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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, quantify the seed- seedling pathogen transmission and assess the effect of plant extracts in reducing plant pathogens in the seeds of the tomato varieties San Marzano and Ipa 6. For 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 seding), in 100 seedlings. The plant (main root, stalk and leaves) tissues were plated in PDA culture medium and assessed after seven days incubation. Aqueous extracts were prepared from cinnamon, basil, neem and eucalyptus with 0.5% concentration and the seeds were immersed for 10 minutes, plated and assessed after seven days. In the health test there was biggest incidence of *Aspergillus fumigatus* (26 %) and *Aspergillus flavus* (26 %) 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 the transmission quantification in the seeds of the two tomato varieties. The basil extract treatment resulted in the least fungus incidence in the transmission quantification of 'San Marzano' tomato seeds, while in the of Ipa 6 seeds it was the eucalyptus treatment. Interference from the treatments was not observed in the tomato seed germination. However, there was decrease in pathogens in the seeds treated with the plant extracts, and differentiated treatment effect was observed according to the pathogen.

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

Introduction

Vegetables in general 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 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), in helping to inhibit the development of several plant pathogens of a fungal nature. More recent studies evaluating the control of diseases in the tomato crop, can also be cited, such as Silva et al. (2017) and Kobayashi and Amaral (2018), both 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, quantify the seed– seedling pathogen transmission and assess the effect of plant extracts on reducing plant pathogens associated to 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, and 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 of *Aspergillus flavus* (26 %) and the lowest was of *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, that accelerates colonization by 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., considered storage fungi, 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, under favorable conditions, invade germinated seed tissues and contribute to their loss of viability.

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 detected 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 th "Ipa 6" tomato seeds, the highest mean transmission rates, at the three times and in the three seedling organs, were for the fungus *Rhizopus stolonifer* with 88.8; 79.8 and 8.,9 %, respectively, for the primary root, stalk and leaves (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 %, respectively, for the primary root, stalks and leaves. Corroborating this study, Casa *et al*. (2006) stated that fungi associated to the seed can cause its deterioration, or be transmitted to the seedling, colonizing the root and canopy organs. Furthermore, Bedendo (2011) and Mazaro *et al.* (2009) stated that in the tomato crop some of these fungi, that may be associated to the seed, especially of the genera *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, that was not observed in the present study.

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

Analyzing the factorial extracts versus varieties, it was only significant for the *Curvularia* sp. where it can be observed that the best extracts for the variety "Ipa 6" were Eucalyptus and Basil, because they controlled the presence of this microorganism, already for the variety "San Marzano" the Neem, the Cinnamon and the Basil, were the one that controlled better (figure 2).
For the other fungi, there was no significance in the joint analysis, with this, the data are presented for each variety separately in table 2, where it can be ob- served that to the results obtained, all the aqueous extracts tested in the present study were able to control most of the fungi present on the "San Marzano" tomato seeds, and the potential of the basil and cinnamon extracts was outstanding. Cinnamon, although it was the only extract that did not differ significantly from the control in relation to the fungus *Aspergillus* sp., obtained 100 % control of the fungi found *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 to seeds. It is emphasized that, similar to basil, cinnamon is also rich in eugenoland 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.

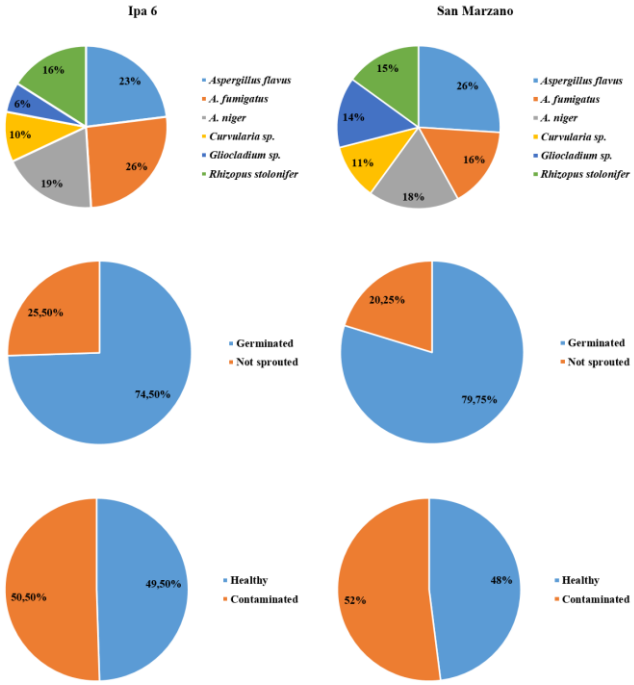

Fig 1. Health assessment of seeds of the tomato varieties San Marzano and Ipa 6 by the Blotter Test. São Luís, UEMA, 2014.

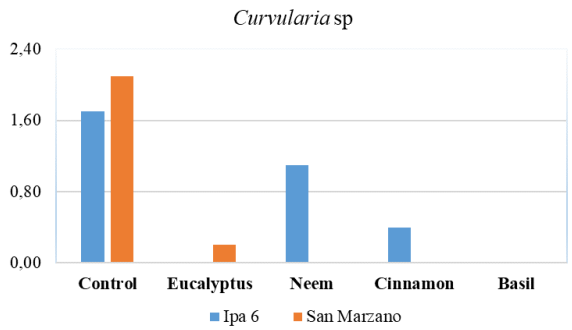

Table 2. Assessment of plant pathogen incidence and control in 'Ipa 6' and in 'San Marzano' tomato seeds by *in vitro* treatment with plant extracts. São Luís, UEMA, 2014

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 (%)	
Tratamentos	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, and were not significantly different from the control (table 2). It was also found that the neem treatment not differ from the control nor from the cinnamon treatments, in relation to *Curvularia* sp. Incidence (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 effects observed probably result from 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 & Brito (1999), this is because 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, 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* sp., *Cercospora kikuchii*, *Colletotrichum* sp., *Fusarium solani* and *Phomopsis* sp., thus 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 by more than 90% the germination of spores of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Regarding the seed germination of the two tomato varieties, taking as standard 80% germinated seed, the plant extracts used not interfere in the seed physiology (table 3). Contrary results were reported by Ferreira *et al*. (2007) who verified the effects of *C. citriodora* extracts on *Bidens pilosa* L. (black-jack) seeds, that reduced the IVG of these seeds. Studies by Khan & Kumar (1993) on wheat seeds showed that the use of medicinal plant extracts in prior seed treatment promoted reduced microflora and increased the germination power.

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

Materials and Methods

Plant materials

Extraction from the plants! (must be detailed how the plant extractions were done and how all have been the same concentraton of 0.5% and how authors think that this concentration would have been sufficenit to control fungi (compare to chemical).

I think authors should have used a negative control (water) and positive control (such as one of the polular fungicides) to compare the efficiency of plant extracts with them.

Experiment location

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.

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 pathogens was then determined using the formula by Goulart (1996):

$$\text{Of the fungus transmission (\%)} = \frac{\text{\% Seedlings with determined pathogen}}{(100)} \times$$

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 a 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.

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References

Aquino LCL, Santos GG, Trindade RC, Alves JAB, Santos PO, Alves PB, Blank AF, Carvalho LM (2010) Atividade antimicrobiana dos óleos essenciais de erva-cidreira e manjericão frente a bactérias de carnes bovinas. Alimentos e Nutrição Araraquara, 21 (4): 529-535.

Bedendo IP (2011) Damping-off. In: Bergamin Filho A, Kimati H, Amorim L (Ed.). Manual de fitopatologia: princípios e conceitos. 3.ed. São Paulo: Agronômica Ceres. (1): 820-828.

Braga MP, Olinda RA, Homma SK, Dias CT (2010) Relações entre tratamento térmico, germinação, vigor e sanidade de sementes de tomate. Revista Brasileira de Sementes, 32 (1): 101-110.

BRASIL, Ministério da Agricultura e Reforma Agrária (2009) Regras para análise de sementes. Brasília: SNDA/DNVD/CLAV.

Camatti-Sartori V, Magrini Fe, Crippa LB, Marchett C, Venturin L, Silva-Ribeiro RT (2011) Avaliação in vitro de extratos vegetais para o controle de fungos patogênicos de flores. Revista Brasileira de Agroecologia. 6 (2): 117- 122.

Casa RT, Reis EM, Nerbass FR (2006) Implicações epidemiológicas da transmissão de fungos em sementes de milho. In: Manejo de doenças de grandes culturas: feijão, batata, milho e sorgo. Lavras: UFLA, p.202-212.

Celoto MIB, Papa MSF, Sacramento LVS, Celoto FJ (2008) Atividade antifúngica de extratos de plantas a *Colletotrichum gloeosporioides*. Acta Scientiarum, 30 (1): 1-5.

Ferreira MC, Souza JRP, Faria TJ (2007) Potenciação alelopática de extratos vegetais na germinação e no crescimento inicial de picão-preto e alface. Ciência e Agrotecnologia, 31: 1054-1060.

Flávio NSDS, Sales NLP, Aquino CF, Soares EPS, Aquino LFS, Catão CRM (2014) Qualidade sanitária e fisiológica de sementes de sorgo tratadas com extratos aquosos e óleos essenciais. Semina: Ciências Agrárias, 35 (1): 7-20.

Goulart ACP (1996) Transmissão de *Bipoloaris sorokiniana* de sementes ao colóptilo do trigo. Summa Phytopatologica. 22 (1): 5-9.

Itako AT, Schwan-Estrada KRF, Stangarlin JR, Tolentino JR JB, Cruz M (2009) Controle de *Cladosporium fulvum* em tomateiro por extratos de plantas medicinais. Arq. Inst. Biol., São Paulo. (76): 75-83.

Kobayashi BF, Amaral DR (2018) Efeito de extratos vegetais de plantas do Cerrado para controle de pinta-preta em tomateiro. Summa Phytopathol, Botucatu, 44(2): 189-192,

Khan MI, Kumar R. (1993) Antifungal activity of leaf extracts of neen on seed mycoflora of wheat. Seed Pathology and Microbiology, 5:13-14.

Leite CD, Maia AJ, Botelho RV, Faria CMDR, Machado D (2012) Extrato de alho no controle in vitro e in vivo da antracnose da videira. Revista brasileira de plantas medicinais, Botucatu, 14:556-562.

Marassi AC, Barbosa TS, Keller LAM, Rodrigues MAA, Kruger CD, Rosa CAR (2008) Microbiota isolada de amostras de arroz provenientes do Estado do Maranhão destinadas ao consumo humano, em áreas de ocorrência de beribéri. Revista Ciência Vida. Seropédica, RJ, EDUR, 28.

Mazaro SM, Wagner JR A, Santos I, Citadin I, Possenti JC, Gouvêa A, (2009) Controle do tombamento de plântulas de beterraba e tomate pelo tratamento de sementes com quitosana. Pesquisa Agropecuária Brasileira, 44:1424-1430.

Oliveira MDM, Nascimento LC, Alves EC, Gonçalves EP, Guedes RS (2009) Tratamentos térmico e químico em sementes de Mulungu e efeitos sobre a qualidade sanitária e fisiológica. Revista Caatinga, 22 (3): 150-155.

Pinto NFJA (2005) Análise sanitária na produção de sementes de grandes culturas. In: Zambolin L, Sementes qualidade fitossanitária. Viçosa: Universidade Federal de Viçosa, 295-332.

Piveta G, Menezes VO, Pedroso DC, Muniz MFB, Blume E, Wielewicki AB (2010) Superação de dormência na qualidade de sementes e mudas: influência na produção de *Senna multijuga* (L. C. Rich.) Irwin & Barneby. Acta Amazônica, 40 (2): 281-288.

Ribeiro, VV (2008) Efeitos de fungicida e produtos naturais sobre o desenvolvimento de *Fusarium oxysporum* f. sp. *tracheiphilum* em sementes de caupi. Areia: UFPB.

Santos Neto J, Schewan-Estrada, KRF Temporal WM, Andrade LMA, Sena JO (2016) Subprodutos de capim-limão no controle de septoriose do tomateiro cultivados em sistema de produção orgânico. Revista Brasileira de agroecologia. 11(1):35-44.

Silva EO, Alves E, Ferreira TC, Albuquerque,CAC (2017) Óleos essenciais no controle da pinta bacteriana e na ativação de respostas bioquímicas em tomateiro. Summa Phytopathol, Botucatu, 43(3):212-217.

Silva MSBS, Rodrigues AAC, Oliveira LJMG, Silva EKC, Pereira TS (2014) Sanidade de sementes de arroz, biocontrole, caracterização e transmissão de *Curvularia lunata* em semente-plântula de arroz. Revista Ceres, 61 (4): 511-517.

Silva JAB (2015) Regulação da biossíntese da vitamina E em tomateiro (Solanum lycoersicum L.): da diversidade natural à manipulação do metabolismo. São Paulo: Instituto de Biociência, Universidade de São Paulo.

Souza MAA, Borges RSOS, Stark MLM, Souza SR (2002) Efeito de extratos aquosos, metanólicos e etanólicos de plantas medicinais sobre a germinação de sementes de alface e sobre o desenvolvimento micelial de fungos fitopatogênicos de interesse agrícola. Revista Universidade Rural (22):181-185.

Tomazoni EZ, Giani SG, Ribeiro RTS, Pauletti GF, Schwambach J (2013) Atividade antifúngica do óleo essencial de *Cinnamomum zeylanicum* Ness sobre fungos

fitopatogênicos do tomateiro (Lycopersicon esculentum Mill.). Cadernos de Agroecologia, (11) 1-5.
Venturoso LR, Bacchi LMA, Gavassoni WL, Conus LA, Pontim BCA, Bergamin AC (2011) Atividade antifúngica de extratos vegetais sobre o desenvolvimento de fitopatógenos. Summa Phytopathologia, 37 (1): 18-23.
Vitti MAS, Brito JO (1999). Avaliação do rendimento e do teor de citronelal do óleo essencial de procedência e raças locais de *Eucalyptus citriodora*. Scientia Florestalis, (56): 145-154.