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RESEARCH ARTICLE

SUNFLOWER SEEDS QUALITY STORED IN UNCONTROLED CONDITIONS, TREATED WITH HYDROLATS AND VEGETABLE EXTRACTS

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ABSTRACT

Plant secondary metabolite allows the fungus control without inhibiting seed germination. Thus, the aim of this work was to evaluate the action of hydrolates and plant extracts in the control of fungi and their effects on quality of sunflower seeds stored at room temperature. The seeds were stored for 12 months in sacks. During storage, sanity test was carried out, "Blotter test", to determine which fungi infested the sunflower seeds. At the end of storage, the seeds undergone throughout fungicide treatment, with hydrolats and with vegetable extracts. After that, the sanity test was carried out again to determine the effect of seed treatment in reducing the infestation of fungi and the physiological quality (first and final count germination, GSI, hypocotyl and radicle length) of the seeds treated with the same products. The experimental design was a completely randomized design. The *Fusarium semitectum, Aspergillus* spp. *and Penicillium* spp. were the fungi with the highest incidence in the seeds during storage. The physiological seed quality was reduced with storage. Treatment with Captan 750 TS and *P. nigrum, S. aromaticum and C. zeylanicum* extractsreduce the fungi infestation and do not compromise the sunflower seeds physiological quality. For sunflower seeds stored in uncontrolled conditions, treatment is recommended.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) has a high capacity to adapt to different soils and climatic conditions, reflecting on agronomic traits, such as drought resistance, cold, excessive heat and little influence of latitude, altitude and photoperiod (Grisi *et al*., 2009). Among vegetable oils, sunflower oil stands out for its excellent physical, chemical and nutritional characteristics, being used mainly for human and animal consumption as well as raw material for biofuel production.

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To know the quality of the seeds before sowing is the most correct and safe procedure to prevent increases on the production cost of the crop. Thus, the use of seeds that have high quality becomes a major factor in the establishment of the crop (Catão *et al*., 2013). In that context, it is essential that producers have available, high quality seeds for the establishment of commercial plantations, enabling rapid development of plants, essential conditions for obtaining crops with high yields. Research with sunflower, particularly in seed quality control area, are essential for crop establishment and are justified by the potential of the specie. Many pathogens can compromise the quality of the seeds and those can be divided into two groups:

Those of the field and those of the storage. However, regardless of where the inoculum is acquired, may result in a progressive increase of a given disease and may reduce the commercial value of the crop. Furthermore, infected seeds can introduce important pathogens in areas previously free of certain diseases. Seed treatment with high doses and with not recommended products for the culture brings serious problems to the humans' health, animals and the environment, and does not efficiently control diseases and pests. To minimize that impact, an alternative on the plant pathogens control is the use of compounds of the secondary metabolism of plants (Aquino *et al*., 2014). It is necessary for a substance with potential for fungal seed treatment not only to present the fungicidal effect, but also does not cause inhibitory effect of physiological quality. In that context, medicinal and condiment plants are given special attention because of the different potential effects such as fungicides and insecticides. In recent years, the use of plant extracts is becoming increasingly important on scientific circles as an alternative method to control plant diseases caused by fungi (Flávio *et al*., 2014). However, there is a wide range of medicinal and condiment plants that have not been investigated, and exist a lack of information about the action of their chemical compounds in the physiological and sanitary quality of seeds. Thus, this work aimed to evaluate the action of hydrolates and plant extracts in the control of fungi and their effects on quality of sunflower seeds stored at room temperature.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Plant Pathology and Seeds Analysis of the Federal University of Minas Gerais, Montes Claros campus, from September 2013 to September 2014. Sunflower seeds cultivar Catissol were used in the experiments. Prior to storage the water content of sunflower seeds was determined by the oven-dry method of at 105 ± 3 ° C during 24 hours, with two samples per cultivar (Brasil, 2009). The seeds were also classified according to size by shaking them for one minute in an oblong hand sieve. The used dimensions were of 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; 7,144 x 19,050 mm; 6,747 x 19,050 mm; 6,350 x 19,050 mm; 5,953 x 19,050 mm; 5,556 x 19,050 mm and bottom). Subsequently, the seeds retained on the sieve 17/64 " x 3/4 " were used for the health and physiological quality assessments, in order to achieve standardization of seeds and seedlings during analysis. Then the seeds were stored for 12 months in sacks in uncontrolled conditions of temperature and air relative humidity. The monthly average temperatures and relative humidity of the air of the storage shed were monitored by a thermohidrograph.

The hydrolats were obtained from alecrim-pimenta leaves (*Lippia sidoides*), capim-santo (*Cymbopogon citratus*) and alfavaca-cravo (*Ocimum gratissimum*). The material was randomly collected throughout the length of matrix plants on four individuals per specie. Then the material was separately packaged in semi-impermeable. After that period, the materials were washed in a triple washing process in running water to remove hypochlorite excess, and then the materials were dried on paper towel for 24 hours. For the hidrolats extraction, 5 kg of leaves of each plant species were utilized, and the distillation of drag by steam method was used, employing a pilot distiller Linax, Model D20. The time for the complete extraction process was 3 uninterrupted hours for each plant material. At the end of the process, the oil was separated from the hydrolat by liquid-liquid partition, and the hydrolats stored in amber vials type, and kept for three days in the freezer at a not controlled temperature and humidity. The extracts were obtained from black pepper seeds (*Piper nigrum*), ginger rhizome (*Zingiber officinale*), clove dried flower buds (*Syzygium aromaticum*), and cinnamon bark (*Cinnamomum zeylanicum*). The extracts were produced with the addition of 50 grams of each plant species to 500 ml of sterile distilled water. The material was grounded for 2 minutes in a domestic blender for extraction of active principles. After milling, 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 seeds storage period, a sanity check was performed every 3 months to verify fungal infestation on the sunflower seeds. The test was conducted through the "Blotter test" with freezing. The seeds were arranged in gerboxes on two sheets of blotting paper moistened with water-agar medium at 10%. Sunflower seeds were treated by immersion for 30 minutes in extracts, or in hydrolats mentioned above, and then placed on sterilized filter paper to dry for 30 minutes (Viegas *et al*., 2005). Another treatment performed with the fungicide Captan 750 ST at the dose of 150 g ai /100 Kg of seeds.

After the treatment, the seeds were incubated for 24 hours, with 12-hour photoperiod and a temperature of 20 ± 2 °C. Thereafter, the boxes were placed in freezing for 24 hours at a temperature of -20°C, and again put in the same initial conditions of incubation. A completely randomized design was used (CRD), and for each treatment were used 200 seeds, divided into 8 repetitions of 25 seeds. After seven days of incubation, the numbers of infected seeds of different genera or species of fungi were assessed. Identification was made from macroscopic and microscopic observations of its features and structures.For the evaluation of the physiological quality, seeds stored for twelve months were used, which were treated with the same statements for hydrolats and fungicide sanity check. After treatment, the seeds were distributed in rolls of 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 Rules and recommendations for Seed-Analysis-RAS (Brasil, 2009).

Seeds were considered germinated by the occurrence of root protrusion in 5 mm. On the fourth day after sowing the first germination count (FGC) was evaluated and at 10 days the number of normal seedlings, thus establishing the germination percentage (G). Daily counts of radicle emission were carried out to evaluate the germination speed index (GSI). To calculate the GSI, the formula suggested by Maguire (1962) was used. A completely randomized design (CRD) was used and for each treatment were employed 200 seeds, divided into 8 repetitions of 25 seeds. The seedlings were evaluated by measuring the length of hypocotyl and radicle, with the aid of a digital caliper, and the results are expressed in centimeters. For that, eight replications of 25 seeds were treated as described above and plated on rolls of papers, those being kept in a semi-impermeable plastic bag, wrapped in the same conditions of light and temperature as the germination test. The evaluation was performed seven days after sowing. Data were subjected to variance analysis and means compared by the Scott-Knott test ($p \leq 0,05$) probability. The quantitative data of physiological quality (germination and first count) and sanitary

(major incidents fungi in the seeds) were submitted to polynomial regression (p = 0,05). A linear correlation analysis of Pearson between the incidence of fungi, physiological quality of seeds and seedlings length was also performed. The significance of the coefficients correlation was checked by the F test ($p \leq 0,05$) probability. The percentage data were transformed to y = arc sen ($\sqrt{\times/100}$).

RESULTS AND DISCUSSION

The average water content of sunflower seeds before storage was 7.2% with a maximum variation of 1%. The average temperature during storage was 18.6°C, and the averages for maximum and minimum temperature of 31.2°C and 17.8°C. The average relative humidity of the air during the storage period was 77.1%, and the maximum 85% and minimum 67%. The incidence of five fungi genera and one *Fusarium* specie on sunflower seeds after 12 months of storage was observed (Figure 1A). However, *Fusarium semitectum*, *Aspergillus* spp. and *Penicillium* spp., were the main fungi with a greater incidence in the seeds throughout the storage period (Figure 1B).

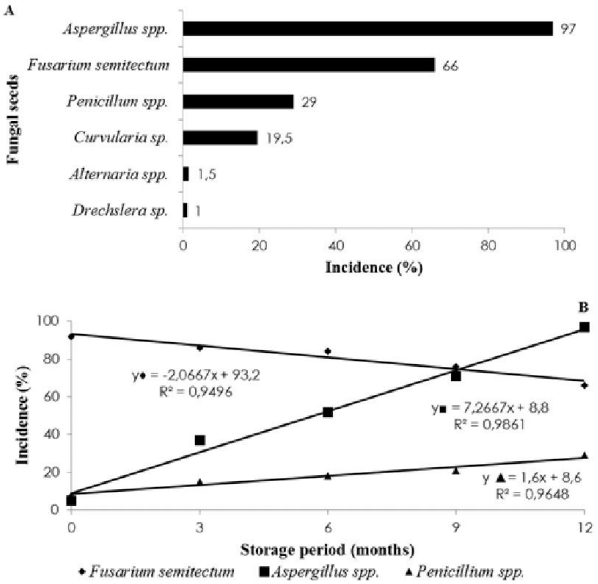

Figure 1. Fungi Effect on sunflower seeds stored for 12 months (A) and fungi with a higher incidence over 12 months of storage (B)

After twelve months storage, it was found that the incidence of *F. semitectum* reduced to 66%, while *Aspergillus* spp. and *Penicillium* spp increased the incidence of 97% and 29%, respectively (Figure 1B). Gomes *et al*. (2008) reported that the major field fungal incident on sunflower seeds were *Fusarium* spp., *Alternaria* spp., *Curvularia* sp. and *Dreschelera* sp. while, *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* were the storage fungi. Because the fact that seeds were stored for 12 months in a not controlled environment there was verified an increase in the incidence of *Aspergillus* spp. and *Penicillium* spp. (Figure 1B). Considered important storage fungi, besides deteriorating grains and seeds are producers of mycotoxins that are highly toxic to humans, animals and plants. The lower incidence (66%) of *Fusarium semitectum* in sunflower seeds occurred after 9 months of storage (Figure 1B). It was reported that the genus *Fusarium* spp. could produce mycotoxins, cause reduced germination, discoloration or staining, rotting, molds and biochemical changes in the seeds (Souza *et al*., 2007).

Despite having presented a low incidence in the seeds, *Alternaria* sp. is the main phytosanitary problem of sunflower cultivation. That pathogen can cause blight in all plant development stages, being common in leaves, petioles, stems, and flowers. In general, all treatments were also effective in reducing pathogens, especially Captan 750 TS, *P. nigrum, S. aromaticum* and *C. zeylanicum*, with control levels above 87% for *Fusarium semitectum*, 62% for *Aspergillus spp*. and 96% for *Penicillium spp*. compared to the control treatment. The Z. *officinale* extract also significantly reduced the incidence of *Penicillium* spp. presenting 100% control of the pathogen compared to the control treatment (Table 1).

The Captan 750 TS has been reported as an effective fungicide to control fungi associated with the seeds of other crops (Vazquez *et al*., 2014). That product also appeared effective in controlling fungi in sunflower seeds (Table 1). The *P. nigrum* extract significantly reduced infestation on seeds. *Piper nigrum* is an aromatic plant from the Piperaceae family and its oil is rich in dillapiol with proven fungicide properties, molluscicide, acaricide, bactericide and larvicide with the advantage of being a biodegradable product. According to Lobato *et al*. (2007), *Piper aduncum* reduced the infestation of *Aspergillus flavus, Penicillium spp*., *Fusarium spp*., *Rhizoctonia solani* and *Macrophomina phaseolina* associated with cowpea beans (*Vigna unguiculata*). Costa *et al*. (2011) reported the action of S. aromaticum in the control of *R. solani, F.oxysporum, F. solani* and *M. phaseolina*. Inhibition of fungi can be explained by the high percentage of eugenol, phenolic antiseptic compound with an already known action. The *C. zeylanicum* extract, showed similar averages to the fungicide Captan 750 TS with the same control efficiency levels (Table 1). Flávio *et al*. (2014) showed that the cinnamon extract was effective in controlling *Curvularia sp*. in sorghum seeds reducing by 61% the infestations. Viegas *et al*. (2005) also reported that the essential oil of cinnamon has antifungal properties on the development of *A. flavus*. In this work could be proven fungitoxic effects of the aqueous extract of cinnamon, which suggests the presence of the above-mentioned compounds or the interaction thereof with other compounds on the fungi present in sunflower seeds.

The hydrolats of *O. gratissimum, C. citratus* and *L. sidoides* did not show the same efficiency of the plant extracts in the fungi control. However, it has been reported in other studies the effectiveness of the active ingredients contained in these plants in the eradication of fungi (Aquino *et al*., 2014). It is noteworthy that eugenol is the major component of *O. gratissimum*, the citral in *C. citratus* and thymol in *L. sidoide*s and this compounds have antifungal, antibacterial, antidiarrheal, hypoglycemic and anti-inflammatory properties (Aquino *et al*., 2014). Sunflower seeds when treated with Captan 750 TS, *P. nigrum, Z. officinale, S. aromaticum* and *C. zeylanicum* showed equal or superior than 75% germination (Table 1), considered the minimum germination pattern of the species. Treatment with Captan 750 TS, was the largest contributor for the seeds to express the most of their viability, showing a germination of 83%. Despite the *C. zeylanicum* extract did not reduce seed germination, it has been reported causing reduction in the first germination count, germination and speed germination index of sorghum seed germination (Flávio *et al*., 2014). It is Important to remark that *C. zeylanicum* has in its constitution eugenol (phenol) and is not toxic in quantity and normal conditions, however, in high concentrations can become.

Table 1. Incidence of fungi, first count (FGC), germination (G), germination speed index (GSI), shoot length (A) and radicle (R) seedlings originated from stored sunflower seeds for twelve months in uncontrolled conditions

Treatments	Fungi			FGC (%)	G (%)	GSI	A(cm)	R (cm)
	Fus	*Asp*	*Pen*					
Control	66,00 a	97,00 a	29,00 a	25 b	32 c	2,54 c	2,12 c	1,34 c
Captan 750 TS	2,50 c	3,00 d	1,00 c	33 a	83 a	7,45 a	8,90 a	5,30 a
Hidrolats								
O. gratissimum	30,00 b	91,52 a	27,52 a	22 b	33 c	2,83 c	3,37 c	1,28 c
C. citratus	34,00 b	78,52 b	7,00 b	26 b	63 b	4,62 b	6,08 b	1,59 c
L. sidoides	27,50 b	72,00 b	10,52 b	28 b	65 b	4,81 b	5,72 b	2,15 b
Extracts								
P. nigrum	8,00 c	4,52 d	0,00 c	21 b	76 a	6,71 a	4,96 b	2,19 b
Z. officinale	20,00 b	40,52 c	0,00 c	37 a	75 a	5,49 a	7,98 a	3,78 b
S. aromaticum	6,00 c	36,48 c	2,00 c	42 a	79 a	8,64 a	8,95 a	4,65 a
C. zeylanicum	4,00 c	0,52 d	0,00 c	35 a	77 a	6,89 a	8,48 a	4,65 a
CV (%)	44,78	33,88	45,87	7,59	6,38	12,39	14,06	19,39

*Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. Fus: Fusarium semitectum; Asp: Aspergillus spp.; Pen: Penicillium spp.

Table 2. Linear coefficient of Pearson (r) between the averages of incidence of *F. semitectum*, *Aspergillus spp*. and *Penicillium spp*., first germination count (FGC), germination (G), germination speed index (GSI) of seeds stored for twelve months in uncontrolled conditions and shoot length (A) and radicle (R) sunflower seedlings

Fungi	FGC (%)	G (%)	GSI	A (cm)	R (cm)
	r	r	r	r	r
Fusarium semitectum	-0,4781^{ns}	-0,8212	-0,8547	-0,7752	-0,6950
Aspergillus spp.	-0,4702^{ns}	-0,9779	-0,8577	-0,7606	-0,8276
Penicillium spp.	-0,5582	-0,8419	-0,8683	-0,8566	-0,7200

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

The seeds of the control (32%) and the seeds treated with *O. gratissimum* hydrolat´s (33%), *C. citratus* (63%) and *L. sidoides* (65%) had the lowest germination percentages (Table 1). Consequently, also showed a low germination at first count and a lower germination speed index (GSI), and therefore less vigorous seeds. On the essential oil extracted from *O. gratissimum* compounds are found the following: 1.8 cineole, eugenol, methyl eugenol, thymol, p-cymene, cis-ocimene, and cis-caryophyllene (Biasi *et al*., 2009). The eugenol, monoterpenes (1.8 cineole and cis-ocimene), and terpenes (Thymol and cis-caryophyllene) may also have influenced the reduction of the sunflower seed germination because they cause extensive damage to membranes and respiratory process of cells. These components are the majority of essential oils of a large number of species and has been reported as effective allelochemicals by the toxic effect on seed germination (Flávio *et al*., 2014). *Cymbopogon citratus* and *Lippia sidoides* are medicinal species, with recognized production capacity of secondary compounds, which have potential for use as bio-herbicides. Magalhães *et al*. (2013) reported that the essential oil of *Cymbopogon citratus* reduced the germination and germination speed index of lettuce achenes compared to the essential oil of *Lippia sidoides*. However, it seems that these compounds apparently do not provided phytotoxic effect on castor seeds. The allelochemicals action is checked to a lesser extent on the final percentage of germination, being the most common. In the present study, negative effects on germination and germination rate were observed (Table 1). Weir *et al.* (2004) suggest that two mechanisms may be involved, the stoppage of mitochondrial respiration and disturbance of enzymes of the Krebs cycle. In fact, during seed germination there is a rapid increase in glycolytic activity linked to an increased respiration rate. This glycolytic activity is needed to mobilize stored carbohydrates, provide to the seed reducing power, ATP and carbon products necessary for the biosynthesis of roots and shoots of emerging seedlings, but if the process of respiration is compromised, consequently the germination is affected.

Another mechanism involved is due to disruption of the metabolic enzymes activity that are involved in glycolysis and oxidative pentose phosphate pathway (OPPP) (Muscolo *et al*., 2001). The lengths of hypocotyl and radicle showed greater development when treated with Captan 750 TS, *Z. officinale S. aromaticum* and *C. zeylanicum* (Table 1). The *P. nigrum* extract even without jeopardizing the physiological seed quality may have inhibited the radicle and hypocotyl due to the caused toxicity. The smallest development of hypocotyls (3.37 cm) and radicle (1.28 cm) was recorded using the hidrolact of O. *gratissimum*. In the other hand hydrolats of *C. citratus* and *L. sidoides* only inhibited the radicle of sunflower seedlings, 1.59 cm and 2.15 cm, respectively. However, Magalhães *et al*. (2013) reported that extracts of *Cymbopogon citratus* and *L. sidoides* not limited the growth of the lettuce seedling´s radicles, however, highlighted that increasing the concentration of these oils might cause a linear decrease in the length of the seedlings. In this study, it was found that the concentrations had differentiated action on radicle and hypocotyl development (Table 1). The action of allelochemicals may vary depending on the plant organ in which they operate, being capable of causing inhibitions in certain areas and increases in others, also being possible to occur hormesis occur on those tissues.

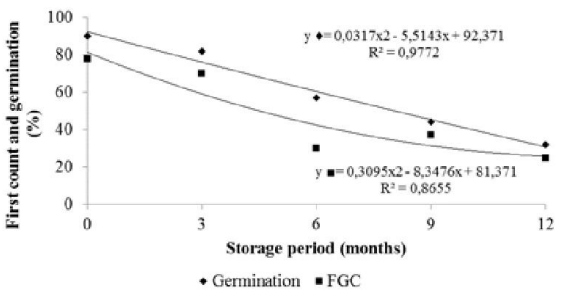

Figure 2. First count (FGC) (%) and Germination (%) of Sunflower seeds stored for 12 months in non-controlled conditions

According to Belz *et al*. (2011) some substances can be toxic at high doses, beneficial or stimulatory at low concentrations. On the work performed by Pina *et al*. (2009) the phytotoxicity varied according to the organ of the seedlings, and in some cases strongly influenced by the concentration of the compound. During storage it was also verified the reduction of physiological seed quality. Before storage, it was found that the sunflower seeds showed germination of 90%, while the first counting was 78% (Figure 2). However, with the storage of seeds period, it was observed a growing reduction in the physiological quality of castor bean seeds, where the regression equations presented quadratic behavior and high coefficient of determination. That reduction may possibly have been caused by the effects of fungi in the seeds and the storage conditions under uncontrolled conditions during 12 months. Another factor to be noted is on relation to plants treatments that did not had control over fungal incidence (Table 1). On those treatments was found that the incidence of fungi was high and germination and seed vigor were low. This became more evident when it was held the linear correlation test of Pearson between fungi (*Fusarium oxysporum, Aspergillus spp. and Penicillium spp*) and seed physiological quality (Table 2). The same happened for the correlations between the fungi with the GSI, hypocotyl length and length of radicle. It was found that there is a high negative correlation between those factors. By the linear correlation could be verified that *Fusarium semitectum*, *Aspergillus* spp. and *Penicillium* spp. jeopardized seeds quality and seedlings development. This can be seen more clearly in the control treatment, in which the seeds showed a high fungal incidence, a low physiological quality and consequently a low seedling development. The *F. semitectum, Aspergillus* spp. and *Penicillium* spp. negatively affected (r = -0.8212, r = -0, 9779; and r = -0.8419 respectively) the germination of seeds (%), thus compromising their development. Thus, it can be inferred that the presence of pathogens during storage helps to reduce the physiological quality as observed in 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. However, Galli *et al*. (2005) stated that there was a negative correlation between the germination of soybean seeds and incidence of pathogens, which caused direct influence on the physiological seed quality. Thus, there are conflicting results when it comes to the pathogen effect on the physiological quality of seeds, because the seeds can be only infested instead of being infected. Additionally, it should take into account the level of contamination, the conditions and time of storage of the seeds (Catão *et at*. 2013).

Conclusion

Treatment with Captan 750 TS and *P. nigrum*, *S. aromaticum* and *C. zeylanicum* reduce the fungi infestation and do not compromise physiological quality of sunflower seeds. For sunflower seed stored in uncontrolled conditions, it is recommended seed treatment.

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