Plant extracts in the control of plant pathogens seeds and fusariosis in okra[1]

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ABSTRACT

The objective of this study was to assess seed health and the effect of plant extracts on *Fusarium oxysporum* f. sp. *vasinfectum* mycelial growth and sporulation, plant pathogen reduction in seeds and fusariosis control in okra seedlings. Health was assessed by the *Blotter Test*, after immersing the seeds in natural extracts and plating. To verify the mycelial growth of the plant pathogen, the extracts were incorporated in PDA culture medium and the pathogen was added and assessed, including the spore count. In the greenhouse, the seeds were immersed in the extracts, sown and at 15 days they were sprayed with extracts, at 21 days inoculated with the pathogen and disease severity on the seedlings was assessed 7 days later. There were higher incidences of *Fusarium* sp. and *Rhizopus stolonifer* in the seeds of the cultivars Valença and Santa Cruz 47. The extracts that resulted in lower pathogen incidence was basil, in cv. Valença and cinnamon and neem, in cv. Santa Cruz 47. The neem treatment presented the best inhibition percentage and the lowest sporulation mean in mycelial growth of the pathogen. Neem presented higher disease incidence control in the seedlings of cv. Valença and basil on the seedlings of cv. Santa Cruz 47.

Keywords: *Abelmoschus esculentus*; *Fusarium oxysporum* f. sp. *Vasinfectum*; Alternative control; Seed treatment.

INTRODUCTION

The okra (*Abelmoschus esculentus* (L.) Moench) is a vegetable belonging to the family Malvaceae. Due to its nutritional value and wide market acceptance, the crop has expanded significantly in Brazil (Paes *et al.,* 2012).

It is known that most vegetables are seed propagated, including the okra, and the association of plant pathogen fungi in seeds has become a concern, as it is harmful at all crop production stages because it damages the seed health quality (Santos *et al.,* 2016). This quality is fundamental in guaranteeing successful production of this vegetable. A very important aspect is the control of diseases transmitted by these seeds, because transmission over long distances needs to be reduced and prevented, which can be achieved by seed health treatment that can reduce and control the causal agents of these diseases without damaging the seed physiological potential (Braga *et al.,* 2010; Flávio *et al.,* 2014).

For the control of these pathogens, the activity of secondary plant compounds for the control of pathogens has become an alternative, but this is only possible because

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several plants have a variety of substances with toxic effects on pathogens (Pimenta *et al.,* 2019).

Studies in the literature report the efficiency of plant extracts obtained from various species, including basil (*Ocimum basilicum* L.) (Dourado *et al*., 2020), garlic (*Allium sativum* L.), rosemary (*Rosmarinus officinalis* L.) (Leite *et al.,* 2012), citronella (*Cymbopogon nardus* (L.) Rendle) (Baseggio *et al.,* 2019) on the inhibition of fungal plant pathogen development.

Thus, the objective of the present study was to assess seed health and the effect of plant extracts on the mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *vasinfectum,* reduction of plant pathogens in seeds and fusariosis control in okra seedlings.

MATERIAL AND METHODS

Experiment Location

The experiments were carried out in the Plant Pathology Laboratory and in a greenhouse at the State University of Maranhão, Brazil – UEMA, using commercial okra seeds of the cultivars Valença and Santa Cruz 47 and extracts of neem (*Azadirachta indica* A. Juss), eucalyptus (*Eucalyptus citriodora* Hook), cinnamon (*Cinnamomum zeylanicum* Blume) and basil (*Ocimum basilicum* L.). The *Fusarium oxysporum* f. sp. *vasinfectum isolate* was obtained from the plant pathogen collection, "Prof. Gilson Soares da Silva"/ UEMA - São Luís- MA, Brazil.

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

Okra seed health was assessed by the *Blotter Test.* The seeds were first disinfected for five minutes by immsersing in sodium hypochloride (NaOCl) solution at 1.5% active chlorine and then washed twice in distilled water.

The seeds were then placed on disinfected Petri dishes containing three layers of sterilized filter paper moistened with sterilized distilled water. Four hundred seeds were plated following the Rule for Seed Analysis AS (Brasil, 2009), placing 20 seeds per Petri dish. The seeds were incubated under conditions of a 12-hour light period, at approximately 26 °C, for seven days. The fungus population on the non-germinated seeds and seedlings was assessed using a stereoscopic microscope (40x magnification), seven days after plating. The colonies that developed on the seeds and seedlings were transferred to Potato Dextrose Agar culture medium (BDA) for identification using microcultures.

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

The methodology by Silva *et al.* (2019) was used to obtain the aqueous extracts from neem, eucalyptus, cinnamon and basil leaves. The aqueous extracts were prepared at 5% concentration and the seeds were immersed for 10 minutes. After treatment the seeds of each variety were plated by the *Blotter Test* method on Petri dishes containing PDA and incubated at 22 ± 2 °C, under 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 completely randomized design was used, with four types of plant extracts and six replications, and each Petri dish with 20 seeds constituted an experimental unit. The control consisted only of seeds immersed in sterilized distilled water.

Effects of the plant extracts on Fusarium oxysporum f. sp. Vasinfectum mycelial growth

Ten milliliters of the aqueous extract preparations at 5% concentration were added to 200 ml previously autoclaved BDA and placed on Petri dishes. After solidification, a culture medium disc was placed in the center of each dish, containing structures of the *F. oxysporum* f. sp. *Vasinfectum* colony. The plates were then incubated in a BOD chamber at 26 ºC, with 12 hours light/ 12 hours dark. The assessment was made on the 7th day of incubation by measuring the colony diameter in two diametrically opposite directions using a millimetric ruler. A mean was defined for each colony and compared with the control. The control consisted of only the pathogen in PDA, where each dish was an experimental unit. A completely randomized design was used with five treatments (control and four aqueous plant extracts) and six replications.

The growth inhibition percentage (PIC) was calculated from the mean of the six replications of each treatment, using the equation (Menten *et al.,* 1976):

$$PIC = \frac{Control\ growth\ -\ Treatment\ growth\ x\ 100}{Control\ growth}$$

Effect of plant extracts on Fusarium oxysporum f. sp. vasinfectum conidia production

The conidia production inhibition test was carried out at the end of the mycelial growth inhibition test for *F. oxysporum* f. sp. *vasinfectum,* after seven days' incubation. For this a spore suspension was prepared by adding 10 mL sterilized water to the dishes, then the colony was scraped using a microscope slide to release the conidia and filtered through gauze, and the number of spores/ml was determined using a Neubauer chamber coupled to the optical microscope. The means were compared by the Tukey test at the level of 5% probability.

Assessment of the effect of different plant extracts on fusariosis control in okra seedlings

The seeds of each cultivar studied were immersed in aqueous plant extract of neem, cinnamon, basil and eucalyptus and then sown in plastic pots, containing sterilized soil, and thinned to two plants per pot. Five days before inoculating the plant pathogen, the aqueous plant extracts were sprayed on the plants.

The okra plants were inoculated 21 days after germination, using the method of "half moon" root wounding, making a groove on one of the side of the root system with a scalpel, and then 20 mL of the inoculum suspension 1 x 10^6 conidia.ml^{-1} were applied to each plant (Menezes, 1972). Disease severity was assessed seven days after inoculation, using the scores according to Cia & Salgado (1997).

A completely randomized experimental design was used with five treatments and five replications, and one replication consisted of two plants per pot.

The means obtained in the methodologies above were compared by the Tukey test at the level of 5 % probability, using the InfoStat program (2018).

RESULTS

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

In the health quality assessment, the okra seeds cv. Valença obtained 64.89% germination percentage with 50.2% contaminated seeds and 49.8% healthy seeds. The fungus species with highest incidence was *F. oxysporum* (58.5%), while the lowest incidence was of *Aspergillus flavus* (1.7%), as shown in Table 1.

The germination percentage of the cv. Santa Cruz 47 okra seeds in the health test was 59.5%, with contaminated and healthy seed percentages of 52.86% and 47.14%, respectively. *Rhizopus stolonifer* was the fungus species with highest incidence (28.40%) and the species with the lowest incidence was *Aspergillus fumigatus* (6.72%), shown in Table 1.

Table 1: Germination percentage and plant pathogenic fungus incidence in okra seeds by the *Blotter Test* method

Fungi (%)	**Cultivars**	
	Valença	**Santa Cruz 47**
Alternaria sp.	-	7,02
Aspergillus sp.	3,87	-
A. flavus	1,57	9,5
A. fumigatus	2,0	6,72
A. niger	1,61	12,0
Chaetomium globosum	1,50	-
Curvularia sp.	5,66	26,50
F. oxysporum	58,35	16,03
Macrophomina sp.	3,0	-
Rhizopus stolonifer	13,43	28,40
Germinated (%)	64,89	59,5
No Germinated (%)	35,11	40,5
Healthy (%)	49,8	47,14
Contaminated (%)	50,2	52,86

Assessment of the incidence and control of plant pathogens in okra seeds of the cultivars Valença and Santa Cruz 47 by in vitro treatment with plant extracts

According to the results obtained (Table 2), all the treatments differed statistically for *R. stolonifer* incidence in cv. Valença, with 100% percentage pathogen control. For the fungus *F. oxysporum* the treatments differed from the control, except for the neem extract, that resulted in a control percentage of only 24% while for the other treatments the percentage ranged from 84 to 89% control. For the other pathogens, the treatments did not differ from the control for incidence. The treatment that resulted in the lowest incidence was basil, with 0.23 mean rate colony/treatment.

Table 2: Assessment of plant pathogen incidence and control in okra seeds cv. Valença by treatment with plant extracts

Incidence (Medium n° Pathogens)		Tratamentos				
		Control	Eucalyptuus	Basil	Cinnamonn	Neem
Alternaria sp.	INC	0.92 a	0.89 a	0.28 a	0.00 a	0.68 a
	(%)		17	83	100	50
Aspergillus Flavus	INC	0.55 a	0.00 a	0.00 a	0.00 a	0.00 a
	(%)		100	100	100	100
A. fumigatus	INC	1.17 a	0.00 a	0.00 a	0.00 a	0.00 a
	(%)		100	100	100	100
A. niger	INC	1.26 a	0.00 a	0.00 a	0.00 a	0.20 a
	(%)		100	100	100	95
Curvularia sp.	INC	1.26 a	0.69 a	0.62 a	0.89 a	0.35 a
	(%)		70	75	50	85
F. oxysporum	INC	2.97 a	0.63 b	0.77 b	0.91 b	2.59 a
	(%)		89	89	84	24
Rhizopus stolonifer	INC	4.14 a	0.00 b	0.00 b	0.00 b	0.00 b
	(%)		100	100	100	100
Média Inc.			0.31	0.23	0.25	0.54

Table 3 shows that for seeds of the okra cultivar Santa Cruz 47, there was significant difference among the treatments compared to the control for incidence of the fungi *A. fumigatus*, *F. oxysporum* and *R. stolonifer*, with 100% control percentage over most of these pathogens. For the incidence of the fungi *A. niger* and *Alternaria* sp., there was no statistical difference between the treatments and the control.

The treatments showed significant difference for *Curvularia* sp. when compared to the control and only the eucalyptus treatment did not differ from the control or the other treatments. The treatments that resulted in the least incidence were cinnamon and neem, with mean 0.6 rate colonies/ treatment.

Effect of different plant extracts on Fusarium oxysporum f. sp. vasinfectum mycelial growth and conidia production

Regarding the effect of the different plant extract treatments on *Fusarium oxysporum* f. sp. *vasinfectum* mycelial growth and conidia production, Table 4 shows that all the treatments differed significantly when compared to the control, but not amongst each other, except for the neem treatment that differed from the control and the other plant extracts, with 55% inhibition percentage.

It was observed for sporulation of the extract-treated pathogen that all the treatments differed statistically from the control but did not differ amongst each other. The neem extract treatment presented the best inhibition percentage with 0.17 sporulation mean.

Table 3: Assessment of the incidence and control of plant pathogen fungi in okra seeds cv. Santa Cruz 47 by treatment with plant extracts

Incidence (Medium n° Pathogens)		Treatments				
		Control	**Eucalyptus**	**Basil**	**Cinnamon**	**Neem**
Alternaria sp.	INC	0.48 a	0.00 a	0.00 a	0.00 a	0.00 a
	(%)		100	100	100	100
Aspergillus fumigatus	INC	1.42 a	0.20 b	0.00 b	0.00 b	0.00 b
	(%)		94	100	100	100
A. niger	INC	1.86 a	0.49 a	0.28 a	0.40 a	0.20 a
	(%)		79	93	86	97
Curvularia sp.	INC	1.72 a	0.57 ab	0.00 b	0.00 b	0.00 b
	(%)		84	100	100	100
F. oxysporum	INC	2.01 a	0.20 b	0.20 b	0.00 b	0.20 b
	(%)		97	97	100	97
Rhizopus stolonifer	INC	3.84 a	0.00 b	0.00 b	0.00 b	0.00 b
	(%)		100	100	100	100
Trichoderma sp.	INC	0.75 a	0.00 a	0.00 a	0.00 a	0.00 a
	(%)		100	100	100	100
Média Inc.			0.21	0.07	0.06	0.06

Table 4: Effect of different plant extracts on *Fusarium oxysporum* f. sp. *vasinfectum* mycelial growth and conidia production, on the 7th day of assessment

Treatments	**CM**	**PIC (%)**	**Sporulation**
Control	7,78 a	-	1,98 b
Basil	4,98 b	25	0,66 a
Eucalyptus	5,29 b	20	0,67 a
Neem	3,32 c	55	0,17 a
Cinnamon	4,88 b	27	0,89 a
CV (%)	8,85		53,18

CM= Mycelial Growth; PIC= Inhibition Percetage of Mycelial Growth. Values followed by the same letter in the column do not differ by the Tukey test at 5%.

Assessment of the effect of different plant extracts on fusariosis control in okra seedlings

The results presented in Table 5 show that in the cultivar Valença, the basil, eucalyptus and cinnamon treatments differed from the control, but not amongst each other. But the neem extract was outstanding, differing from both the control and the other treatments used, presenting 79.2% control of the disease incidence in the treated seedlings.

The Disease Index, presented in Table 5, varied from 20 to 28%, but the treatment with neem was outstanding with the lowest disease index of 20%.

For the effect of different plant extracts on cultivar Santa Cruz 47 okra seedlings, shown in Table 5, all the treatments differed significantly from the control, but not amongst each other.

The Disease Index ranged 18-10%, presenting 60-70% percentage control, and the treatment with basil plant extract was outstanding with 70.9% control.

Table 5: Effect of different plant extracts on okra seedlings of the cultivars Valença and Santa Cruz 47, inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*

Cultivars		Treatments					CV(%)
		Control	Basil	Eucalyptus	Neem	Cinnamon	
Valença	Notas	3,0 a	1,2 b	1,4 b	0,4 c	1,2 b	26,25
	ID(%)	48	24	28	20	26	
	Ctr(%)	-	60,5	58,3	79,2	58,3	
Santa Cruz 47	Notas	2,8 a	1,3 b	1,5 b	1,2 b	1,2 b	20,73
	ID(%)	24	18	20	18	20	
	Ctr(%)	-	70,9	60,8	65,2	60,8	

ID = Disease Index. Values followed by the same letter in the column do not differ by the Tukey test at 5%.

DISCUSSION

It is known that the genus *Fusarium*, that presented the highest incidence in cv. Valença okra seeds, includes the species *F. oxysporum* f. sp. *vasinfectum* that causes of the main disease in okra, fusaiosis. According to Bedendo (2018), the species of this genus are characterized as facultative parasite fungi that can destroy new tissues produced by germination because they are highly aggressive.

Rhizopus stolonifer, the fungus that presented the highest incidence in the cv. Santa Cruz 47 okra seeds, is considered a contaminating fungus and according to Juliatti *et al.* (2011) can deteriorate stored seeds, reducing the seed germination percentage and vigor.

Results similar to the present study for fungal species incidence in seeds were reported by Silva *et al*. (2019), who assessed okra seeds and observed a higher incidence of fungi of the genus *Fusarium* sp. Cunha *et al.* (2017) identified the presence of fungi of different genera associated to pumpkin seeds (*Cucurbita moschata* Duchesne ex Poir), including *Fusarium* sp. and *Rhizopus* sp., and the latter was one of the genera that with highest incidence in the okra seeds used in the present study. This shows the enormous potential of seeds as plant pathogen fungus transporters, and consequently, of plant diseases, so that seeds without contaminating microorganisms should be used, and healthy seeds are the most viable option. However, from the identification of the plant pathogens associated to determined seeds, the treatment method can be chosen so that physiological quality loss from the seeds in question is minimized.

In the treatment of okra seeds with plant extracts, basil, neem and cinnamon were outstanding as the best treatments for cultivars Valença and Santa Cruz 47, respectively. There are several reports in the literature on the antimicrobial effects of basil and the fungicide potential of neem and cinnamon. According to Koroch *et al.* (2017), the effects of basil may be associated to the presence of compounds such as eugenol and linalol, that act in synergy on fungus and bacteria inhibition. And according to Silva *et al.* (2012) and Gomes *et al.* (2016), cinnamon has antifungal and antibacterial activity and its main is compound eugenol, a substance with high antimicrobial power. Neem contains azadirachtin, considered a very important compound to which are attributed insecticide and antifungal actions, in synergy with other triterpenoids, geduinas, neembinm and limonoides (Maciel *et al.,* 2010; Kasper *et al.,* 2018).

Dourado *et al.* (2020) reported results similar to those of the present study for the microbial potential of basil extract when assessing bell pepper seeds cv. All Big. It was observed that the treatment with aqueous basil extract (5%) achieved best control of the fungi present in the seeds. Flávio *et al.* (2014) studied the health quality of sorghum seeds and observed that seeds treated with cinnamon extract showed reduced incidence of the fungi *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. Cardozo & Pinhão Neto (2019) reported that the use of aqueous neem leaf extract was efficient in treating cv. Super Marmande tomato seeds that corroborates the results of the present study.

There are several reports in the literature regarding the inhibitory activity of plant extracts on plant pathogens, both *in vitro* and *in vivo*. For example, Brito & Nascimento (2015) observed the efficaciousness of the inhibitory action of neem and ginger extracts on *Curvularia eragrostidis* (P. Henn.) on mycelial growth and sporulation. Silva *et* *al.* (2012) studied the antifungal activity of plant extracts and observed that pepper and neem extracts provided fungitoxicity on *Fusarium oxysporum* f. sp *vasinfectum* and *Pyricularia oryzae*, respectively. Further, Dourado *et al.* (2020) managed anthracnosis in bell pepper plants and observed the efficiency of basil extract in reducing the disease severity in the plants, thus ratifying the results of the present study for plant extract efficiency in the control of disease causing fungi.

CONCLUSIONS

In the assessment of seed health quality, there were higher incidences of *Fusarium* sp. and *Rhizopus stolonifer* in the seeds of the cultivars Valença and Santa Cruz 47, respectively.

The extracts that provided the lowest plant pathogenic fungus incidence in the treated seeds were basil, in cv. Valença; and cinnamon and neem, in cv. Santa Cruz 47.

The neem plant extract presented the highest inhibition percentage for mycelial growth of the fungus *Fusarium oxysporum* f. sp. *vasinfectum*.

The plant extracts of neem, for cv. Valença, and basil, for cv. Santa Cruz 47 were outstanding for control of disease incidence in okra seedlings.

The plant extracts tested in the present study showed great potential in plant pathogenic fungi control, and can be considered a viable alternative to chemical control. They are sustainable and easily accessible to family farmers.

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