Australian Journal of Crop Science

AJCS

AJCS 13(04):635-641 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.04.p1680

Health quality and reduction of pathogenic transmission in tomato seeds using plant extracts

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Abstract

The objective of the present study was to assess the seed health quality, quantification of seed-seedling pathogen transmission and the effect of plant extracts in reducing plant pathogens in the seeds of the tomato varieties San Marzano and Ipa 6. For the seed health, the samples were disinfested, plated and assessed after seven days, according to the Brazilian Seed Analysis Rule. For the transmission rate, 12 trays were prepared with 100 seeds each and assessed at 7, 14 and 21 d.a.s. (days after seeding) using 100 seedlings. The plant (main root, stalk and leaves) tissues were plated in PDA culture medium and assessed after seven days of incubation. Aqueous extracts were prepared from cinnamon, basil, neem and eucalyptus with 0.5% concentration and the seeds were immersed in each solution for 10 minutes. Then, they plated and assessed after seven days. The health test showed that biggest incidences of *Aspergillus fumigatus* (26 %) and *Aspergillus flavus* (26 %) were occurred in the seeds of the varieties Ipa 6 and San Marzano, respectively. The fungi *A. flavus*, *A. fumigatus*, *A. niger*, *R. stolonifer* and *Curvularia* sp. were detected in quantification of transmission in the seeds of the two tomato varieties. The treatment with basil extract resulted in the least fungus incidence in the transmission quantification of 'San Marzano' tomato seeds, while on Ipa 6 seeds the eucalyptus treatment performed better. The interference of treatments was not observed in tomato seed germination. However, there was decrease in incident of pathogens in seeds treated with the plant extracts and different effects was observed based on type and species of the pathogen.

Keywords: *Solanum lycopersicon*, transmissibility, alternative treatment, cinnamon, basil, neem, eucalyptus.

Introduction

In general, vegetables are subject to several diseases, especially those vectored by seeds. According to Piveta et al. (2010), seed health quality is one of the most important aspects related to healthy seedling production, because microorganisms can cause abnormalities and lesions in the seedlings and seeds.

The tomato (*Solanum lycopersicon* L.) is one of the vegetables with biggest economic importance worldwide (Silva, 2015). It is present throughout the world and is one of the most consumed vegetables (Tomazoni et al., 2013). The crop is attacked by several diseases and fungi are the most usual disease-causing microorganisms. Tomato seeds can shelter several types of microorganisms, either disease causers or not. Some deteriorate the seeds reducing germination and vigor, and can further be a means of disease dissemination in the field (Brasil, 2009). The importance of the tomato crop and the commercial value of its seeds justify efforts to increase options for treatments that contribute to the quality of its seeds (Braga et al., 2010). With this, it is necessary to treat seeds, to obtain a reduction in the disease- causing plant pathogens.

The use of large products has been successful in the selection of resistant pathologies (Santos Neto et al., 2016). However, it is known that indiscriminate use can lead to various problems for the environment. So, there is a need to search for alternatives to the chemical method. Technologies geared to environmental sustainability have gained space, an example of which is the use of crude extract and essential oil derived from medicinal plants (Itako et al., 2009).

The literature reports the efficiency of plant extracts obtained from several plants including garlic (*Allium sativum* L.), rosemary (*Rosmarinus officinalis* L.) (Leite et al., 2012), cinnamon (*Cinnamomum zeylanicum* Blume), eucalyptus (*Corymbia citriodora* L.), bitter melon (*Momordica charantia* L.) and neem (*Azadirachta indica* A. Juss). (Venturoso et al., 2011), to inhibit the development of several plant pathogens of a fungal nature. Silva et al. (2017) and Kobayashi and Amaral, (2018), studied the use of different plant extracts in the control of *Pseudomonas syringae* pv. tomato- Pst and *Alternaria solani*.

Thus the objective of the present study was to assess the seed health quality, quantification of seed to seedling pathogen transmission and to assess the effect of plant extracts on reducing plant pathogens associated with tomato seeds.

Results and Discussion

Detection, identification and assessment of plant pathogen incidence in tomato seeds

The germination percentage in the seeds of the "Ipa 6" and "San Marzano" tomatoes was 74.5 % and 79.75 %, respectively. In the health test, there was incidence of fungi belonging to the genera *Aspergillus* spp., *Curvularia* sp., *Rhizopus* spp. and *Gliocladium* sp. (Figure 1).

In the Ipa 6 variety seeds, 50.5% infected seeds and 49.5% healthy seeds were observed. Furthermore, there was highest incidence of *Aspergillus fumigatus* (26 %) and least occurrence of *Gliocladium* sp. (6 %). In the San Marzano variety seeds, the highest incidence was observed for *Aspergillus flavus* (26 %) and the lowest was for *Curvularia* sp., with only 11% incidence, and there were 52% infected seeds and 48% healthy seeds. The results confirmed the potential of the seeds as main disseminating vectors of plant pathogen microorganisms. According to Flávio et al. (2014), the seed is the most efficient medium for pathogen dissemination, favoring introduction of diseases in new areas and reducing the production of determined crops. Marassi et al. (2008) reported that the high incidence of plant pathogens in seeds is related to the conditions of a tropical climate, which is characterized by high temperatures and air relative humidity levels, accelerating colonization of fungi. Similar results to those of the present study were reported by Braga et al. (2010) who studied two batches of tomato cultivar UC-82 seeds and obtained incidences for the fungi *Aspergillus* spp. (45.45 %), *Penicillium* spp. (37.71 %), *Fusarium* spp. (5.72 %) and *Colletotrichum* spp. (6.39 %) in the health test.

The genera *Aspergillus* sp. and *Rhizopus* sp., were considered storage fungi. These are common in vegetable seeds and damage germination and vigor. Oliveira et al. (2009) stated that these storage fungi, called saprophytes, are opportunistic because they can invade germinated seed tissues and contribute to their loss of viability under favorable conditions.

Quantification of the transmission rate of the main fungi associated to the tomato seeds

Quantification of the transmission rate in the seeds of the two tomato varieties, San Marzano and Ipa 6, showed that the fungi *A. flavus*, *A. fumigatus*, *A. niger*, *R. stolonifer* and *Curvularia* sp. were present in all the seedling organs at 7, 14 and 21 (d.a.s.). The highest mean transmission rates, in the three assessment periods, in the seed of the tomato variety San Marzano were 80.3 %, for *A. flavus* in the main root; 87.1 % for *A. fumigatus* and *A. niger* in the stalk and this last fungus had 88 % transmission for the leaves. In "Ipa 6" tomato seeds, the highest mean transmission rates, at three times and three seedling organs were for the fungus *Rhizopus stolonifer* with 88.8; 79.8 and 8.9 %, for the primary root, stalk and leaves, respectively (Table 1). These results are in agreement with Casa et al. (2006), who stated that the pathogen makes use of organs or parts of the host for its survival and dissemination.

Silva et al. (2014) observed results similar to those of the present study in rice seeds when they reported that the fungus *Curvularia lunata* was detected in all the seedling organs at the three assessment times, where the mean transmission rates in the three periods were 51.75, 44.16 and 73.12 %, for the primary root, stalks and leaves, respectively. Corroborating this study, Casa et al. (2006) stated that fungi associated with the seed can cause deterioration, or be transmitted to the seedling, colonizing the root and canopy organs. Furthermore, Bedendo (2011) and Mazaro et al. (2009) stated that in tomato crop some of these fungi, which are possibly associated with seed, especially *Rhizoctonia*, *Pytium*, *Phytophtora*, *Colletotrichum*, *Phoma*, *Fusarium*, *Helmithosporium*, *Cercospora* and *Botrytis*, can cause seedling rot, also known as *damping-off*, resulting in depressed regions in the young plants tissues that provoke stalk cracking or constriction, leading to the fall of the seedling. However, it was not observed in the present study.

Assessment of plant pathogen control in tomato seeds using in vitro treatment with plant extracts

Analysis of factorial extracts versus varieties, we observed that only Eucalyptus and Basil could significantly control the presence of *Curvularia* for the best extracts on "Ipa 6", while for the variety "San Marzano" the Neem, the Cinnamon and the Basil, were the best controllers (Figure 2).

For the other fungi, there was no significance in the joint analysis. The data presented separately for each variety are Table 2, where it can be observed that to the results obtained, all the aqueous extracts tested in the present study were able to control most of the fungi on the "San Marzano" tomato seeds, where the potential of the basil and cinnamon extracts was outstanding. Although, cinnamon was the only extract that did not differ significantly to control *Aspergillus* sp., it could perform 100 % to control *A. flavus*, *A. fumigatus*, *A. niger*. and *R. stolonifer*. Basil, like cinnamon, also controlled the fungi *Aspergillus* sp., *A. fumigatus*, *A. niger* and *Curvularia* sp., with 100% control. Significant statistical difference was observed for the other fungi among all the treatments and the control, but for *R. stolonifer* none of the extracts was able to obtain this difference (Table 2).

The basil treatment resulted in the lowest mean fungus incidence in the San Marzano tomato variety seeds, with a mean of 0.1 colonies/treatment. Aquino et al. (2010) stated that basil is rich in eugenol, identified as an effective component in fungus inhibition. Similar results were reported by Ribeiro (2008), who verified that clove extract inhibited the growth of *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* sp., thus confirming the potential of plant extracts to reduce plant pathogens associated with seeds. It is emphasized that, similar to basil, cinnamon is also rich in eugenoland which its fungicide potential is well known in the literature. This substance has been reported with anti-

Table 1. Pathogen transmission percentage from tomato seeds to seedlings in the varieties San Marzano and Ipa 6 at 7, 14 and 21 days after sowing). São Luís, UEMA, 2014.

D.a.s	Transmission %														
	San Marzano														
	A. Flavus			*A. fumigatus*			*A. niger*			*Rhizopus Stolonifer*			*Curvularia* sp.		
	R	C	F	R	C	F	R	C	F	R	C	F	R	C	F
7 d.a.s.	64.1	69.5	82.1	80.1	82.8	75	78.3	73.6	88.9	51.3	64.3	62.2	58.3	54.8	72.7
14 d.a.s.	100	88.7	89.4	81	96.2	72.7	92.5	92.6	90.4	98.7	76.9	62	100	81.6	63.4
21 d.a.s.	76.9	96.2	71.7	62.5	82.2	95.3	67.9	95	84.7	88.9	96.5	100	50.5	71.8	92.5
Average	80.3	84.8	81.1	74.5	87.1	81	79.6	87.1	88	79.6	79.2	74.7	69.6	69.4	76.2
Ipa 6															
	R	C	F	R	C	F	R	C	F	R	C	F	R	C	F
7 d.a.s.	58.5	39.5	65.4	55.4	21.8	49.6	96.1	71.7	84.8	78.1	99.4	80.6	57.6	90.9	86
14 d.a.s.	89.7	42.2	67.9	45.9	85.8	53.7	85.8	56.2	84.8	95.1	42.4	83.7	43.4	48.5	53.7
21 d.a.s.	90.8	62.8	81.9	74.6	83.4	44.5	47.1	69.7	30.5	93.2	97.8	90.5	74.6	48.1	86.9
Average	79.7	48.2	71.7	58.6	63.7	49.3	76.3	65.8	66.7	88.8	79.8	84.9	58.5	62.5	75.5

*D.a.s = days after sowing; R = primary root; C = stalks; F = Leaf.

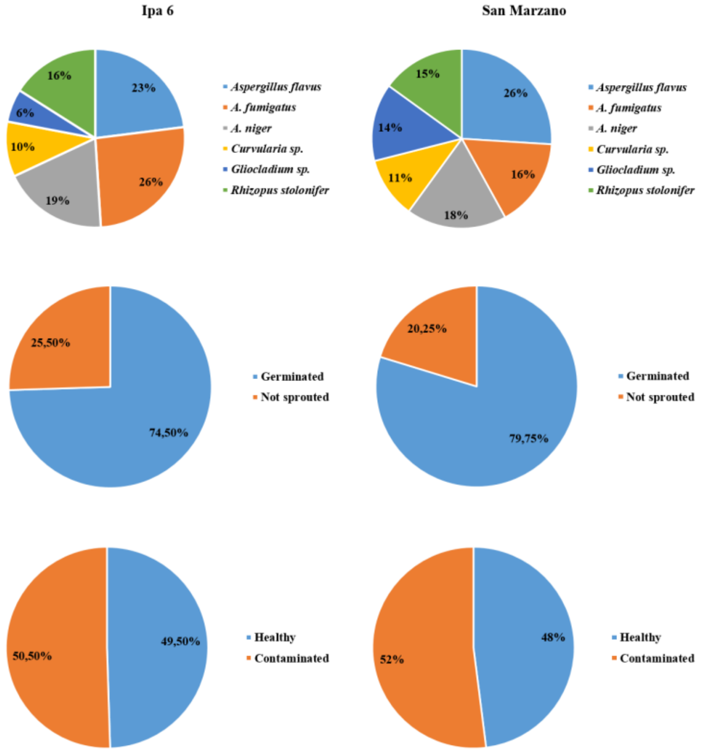

Fig 1. Health assessment of seeds of the tomato varieties San Marzano and Ipa 6 by the Blotter Test. São Luís, UEMA, 2014.

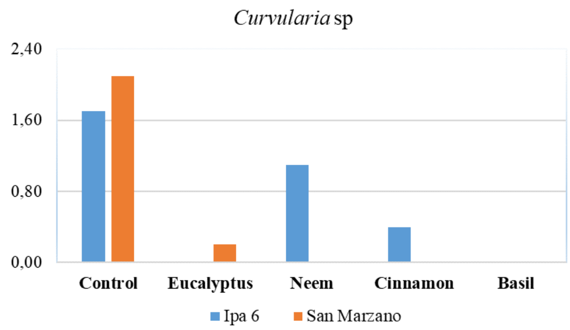

Fig 2. Effect of eucalyptus, neem, cinnamon and basil extracts on incidence of *Curvularia* sp.

Table 2. Assessment of plant pathogen incidence in 'Ipa 6' and in 'San Marzano' tomato seeds after *in vitro* treatment with plant extracts. São Luís, UEMA, 2014.

San Marzano						
Incidence		Treatments				
		Control	Eucalyptus	Neem	Cinnamon	Basil
Aspergillus sp.	INC	1.30 a	0.20 b	0.20 b	0.90 ab	0.00 b
	Ctr (%)		90	90	20	100
A. Flavus	INC	2.40 a	0.00 b	0.00 b	0.00 b	0.40 b
	Ctr (%)		100	100	100	60
A. Fumigatus	INC	1.70 a	0.20 b	0.00 b	0.00 b	0.00 b
	Ctr (%)		94.1	100	100	100
A. niger	INC	2.30 a	0.00 b	0.00 b	0.00 b	0.00 b
	Ctr (%)		100	100	100	100
Colletotrichum sp.	INC	0.50 a	0.00 a	0.40 a	0.00 a	0.00 a
	Ctr (%)		100	25	100	100
Rhizopus stolonifer	INC	2.10 a	0.20 b	0.00 b	0.00 b	0.00 b
	Ctr (%)		96.2	100	100	100
Average		1.50 a	0.20 a	0.50 a	0.00 a	0.20 a
Ipa 6						
Incidence		Treatments				
		Control	Eucalyptus	Neem	Cinnamon	Basil
Aspergillus sp.	INC	1.30 a	0.00 b	0.70 ab	0.20 ab	0.00 b
	Ctr (%)		100	20	80	100
A. Flavus	INC	1.40 a	0.00 b	0.00 b	0.00 b	0.50 ab
	Ctr (%)		100	100	100	46.7
A. Fumigatus	INC	2.20 a	0.00 b	0.20 b	0.00 b	0.00 b
	Ctr (%)		100	96.4	100	100
A. niger	INC	2.40 a	0.30 b	0.90 b	0.00 b	1.20 ab
	Ctr (%)		90	76.7	100	56.7
Colletotrichum sp.	INC	1.70 a	0.00 c	1.10 ab	0.40 bc	0.00 c
	Ctr (%)		100	52.9	82.3	100
Rhizopus stolonifer	INC	1.50 a	0.00 b	0.00 b	0.00 b	0.00 b
	Ctr (%)		100	100	100	100
Average		1.57	0.06	0.42	0.10	0.23

Values followed by the same letter do not differ by the Tukey test at 5%. Values transformed by √x).

Table 3. Effect of plant extracts on tomato seed germination. São Luís, UEMA, 2014.

	Germination (%)	
Tratatments	San Marzano	Ipa 6
Control	84	83
Eucalyptus	82	81
Neem	80	79
Cinnamon	81	80
Basil	83	82

fungus activity, disturbing the cytoplasmic membrane and interrupting the proton motor force, electron flow, active transport and coagulation of the cell content in filamentous fungi (Abbaszadech et al. 2014).

It was observed in the Ipa 6 variety seeds that the treatments provided more than 50% control of most of the fungi, except for the neem and basil treatments that obtained a control percentage of only 20 % and 46.7 % over the fungi *Aspergillus* sp. and *A. flavus*, respectively, which were not significantly different from the control (Table 2). It was also found that the neem treatment do not differ from the control nor from the cinnamon treatments to control incidence of *Curvularia* sp. (Figure 2). For the same pathogen, the cinnamon, eucalyptus and basil treatments differed from the control. However, it is known that neem affects various species of organisms, including fungi and bacteria. Souza (2002) stated that the observed effects were probably due to various substances, including azadirachtin.

There was statistical difference between all the treatments regarding *R. stolonifer* and *A. fumigatus* incidence compared to the control. The eucalyptus aqueous extract resulted in the lowest mean fungus incidence, with 0.06 colonies/treatment, with 100% control of most of the pathogens such as *Aspergillus* sp., *A. flavus*, *A. fumigatus,* and *R. stolonifer*, (Table 2). According to Vitti and Brito (1999), the eucalyptus (*E. citriodora*) is rich in citronellal of the aldehyde group that has anti-fungus and bacteriacide properties. Similar results were reported by Camatti-Sartori et al. (2011) in an *in vitro* test, where extracts of horse tail (*Equisetum* ssp.), bay (*Laurus nobilis* L.), mint (*Mentha* spp.) and eucalyptus at 50% concentration inhibited the fungus *Botrytis* sp. Corroborating the results of the present study, *sp., Cercospora kikuchii, Colletotrichum sp., Fusarium solani and Phomopsi*s sp., confirming the anti-fungal potential of plant extracts. Studies in the literature confirm the findings of the present study regarding the anti-fungal capacity of aqueous extracts. Celoto et al. (2008) reported that the aqueous extract of loofah (*Luffa acutangula* Mill.), Eucalyptus (*Eucalyptus citriodora*), erva-santa-maria (*Chenopodium ambrosioides* L.) and bauhinia (*Bauhinia* spp.) inhibited the germination of spores of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc more than 90%.

Regarding the seed germination of the two tomato varieties, taking as standard 80% germinated seed, the plant extracts did not interfere the seed physiology (Table 3). In the contrary Ferreira et al. (2007) verified the effects of *C. citriodora* extracts on *Bidens pilosa* L. (black-jack) seeds, that reduced the IVG of these seeds. Studies by Khan and Kumar (1993) on wheat seeds showed that the use of medicinal plant extracts as seed pre-treatment reduced harmful microflora and increased the germination power.

In the present study, the incident of pathogens was decreased in the seeds when they were treated with plant extracts, but the effects of the treatments were different according to the pathogen type.

Materials and Methods

Plant materials

The experiments were carried out in the Plant Pathology Laboratory at the State University of Maranhão – UEMA, Brazil. Untreated commercial tomato seeds were used of the varieties San Marzano and Ipa 6, and six plant extracts of basil, cinnamon, eucalyptus and neem, obtained from the leaves.

Tomato seed health assessment by the Blotter Test

The seed samples were first disinfested for five minutes by immersing in a sodium hypochloride solution (NaOCl), with 1.5 % active chlorine and then washed twice in distilled water.

These seeds were then placed and spread on previously disinfected Petri dishes containing three layers of sterilized filter paper moistened with distilled water. A total of 400 seeds were plated, following the Pre-established Seed Analysis Rules (Brasil, 2009), divided into eight replications of 20 seeds each. The seeds were incubated in conditions of a 12-hour light period at a temperature of approximately 26±5 ^{0}C, for seven days (Pinto, 2005). The fungus population of the non-germinated seeds and seedlings was verified using a stereoscopic microscope (magnification 40x), seven days after plating. The colonies developed on the seeds and seedlings were transferred to PDA (Potato–Dextrose–Agar) culture medium for identification using micro-cultures.

Venturoso et al. (2011) reported that the extracts of clove, garlic and cinnamon were the most promising for reducing growth of the plant pathogens *Aspergillus* sp., *Penicillium*

Quantification of the fungus transmission rate in tomato seeds

The seeds were sown on 12 trays containing substrate consisting of a mixture of autoclaved soil, coarse sand and vermicullite at the proportion 3:1:1. One hundred seeds were sown on each tray. Soil moisture was kept at field capacity and the assessments were made at three times, at 7, 14 and 21 days after sowing (d.a.s.).

At each time 100 seedlings were collected randomly, washed in running water to remove excess soil adhering to the root system and taken to the Plant Pathology Laboratory. Structures were removed from each seedling (main root, stalk and leaves) and then the material was cleaned in sodium hypochlorite (1 %) for 3 min, and washed in sterilized distilled water. The plant tissues were plated on Petri dishes, containing PDA culture medium with the addition of the antibiotic ampicillin at 200 mg/l. The material was incubated for seven days in BOD, at 25 ºC and 12 h light period. The organ was considered infected, where the fungus colony and/or structures were identified under stereoscopic microscope

The data were expressed as transmission rate of the fungus from the seed, for each organ of the seedling, according to their incidence in the seed and the respective structures were assessed at different times. The transmission percentage of each pathogen was then determined using the formula by Goulart (1996):

$$\text{The fungus transmission (\%)} = \frac{\text{\% Seedlings with determined pathogen}}{\text{Incidence of these pathogens in the seeds}} \times (100)$$

Assessment of plant pathogen control in tomato seeds by in vitro treatment with plant extracts

The aqueous extracts were obtained from neem, eucalyptus, cinnamon and basil (*Ocimum basilicum* L.) leaves that were dried, ground and immersed in distilled water for 24 hours to extract the compounds, then filtered through gauze, centrifuged for two minutes at 1800 rpm and filtered again through 22 µm cellulose membrane, attached to a 20 ml syringe.

Aqueous extracts were prepared at 0.5% concentration, in which the seeds were immersed for 10 minutes. After treatment, the seeds of each variety were plated in Petri dishes containing PDA culture medium and incubated at 22±2 °C under an illumination regimen of 12 hours light/12 hours dark. Pathogen incidence was assessed after seven days, by examining the seeds individually under a stereoscopic microscope to observe the plant pathogen incidence. A complete randomized experimental design was used with five treatments and five replications, where each plate contained 20 seeds and constituted an experimental unit.

Acknowledgments

The authors thank CAPES, for the study grant provided to the first author, the Maranhão Foundation for Support for Research and Scientific Development - FAPEMA and the graduation program in agroecology at the State University of Maranhão.

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