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Hydrolats and extracts vegetable action on quality of stored castor bean seeds in non-controlled conditions

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ABSTRACT: This study aimed to evaluate the action of hydrolats and extracts of medicinal plants and herbs on the quality of castor bean seeds stored in uncontrolled conditions. The seeds used in the experiment were packed in cotton bags and stored for 12 months. During storage was carried sanity test, "Blotter test" to determine the sanitary quality of castor beans seeds. After storage, the seeds were treated with fungicide, hydrolats, and extract of medicinal plants and herbs. After that, we proceeded the sanity test again to determine the effect of seed treatment on reducing the infestation of fungi and evaluate the physiological quality of treated seeds. The experiments were set up in an entirely randomized. *F. oxysporum*, *Aspergillus* spp. and *Penicillium* spp. were observed in the seeds on storage. The physiological quality of seeds reduced with storage. Treatment with Captana, hydrolate of *L. sidoides* and *C. zeylanicum* extract drastically reduced the fungal infestation. *C. zeylanicum* extracts diminished the germination and vigor of castor bean seeds. The *Zingiber officinale* extract enables the control of *Fusarium oxysporum* and does not affect the quality of castor bean seeds.

Key words: alternative control; phytopathogenic fungi; *Ricinus communis*

Hidrolatos e extratos vegetais sobre a qualidade de sementes de mamona armazenadas em condições não controladas

RESUMO: Neste trabalho, objetivou-se avaliar a ação de hidrolatos e extratos de plantas medicinais e ervas sobre a qualidade de sementes de mamona armazenadas em condições não controladas. As sementes utilizadas no experimento ficaram armazenadas por 12 meses em sacarias. Ao longo do armazenamento foi realizado teste de sanidade, "Blotter test", para verificar quais fungos infestavam as sementes de mamona. Ao fim do armazenamento as sementes passaram por tratamento: fungicida, com hidrolatos e extratos plantas medicinais e condimentares. A seguir, realizou-se novamente o teste de sanidade para verificar o efeito do tratamento de sementes na redução da infestação dos fungos e avaliou-se também a qualidade fisiológica das sementes tratadas. Os experimentos foram instalados em delineamento inteiramente casualizado. *F. oxysporum*, *Aspergillus* spp. e *Penicillium* spp. foram os fungos observados nas sementes no armazenamento. A qualidade fisiológica das sementes reduziu com o armazenamento. O tratamento com Captan, com o hidrolato de *L. sidoides* e o extrato de *C. zeylanicum* reduziu drasticamente a infestação fúngica. O extrato de *C. zeylanicum* reduziu a germinação e o vigor das sementes de mamona. O extrato de *Z. officinale* permite o controle de *F. oxysporum* e não afeta a qualidade das sementes de mamona.

Palavras-chave: controle alternativo; fungos fitopatogênicos; *Ricinus communis*

Introduction

Production of oilseeds plants has been prominent on the national scene due to the growing interest in the production of oil for the biodiesel production. Among the cultivated oilseeds, the castor bean (*Ricinus communis* L.) has advantages for producing good quality oil to the manufacture of various industrial products, castor beans press cake is used as organic fertilizer with high C/N ratio of 11:1 (high content of nitrogen) and efficient control of some pests (Martins et al., 2011; Lins et al., 2013).

The utilization of seeds that have high genetic, physical, physiological and sanitary quality constitutes a significant factor for the establishment of crops, which enables higher productivity and income by field area (Catão et al., 2013). Thus, due to the culture of expansion potential, there is increasing demand for quality seeds. Therefore, it is extremely important to evaluate the occurrence of pathogens and ways for controlling them. Many pathogens can compromise the quality of the seeds and the inoculum; which may result in a progressive increase of diseases in the field and therefore reducing the commercial value of the crop. Furthermore, infected seeds can introduce pathogens that cause important diseases in unaffected areas.

Seed treatment with high doses and non-recommended product for management field crops brings severe problems to the environment and the health of humans and animals. Moreover, does not efficiently control diseases and pests. To minimize this impact, an alternative to control plant pathogens is the use of compounds of the secondary metabolism of plants (Aquino et al., 2014). A substance with potential users in fungal seed treatment should not only present fungicidal effect but also does not cause the inhibitory effect of physiological quality. In that context, medicinal plants and herbs have been received attention for their different potential activities such as fungicides, herbicides, insecticides, and nematicides.

The use of plant extracts is becoming increasingly important for scientific research as an alternative method to control plant diseases caused by fungi (Aquino et al. 2014; Flávio et al., 2014). However, there is a wide range of medicinal plants and herbs that have not been surveyed, and lacking information about the action of their chemical compounds in the physiological and sanitary quality of seeds. Thus, this study aimed to evaluate the action of hydrolats and extracts of medicinal plants and herbs on the quality of castor bean seeds stored in uncontrolled conditions.

Material and Methods

The experiment was conducted in the Laboratory of Pathology Plant and the Laboratory of Seeds Analysis at the Universidade Federal de Minas Gerais, Montes Claros campus, from September 2014 to September 2015). In the experiments were used the IAC-2028 castor bean cultivar produced in the 2013/2014 harvest (working seed lot). The water content of the castor bean seeds was determined by the greenhouse gases method at 105 ± 3°C for 24 hours with two samples per crops before storage (Brasil, 2009b). The seeds were also classified according to size by shaking for one minute in an oblong sieve hand, the dimensions 19/64'' x $^{3}/_{4}$'', 18/64'' x $^{3}/_{4}$'', 17/64'' x $^{3}/_{4}$'', 16/64'' x $^{3}/_{4}$'', 15/64'' x $^{3}/_{4}$'', 14/64'' x $^{3}/_{4}$'' and bottom (respectively 7.541 x 19.050 mm x 19.050 mm 7.144; 6.747 x 19.050 mm x 19.050 mm 6,350; 5,953 x 19.050 mm 5.556 x 19.050 mm and bottom). Subsequently, the retained seeds on the sieve 17/64'' x $^{3}/_{4}$'' were used for the health and physiological quality assessment, to achieve standardization of seeds and seedlings during analysis. Then, the seeds were packed in cotton bags and stored for 12 months under uncontrolled conditions of temperature and relative humidity. The monthly average temperature and relative humidity of the air storage shed were monitored using a thermohygrograph.

The hydrolats were obtained from leaves of *Lippia sidoides*, *Cymbopogon citratus* and *Ocimum gratissimum*, collected randomly in the matrix plants in four individuals per species and separately packaged in semi-impermeable plastic bags. All plants material were collected after the rinsed in tap water and disinfected with 0.5% of sodium hypochlorite for thirty minutes to eliminate microorganisms on the surface. After this period, the materials were washed with triple washing in running water to remove excess hypochlorite and dried on a paper towel for 24 hours. For the hydrosols extraction, 5 kg leaves were used for each plant species employed and the distillation of water by steam, using pilot distiller Linax (Model D20). The time for the complete extraction process was three uninterrupted hours for each plant material. At the end of the process the oil was separated from hydrolate by liquid-liquid partition, and the hydrolats stored in amber vials type and kept for three days in the freezer under non-controlled temperature and humidity (Martins et al., 2002).

The extracts were obtained from black pepper seeds (*Piper nigrum*), ginger rhizome (*Zingiber officinale*), a clove of dried flower buds (*Syzygium aromaticum*), and cinnamon bark (*Cinnamomum zeylanicum*). The extracts were produced with the addition of 50 grams of each plant species 500 mL of sterile distilled water; the material was crushed for 2 minutes in a domestic blender for extraction of active principles. After shredding each extract was filtered on sterile filter paper and kept in a clean and sterile container for seed treatment following the methodology proposed by Coelho et al. (2011).

During the storage period, it was held every 3 months the sanity test to verify the fungal occurrence on castor bean seeds. The test was conducted through "Blotter test" with freezing, the seeds being arranged in gearboxes on two sheets of paper blur kills moistened with the water-agar medium at 10%. Castor bean seeds were treated by immersion for 30 minutes in the extracts or the hydrosols mentioned above and then placed on sterilized filter paper to dry for 30 minutes. Another treatment was performanced

with the fungicide Captana 750 TS at the dose of 150 g i.a/100 kg seeds. After the treatment, the seeds were incubated for 24 hours, 12 hours of photoperiod and a temperature of 20±2°C. After that, the boxes were placed in freezing for 24 hours at a temperature of -20°C, and again packed in the same initial conditions of incubation (Brasil, 2009a). We used a completely randomized design and for each treatment 200 seeds were used, divided into 8 repetitions of 25 seeds. After seven days of incubation, it was assessed the number of infected seeds of different genera or species of fungi. Identification was made from macroscopic and microscopic observations of its features and structures.

For the evaluation of the physiological quality were used the seeds stored for twelve months, which were treated with the same extracts, hydrolats and fungicide sanity test. After the treatment, the seeds were distributed in rolls germitest paper moistened with distilled water in an amount equivalent to 2.5 times the weight of paper and placed in a germination chamber with 25°C temperature, performed according to the recommendations for Rules to Analysis of Seed-RAS (Brasil, 2009b).

The seeds were considered germinated by the occurrence of root protrusion 5 mm. At 7 days after sowing it was evaluated the first count germinated (FCG) and 14 days the number of normal seedlings, thus establishing the germination percentage (G). Daily counts radicle emission was carried out to evaluate the germination speed index (GSI). To calculate the GSI was used in the formula suggested by Maguire (1962). We used a completely randomized design (and for each treatment were used 200 seeds, divided into 8 repetitions of 25 seeds.

The evaluation of the seedlings consisted by measuring the length of hypocotyl and radicle for this, eight replications of 25 seeds were treated as described above and plated on rolls, these being kept in a semi-impermeable plastic bags, wrapped in the same light and temperature conditions as the germination test. The evaluation was performed seven days after sowing. The aid of a digital caliper was determined the length of hypocotyl and radicle, and the results are expressed in centimeters.

The data were subjected to variance and means analysis compared by the Tukey test ($p \leq 0.05$) of probability. The quantitative data of physiological quality (germination and first count) and health (significant incidents fungi in the seeds) were submitted to polynomial regression ($p \leq 0.05$). It was also performed in a Pearson's correlation coefficient analysis between the incidence of fungi, physiological quality of seeds and seedlings length. The significance of the correlation coefficients was checked by F test ($p \leq 0.05$) of probability. The percentage data were transformed to $y = \text{arc sin } (\sqrt{x}/100)$.

Results and Discussion

The average water content of castor bean seeds before storage was 6,7% with a maximum variation of 1%. The average temperature during storage was 18,6 °C, and the averages for maximum and minimum temperature was 31,2 °C and 17,8 °C. The average air relative humidity during the storage period was 77,1%, and the maximum 85% and minimum 67%.

It was observed the incidence of five genera of fungi of *Fusarium* species and on castor bean seeds at 12 months of storage (Figure 1A). However, *F. oxysporum*, *Aspergillus* spp. e *Penicillium* spp. were the main fungi with a higher incidence in the seeds throughout the storage period (Figure 1B). After twelve months storage, it was found that the incidence of F. *oxysporum* reduced to 79%, while *Aspergillus* spp. and *Penicillium* spp. increased the incidence of 65% and 24%, respectively (Figure 1B). The main fungi found in castor beans were *Cladosporium* spp., *Bipolaris* spp., *Curvularia* spp., *Aspergillus* spp., *Rhizopus* sp., *Penicillium* sp., *Rhizoctonia* sp., *Verticillium* sp., *Fusarium* sp. and *Arthrobotrys* sp. (Tropaldi et al., 2010).

Because the seeds were stored for 12 months in a non-controlled environment, it was observed an increase in the incidence of *Aspergillus* spp. and *Penicillium* spp. (Figure 1B). They are considered important storage fungi, in addiction to deteriorate grains and seeds; they are producers of mycotoxins are highly toxic to humans, animals, and plants (Reverberi et al., 2010).

The lower incidence (79%) of *F. oxysporum* in the castor bean seeds occurred after 9 months of storage (Figure 1B). The genus *Fusarium* is reported as one of the most important

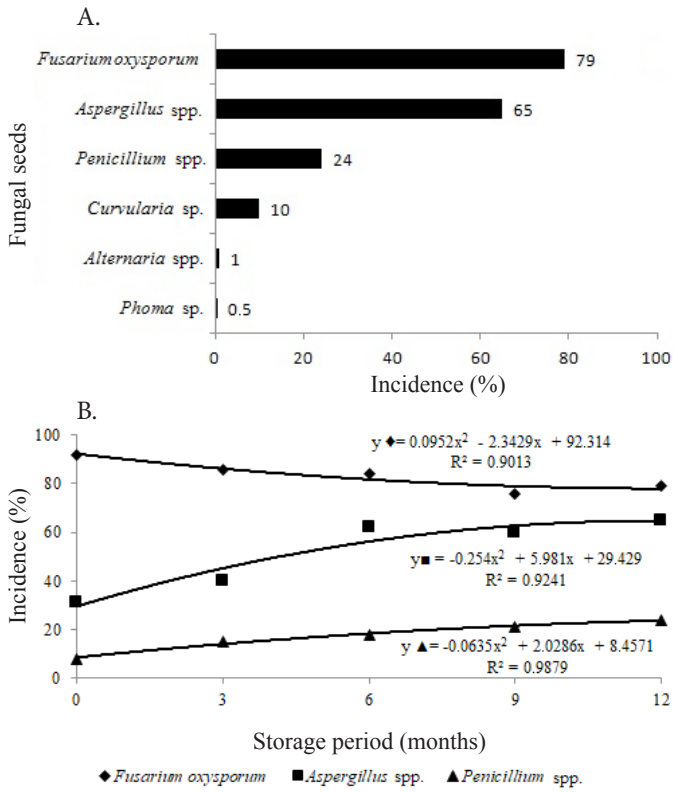

Figure 1. The incidence of fungi in castor bean seeds stored for 12 months (A) and the higher incidence of fungi during 12 months storage (B).

fungi of seeds in the castor bean crops, which can cause up to 80% loss of productivity (Mhaske et al., 2013). *Fusarium* spp. can also produce mycotoxins, reduce germination, cause discoloration or staining, damping-off, and biochemical changes in seeds. Therefore, seed treatment with systemic fungicides or its contact is critical to prevent the spread of the pathogen.

In general, all treatments reduced the percentage of pathogens in castor beans compared to control (Table 1). However, there was a dramatic reduction in the incidence of fungal performed when the treatments with the fungicide Captana, with *L. sidoides* hidrolact and *C. zeylanicum* extract. It is noted worthy that *Z. officinale* e *S. aromaticum* extracts reduced the incidence of *F. oxysporum* significantly. However these extracts did not reduce the incidence of *Aspergillus* spp. e *Penicillium* spp.

The Captana has been reported as an effective fungicide to control fungi associated with the seeds of other crops (Vazquez et al., 2014). This product also presented effective fungi control in castor bean seeds (Table 1). The antifungal effect of *L. sidoides* has also been reported to control *C. gloeosporioides* in vitro, and the main constituent of its oil compound, thymol (Veras et al. 2012; Aquino et al., 2014).

Among the extracts used, it should be noted that *C. zeylanicum* extract showed the same control efficiency of fungicide Captana (Table 1). Flávio et al. (2014) showed that the cinnamon extract was effective to control *Curvularia* sp. in sorghum seeds, reducing by 61% of infestations. Eugenol and cinnamaldehyde compounds assign the main antifungal properties of cinnamon oil. This work has proven fungitoxic effect of the aqueous extract of cinnamon, which suggests the presence of the compounds mentioned above or the interaction thereof with other compounds on the fungi present in the castor bean seeds.

The *O. gratissimu* hydrolate and *Z. officinale* and *S. aromaticum* extracts were not effective in the control of *Aspergillus* and *Penicillium* (Table 1). A similar result was obtained by Silva et al. (2012) using an aqueous extract of *O. basilicum*, and the results were unsuccessful to the mycelial control of in vitro pathogens. The *O. gratissimum* oil has antifungal, antibacterial, antidiarrheal, hypoglycemic and anti-inflammatory proprieties, and the major component of the essential oil is eugenol (Aquino et al., 2014).

Regarding the physiological quality, seed treated with *O. gratissimum* hydrolate (44%), *P. nigrum* extract (61%), *C. zeylanicum* (66%) and the control (49%) had the lowest percentage of germination (Table 1). Consequently, they showed a low germination at first countand a lower germination speed index (GSI). Therefore they were the least vigorous seeds. Hydrolats and other extracts did not affect the germination and vigor of castor bean seeds.

Although cinnamon extract has been effective in controlling fungi, it also reduced the physiological quality of castor bean seeds (Table 1). Flávio et al. (2014) reported that cinnamon extract caused a reduction in the first count germination and the germination speed index of sorghum seeds. The cinnamon extract has eugenol which is a phenylpropene (phenol) sparingly soluble in water and is present in the essential oil of some plants, giving the characteristic aroma of cloves. The phenols are plants common substances, in non-toxic quantity, and in normal conditions, however, they can be at high concentrations (Flávio et al., 2014).

The compounds that were found in essential oil extracted from *O. gratissimum* are the following: 1.8 cineole, eugenol, methyl eugenol, thymol, p-cymene, cis-ocimene and cis-caryophyllene (Biasi et al., 2009). Eugenol, monoterpenes (1.8 cineole and cis-ocimene), and terpenes (thymol and cis-caryophyllene) may also have influenced the reduction of the castor seed germination because they cause extensive damage to membranes and respiratory cells process. These components are most of the essential oils of a large number of species and have been reported as effective allelochemicals by the toxic effect on seed germination (Souza Filho et al., 2009; Flávio et al., 2014).

C. citratus and *L. sidoides* are medicinal species, with recognized production capacity of secondary compounds (Aquino et al., 2014), which have potential to be used as

Table 1. Incidence of fungi, first count germination (FCG), germination (G), germination speed index (GSI), length of the aerial part (A) and radicle (R) of seedlings from castor beans stored for twelve months in uncontrolled conditions.

Treatments	Fungi			FCG	G	GSI	A	R
	Fus	*Asp*	*Pen*	(%)			(cm)	
Control	79.00 a	65.00 a	24.00 a	45 bc	49 c	4.55 bc	7.05 b	2.30 d
Captana	0.50 e	3.50 d	0.50 d	71 a	96 a	9.69 a	13.59 a	10.08 a
Hidrolacts								
O. gratissimum	34.00 c	63.50 a	11.50 b	33 c	44 c	3.63 c	4.98 c	2.87 d
C. citratus	28.50 c	16.50 c	3.00 cd	80 a	87 ab	8.92 a	10.58 a	9.47 a
L. sidoides	7.00 d	11.50 c	3.50 cd	71 a	83 ab	8.82 a	8.18 ab	7.07 b
Extracts								
P. nigrum	42.50 b	29.50 bc	2.50 c	60 b	61 b	5.83 bc	6.96 b	5.11 c
Z. officinale	0.00 d	48.00 ab	25.50 a	76 a	81 ab	9.27 a	13.03 a	10.27 a
S. aromaticum	7.50 d	61.50 a	14.50 b	64 b	84 ab	7.16 ab	8.24 ab	7.01 b
C. zeylanicum	0.00 e	3.50 d	0.00 d	60 b	66 b	6.94 ab	6.84 b	6.05 bc
CV (%)	34.29	29.85	36.49	8.23	5.84	10.42	15.60	16.52

* Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. Fus: *Fusarium oxysporum*; Asp: *Aspergillus* spp.; Pen: *Penicillium* spp.

a bioherbicide. Magalhães et al. (2013) reported that the essential oil of *C. citrates* decreased the percentage and germination speed index of lettuce achenes compared to the *L. sidoides*. However, those compounds apparently do not provide a phytotoxic effect on castor seeds.

The action of allelochemicals is verified in lower proportion on the final percentage of germination and is the most common negative or positive effect on vigor and seedling development (Wandscheer & Pastorini, 2008). In the present study, we observed adverse effects on germination and germination rate (Table 1). Two mechanisms may be involved, the inactive of mitochondrial respiration and disturbance of Krebs cycle enzymes. In fact, during seed germination, there is a rapid increase in glycolytic activity linked to the increased respiration rate (Podesta & Plaxon, 1994). This glycolytic activity is necessary to mobilize stored carbohydrates, to provide the seed reducing power ATP and carbon products for the biosynthesis of roots and aerial part of emerging seedlings, but if the respiration process is compromised, consequently the germination is affected.

Another mechanism involved is due to disruption of the metabolic activity of enzymes that are involved in glycolysis and oxidative pentose phosphate pathway (OPPP) (Muscolo et al., 2001). The treatment with *C. citratus*, Captana, and *Z. officinale* did not inhibit the development of hypocotyl and radicle length (Table 1). The *C. citratus* essential oil did not limit the growth rootlet of seedlings of lettuce when it was compared to *L. sidoides*oil (Magalhães et al., 2013). However, the same authors point out that the concentration of these oils can cause a linear decrease in hypocotyl length of seedlings.

In this study, it was found that the concentration had differentiated action to the development of the radicle and hypocotyls (Table 1). The action of allelochemicals may vary depending on the plant organ in which they operate, can cause inhibitions in specific areas and increases in others, also being possible occur hormesis in these tissues. According to Belz et al. (2011), some substances can be toxic at high doses, beneficial or stimulatory at low concentrations. The work performed by Pina et al. (2009) the phytotoxicity varied according to the seedlings organ, and in some cases strongly influenced by the concentration of the compound.

During storage, it was also verified the reduction of seed physiological quality. Before storage, it was found that castor seeds showed good germination (95%) while the first counting was 80% (Figure 2). However, the storage period of

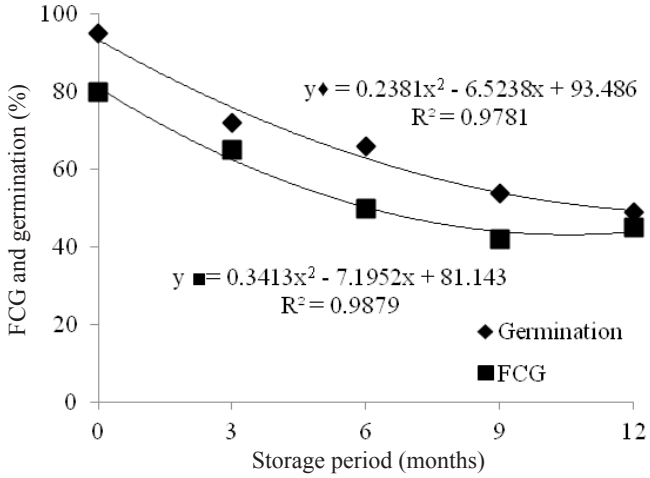

Figure 2. First counting (FCG) and germination (%) of castor bean seeds stored for 12 months in uncontrolled conditions.

seeds was observed a high reduction in physiological quality of castor bean seeds, and the regression equations presented a quadratic function and high coefficient of determination. This reduction may have been caused by the fungi effects in the seeds and the storage conditions under uncontrolled conditions for 12 months.

Another observed fact is about plants treatments that did not have control of fungal incidence (Table 1). In those treatments the incidence of fungi was high, and the germination and seed vigor were low. Pearson's linear correlation shows that fungi and physiological quality have a negative correlation (Table 2).

F. oxysporum and *Aspergillus* spp. negatively affected (r = -0.6627, r = -0.6216, respectively) the seeds germination, thus compromising their development. Therefore, it can be inferred from the fungal presence during storage, reducing the physiological quality (Figure 2). According to Catão et al. (2013), the fungi *F. moniliforme*, *Aspergillus* spp. and *Penicillium* spp. do not compromise the physiological quality of corn seeds by performing the linear correlation analysis. According to these authors, there are still controversial results when it comes to the antifungal effect on the physiological quality of seeds because the seeds can only be infested instead of being infected, beyond the level of contamination, the conditions and time of storage.

There were also negative correlations between fungi with GSI, length of hypocotyl and radicle length. It is noted the correlation between *F. oxysporum*, and the length of the radicle, through the reduction of the radicle by the

Table 2. Pearson's correlation coefficient (r) between the mean incidence of *F. oxysporum*, *Aspergillus* spp. e *Penicillium* spp., first count germination (FCG), germination (G), germination speed index (GSI) of seeds stored for twelve months in uncontrolled conditions and aerial part (A) and radicle (R) castor bean seedlings.

Fungi	FCG (%)	G (%)	GSI	A (cm)	R (cm)
	r	r	R	r	r
F. oxysporum	-0.5996	-0.6627	-0.7745	-0.6361	-0.8517
Aspergillus spp.	-0.6104	-0.6216	-0.6814	-0.6091	-0.6199
Penicillium spp.	-0.2697 ns	-0.1306 ns	-0.2983 ns	-0.0697 ns	-0.2407 ns

* Not significant (ns) level of 5% probability by F test.

pathogen (r = -0.8517). There was no significant correlation between seed quality and *Penicillium* spp. at 5% by F test. The negative correlation between soybean seed germination and incidence of fungi indicates the direct influence on the physiological quality of seed quality (Galli et al., 2005).

Conclusions

The treatments with Captana, *L. sidoides* hidrolact, and the *C. zeylanicum* extract reduce the fungus infestation on castor bean seed.

Cinnamomum zeylanicum extract presented phytotoxic effect reducing the viability and vigor of the seeds.

The *Zingiber officinale* extract enables the control of *Fusarium oxysporum* and does not affect the quality of castor seeds.

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