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Incidence of *Cladosporium cladosporioides* on Jatropha seeds and its effect on germination

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Abstract: The objective of this study was to estimate the incidence and perform morphological characterization of *Cladosporium* sp. found on the seeds and analyze their interference in the germination of *Jatropha curcas*. Therefore, jatropha seeds were sown in acrylic box gerbox type and incubated at 25°C for 14 days after sowing (DAS) to evaluate the fungus incidence. For the confection of semi-permanent slide mountings, fungus structures were removed with platinum handle and the measurement performed under a microscope. Another experiment was conducted in paper roll to evaluate germination 7 DAS. The occurring fungus specie was identified as *Cladosporium cladosporioides*, which incidence was 98.0%. In the test using paper roll, there was a percentage of normal seedlings of 77.0%, abnormal seedlings of 15.0% and dead seeds of 8.00%. The germination rate is not affected by the high incidence of the fungus in the seeds.
Keywords: *Jatropha curcas*; seeds pathology, mycology.

Introduction

In Brazil, jatropha has emerged as a promising specie of the biodiesel production from its seeds, due to its high oil yield (Andréo-Souza et al., 2010). The jatropha (*Jatropha curcas* L.) is a bush that reaches up to four meters of height, belongs to the Euphorbiaceae family, and is widely distributed around tropical and subtropical areas (Sujatha et al., 2008), it is considered a rustic crop, adapted to the most diverse edaphoclimatic conditions, surviving in a great diversity of soils, ranging from low to high fertility (Arruda et al., 2004; Oliveira et al., 2012).

The jatropha seeds are used for species propagation and seedling formation. The plant produces, on average, 100, 500, 2.000 and 4.000 g of seeds in the first, second, third and fourth years of cultivation (Tominaga et al., 2007). Thus, in order to obtain good quality seeds, especially as regard sanity, since occurring pathogens in seeds may interfere with germination and, consequently, production of healthy seedlings (Benetti et al., 2010; Carvalho et al., 2014). Among these pathogens, species of *Cladosporium* sp. genus stand out, which have already been reported as contaminants in jatropha seeds, causing damage to seeds germination (Silva & Silva, 2000; Goldfarb et al., 2010).

Another important reason is that contaminated seeds are considered one of the most efficient dissemination vehicle for introducing new pathogens in distant and indene areas, besides being an efficient means of survival of pathogens in nature (Agrios, 2005; Corrêa et al., 2008).

As a result of not having much research on the jatropha crops, there is a high demand for studies addressing pathology of seeds of this important species. The objectives of this study were to estimate *Cladosporium* sp. incidence in jatropha seeds, to verify the fungal effect on germination of contaminated seeds and to identify which species the pathogen belongs.

Methods

Obtaining seeds

Jatropha seeds were collected on September 28th, 2014, in experimental areas at Universidade Estadual de Goiás (UEG), Ipameri Câmpus (17º43'00.38''S, 48º08'40.96''W, 796 m), extracted from the fruits on the same day of collection and stored in a plastic bag for two months until the subsequent tests were performed. The

seeds were from 3-year-old jatropha plants, corresponding to the third year of harvest.

Sanitary analysis

After storage, the seeds were submitted to sanitary analysis by the *blotter test* method (Brasil, 2009), which were used 128 untreated seeds placed in 8 transparent acrylic boxes (gerbox type) (11,0 x 11,0 x 3,5 cm) previously disinfested with 70% alcohol and lined with two sheets of blotting paper moistened with sterile distilled water 2.5x its weight. In each gerbox (repetition), 16 seeds were placed. The design was completely randomized (CRD), with eight replications. After 7 days at 25ºC, the incidence of *Cladosporium* sp. was evaluated under a light microscope, confirming the phytopathogen genus by making semi-permanent slide mountings.

Germination test

A germination test was conducted using the same seed lot. For this test the paper roll method was used with tree germitest sheets and alternating temperature of 20-30ºC, taking as reference the recommended one for castor bean (*Ricinus communis* L.). In the experiment, 128 seeds (16 seeds per paper roll) were used. The germitest paper rolls were moistened with sterile distilled water 2.5x its weight and evaluations were performed at 7 and 14 days after sowing (Brasil, 2009).

Morphological characterization of Cladosporium sp.

Semi-permanent slide mountings containing biological material removed directly from the seeds were used to record images and perform the morphological characterization of the conidia, conidiophore and their structures, by obtaining 90 measurements of each component using Leica DM500 light microscope, with help from LAS EZ 2.0 (100x) software. The microscopic preparations were deposited in the collection of Phytopathology Laboratory of UEG, Ipameri Câmpus.

Statistical analysis

The results concerning sanitary analysis, seeds germination and fungal structures measurements were submitted to analysis of variance using the Sisvar 5.3 statistical software (Ferreira, 2011).

Results and discussion

In the presente study, observations were made about presence of *Cladosporium* sp. fungus in jatropha seeds, which had spots or growth of greenish-colored mycelium on their surface. The morphological makers that most aided the identification of the fungus at genus and species level were the prominent scar in conidia, conidiophores without nodules, conidia without septa or only one septum and, mainly, the ellipsoid or limoniform shape of conidia (Figure 1). After measuring the *Cladosporium* conidia obtained from seeds, it was found the following dimensions: 2.2 – 11.5 x 1.8 – 5.5 µm (5.0 x 3.2 µm) (Table 1) and length/width ratio of 0.7 – 4.7 µm (1.7). The conidiophores had dimensions of 62.0 – 123.4 x 3.1 – 5.5 µm (70.2 x 3.7 µm).

According to the characteristics described above, the species was identified as *Cladosporium cladosporioides* and had an incidence in the seeds of 98.0%. After the germination test were verified at 7 days after sowing on paper roll a percentage of 77.0% of normal seedlings, 15.0% of abnormal seedlings and 8.0% of dead seeds.

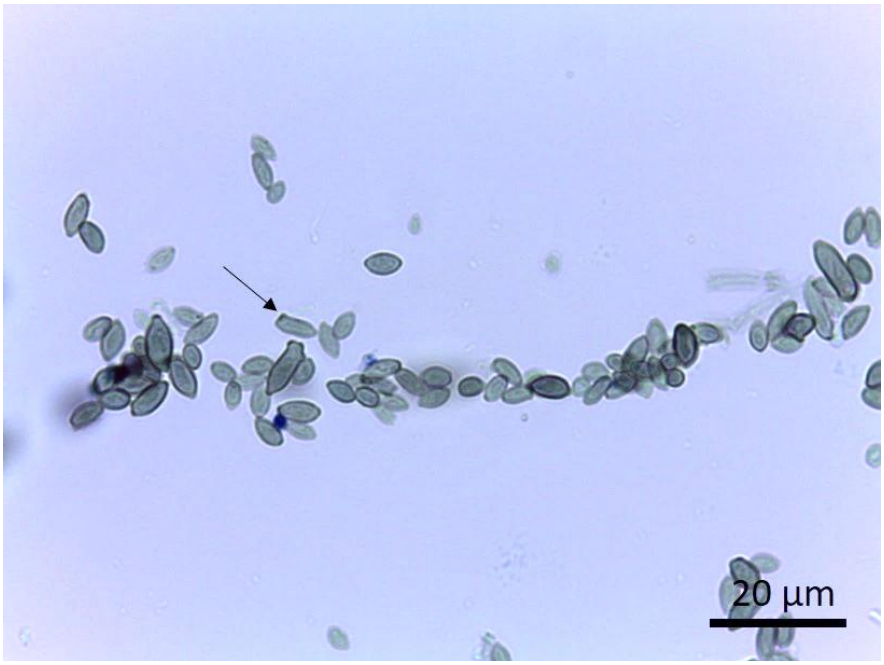

Figure 1. *Cladosporium cladosporioides* from jatropha seeds. Arrow shows a “scar” on conidia.

Table 1. Morphological characteristics of *Cladosporium* spp. conidia, including the species found in jatropha seeds.

Cladosporium species[1]	Conidium size (µm)	
	Length	Width
C. chlorocephalum	6.0 – 14.0	4.0 – 9.0
C. cladosporioides	3.0 – 11.0	2.0 – 5.0
C. oxysporum	5.0 – 30.0	3.0 – 6.0
C. colocasiae	15.0 – 20.0	6.0 – 8.0
C. herbarum	8.0 – 15.0	4,0 – 6.0
C. variabile	5.0 – 30.0	3.0 – 13.0
C. macrocarpum	15.0 – 25.0	7.0 – 10.0
C. spongiosum	5.0 – 40.0	2.5 – 7.0
C. carcophilum	12.0 – 20.0	4.0 – 5.0
C. musae	6.0 – 22.0	3.0 – 5.0
C. cucumerinum	4.0 – 9.0	3.0 – 5.0
C. cladosporioides[2]	2.2 – 11.5	1,8 – 5.5

[(1)]Ellis (1971); [(2)]Species found in the jatropha seeds.

The seeds obtained from experimental areas at UEG Ipameri Câmpus, Goiás, presented a relevant percentage of germination (77,0% of normal plants). Otherwise, (Pasquali et al. 2012) evaluated the jatropha seeds germination under different temperatures and verified that the constant temperature of 25ºC reduced the germination of seeds, however Mota et al. (2012) verified that jatropha seeds presented significant germination when submitted from 25 to 30ºC. Thus, the adoption of the alternating temperature of 20-30ºC, taking as reference the recommended for castor bean (*Ricinus communis* L.) (Brasil, 2009), contributed to the high percentage of normal seedlings.

Concerning the paper roll substrate used for germination test, Martins et al. (2008) reported that this is most appropriate for the jatropha in germination analysis in laboratory. It should be noted that the first count on the seventh day after sowing of the germination test could already be considered, since the germination speed is reduced as the deterioration progress caused by *C. cladosporioides*, which interfered negatively in the second count at fourteenth day.

The morphological characteristics verified in the present study indicated that the species present in jatropha seeds was *C. cladosporioides* (Figure 1). To confirm this result, morphological characteristics of other *Cladosporium* species reported by Ellis (1971) were used for comparison. The dimensions of the conidia analyzed in the present work are close to those described by Ellis (1971), who considered for *C. cladosporioides* as having conidia with dimensions of 3.0 – 11.0 x 2.0 – 5.0 µm and conidiophores that can reach 350 µm of length and had 2.0 – 6.0 µm of width. In addition, according to the same author, it was possible to observe conidiophores without pigmentation or moderately olivaceous brown colored with smooth surface and ellipsoid to limoniform conidia without pigmentation or moderately olivaceous brown with presence of catenulation, presenting smooth surface, besides conidiophores macronematosus, archaic or moderately olivaceous with smooth surface.

In contrast, it was possible to verify several species among those reported by Ellis (1971), such as: *C. chrolocephalum* (6.0 – 14.0 x 4.0 – 9.0 µm), *C. oxysporum* (5.0 – 30.0 x 3.0 – 6.0 µm), *C. colocasiae* (15.0 – 20.0 x 6.0 – 8.0 µm), *C. herbarum* (8.0 – 15.0 x 4.0 – 6.0 µm), *C. variabile* (5.0 – 30.0 x 3.0 – 13.0 µm), *C. macrocarpum* (15.0 – 25.0 x 7.0 – 10.0 µm), *C. spongiosum* (5.0 - 40.0 x 2.5 – 7.0), *C. carcophilum* (12.0 – 20.0 x 4.0 – 5.0 µm), *C. musae* (6.0 – 22.0 x 3.0 – 5.0 µm) and *C. cucumerinum* (4.0 – 9.0 x 3.0 – 5.0 µm). Generally, it can be concluded that all species mentioned above were shown with conidia measurements higher than that found in the present study (2.2 – 11.5 x 1.8 – 5.5 µm).

During the statistical analysis of the conidia dimensions of *C. cladosporioides*, attention was given to the variability of the measures. Therefore, it was verified that the coefficient of variation for the length and width of conidia were 33.0 and 24.0%, respectively, differently from *C. herbarum* occurring in common bean seeds, whose variability of the data referring to the measurements of all fungus structures did not exceed 10% (Guimarães & Carvalho, 2014). The explanation lies not only in the difference of species, but in the number of structures

measured, since Guimarães & Carvalho (2014) measured only 30 conidia and not 90.

The *C. cladosporioides* fungus, when occurring in jatropha, has been reported with variable incidences, that is, ranging from 4.5% to 85% in seeds (Kobayasti et al., 2011). High incidences reported for this species of fungus in jatropha as well as other crops, support the incidence verified in the present study. For example, can be mentioned the work of Pereira et al. (2005) who characterized the colonization dynamics of *C. cladosporioides* with incidence of 60% in coffee seeds (*Coffea arabica* L.). Meanwhile, Machado et al. (2003), according to the present study, reported incidences from 66.0 to 75% in soybean seeds (*Glycine max* L.)

Finally, it is worth mentioning that the seed lot evaluated, even with high incidence of *C. cladosporioides*, presented a high number of normal seedlings, indicating, according to Vanzolini (2010), that this pathogen exerts slight influence in germination.

Conclusions

The fungus found in seeds of jatropha is *Cladosporium cladosporioides*.

The germination rate is not affected by high incidence of *C. cladosporioides* in seeds.

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