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PATHOLOGY OF ANNATTO (*Bixa orellana* L.) SEEDS[1].

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ABSTRACT: Annatto is a widespread species in the Tropics. Among the diseases which affect the crop, damping-off, caused by the fungi *Rhizoctonia solani*, *Colletotrichum* sp. and *Alternaria* sp., are seed transmitted. This research was conducted to determine seed borne pathogens and germination of 13 seed lots. The annatto seeds were obtained from a germplasm collection maintained by APTA Regional Centro Norte – Pindorama – SP – Brazil. The pathogens were analyzed utilizing the blotter test, with and without surface disinfestation and germination test. The results showed that seed surface disinfestation did not decrease frequency of fungal occurrence. *Cladosporium* sp., *Phoma* sp., *Alternaria* sp. and *Colletotrichum* sp. were the most common fungi observed in the seeds, with frequency of, 62%, 54%, 49% and 4% respectively, in disinfested seeds. Annatto is typically a backyard plant, cultivated as a single bush for domestic use, and is commonly propagated, through seeds, which are exchanged within and among local communities, thus can spread important fungi, such as *Alternaria* and *Colletotrichum*, to other area.

INDEX TERMS: Seed Pathology, Germination.

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PATOLOGIA DE SEMENTES DE URUCUM (*BIXA ORELLANA* L.)

RESUMO: O urucum é uma espécie amplamente distribuída nos trópicos. Entre as doenças que afetam a cultura, o "damping-off", causado pelos fungos *Rhizoctonia solani*, *Colletotrichum* sp. e *Alternaria* sp., é transmitido por sementes. O objetivo desta pesquisa foi avaliar a patologia de sementes de 13 lotes de sementes de urucum, obtido na coleção de germoplasma da APTA Regional Centro Norte – Pindorama – SP – Brasil. As sementes de cada árvore de polinização aberta foram colhidas e submetidas ao "Blotter test" com e sem desinfestação superficial com solução de 1% de hipoclorito de sódio por três minutos, e ao teste de germinação. Os resultados mostraram que a desinfestação superficial das sementes não diminuiu a porcentagem de fungos. *Cladosporium* sp., *Phoma* sp., *Alternaria* sp. e *Colletotrichum* sp. foram os fungos mais comumente observados nas sementes, com os percentuais nas sementes desinfestadas de 62%, 54%, 49% e 4%, respectivamente. O urucum é uma planta típica de quintais, cultivado como única árvore para o uso doméstico de suas sementes. É um procedimento comum propagar essas sementes dentro e entre comunidades locais, o que pode disseminar fungos importantes, como *Alternaria* e *Colletotrichum*, para outras áreas.

TERMOS PARA INDEXAÇÃO: Patologia de Sementes, Germinação.

Annatto (*Bixa orellana* L.), called "urucum" in Brazil, is a widespread in the Neotropics, and belongs to the native flora of Brazil, Paraguay, Colombia, Dominican Republic, Haiti, Trinidad, Mexico, Panama and Hawaii (RAMALHO; PINHEIRO; DINIZ, 1987). The original geographic range of annatto in Brazil includes Northern and Northeastern parts of the country, encompassing the States of Amazonas, Pará, Maranhão, Piauí, Ceará, Paraíba and Bahia (LINGERFELT, 1984). The reddish-yellow pigment coating the seeds is still used by Brazilian indigenous people to paint their bodies, and to dye their breechcloths (PLOTKIN, 1993).

The annatto pigment contains bixine and norbixine, valuable natural colorants used for industrial food and beverages, cosmetics, pharmaceutical products and for textiles (PÓVOA, 1992). Restrictions imposed on the use of synthetic additives in the food industry have intensified the investigation on biochemical properties of annatto plants and seeds (PRENTICE- HERNANDEZ; RUSIG, 1992), and raised interest in the evaluation of genetic variability in the species.

Among the diseases that affect the annatto crop, damping-off, caused by *Rhizoctonia solani* Kühn and some species of *Colletotrichum* and *Alternaria*, are seed transmitted.

Seeds are regarded as a highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agricultural literature for the international spread of

plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens. Various fungi are commonly associated with seeds of many tree species, and these can include pathogens and saprophytes. Seeds may be infected internally, often resulting in the destruction of endosperm and embryo, or simply contaminated, whereby the pathogen is associated primarily with the seedcoat. The development of quarantine programs for seeds requires biological and ecological information about seed-borne pathogens, the ability to detect their presence, knowledge about the inoculum type and its location on seeds, and effective means for control. With this information, risks associated with the importation of seeds of particular tree species can be evaluated more accurately (FRAEDRICH, 2001).

Damages caused by seedborne pathogens, highly vary according to the pathogen involved, initial inoculum and environment conditions during crop growth (LUCCA, 1985).

Due to the lack of information about the occurrence of pathogenic fungi in annatto seeds, the present work was done to survey incidence of fungi in annatto seeds, and verify their influence on seed germination.

The seeds were obtained from germoplasm collections maintained by APTA Regional Centro Norte, in Pindorama-SP-Brazil.

Fruits of each open-pollinated tree were harvested in june/2006, when two or three fruits in a bunch turned reddish-brown (PIMENTEL, 1985) and started to dehiscence. After the harvest, fruits from three trees of each of 13 progeny, were threshed manually and cleaned through 2 mm screen. Seeds were tested for germination and for external and internal fungal contamination

Blotter test was used to determine fungal contamination. To determine internal fungi, seeds were surface disinfested by soaking for three minutes in a 1.0% solution of sodium hypochloride and surface disinfestation was omitted to determine external fungi. (SAUER, BURROUGHS, 1986). Seeds were than placed on three layers of moist blotter paper in Petri dishes. Eight Petri dishes containing 25 seeds each, evenly spaced to avoid contact with each other were used in each treatment. The incubation was done for seven days at 20 $\pm$ 2^{O}C, under NUV light (12 h photoperiod). The number seeds yielding fungi was counted and the fungi were identified.

Data were transformed in arc sen $(x/100)^{1/2}$, for statistical analysis. Analysis of variance was conduct in a completely randomized design, in factorial outline 13 x 2 (seed lots x seeds with and without surface disinfection), and the means were compared by Tukey test, at 5% (P = 0.05). All treatments were replicated eight times.

Seed germination test was performed as described in Seed Analysis Rules (BRASIL, 1992), with some modifications. Four replications of 50 seeds each were placed on moist towel papers, which were rolled and kept at 20-30^{O}C. The evaluations were done

after 10 and 19 days, by counting the number of normal seedlings and dormant seeds. The experimental design was completely randomized, with 13 treatments (seed lots) and four replications. The means were compared by Tukey test (p = 0.05).

Cladosporium sp., *Phoma* sp., *Alternaria* sp. and *Colletotrichum* sp. were the most common fungi isolated from annatto seeds (Table 1). *Aspergillus* sp., *Epicoccum* sp. and *Penicillium* sp. also were isolated from some seed lots, with low frequency. *Alternaria* sp. and *Colletotrichum* sp. are important fungal species that cause damping-off in annatto (FRAEDRICH, 2001).

The seed disinfestation did not decrease fungal recovery. However, the disinfestation increased the recovery rate of *Alternaria* sp. and decreased that of *Colletotrichum* sp. from lot 8, and of *Phoma* sp., from lots 8, 9 and 13 (Table 1).

The percentage of germination and dormant seeds are shown in Table 2. There were variations among seed lots, with germination ranging from 9.6% (lots 4 and 6) to 72.0% (lot 12). Custódio et al. (2002) reported that annatto seed germination is low because of seed coat dormancy, and in seeds without dormancy breakdown treatment, the germination percentage ranges from 1.0 to 12.0%, contrary to the data of this study where some seeds lots had higher germination.

Belfort, Kato e Kato (1992) reported high germination (96.0%) of undissected annatto seeds, sown soon after extraction from fruits, with 64.6% moisture content. In this study the seeds were maintained inside fruits until the germination test.

According to the Tables 1 and 2, the fungi may not have interfered with seed germination. There was high occurrence of *Alternaria* sp., *Cladosporium* sp. and *Phoma* sp. in lots with high and low germination. Gure (2004) studied the impact of seed-borne fungi on seeds and seedlings of the tree *Podocarpus falcatus* and *Prunus africana* and found variable effects on seeds and seedlings. The fungi could be grouped into five categories namely, I) isolates that were pathogenic only to seeds and had no visible impacts on seedlings; II) isolates that were pathogenic only to seedlings; III) isolates that were pathogenic both to seeds and the emerging seedlings; IV) isolates that were moderately harmful to both seeds and seedlings; and V) isolates that were germination promoters. These results showed the difficulty of studying seed pathology of native tree species.

In Brazil, annatto is typically a backyard plant, cultivated as single plant or ornamental shrub for domestic use, in cuisine and folklore medicine. It is generally propagated through seed that are also exchanged among communities, which can spread fungi, such as *Alternaria* sp. and *Colletotrichum* sp., to the other areas. The data we present indicate that *Cladosporium* sp., *Phoma* sp., *Alternaria* sp. and *Colletotrichum* sp. were the most common fungi recovered from seeds; and these fungi did not affect the seeds germination.

Table 1 – Percentage of fungi in annatto seeds, without and with superficial disinfection.

Lots	*Alternaria* sp.				*Cladosporium* sp.			*Colletotrichum* sp.				*Phoma* sp.			
	w/d		d		w/d	d		w/d		d		w/d		d	
1	2	Acd[12]	4	Aef	49	47	ab	4	Aab	1	Aa	46	Aab	43	Aabc
2	3	Abcd	5	Acdef	15	17	c	2	Aab	0	Aa	51	Aa	28	Bcd
3	5	Abcd	5	Acdef	58	51	ab	0	Ab	0	Aa	46	Aab	32	Aabcd
4	2	Acd	1	Af	13	15	c	1	Ab	2	Aa	45	Aab	33	Aabcd
5	1	Ad	2	Aef	11	13	c	0	Ab	0	Aa	59	Aa	51	Aab
6	16	Aab	16	Abcd	54	59	ab	1	Ab	0	Aa	54	Aa	54	Aa
7	15	Aabc	22	Aabc	42	45	ab	3	Aab	1	Aa	39	Aab	36	Aabc
8	2	Bcd	28	Aab	36	46	b	11	Aa	0	Ba	46	Aab	16	Bcd
9	2	Bcd	15	Abcde	57	59	a	0	Ab	1	Aa	42	Aab	26	Abcd
10	1	Ad	1	Af	10	18	c	4	Aab	4	Aa	37	Aab	21	Acd
11	4	Abcd	5	Adef	47	38	ab	1	Ab	3	Aa	23	Ab	20	Acd
12	23	Aa	16	Abcd	50	47	ab	0	Ab	1	Aa	34	Aab	6	Bd
13	26	Ba	49	Aa	49	62	ab	0	Ab	0	Aa	47	Aab	21	Bcd
F (lots)	17.775**				26.536**			2.507**				6.641**			
F (dis.)	12.901**				1.063NS			3.210**				42.601**			
F (l x d)	3.137**				0.807NS			2.295*				1.948*			
CV(%)	37.15				25.51			62.38				31.71			

w/d = without disinfection d = disinfection; [1]Averages followed by the same letters, capital in lines and minuscule in columns, have no difference (Tukey test, 5% probability); [2]Analisys of data transformed in arc sen $(x/100)^{1/2}$; *Significative, 1% of probability; ** Significative, 5% of probability; NS No significative.

Table 2 - Percentage of annatto seed germination and dormancy.

Lot	Germination		Dormancy
12	72.0	a[1,2]	12.0
9	60.0	ab	4.0
10	50.7	abc	2.7
5	40.0	bcd	38.7
8	36.0	bcd	20.0
11	36.0	bcd	26.7
2	32.0	cd	52.0
3	30.7	cde	26.7
1	28.0	cde	20.0
13	28.0	cde	52.0
7	21.3	de	41.3
4	9.3	e	89.3
6	9.3	e	52.0
F	14.22**		
CV(%)	14.93		

[1]Averages followed by the same letters have no difference (Tukey test, 5% probability); [2]Analisys of data transformed in arc sen $(x/100)^{1/2}$;** Significative, 5% of probability.

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