Analysis*, v. 24, n. 2, p. 166-174.
- [3]. Urben AF. et al. (1987). Fungos associados a manchas de folhas em plantas de uso medicinal. *Fitopatologia Brasileira*, v. 12, n. 4, p. 390-394.
- [4]. Correa-júnior C, Ming LC, Scheffer MC. (1994). Cultivo de Plantas Medicinais, Condimentares e Aromáticas. 2 ed. Jaboticabal, SP: FUNEP. p.162.
- [5]. Neergaard P. (1979). Seed pathology. Vol. 2. London: Mac Millan Press, 829 p.
- [6]. Lazzari FA. (1997). Umidade, fungos e micotoxinas na qualidade de sementes, grãos e rações. 2. ed. Curitiba, p. 148.
- [7]. Maziero, MT, et al. (2010). Micotoxinas em alimentos produzidos no Brasil. *Revista Brasileira de Produtos Agroindustriais*, v.12, n.1, p.89-99.
- [8]. Pedroso, DC, et al. (2010). Métodos de inoculação de *Alternaria* e *A. dauci* em sementes de salsa e sua influência na qualidade fisiológica. *Revista Brasileira de Sementes*, v. 32, n. 3, p. 080.
- [9]. Barnett HL, Hunter BB. (1972). Illustrated genera of imperfect fungi. Burgess Publishing Company. 3. ed. Minneapolis, p. 241.
- [10]. Embrapa - Empresa Brasileira De Pesquisa Agropecuária. (2013). Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos. 3. ed. rev. amp. - Brasília, DF: EMBRAPA. 353p.
- [11]. Katan J, et al. (1976). Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. *Phytopathology*, v. 66, n. 5, p. 683-688.
- [12]. Leslie JF, Summerell BA. (2008). The *Fusarium* laboratory manual. John Wiley & Sons.
- [13]. Goulart ACP. (2004). Fungos em sementes de soja: detecção, importância e controle. Dourados: Embrapa Agropecuária Oeste.
- [14]. Lopez AMQ. (2001). Taxonomia, patogênese e controle de espécies do gênero *Colletotrichum*. *Revisão Anual de Patologia de Plantas*, v. 9, n. 1, p. 291-338.
- [15]. Gaetan SA, Gally ME. (1993). Anthracnose of peppermint (*Mentha piperita* L.) caused by

- Colletotrichum gloeosporioides* (Penz.) Sacc. Boletim de Sanidade Vegetal, v.19, n. 4, p. 673-676.
- [16]. Garibaldi A, Gullino ML, Minuto G. (1997). Diseases of basil and their management. Plant Disease, v. 81, n. 2, p. 24-132.
- [17]. Cherobini EAI, et al. (2008). Avaliação da qualidade da semente e mudas de cedro. Ciência Florestal, v. 18, n. 1, p. 65-73.
- [18]. Martins J. (2015). Contaminação fúngica em sementes de Chia comercializadas no município de Campo Mourão. 27f. Monografia (Universidade Tecnológica Federal do Paraná), Campo Mourão-PR.
- [19]. Trigo MFOO, et al. (1997). Fungos associados às sementes de coentro (*Coriandrum sativum* L.) no Rio Grande do Sul. Revista Brasileira de Sementes, v. 19, n. 2, p. 214-218.
- [20]. Jiménez IAR, et al. (2015). Detección e identificación de hongos en semillas de chía. II Jornadas Paraguayas de Botánica – Sesión Micología y Liquenología. Revista Steviana, v. 7, n. 21767, p. 87.
- [21]. Almeida TL, et al. (2016). Qualidade sanitária e fisiológica de sementes de Chia. Enciclopédia Biosfera, v.13 n. 23, p. 1117-1122.
- [22]. González V, Martínez V, Muñoz L, Ploper LD. (2010). Patógenos detectados en el cultivo de chía (*Salvia officinalis* L.) en las provincias de Tucumán y Salta. Journal Avance Agroindustrial, v. 31, n. 4, p. 36-39.
- [23]. Machowicz-stefaniak Z, Zalewska E, Zimowska B. (2002). Fungi colonizing various organs of lemon balm (*Melissa officinalis* L.) cultivated in South-East Poland. Plant Protection Science, v. 38, n. 2, p. 347-350.
- [24]. Machowicz-stefaniak Z, Zalewska E, Zimowska B. (2004). Fungi colonizing above-ground parts of lemon balm (*Melissa officinalis* L.) and thyme (*Thymus vulgaris* L.) cultivated in the Lublin Region. Folia Universitatis Agriculturae Stelinensis, v. 95, p. 229-232.
- [25]. Kobayashi L, et al. (2011). Levantamento da incidência de fungos potencialmente patogênicos em sementes de pinhão manso. Pesquisa Agropecuária Tropical, v.41, n.3, p. 385-390.
- [26]. Pádua GP, Vieira RD. (2011). Deterioração de sementes de algodão durante o armazenamento. Revista Brasileira de Sementes, v. 23, n. 2, p. 255-262.
- [27]. Ellis RH. (1989). The effects of differences in seed quality resulting from priming or deterioration on the relative growth rate of onion seedlings. Acta Horticulturae, v.1, n. 253, p. 203-212.