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Aqueous Extracts of Plants on the Physiological and Sanitary Quality of Chenopodium Quinoa Seeds as an Alternative to Conventional Seed Treatment

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Abstract

This work evaluated the effect of aqueous plant extracts from chrysanthemum (*Dendranthema grandiflora* Tzvelev), cinnamon (*Melia azedarach* L.) and clove (*Syzygium aromaticum* L.) in the physiological and sanitary quality of germinating *Chenopodium quinoa* Willd (quinoa) seeds, as an alternative to seed treatment. The experiment was carried out under laboratory conditions in the year 2018. The experimental design was completely randomized in a 2×10 factorial scheme (two lots of quinoa seeds × ten doses of concentrated plant extracts), with four replicates each. The quinoa seeds were exposed to the plant extracts separately for ten minutes by submersion at the concentrations of 0, 1, 5 and 10%. The variables evaluated were germination, first germination count, field emergence, germination and emergence speed index, seedling length and sanity. The aqueous plant extracts of *Dendranthema grandiflora Tzvelev*, *Melia azadarach* L. and *Syzygium aromaticum* L. used in the quinoa seed treatment raised the emergence speed and the fungi control of these seeds within the variations and situations of each batch. The D. grandiflora extract, in all concentrations used, improved seed germination index, obtaining the highest rate of 70% in seeds treated with 5% concentration compared to the control treatment, which obtained 59%. *M. azedarach* (10% concentration) is the best treatment for emergence speed improvement, while *S. aromaticum* (10% concentration) provides the highest control of pathogens: 28% in relation to the control treatment that obtained 75%. These results highlighted the viability of the use of these species with low toxicity to man and the environment as treatment of quinoa seeds.

Keywords: quinoa, sanity of seeds, dendranthema grandiflora tzvelev, melia azedarach l, syzygium aromaticum l

1. Introduction

The Chenopodium quinoa Willd (quinoa) is a pseudo cereal from the Chenopodiaceae family, native to the Andes mountains and introduced in Brazil during the 90s. Peru is the world leading grower of quinoa with around 50 thousand tons year^{-1}, corresponding to 50.4% of the South American production (Faostat, 2014). In Brazil, however, low production levels have so far constrained the growing and trading of this pseudo cereal, demanding a considerable amount of imports in order to meet the national demand (Borges et al., 2010).

Its use as human food has grown due to its high nutritional value, low cholesterol levels and, especially, absence of gluten (Gewehr et al., 2012; Jellen et al., 2014; Strenske et al., 2015; Souza, 2016). Furthermore, quinoa seeds display fast germination in high-rainfall environments and no dormancy (Spehar and Santos, 2002), allowing its cultivation on commercial scale. Regarding production problems, quinoa seeds are particularly susceptible to pathogens attacks and the diseases resulted therein. Fungi of the genera *Aspergillus*, *Alternaria* and *Fusarium* are among the most relevant and prevalent ones, with high potential to affect the development of the plants and, consequently, the yield of quinoa seeds (Silva, 2009).

Ongoing advances in the use of chemical products (e.g. pesticides) for seed pathogens control have led to quick and significant improvements in the control of agricultural diseases, consequently increasing the overall sanitary quality of crop seeds. However, the excessive and incorrect use of these products ended up favoring the selection of pathogen strains resistant to these mechanisms (Martins et al., 2009), raising the demand for alternatives to conventional pesticides. In this context, certain substances obtained from plant extracts have been often used to control plant pathogens in seeds due to its high effectiveness and low toxicity to human health and natural environments (Venturoso et al., 2011; Medeiros et al., 2015).

Among plant species with natural insecticidal and fungicidal properties, the azedarachti of *M. azedarach* (Lovatto et al., 2012), the eugenol and beta-caryophyllene of *S. aromaticum* (Cardoso et al., 2007) and the pyrethrin of *D. grandiflora* (Gazola et al., 2009) stand out. These substances can be obtained from plants in a relatively simple way and in sufficient amount to be used in the control of fungi and insects, through extraction with aqueous or organic solvents, distillation or steam (Balandrin et al., 1985; Vivan, 2005). Several methods, however, are cited on literature for the extraction of these compounds, with the choice relying on economic viability among other factors (Franzen et al., 2018).

In this context, the aim of this work, which was to evaluate the effect of aqueous plant extracts from *D. grandiflora, M. azedarach and S. aromaticum* in the physiological and sanitary quality of quinoa seeds (C. quinoa) as an alternative to seed treatment with pesticides, has not been found in any studies. In relation to other species used as pathogen control in plants mentioned in the literature, the species chosen have in many studies been found to be highly effective and to have low toxicity to humans and the environment, besides the ease of finding them in the nature or at a relatively low cost, being easily available to agricultural producers.

2. Materials and Methods

The experiment was carried out during 2018 in a completely randomized experimental design, with a 2×10 factorial scheme (two lots of quinoa seeds × ten doses of concentrated plant extracts), and four replicates each. The lots of quinoa seeds came from an experimental cultivation area at the Santa Maria Federal University (UFSM), in 2017. The seeds that formed Lots 1 and 2 (genotypes Q-1303 and Q-1331, respectively) were harvested, cleaned and stored for five months in a cold chamber (15 °C and 40% HR), with initial moisture of 12.2%.

2.1 Plant Extracts

The treatments with plant extracts were collected from *S. aromaticum* flower buds, *M. azedarach* leaves and *D. grandiflora* leaves, according to Mazaro et al. (2008) with adaptations. The leaves of each species were grounded, separately, in a processor with 100 mL of distilled water in the concentrations of 0% (experimental), 1% (1 g 100 mL^{-1} distilled water), 5% (5 g 100 mL^{-1}distilled water) and 10% (10 g 100 mL^{-1} distilled water). The liquid obtained in each extract passed by storage process, decanting for 24h in closed locker without light and at room temperature. After the time elapsed, the filtration was held, separately, in Wathamann °1 paper and each material were identified. After the filtration process and the identification of the aqueous plant extracts of each species, the quinoa seeds were treated by submersion for 10 minutes at concentrations of 0, 1, 5 and 10% of each plant extract, and evaluated throughout germination process for physiological quality assessment.

The evaluated variables were the germination pattern test, first germination count, field emergence, germination and emergence speed index, seedling length (root and shoot) and sanity, according to the following methodologies:

2.2 Germination Patter Test and First Germination Count

Four replicates of 100 seeds were used, which were prepared in Gerbox® boxes with three sheets of filter paper moistened with distilled water, corresponding to 2.5 times their weight, and settled in a B.O.D. (Box Organism Development) germination chamber at 20 °C for six days, with photoperiod of 16 h. Vigor and germination assessments were done four and six days after sowing (DAS), and then the count of normal seedlings was carried out. Seedlings that presented more than 1.5 cm and above-ground part and root system well developed were considered normal. The results were expressed as percentage of normal seedlings, damaged abnormal seedlings, infected abnormal seedlings and total dead seeds (Brazil, 2009a). Additionally, daily evaluations were held in order to determine the germination speed index (GVI), according to the methodology proposed by Maguire (1962).

2.3 Field Emergence and Germination Speed Index

Four replicates of 50 seeds were used. Each replicate were sown in substrate, on 1 m-rows spaced 0.2 m and 0.03 m deep. The emergence speed index was determined by daily evaluations according to the methodology proposed by Maguire (1962). The emergence evaluation was held at 14 days after sowing (DAS).

2.4 Seedling Length

Four replicates of 50 seeds were used, each one were sown in Gerbox® and kept in the same germination conditions mentioned above. The length of the above-ground part and of the radicle was assessed for ten normal seedlings per replicate, at each day after sowing (DAS) (Nakagawa, 1999).

2.5 Sanity Test on Filter Paper

After submersion in each plant extract concentration, four replicates of 50 seeds each were incubated on paper substrate (Blotter Test), following the same methodology described above. Germination was inhibited by the freezing method for 24h. The seeds remained in the B.O.D for five days, at 20±2 ℃ and photoperiod of 12 h. The percentage of infested seeds and the genera of infesting pathogens were assessed with the aid of a magnifying glass (microscope stereoscope) (Brazil, 2009b).

2.6 Statistical Analysis

The data expressed as percentages were transformed into $\sqrt{x/100}$ arc sine. The analyses of data variance and mean comparison through Tukey's test ($P>0.05$) were carried out in the statistical program R Core Team (2018).

3. Results and Discussion

3.1 Germination

In the initial test, the quinoa seeds presented 59% of germination in lot 1 and 61% in lot 2. Seed vigor (i.e. first germination count) was not significantly affected by the use of plant extracts, and germination did not differ between the tested lots. However, when considering the conditions of each lot, there was an increase in germination potential when the seeds were treated with *D. grandiflora* plant extract at different concentrations, highlighting the ones treated with a 5% concentration, where 8% of germination increase was obtained. Hüller and Schock (2011) point out that germination is one of the most used parameters to identify allopathic effects, since its determination is considered of simple evaluation.

Vigor and germination of abnormal seedlings (including damaged seedlings and the ones infested in the germination test) was not significantly affected by the extracts and concentrations evaluated (Table 1). Nonetheless, when comparing each specific lot, the use of plant extracts provided a significant decrease in the number of infected and damaged abnormal plants (Table 1). The lowest indices were obtained in the seeds of lot 1, where the concentrations of 5% of *S. aromaticum* and 1% of *M. azedarach* resulted in 18% and 20% of damaged abnormal plants, respectively, when compared to the control treatment with 35% of damaged abnormal plants.

Table 1. First germination count, germination, abnormal seedlings, total dead seeds, germination speed index, emergence, emergence speed index, root length and above-ground part length of two lots of quinoa seeds (*C. quinoa*) treated with different plant extracts

Extract (%)	First germination count (%)			Germination (%)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	53^{ns}	59	56a	59^{ns}	61	60a
D. grandiflora 1%	55	57	56a	68	62	65a
D. grandiflora 5%	64	58	61a	70	60	65a
D. grandiflora 10%	55	61	58a	67	63	65a
M. azedarach 1%	64	56	60a	72	57	64a
M. azedarach 5%	56	57	57a	58	59	58a
M. azedarach 10%	57	58	58a	63	59	61a
S. aromaticum 1%	60	46	53a	63	48	55a
S. aromaticum 5%	56	52	54a	68	54	61a
S. aromaticum 10%	57	54	56a	65	56	60a
Average	58A	56A		65A	58B	
CV (%)		6.68			6.75	
Dp:		3.81			4.15	
	Abnormal seedlings (%)			Total dead seeds (%)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	35Aa	35Aab	35	7^{ns}	5	6b
D. grandiflora 1%	23Babc	27Ab	25	10	12	11ab
D. grandiflora 5%	22Bbc	28Ab	25	9	12	11ab

D. grandiflora 10%	26Babc	31Aab	28	8	7	8ab
M. azedarach 1%	20Bc	34Aab	27	9	10	10ab
M. azedarach 5%	33Aab	29Ab	31	10	12	11ab
M. azedarach 10%	23Babc	27Ab	25	15	15	15a
S. aromaticum 1%	21Bbc	45Aa	33	17	8	13a
S. aromaticum 5%	18Bc	37Aab	27	10	9	10ab
S. aromaticum 10%	26Babc	31Aab	28	10	14	12a
Average	25	32		11A	10A	
CV (%)		14.74			29.04	
Dp:		4.20			3.04	

	Germination speed index			Emergence (%)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	63^{ns}	82	72a	57	54	55b
D. grandiflora 1%	62	87	74a	65	69	67ab
D. grandiflora 5%	63	81	72a	65	60	62ab
D. grandiflora 10%	62	78	70a	67	63	65ab
M. azedarach 1%	65	79	72a	68	64	66ab
M. azedarach 5%	70	78	74a	73	69	71a
M. azedarach 10%	63	78	71a	67	67	67ab
S. aromaticum 1%	62	88	75a	66	62	64ab
S. aromaticum 5%	61	76	68a	58	69	63ab

S. aromaticum 10%	59	78	68a	68	63	65ab
Average	64A	81B		65A	64A	
CV (%)		9.82			7.14	
Dp:		7.11			4.60	

	Emergence speed index			Root length (mm)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	37Bc*	40Acd	38	1.2	2.2	1.7a
D. grandiflora 1%	48Aab	47Aabc	48	0.7	1.5	1.1b
D. grandiflora 5%	55Aa	44Babcd	50	0.6	1.5	1.0b
D. grandiflora 10%	43Abc	42Abcd	43	0.5	1.2	0.8b
M. azedarach 1%	50Aab	52Aa	51	0.6	1.5	1.0b
M. azedarach 5%	51Aab	45Babc	48	0.8	1.4	1.1b
M. azedarach 10%	48Bab	50Aab	49	0.5	1.8	1.1b
S. aromaticum 1%	45Abc	35Bd	40	0.6	1.9	1.2ab
S. aromaticum 5%	35Bc	42Abcd	39	0.7	1.8	1.2ab
S. aromaticum 10%	47Aab	39Bcd	43	0.6	1.6	1.1b
Average	46	44		0.680A	1.640B	
CV (%)		14.25			25.39	
Dp:		6.41			0.29	

	Above-ground part length (mm)		
	Lot 1	Lot 2	Mean

Control	1.8Ba	2.5Aab	2.1
D. grandiflora 1%	1.9Ba	2.1Acde	2
D. grandiflora 5%	1.7Babc	2.3Abcd	2
D. grandiflora 10%	1.8Bab	2.6Aa	2.2
M. azedarach 1%	1.4Bc	2.5Aab	1.9
M. azedarach 5%	1.6Aabc	1.9Aef	1,7
M. azedarach 10%	1.5Bbc	2.5Aabc	2
S. aromaticum 1%	1.6Babc	2.4Aabc	2
S. aromaticum 5%	1.7Babc	2.1Adef	1.9
S. aromaticum 10%	1.8Aabc	1.8Af	1.8
Average	1.680	2.270	
CV (%)		12.31	
Dp:		0.24	

Note. *Significant interaction and ^{ns} non-significant interaction of factors; the average not followed by the same letter, uppercase in the row and lower case in the column, differ by the Tukey test. CV: coefficient of variation.

The length of the root and above-ground parts of the seedlings were negatively affected by the plant extracts at 10% concentration (Table 1). Iganci et al. (2006) described that compounds with allopathic effects can inhibit germination and growth, negatively participating in cell division, in the activation of enzymes and absorbance of membranes. Although toxicity levels in the plant extracts were not detected in the tests, the reductions observed in root length and above-ground part length can be attributed to the conclusions of Rabêlo (2010) who observed that S. aromaticum essential oil is highly toxic to larvae of Artemiasalina, due to the presence of eugenol.

Emergence speed index (ESI) in the two lots of seeds was significantly improved by the plant extracts, at all concentrations. For the seeds in lot 1, the most significant value was observed in the seeds treated with *D. grandiflora* (5%), obtaining the index of 55.55 in relation to the control, which presented 37.1.

Germination speed index (GSI) did not show a significant difference in both seed lots tested. when considering the interactions within each lot, the lowest index was verified in the seeds of lot 1, which presented the value of 59 when treated with *S. aromaticum* (10%), in relation to the control, which presented 62.95 (Table 1). Similar results were found by Gonçalves et al. (2003), who concluded that seeds treated with *S. aromaticum* (10%) and subsequently stored in metal packages showed reduction of GSI.

3.2 Plant Pathogen Control

All treatments presented lower fungi incidence than the control treatment in both tested lots, but inferior results were obtained in lot 1 when compared to lot 2. Phytopathogens infested 75 and 25% of the quinoa seeds from lots 1 and 2, respectively, with predominance of the genera *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Sclerotinia* spp. (Table 2).

The statistical analysis for sanitary test differed significantly between the two lots. Except for the treatments with *M. azedarach* extracts (1%, 5% and 10%), quinoa seeds from lot 1 obtained a favorable sanitary quality index in relation to the control plot, which presented 75% of phytopathogen incidence. When the seeds were treated with *S. aromaticum* (10%) and *D. grandiflora* (10%), sanitary indexes of 28% and 33% were obtained, respectively. Medeiros et al. (2015) concluded that efficient fungi control contributes to the reduction of microflora in the surface of the seeds, allowing the increase of germination percentage under field and laboratory conditions.

Out of all pathogen genera identified, the genus *Aspergillus* spp. was the one that presented the highest incidence in the seeds of lots 1 and 2, with 45% and 61% respectively, indicating that the plant extracts tested do not inhibit the growth of *Aspergillus* spp. No significant results were found regarding growth inhibition of *Sclerotinia* spp. (Table 2) in the comparison between the two lots of seeds.

Table 2. Incidence of phytopathogens from the genera Aspergillus, Fusarium, Penicillum and Sclerotinia on the two lots of quinoa seeds (C. quinoa) treated with different plant extracts

Extract (%)	Infested seeds (%)			Aspergillus spp. (%)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	75Aa*	25Bcd	50	16Bc*	62Aa	39
D. grandiflora 1%	73Aab	16Bd	45	33Bb	74Aa	53
D. grandiflora 5%	69Aab	34Bbc	52	43Bab	67Aa	55
D. grandiflora 10%	33Ade	31Abc	32	40Bab	63Aa	51
M. azedarach 1%	44Acd	42Aab	43	47Aab	49Aa	48
M. azedarach 5%	74Aa	33Bbc	54	51Aab	52Aa	51
M. azedarach 10%	74Aa	28Bbc	51	55Bab	60Aa	57
S. aromaticum 1%	68Aab	56Ba	62	63Ba	68Aa	65

S. aromaticum 5%	53Abc	25Bcd	39	66Aa	58Ba	62
S. aromaticum 10%	28Ae	22Bcd	25	36Bb	60Aa	48
Average	59	31		45	61	
CV (%)		16.95			23.37	
Dp:		7.63			12.37	
	Fusarium spp. (%)			*Penicillum* spp. (%)		
	Lot 1	Lot 2	Mean	Lot 1	Lot 2	Mean
Control	43^{ns}	35	39a	32Aa*	2Ba	17
D. grandiflora 1%	39	16	27a	18Aabc	0Ba	9
D. grandiflora 5%	26	21	23a	10Abc	4Ba	7
D. grandiflora 10%	32	28	30a	7Ac	4Ba	5
M. azedarach 1%	29	25	27a	17Aabc	6Ba	11
M. azedarach 5%	27	35	31a	20Aab	2Ba	11
M. azedarach 10%	22	25	23a	18Aabc	1Ba	9
S. aromaticum 1%	28	16	22a	8Abc	6Ba	7
S. aromaticum 5%	18	27	22a	9Abc	2Ba	5
S. aromaticum 10%	47	22	34a	8Abc	4Ba	6
Average	31A	25B		14	3	
CV (%)		28.37			68.90	
Dp:		7.94			5.86	

	Sclerotinia spp. (%)		
	Lot 1	Lot 2	Mean
Control	9Aab*	2Bb	5
D. grandiflora 1%	10Aab	11Aab	10
D. grandiflora 5%	21Aa	8Bab	14
D. grandiflora 10%	21Aa	5Bab	13
M. azedarach 1%	7Bab	19Aa	13
M. azedarach 5%	2Bb	10Aab	6
M. azedarach 10%	4Bb	14Aab	9
S. aromaticum 1%	1Bb	10Aab	5
S. aromaticum 5%	7Bab	13Aaba	10

S. aromaticum 10%	9Bab	14Aab	11
Average	9	10	
CV (%)		69.29	
Dp:		6.58	

Note. *Significant interaction and ^{ns} non-significant interaction of factors; averages followed by the same uppercase letter in the row and lowercase in the columndo not differ by Tukey's test (P>0.05). CV: coefficient of variation.

The use of plant extracts at certain concentrations reduced infestation by *Fusarium* spp. Quinoa seeds from lot 2 treated with *D. grandiflora* (1%) and *S. aromaticum* (1%) presented incidence of 16% in both tests, against 35% of fungi incidence in the control plot. These results attest the benefits of seed treatment with plant extracts, providing improvement in the sanitary quality of the seeds and, consequently, in their physiological development.

No significant control of Penicillium spp. was observed in the quinoa seeds from lot 2. Yet, Seeds from lot 1 showed significant control, with fungi incidences of 8, 9 and 8% when treated with plant extracts of *S. aromaticum* at 1%, 5% and 10% concentration, respectively, against 32% of incidence in the control plot. Amaral et al. (2005) concluded that the use of *S. aromaticum* provides significant control of seed phytopathogens at concentrations of 0.5% to 0.1%. Overall, the sanitary tests revealed 75% and 25% of phytopathogen incidence in the quinoa seeds from lots 1 and 2, respectively. The use of aqueous plant extracts as seed treatment, at certain concentrations, provides a significant reduction in the infestation by fungi, improving the development of quinoa seeds.

The two lots differed statistically from each other, and it is worth highlighting that the quinoa seeds from lot 1 displayed less pathogen incidence than the control plot (75%) for all plant extracts, except *M. azedarach*. Seeds treated with S. aromaticum (10%) and D. grandiflora (10%) presented sanitary indexes of 28% and 33%, respectively. Accordingly, Medeiros, et al. (2015) states that efficient fungi control contributes to the reduction of microflora in the surface of the seeds, improving germination under field and laboratory conditions.

4. Conclusion

The aqueous plant extracts of clove (Syzygium aromaticum L.), cinnamon (Melia azadarach L.) and chrysanthemum (Dendranthema grandiflora Tzvelev) used in the quinoa seed treatment raised the emergence speed and the fungi control of these seeds within the variations and situations of each batch. The D. grandiflora extract, in all concentrations used, improved seed germination index, obtaining the highest rate of 70% in seeds treated with 5% concentration compared to the control treatment, which obtained 59%. M. azedarach (10% concentration) is the best treatment for emergence speed improvement, while S. aromaticum (10% concentration) provides the highest control of pathogens: 28% in relation to the control treatment that obtained 75%. These results highlighted the viability of the use of these species with low toxicity to man and the environment as treatment of quinoa seeds.

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