

Sanity, Pathogenicity, and Transmission of Fungi associated with Seeds of Chia coming from the Southern Region of Tocantins

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Abstract— A seed of Chia (*Salvia hispânica* L.) has been growing, assuming significant values of consumption in the current society, due to its high nutritional power. However, its seeds can be targeted by the presence of fungi. The aim of this study was to evaluate the health, pathogenicity, and transmission of fungi associated with seeds in Chia culture in the southern Tocantins. For the work, Chia seeds were collected from the UFT experimental unit, Gurupi-TO, produced from the combination of five levels of phosphate fertilization (0, 30, 60, 90 and 120 kg ha⁻¹) and five levels of nitrogen fertilization, 30, 60, 90 and 120 kg ha⁻¹). The filter paper method (blotter test) was used to perform the mycobacterial survey. The pathogenicity and transmissibility of fungi associated with seeds were also tested. The genus detected in the superficial part of the seeds were: *Cladosporium* sp., *Phoma* sp., *Fusarium* sp., *Alternaria* sp. and *Curvularia* sp. The Chia seeds of the municipality of Gurupi-TO presented low incidence of fungi. There was no pathogenicity and transmissibility of the fungi detected in Chia seeds in the seedlings.

Keywords— *Salvia hispânica* L., pathogen, quality, disease.

I. INTRODUCTION

Salvia hispânica L. is an herbaceous plant, originating from the mid-west region of Mexico to northern Guatemala. Culture with great potential, because it is considered a seed rich in fatty acids, omega-3, omega-6, carbohydrates, fiber and minerals, known mainly with the name Chia, belonging to the large group of the family Lamiaceae which are often medicinal, aromatic and part of the herbs used in cooking [1, 2].

Recently, the interest in Chia has been growing, assuming significant consumption values in today's society, particularly in Mexico and the southwestern United States, and still in South America. Although Chia is not widely known in Brazil, for the little time that Chia seeds

were introduced in the Portuguese market. However, the consumption of this seed has aroused a growing interest in Brazil, mainly in the regions of the West Paranaense and northwest of Rio Grande do Sul began to gamble and to plant the culture of Chia in the last harvests, intending to present good Results [3–5].

The propagation of Chia culture is especially due to seeds, so the sanitary quality is a factor of great relevance, for better viability, germination, vigor, and production of Chia seeds [6]. Also, considering that better sanitary quality can reduce the incidence of diseases in culture. Among the existing records regarding agronomic management, the possible diseases caused by fungi are

scarce due to the fact that it is a poorly explored culture. However, there are reports of diseases caused by fungi of economic importance that have affected the crop in the last harvests [7–9].

The presence of fungi in seeds, besides allowing the introduction of pathogens in areas of cultivation and causing diseases to agricultural crops, can also cause alterations in the metabolism of plants compromising their therapeutic properties and flavor.

Several species of fungi cause physicochemical alterations in the tissues of the seeds, causing loss of lipids, carbohydrates, proteins, and fatty acid increase, besides influencing seed germination [10, 11].

The detection of occurrence, associated pathogenicity, and transmission of fungi in seeds can facilitate the choice of disease control measures, enabling the elaboration of identification and control methodologies. However, there are few related studies on the presence of fungi in Chia seeds produced in Brazil. For this reason, the present work had as objective: to evaluate the sanity, pathogenicity, and transmission of fungi associated with seeds in Chia culture produced in the southern region of Tocantins.

II. MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Phytopathology and greenhouse of Universidade Federal do Tocantins (UFT), Gurupi-TO.

The tests were made approaching 1 — Sanity, 2 — Pathogenicity, 3 — Transmission of fungi associated with seeds. Therefore, the seeds were collected in the experimental field of the UFT located at the coordinates with 11° 43' 45"S and 49° 15'W average altitude of 285 m. The climate of the region is of the mega thermic type with summer rains and dry winter [12].

For the tests, 25 samples of Chia seeds produced from the combination of five levels of phosphate fertilization (0, 30, 60, 90 and 120 kg ha⁻¹) and five levels of nitrogen fertilization (0, 30, 60, 90 and 120 kg ha⁻¹) were used in the crop 2015/2016 in the municipality of Gurupi-TO.

Test 1 – Sanity 1

For this assay, we used the filter paper method (*blotter test*) according to the Manual of Sanitary Analysis of Seeds (Brazil, 2009) [13]. The experimental design was completely randomized (CRD) with 4 replications, each one in a Petri dish containing 50 seeds of each treatment. The Petri dishes containing the seeds were placed in the freezer for 12 hours to inhibit the germination. After this period, the Petri dishes were transferred to incubation chamber at 25 ± 2°C and photoperiod of 12 hours for 7

days. At the end of this period, the fungi were surveyed from the individual analysis of the seeds with the aid of the stereoscopic optical microscope visualizing the morphological characteristics of the fungal structures, the fungi were identified as Based on specialized literature such as Ellis (1971) [14], Barnett & Hunter (1998) [15] and Watanabe (2010) [16]. The fungi found were isolated and cultivated in potato-dextrose-agar culture medium (PDA) for the pathogenicity assay. The fungi incidence data were expressed as percentages (%).

Test 2 – Pathogenicity

The pathogenicity of the fungi transported by the seeds was evaluated by inoculations in the aerial part of seedlings. To obtain the seedlings, Chia seeds were sown in pots of 8 dm³ containing as substrate the mixture of soil and sand autoclaved at 120°C for 1 hour, in the proportion 2:1. Each vase with 4 seeds, at 45 days after sowing, with the aid of a manual spray was made the inoculation of the suspension 10⁶ spores/ml resulting from each fungal isolate (*Cladosporium* sp., *Phoma* sp., *Fusarium* sp., *Curvularia* sp. and *Alternaria* sp.), adjusted with Neubauer chamber. The suspensions were prepared from pure colonies of fungi cultivated in culture medium (PDA) incubated 7 days, under a temperature of 25 ± 2°C and photoperiod of 12 hours. Similarly, disc inoculations were made on the stems of the plants. Using as a witness a plant sprayed only with sterile water. The inoculated seedlings were packed in a moist and dark chamber for 48 hours. After this period, they were transferred to a greenhouse, where they remained for 10 days.

The evaluations of the symptoms were performed ten days after inoculation. Subsequently, indirect isolation of the fungus was performed, according to Alfenas & Mafia (2007) [17] with the purpose of confirming the causal agent, fulfilling Koch postulates. The experiment was assembled according to a completely randomized design (CRD) with 6 treatments (5 fungi + control), and 4 replications. Each repetition consisted of a plant.

Test 3 – Transmission of seed-associated fungi.

For the transmission test, 50 seeds of each treatment were sown in styrofoam trays. Previously disinfected with 1% sodium hypochlorite, one seed per cell, containing Nutrimax commercial substrate, autoclaved at 120°C for 1 hour. After sowing, the trays were transferred in a greenhouse and irrigated daily. The seedling evaluation was performed at 5, 10, 15, 20, 25 and 30 days. After germination, none of the seedlings presented symptoms characteristic of the diseases, so the Koch postulated was

not made, which is a requirement solely for confirmation of the existing pathogen.

III. RESULT AND DISCUSSION

In the sanity test, a total of five fungi genera (*Cladosporium* sp., *Phoma* sp., *Fusarium* sp., *Alternaria* sp., and *Curvularia* sp.) associated with Chia seeds were detected. The results of fungal incidence are presented as percentages in Table 1.

Table 1. Percentage of fungi associated with Chia seeds (*Salvia Hispânica L.*) detected by the filter paper method (blotter test) according to the Handbook of Sanitary Analysis of Seeds (Brazil, 2009).

SAMPLE (N-P)	CLA	FUS	ALT	CUR	PHO
0-0	15	2	1	1	0
0-30	8	2	1	2	0
0-60	5	2	0	0	1
0-90	8	0	1	0	0
0-120	10	2	0	0	0
30-0	30	5	0	1	1
30-30	16	4	2	5	0
30-60	9	4	0	1	0
30-90	16	0	2	0	0
30-120	20	3	0	1	1
60-0	15	2	1	2	0
60-30	15	0	3	1	0
60-60	10	1	1	2	0
60-90	14	9	1	0	0
60-120	15	1	0	2	0
90-0	14	3	1	1	1
90-30	31	1	1	0	0
90-60	14	2	1	1	0
90-90	34	5	2	1	1
90-120	36	5	2	3	0
120-0	16	6	0	0	0
120-30	21	4	0	0	0
120-60	15	3	1	1	0
120-90	6	6	0	0	0
120-120	8	2	3	3	0

In that: CLA: *Cladosporium* sp., FUS: *Fusarium* sp., ALT: *Alternaria* sp., CUR: *Curvularia* sp. e PHO: *Phoma* sp. N: Nitrogen P: Phosphorus.

Similar studies were reported by Almeida et al., (2016) [6] when evaluating samples of commercial Chia seeds, verified the presence of fungi: *Fusarium* sp., *Cladosporium* sp., *Bipolaris* sp., and *Aspergillus* sp. Jiménez et al., (2015) [18] when studying the same species *Salvia hispânica L.* also determined the presence of contaminating filamentous fungi in seven commercial samples of seeds evaluated observing the presence of the following fungi genera: *Aspergillus* sp., *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Cladosporium* and *Curvularia* sp.

In Brazil, the genera of fungi that most frequently attack the seeds of medicinal, condiment and aromatic plants,

highlight the *Aspergillus*, *Epicoccum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* considered contaminants in seeds, *Cladosporium*, *Curvularia* and *Pestalotiopsis*, considered contaminants also however in some cases can cause diseases, *Bipolaris*, *Alternaria*, *Fusarium*, and *Phoma*, which have species that can cause important diseases, as the host involved [19].

For this essay, it was observed that in the Chia seed samples, from high and low Nitrogen (N) and Phosphorus (P) doses, the genus *Cladosporium* sp. presented the highest percentages of fungi. The fact that it was detected in 100% of the samples analyzed and the incidence of this pathogen in the seeds ranged from 5% to 36%. Observing

the highest incidence in the 90 doses of N and 120 P (Table 1).

Reis et al., (2007) [20], when evaluating six lots of commercial seeds belonging to the family of Lamiaceae, also evidenced the presence of *Cladosporium* sp., reaching more than 20% of incidence. Most fungi of the genus *Cladosporium* sp. are cosmopolitan and their distribution is commonly found in plants and seeds [21, 22]. Depending on the fungal species and host plant, there may be a lower or higher incidence and may cause important lesions or death in plants. High incidence levels of *Cladosporium* sp. are common in seeds from the field or during storage, causing damage to germination and vigor, especially when the treatment is not done in seeds [23, 24]. According to Braga et al., (2010) [25], the high or low incidence of *Cladosporium* sp may also be influenced by the drying period, environment conditions or storage period. It is important to highlight the fact that few are the studies addressing the occurrence of *Cladosporium* sp. in Chia seeds.

The second most frequent genus in the seeds evaluated was *Fusarium* sp and the seeds were from the 60-90 dose of N and P that presented the highest incidence (9%). This fungus is constantly associated with seeds of different plant species and its aggressive and rapid growth can cause the death of Seeds [26]. The *Fusarium* is considered pathogenic, because it causes reduction of the germinative capacity and diseases in the plant, this fungus presents a wide range of host and high economic expression, besides being producing mycotoxins [27, 28]. There are reports of the genus *Fusarium* sp. causing diseases in aromatic and medicinal plants (*Mentha piperita* L.) compromising different parts of the plants [29]. Several species of this fungus are sources of soil inoculum, causing problems such as seed rot, root necrosis, rot, mold, biochemical seed transformation, widespread wilting and seedling death [10, 30].

Other important genera found in the sanity test were *Alternaria* sp. and *Curvularia* sp. presenting similar percentages in the samples evaluated, with variation between the samples of 1-5% of incidence. Kruppa & Russomanno (2008) [31] affirm that in Brazil there are few records of diseases in plants of the family Lamiaceae caused by fungi. However, these authors evaluating 117 families found fungi in 71.8% of the samples evaluated, involving 24 genera, among them *Alternaria alternata* with high frequency, *Curvularia eragratides*, *Curvularia inaequalis*, *Curvularia bezel* and *Curvularia* sp. presenting low frequencies in seeds of basil, Sálvia, and Segurelha. These fungi are very common in seeds, most often are considered pathogenic fungi causing important

damage to plants or saprophytes not causing any impairment. According to Goulart (2004) [27], the genus *Alternaria* sp. is very common in seeds, being, in most cases, associated only as a saprophyte and depending on the incidence can cause impairment in seed quality. However several species of *Alternaria* sp. and *Curvularia* sp. are associated with diseases in many plant species [19, 32]. According to Machado (1988) [33], The pathogen maintains its viability and characteristics for a long time, allowing its dissemination in new areas quickly and with a high probability of causing disease after planting.

For the genus *Phoma* sp. In the sanity test, we observed a low percentage occurrence of these fungi in Chia seeds produced in high and low doses of N and P. There was an occurrence less than or equal to 1% among the samples evaluated. Different species of cultivated and wild plants have shown the similarity of fungi of the genus *Phoma* sp. [34]. This fungus has caused diseases in several species of plants, there are reports of species of *Phoma* sp. about symptoms in the stems and leaf in adult specimens of this host [35]. Machowicz-Stefaniak et al., (2008) and Zimowska (2008) [36, 37], evaluating the presence of *Phoma exigua* in sage, thyme, and lemon balm plants, observed inhibition in germination and necrotic stains on the stem and leaves. According to all the work reported above, the percentage of fungi found in Chia culture is low compared to other Lamiaceae families, where the highest incidence of this genus was observed. It was also observed that the control presented percentages not equidistant or equal to the higher doses than seeds produced in low or high doses of nitrogen and phosphorus did not interfere in the percentage of fungi observed. Justifying that the percentages of fungi present in the evaluated samples may be due to favorable climate conditions in their development, such as precipitation during the maturation and harvest periods that often occur in the region, or to successive cultivations of the same species or species belonging to the same family, in the same production field or due to storage. This is why the need for better management of productive fields, harvesting, and storage techniques is evident, in order to reduce the incidence of fungi and thus ensure a better sanitary quality of the seeds in Chia crop.

Pathogenicity of fungi associated with Chia seeds.

Regardless of the part of the plant in which the fungi *Cladosporium* sp., *Phoma* sp., *Fusarium* sp., *Alternaria* sp. and *Curvularia* sp. were inoculated, no pathogenic action was observed (Table 2).

Table 2. Pathogenicity of fungi in plants of Chia (*Salvia hispânica* L.).

Fungus	Pathogenicity
<i>Cladosporium</i> sp.	–
<i>Fusarium</i> sp.	–
<i>Alternaria</i> sp.	–
<i>Curvularia</i> sp.	–
<i>Phoma</i> sp.	–
Proof	–

(-)non-pathogenic; (+)pathogenic.

In the inoculation of *Phomopsis* sp., *Colletotrichum Gloeosporioides* in seedlings of the species Sabiá (*Mimosa caesalpiniaefolia*), no pathogenicity was observed. However, when testing the pathogenicity of *Fusarium Solani* and *Pestalotiopsis* sp., it was observed wilt and white-greyish leaf spots, respectively [38]. Reports of pathogenicity of fungi associated with Chia seeds, such as those described in the present study, were not found in the literature. However, further research on pathogenicity in the culture is necessary to verify these results. According to Aguaysol et al., (2015) [7], there is scarce information available on fungi that may affect Chia. However, these authors evidenced in the Chia culture in the municipality of Tucuman, Argentina the presence of white mycelium, corresponding to *Sclerotinia sclerotiorum*, disseminated pathogen via, seeds, causing symptoms such as Light brown coloration in inflorescences, fruit detachment, stem rot and formation of sclerotia in the inflorescences. González et al., (2010) [9] also observed in the provinces of Salta and Tucuman in Argentina different symptoms in Chia culture and identified their agents cause them, as generalized wilted associated with *Fusarium* sp., chlorosis of leaves produced by *Phytophthora* sp., purple staining in stems caused by *Sclerotinia sclerotiorum* carbonous spots on the stem caused by *Macrophomina phaseolina* and necrosis in leaves produced by *Rhizoctonia Solani*.

Transmission of fungi associated with Chia seeds.

According to Oliveira et al., (2013) [39], phytopathogenic fungi are transmitted via seed, causing seedling death. However, in the transmission test of the twenty-five samples evaluated from high and low doses of N and P, it was observed that 100% of the seeds emerged and did not present symptoms of diseases, indicating that the fungi present in the evaluated seeds did not were transmitted to the seedlings, this can be explained mainly by the low natural infestation of the seeds evidenced by the sanity tests. Suggesting that the presence of these fungi in the

seeds is not directly related to the decrease in the rate of Chia germination. In healthy seedlings, when sectioned and plated in BDA medium, no latent pathogen was detected. Mendes et al., (2005) [38] and Nascimento et al., (2006) [40] evaluating seedlings of *Mimosa Caesalpiniaefolia* and *Pterogyne nitens*, observed that fungi associated with seeds (*Fusarium solani*, *Phomopsis* sp., *Colletotrichum*, *Gloeosporioides*, *Pestalotiopsis* sp., *Aspergillus* sp., *Penicillium* sp., *Fusarium Moniliforme*, *Alternaria alternata*, *Rhizopus* sp., *Cladosporium* sp. and *Phoma* sp.) were not transmitted to the seedlings.

Reports from several studies confirm that the presence of microorganisms, even pathogenic, in the seed, is not sufficient to ensure that it will infect the plant originating from this seed, because, there are several factors that can limit the efficiency of transmission of pathogens present in seeds such as - inoculum quality, soil physical factors, temperature, humidity, oxygen, survival time of the pathogen in the seed [33, 41]. What may have occurred with the pathogenic agents present in Chia seeds, which were not transmitted to seedlings due to lack of favorable conditions, however, the seed-pathogen and possible relationship, allowing the transmission and establishment of the disease in the field [42].

Another factor that may have influenced the absence of transmission of fungi present in the seeds may be due to disinfection made with sodium hypochlorite 1%. It is known that the inhibitory effect of sodium hypochlorite has the ability to penetrate the microbial cell, destroying it [43]. The microorganisms were probably found in the external parts of the seeds, which allowed a decrease in the incidence at the time of the disinfestation.

According to Botelho et al., (2008) [44], the fungi in the asepsis process have less survival capacity, however, for its total elimination, it is indispensable to consider several factors such as - location of the fungus in the seed, conditions in which the concentration of the product and immersion time in the solution.

In general, this report is interesting and has contributed valuable information in research on sanity, pathogenicity, and transmission of Chia seeds, allowing to broaden the information contained in the literature and thus to subsidize future studies such as described in the present study.

IV. CONCLUSION

Chia seeds from the municipality of Gurupi-TO showed a low incidence of fungi.

There was no pathogenicity and transmissibility of the fungi detected in Chia seeds in seedlings.

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