

DIVERSITY OF FUNGI IN SEEDS OF *HYMENAEA STIGNOCARPA* AND *HYMENAEA COURBARIL* BEFORE AND AFTER FUNGICIDE TREATMENTS

INCIDÊNCIA DE FUNGOS NO TRATAMENTO DE SEMENTES DE *HYMENAEA STIGNOCARPA* E *HYMENAEA COURBARIL*

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ABSTRACT: The recovery of degraded areas led to an increase of native forest seedlings production. Jatoba-do-cerrado (*Hymenaea stignocarpa* Mart.) and jatoba-da-mata (*Hymenaea courbaril* L.), native species of the Brazilian Cerrado, are widely used for the recovery of this biome. Although forest species present a diversity of pathogens disseminated by seeds, few studies have been published and no study was found for jatoba. Because the seed is one of the main forms of pathogen dissemination, the aim of our work was to evaluate the natural incidence of fungi in seed lots of jatoba-do-cerrado and jatoba-da-mata and the performance of different fungicides for seed treatment recommendation. The experiment was carried out at the Genetics and Biotechnology Laboratory of the University Center of Patos de Minas. Two chemical treatments were submitted in the seeds and then divided into seven replicates of 15 seeds for each species. The first treatment was with the active principle thiophanate-methyl and fluzazine, commercial name Certeza[®], in the dose of 145 mL of the product in 100 kg of seeds. The second treatment was with fludioxonil, commercial name Maxim[®] XL, in the dose of 100 mL of the product in 100 kg of seeds. There was a third treatment corresponded to the control (without seed treatment). The visual analyzes were performed after eight days on each seed with a magnifier to verify the presence of developing fungal colonies. The growth fungi fructifications were observed using a stereomicroscope. The found fungi species were: *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Rhizoctonia* sp., *Rhizopus* sp. and *Trichoderma* sp. It occurred interaction between seed treatment and species for *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Phomopsis* sp., *Rhizoctonia* sp. and *Trichoderma* sp. For *Penicillium* sp., *Pestalotiopsis* sp. and *Rhizopus* sp., only the isolated factors were significant. Jatoba-do-cerrado seeds had a higher incidence than jatoba-da-mata seeds in all the found fungi. Certeza[®] and Maxim[®] XL have potential in seed treatment for the species. The fungicide Certeza[®] was more efficient to control the pathogenic fungi in seed treatment.

KEYWORDS: Jatoba-do-cerrado. Jatoba-da-mata. Fludioxonil. Thiophanate-methyl. Fluzinazina.

INTRODUCTION

The recovery of degraded areas led to an increase of native forest seedlings production. Because of that, many nurseries began to specialize in the production of forest seedlings. Seeds are collected from natural biomes and then sold to the nurseries (CARVALHO FILHO et al., 2003). Jatoba-do-cerrado (*Hymenaea stignocarpa* Mart.) and jatoba-da-mata (*Hymenaea courbaril* L.), native species of the Brazilian Cerrado, are widely used for the recovery of this biome (BRANDÃO; GAVILANES, 1992).

The jatoba belongs to the Caesalpinaceae family, reaching up a height of 20 meters with a trunk of one meter in diameter, with logging possibility (SAMPAIO et al., 2015). The fruits have a farinaceous pulp that provides flour with protein value equivalent to corn meal, with culinary use (ORSI et al., 2012). It is not very demanding on

fertility and soil moisture, with adaptive ability for different edaphoclimatic conditions, being an important element for the composition of heterogeneous reforestations and in parks afforestation (SCABORA et al., 2011).

The improvement of seedling production techniques has great relevance because of the environment degradation increases. Researches about native forest species for the accomplishment of forest recomposition are necessary (SAMPAIO et al., 2015). Seed sanity is the key component for healthy seedlings formation and helps to prevent the spread of seed pathogens. However, although forest species present a diversity of pathogens disseminated by seeds, few studies have been published (CARNEIRO, 1986; 1990; NETTO; FAIAD, 1995; MUNIZ et al., 2007), and for jatoba, no publications have been found. The knowledge about pathogens disseminated by seeds is the first process for a correct seed treatment.

The seed treatment, eliminating the pathogens of the seeds or protecting them against the action of environmental pathogens, has great importance in the development of vigorous and healthy plants. The chemical seed treatment is economic, easy to perform and considered safe for the human and environment. Due to the small number of products added in the seeds and the direct contact with the target site, this method is not harmful for the environment when compared to conventional disease treatment controls (MITTAR, 1986; MENTEN; MORAES, 2010). Among the seed treatment chemicals, the active ingredients fludioxonil and thiophanate-methyl + fluazinan are widely used in several crops of agricultural interest, like soybean, corn, bean and cotton (AGROFIT, 2003; SILVA et al., 2011).

Because the seed is one of the main forms of plant diseases dissemination, the aim of our work was to evaluate the natural incidence of fungi in seed lots of jatoba (*H. stigonocarpa* and *H. courbaril*) and the performance of different fungicides for seed treatment recommendation.

Contents

The experiment was carried out at the Genetics and Biotechnology Laboratory (GENEB) of the University Center of Patos de Minas (UNIPAM), located in the city of Patos de Minas, Minas Gerais. Seeds of *H. stigonocarpa* and *H. courbaril* were donated by the State Forestry Institute (IEF), located in the same city. The seeds were collected in the years of 2016 and 2017, respectively, and then they were duly conditioned and stored in a cold room (3 °C, 24% RH) before the analysis.

Seed sanity evaluations were adapted for jatoba based on "Regras para Análise de Sementes" prescriptions (BRASIL, 2009). Pathogen detection was determined using the blotter test method. Acrylic gerboxes (11 x 11 x 3.5 cm), previously washed and disinfested with 70% alcohol solution, were used.

Two chemical treatments were submitted in the seeds and then divided into seven replicates of 15 seeds for each species. The first treatment was with the active principle thiophanate-methyl and fluazina, commercial name Certeza[®], in the dose of 145 mL of the product in 100 kg of seeds. The second treatment was with fludioxonil, commercial name Maxim[®] XL, in the dose of 100 mL of the product in 100 kg of seeds. There was a third treatment corresponded to the control (without seed treatment).

The market fungicides are not recommended for forest species treatment, so the fungicide doses were established according to the agricultural crops recommendation. The fungicides were applied dry, then mixed with the corresponding forest seeds into a plastic bag and shaken manually for five minutes.

Three sheets of paper filter were sterilized in an autoclave (120 °C and 1 atm for 20'), placed and moistened with distilled water for each gerbox. Thereafter, the gerboxes were taken into a laminar flow cabinet and the seeds were arranged on the filter paper. The boxes were sealed with transparent acrylic lid and placed in a Biochemical Oxygen Demand (BOD) germination chamber type, set at 20±2 °C and with a 12 hour photoperiod (white fluorescent light).

The visual analyzes were performed after eight days on each seed with the help of a magnifier to verify the presence of developing fungal colonies. In the presence of fungi, the growth fungi fructifications were observed using a stereomicroscope with magnification from 10 to 100X. In some observations, the fragments of the fungal structures were transferred to Petri dishes containing BDA medium for the preparation of microscope slides for optical microscopy to confirm the genus of the fungi. The results were expressed as fungi incidence percentage.

The identified fungus incidence data was adjusted to a Generalized Linear Model (GLM), with a binomial distribution and logit link function. The effects were subjected to deviance analysis (ANODEV) using the Chi-square test (X^2) at 0.05 of significance, and when significant differences were found, the means were compared with Tukey's test at 0.05 of significance.

After the stereomicroscope and optical microscopy evaluations, the following fungi were found: *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Rhizoctonia* sp., *Rhizopus* sp. and *Trichoderma* sp. It occurred interaction between seed treatment and species for *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Phomopsis* sp., *Rhizoctonia* sp. and *Trichoderma* sp. (Table 1). For *Penicillium* sp., *Pestalotiopsis* sp. and *Rhizopus* sp., only the isolated factors were significant (Table 2).

For jatoba-da-mata, the seed treatment with Certeza[®] and Maxim[®] XL were efficient in the control of *Aspergillus* sp. and *Fusarium* sp., preventing fungi proliferation of 16.19% for *Aspergillus* sp. and 45.71% for *Fusarium* sp. The untreated seeds showed incidence of *Mucor* sp., *Phomopsis* sp. and *Rhizoctonia* sp., although the

seeds treated with Maxim[®] XL accentuated fungi incidence. The same behavior was not observed for Certeza[®] product, which did not allow the development of these fungi. For jatoba-do-cerrado, efficiency in seed treatments were identified for *Aspergillus* sp., *Mucor* sp. and *Trichoderma* sp. with

Maxim[®] XL; for *Fusarium* sp. with Certeza[®]; and for *Phomopsis* sp. and *Rhizoctonia* sp. with both products. For *Mucor* sp., a higher incidence with Certeza[®] was observed when compared to the control.

Table 1. Percentage means for fungi incidence of *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Phomopsis* sp., *Rhizoctonia* sp. and *Trichoderma* sp. in jatoba seeds submitted to different seed treatments.

Treatment	<i>Aspergillus</i> sp.		<i>Fusarium</i> sp.	
	<i>H. courbaril</i>	<i>H. stignocarpa</i>	<i>H. courbaril</i>	<i>H. stignocarpa</i>
Certeza [®]	0.00Aa	37.14ABb	0.00Aa	0.00Aa
Maxim [®] XL	3.81Aa	22.85Ab	0.00Aa	11.14Bb
Control	16.19Ba	45.71Bb	24.76Bb	5.71Ba

Treatment	<i>Mucor</i> sp.		<i>Phomopsis</i> sp.	
	<i>H. courbaril</i>	<i>H. stignocarpa</i>	<i>H. courbaril</i>	<i>H. stignocarpa</i>
Certeza [®]	0.00Aa	27.61Bb	0.00Aa	4.76Ab
Maxim [®] XL	9.53Bb	0.00Aa	10.47Ba	10.47Aa
Control	0.00Aa	0.00Aa	0.00Aa	41.90Bb

Treatment	<i>Rhizoctonia</i> sp.		<i>Trichoderma</i> sp.	
	<i>H. courbaril</i>	<i>H. stignocarpa</i>	<i>H. courbaril</i>	<i>H. stignocarpa</i>
Certeza [®]	0.00Aa	14.29Ab	0.00Aa	20.00Bb
Maxim [®] XL	6.67Ba	11.42Aa	9.52Ba	5.71Aa
Control	0.00Aa	46.7Bb	7.62Ba	20.00Bb

Means followed by same letters, uppercase in columns and lowercase in rows, are not different by the Tukey's test (0.05 of significance).

Seed treatments with Certeza[®] and Maxim[®] XL were efficient in *Penicillium* sp. and *Rhizopus* sp. control, reaching a complete control when the incidence was 27.51% for *Penicillium* and 54.54% for *Rhizopus*. For *Pestalotiopsis* sp., a fungus that

was not incident on the control, a small presence was observed applying Maxim[®] XL. Moreover, it was verified that these three genera of fungi were incident only in *H. stignocarpa* (Table 2).

Table 2. Percentage means for fungi incidence of *Penicillium* sp., *Pestalotiopsis* sp. and *Rhizopus* sp. in jatoba seeds submitted to different seed treatments.

Treatment	<i>Penicillium</i> sp.	<i>Pestalotiopsis</i> sp.	<i>Rhizopus</i> sp.
Certeza [®]	0.00A	0.00A	0.00A
Maxim [®] XL	7.00A	4.77B	0.00A
Control	27.51B	0.00A	54.54B

Species	<i>Penicillium</i> sp.	<i>Pestalotiopsis</i> sp.	<i>Rhizopus</i> sp.
<i>H. courbaril</i>	00.01A	0.00A	0.00A
<i>H. stignocarpa</i>	22.35B	0.25A	25.03B

Means followed by same uppercase letters in columns are not different by the Tukey's test (0.05 of significance).

There are few studies about the pathogens transmission by seeds in forest species, despite the great number of pathogens that can be found with them. Many of these pathogens are genera of fungi that cause problems for agronomic crops (SANTOS et al., 2000). Phytopathogens may be associated with seeds on their surface, in their interior or in a

mixture, with the most varied forms of propagation, from spores to resistance structures. Phytosanitary aspects have been difficult because of the lack of research information on the fungi pathogenicity associated with forest species seeds (LAZAROTTO et al., 2013), revealing the importance of these studies.

The major problems associated with diseases occur during the germination and seedlings formation in nurseries, usually caused by a fungus. The fungi frequency occurs because of the fruit traits and seed harvest, processing and storage (NETTO; FAIAD, 1995; SILVA, 2003; SILVA et al., 2011). Due to the inaccessibility of the fruits in tall trees, the seeds harvest is made from fruits or even seeds dropped on the ground. In the soil, under the trees, part of the fruits and seeds are colonized by fungi influencing the germination (SANTOS et al., 2000).

In forest species, there are worldwide reports identifying the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Rhizoctonia*, *Rhizopus*, *Trichoderma* and others (LASCA et al., 1971; CARNEIRO, 1986; MITTAR, 1986; CHARCHAR et al., 2003). Jatoba seed sanity studies are scarce. However, the highest occurrence of diseases was verified in leaves of *H. courbaril* when compared with other six forest species. The genus *Pestalotiopsis* was identified in *H. courbaril* leaves (ASSIS et al., 2010) and *Phomopsis* in *H. stignocarpa* (CHARCHAR et al., 2003). In lumbers of *H. courbaril*, the incidence of *Fusarium* sp., *Penicillium* sp., *Rhizoctonia* sp. and *Trichoderma* sp. was eminent (SACCOMAN et al., 2016). This is the first record about the presence of pathogens in two species of jatoba seeds.

In addition, the genus *Aspergillus* and *Penicillium* were found in pata-de-vaca (*Bauhinia variegata*) (SENEME et al., 2006), paricá (*Schizolobium amazonicum*) (OLIVEIRA et al., 2012), and manduirana (*Senna mancrathera*) (PIVETA et al., 2018); *Fusarium* in pata-de-vaca (SENEME et al., 2006), amendoim-bravo (*Pterogyne nitens*) (NASCIMENTO et al., 2006) and favela (*Dimorphandra mollis*) (ARAÚJO et al., 2009); *Pestalotiopsis* in paricá (OLIVEIRA et al., 2012); *Phomopsis* sp. in favela (ARAÚJO et al., 2009); *Rhizoctonia* in amendoim-bravo (NASCIMENTO et al., 2006) and manduirana (PIVETA et al., 2018); *Rhizopus* in pata-de-vaca (SENEME et al., 2006) and *Trichoderma* in manduirana (PIVETA et al., 2018). This is the first report about the incidence of *Mucor* sp. in species of the Caesalpinaceae family.

Seeds treated with Maxim[®] XL had the incidence higher than the control for *Fusarium* sp. in *H. stignocarpa*; and for *Mucor* sp., *Phomopsis* sp., *Rhizoctonia* sp. and *Trichoderma* sp. in *H. courbaril*. For *Mucor* and *Trichoderma* genera, the result is explained by the chemical peculiarities of

the fungicide that do not control these fungi. Possibly, the fungicide allowed the growth of these fungi due to the great incidence and variability of genera present in the jatoba seeds. The control of *Aspergillus* sp. and *Fusarium* sp. in *H. courbaril* and *Aspergillus* sp., *Mucor* sp., *Phomopsis* sp. and *Rhizoctonia* sp. in *H. stignocarpa*, favored the fungi growth due to the inhibition of competition.

The importance and efficiency of chemicals in forest seeds treatment is subject of study by researchers. Fungicides with active ingredient carboxim-thiram in the dose of 250 mL in 100 kg of seeds, were viable for the control of *Alternaria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp. and *Penicillium* sp. in *Chorisia speciosa* (SILVA et al., 2003); and *Aspergillus* sp., *Curvularia* sp., *Penicillium* sp. and *Trichoderma* sp. in *Acacia mearnsii* (PISSININ et al., 2008).

Silva et al. (2011) showed that chemical molecules captan (120 g in 100 kg of seeds) and thiram (280 g in 100 kg of seeds) reduced the incidence of the genera *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* in *Dalbergia nigra*, *Handroanthus heptaphyllus* and *Handroanthus chrysotrichus*. In the seeds of *Acca sellowiana*, different chemical treatments with captan (160 g), carbendazim (100 mL), carbendazim + thiram (300 mL) and carboxim + thiram (180 g in 100 kg of seeds) had low or no occurrence of *Alternaria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp. and *Phomopsis* sp. (FANTINEL et al., 2015).

Forest seeds pathology deserves a comprehensive scientific space due to the occurrence of fungi that cause damage to quality and seedling production (SILVA et al. 2011), such as *Aspergillus*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Rhizoctonia*, *Rhizopus* and *Trichoderma*, causing seed rot, leading to germination and vigor reduction by deterioration, and death (NETTO; FAIAD, 1995; DHINGRA et al., 2002). Considering the economic and social impacts of several forest species, the chemical seed treatment is an efficient control treatment aimed to reduce diseases in these species.

Jatoba-do-cerrado (*H. stignocarpa*) seeds have a higher incidence than jatoba-da-mata (*H. courbaril*) seeds in all the found fungi. Certeza[®] (thiophanate-methyl + fluazina) and Maxim[®] XL (fludioxonil) have potential in these species seeds treatment. The fungicide Certeza[®] was more efficient to control the pathogenic fungi in seed treatment.

RESUMO: A recuperação de áreas degradadas levou ao aumento da produção de mudas de espécies florestais nativas. Jatobá-do-cerrado (*Hymenaea stignocarpa* Mart.) e jatobá-da-mata (*Hymenaea courbaril* L.), espécies nativas do Cerrado brasileiro, são amplamente utilizadas na recuperação deste bioma. Apesar da diversidade de fungos encontrada em sementes de espécies florestais, poucos são os estudos publicados, e não nenhum relato para o jatobá. Por a semente ser um dos principais meios de disseminação de patógenos, o objetivo do trabalho foi avaliar a incidência natural de fungos em lotes de semente de jatobá-do-cerrado e jatobá-da-mata, e a performance de diferentes fungicidas para a recomendação do tratamento de sementes. O experimento foi realizado no Laboratório de Genética e Biotecnologia do Centro Universitário de Patos de Minas. Dois tratamentos químicos foram realizados nas sementes, que então foram divididas em sete repetições com 15 sementes cada. O primeiro tratamento foi realizado com o princípio ativo tiofanato-metílico e fluazinam, nome comercial Certeza[®], na dose de 145 mL do produto para 100 kg de sementes. O segundo tratamento foi realizado com fludioxonil, nome comercial Maxim[®] XL, na dose de 100 mL do produto para 100 kg de sementes. O terceiro tratamento correspondeu a testemunha (sem tratamento de sementes). Após oito dias, a análise visual foi realizada com o auxílio de uma lupa para verificar a presença de colônias de fungos. As frutificações dos fungos foram observadas com o auxílio de um microscópio óptico. Após as avaliações, os seguintes fungos foram encontrados: *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Rhizoctonia* sp., *Rhizopus* sp. e *Trichoderma* sp. Ocorreu interação entre o tratamento de sementes e as espécies para *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Phomopsis* sp., *Rhizoctonia* sp. e *Trichoderma* sp. Para *Penicillium* sp., *Pestalotiopsis* sp. e *Rhizopus* sp., apenas os fatores isolados foram significativos. Sementes de jatobá-do-cerrado possuíram maior incidência do que as sementes de jatobá-da-mata para todos os fungos encontrados. Certeza[®] e Maxim[®] XL possuem potencial para o tratamento de sementes de jatobá. O fungicida Certeza[®] foi mais eficiente para o controle de fungos patogênicos no tratamento de sementes.

PALAVRAS-CHAVE: Jatobá-do-cerrado. Jatobá-da-mata. Fludioxonil. Tiofanato-metílico. Fluazinam.

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