

Allelopathic effect of aqueous extracts from agro-industrial residues of pecan nut [*Carya illinoinensis* (Wangen) C. Koch] and pinhão (*Araucaria angustifolia*)

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Abstract

Pinhão seed coat and pecan nutshell are residues widely produced in households and industries, which present great potential to be used as source of natural preservatives in the food chain. The objective of the present study is to evaluate the effectiveness of pinhão (*Araucaria angustifolia*) seed coat and pecan nutshell [*Carya illinoinensis* (Wangen) C. Koch] aqueous extracts in inhibiting seed germination. Extracts were obtained in concentration of 10kg/m³, 5kg/m³ and 2.5kg/m³ and tested against lettuce seed germination in vitro tests.

The results show that both aqueous extract presented the significant ($p<0.05$) ability to inhibit seeds germination as well as the germination seed index, in relation to control experiments, when used in concentrations of up to 5kg/m^3 . Extracts did not interfere significantly ($p>0.05$) the germinated plant root size. Thus, aqueous extracts of agro-industrial residues from pinhão seed coat and pecan nutshell presents allelopathic activity, showing up as interesting alternative to be used as natural herbicide, also representing a way of solid waste management by industries and small farmers.

Keywords: Allelochemical. Residues. *Carya illinoiensis* shell. *Araucaria angustifolia* seed coat.

Resumo

Efeito alelopático de extrato aquoso de resíduos industriais de noz-pecã [*Carya illinoiensis* (Wangenh) C. Koch] e de pinhão (*Araucaria angustifolia*)

Casca de pinhão e de noz-pecã são resíduos amplamente produzidos em âmbito doméstico e industrial, que apresentam grande potencial para ser usado como fonte de conservantes naturais na cadeia alimentar. O objetivo do presente estudo é avaliar a eficácia de seus extratos aquosos na inibição da germinação de sementes. Os extratos foram obtidos nas concentrações de 10kg/m^3 , 5kg/m^3 e 2.5kg/m^3 e testados contra a germinação de sementes de alface em ensaios in vitro. Os resultados mostram que os extratos de ambos os resíduos apresentaram a capacidade de inibir significativamente ($p<0.05$) a germinação de sementes e o reduzir o índice de germinação das sementes, em relação ao experimento controle, quando

utilizados em concentrações de até 5kg/m³. Extratos não interferiram significativamente ($p>0.05$) no tamanho da raiz da planta germinada. Assim, extratos aquosos de resíduos agroindustriais de casca de pinhão e de *noz-pecã* apresentam atividade alelopática e se mostram como uma interessante alternativa para serem usados como herbicidas naturais, também representando uma forma de gestão de resíduos sólidos por indústrias e agricultores.

Palavras-chave: Aleloquímicos. Resíduos. Casca de *Carya illinoinensis*. Casca de semente de *Araucaria angustifolia*.

Introduction

The use of chemical herbicides and pesticides has been increasingly questioned worldwide due to public health problems and environmental aspects, which has lead research groups on the study of biocontrol for food production (ALTIERI; NICHOLSON, 2003; GYAWAL; IBRAHIM, 2014). Allelopathy encompasses any direct or indirect interaction among plants due to the release of primary and secondary metabolites leading to stimulatory or inhibitory effects on the germination, growth and development of other plants, depending on the compound concentration (RICE, 1984; CARMO *et al.*, 2007). Among allelopathic agents, several compounds extracted from agro-industrial residues have been identified to present such capability, such as: cinnamic acid, simple phenols, benzoic acid and gallic acid, flavonoids, hydrolyzed and condensed tannins (RICE, 1984; JORGE *et al.*, 2001; TAIZ; ZEIGER, 2004; CARMO *et al.*, 2007; ALMEIDA *et al.*, 2008; SERRANO *et al.*, 2009). Current literature shows that the food byproducts are source of polyphenols (CAXAMBÚ *et al.*, 2016;

SANT'ANNA *et al.*, 2017; PRADO *et al.*, 2014), and consequently they also may show up as source of natural herbicides, although few studies have explored this potential.

Pecan nut [*Carya illinoinensis* (Wangenh) C. Koch], widely produced in southern Brazil, presents approximately 45% of its mass as shell, which is an industrial processing residue. Pecan nutshell (PNS) is a phenolic rich agro-industrial byproduct, whose polyphenolic profile includes high concentration of gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, epigallocatechin and epicatechin gallate (PRADO *et al.*, 2014). Due to the elevated concentration of polyphenols, the consumption of its infusion is associated with antioxidant activity, decreasing liver damage, reduction of total cholesterol and action as natural anxiolytic. Additionally to the benefits to human health, Caxambú *et al.* (2016) found that PNS infusion presents antibacterial activity against important foodborne bacteria, showing up PNS as an interesting alterative to be used as natural preservative in food production (MALICK *et al.*, 2009; PRADO *et al.*, 2014; RECKZIEGEL *et al.*, 2011; MÜLLER *et al.*, 2013; TREVISAN *et al.*, 2014).

Araucaria angustifolia is a tree existing in Argentina, Paraguay, Chile and southern Brazil, whose seeds, known as *pinhão*, are widely consumed during the winter. Several studies have been conducted to evaluate the utilization of *pinhão* starch for industrial applications (DADAUT *et al.*, 2014; DADAUT *et al.*, 2015). Cordenunsi *et al.* (2004) observed that the cooked *pinhão* seed presented higher amounts of polyphenol than the raw seeds, possibly due to migration of the compounds from the coat during the thermal process. Daudt *et al.* (2015) observed that *pinhão* seed coat (PSC) extract is a promising natural ingredient to be incorporate in cosmetic formulations. Results from Sant'Anna *et al.* (2016) indicate that agro-industrial

residues from *pinhão* processing may represent an important source of natural biopreservatives to foods, besides presenting good stability during storage through its shelf-life. In this sense, the *pinhão* coat left during the seed dehulling is an interesting residue from the seed consumption, since it represents about 10% of the total weight of the seed, despite being little explored for food applications.

There is little information in the literature about the inhibitory capability of industry and household food waste as source of allelochemical compounds. Thus, the objective of the present study is to evaluate the effectiveness of PNS and PSC extracts in inhibiting lettuce (*Lactuca sativa* L.) seed germination.

Material and Methods

Milled PNS, from nuts harvest in 2014, was kindly supplied by Agroindústria Pitol (Anta Gorda, RS, Brazil). *Pinhão* seeds (*A. angustifolia*) were purchased in local market (Encantado, RS, Brazil), in 2015, and cooked at 121°C for 15 min in vertical autoclave (model CS-75, Prismatec, Brazil). Seeds were manually peeled with a knife, the internal seed, removed and the external coats, dried at 60°C for 24h and milled in industrial blender (model LI-2N, Skymsen, Brazil). Both residues were sieved to 5 mm particle size and stored at -18°C, protected from light, until use.

Aqueous extract was prepared by maintaining 10 g of PNS or PSC with 1 L of boiling distilled water for 10 min under constant stirring (model 754A, Fisatom, Brazil), and then filtered through Whatman filter paper nº1. The aqueous systems were diluted for

obtainment of extracts with concentration of 10kg/m³, 5kg/m³ and 2.5kg/m³.

The allelopathic activity was evaluated following procedure described elsewhere (ALVES *et al.*, 2004; MAGIERO *et al.*, 2009) by germination and seed vigor, using sterile distilled water as control and the PNS and PSC extracts at concentrations of 2,5, 5 and 10 kg/m³. The tests were conducted using 4 replicates of 50 lettuce (*L. sativa L-* Asteraceae) seeds, arranged between Germitest papers (Germinlab, Brazil) moistened with the aqueous extracts at the rate of two and a half times the weight of the paper (BRASIL, 2009), and placed into transparent polyethylene bags. Assays were performed in germination chamber (model CT-310P, Polimate, Brazil) at 20 ± 2°C for 7 days. After the treatments, seed vigor was evaluated by the germination of seeds, as percentage normal plants after 7 days of experiment, first count (FC) by percentage normal plants after the fourth experiment day (BRASIL, 2009), and germination speed index (GSI), was calculated using Eq. 1 as suggested by Maguire (1962).

$$\text{GSI} = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \frac{N_3}{D_3} + \dots + \frac{N_n}{D_n} \quad (1)$$

In Eq. 1, GSI is the germination seed index, *N* is the normal germinated seeds at the count day and *D*, the number of days after sowing in which the count was performed. In the end of experiments, the root sizes of the germinated plants were measured by caliper ruler. All experiments were conducted four times and averages of two independent tests were calculated and compared by Tukey's test using the software Statistica 10.0 (StatSoft, Tulsa, OK, USA), and differences were considered statistically significant when *p*<0.05.

Results and Discussion

Agro-industrial byproduct extracts have shown up as trend because they may represent a natural source of biopreservatives (AYALA-ZAVALA *et al.*, 2011; GYAWALI; IBRAHIM, 2014), representing a way of industrial waste management, also potentially reflecting on final product costs, due to their potential as low-cost source (SILVEIRA *et al.*, 2011).

The evaluation of the allelopathic activity of PSC aqueous extract is shown Table 1. The results show that the use of PSC aqueous extract in the ratio of 10kg/m³ and 5kg/m³ significantly ($p<0.05$) reduced germination and FC of lettuce seeds compared to control. Similar results were found in relation to the GSI, wherein the tested extracts were effective in significantly reduced ($p<0.05$) the GSI parameter. Use of PSC at concentration of 2.5kg/m³ did not change significantly ($p>0.05$) all evaluated parameters compared to the control. The results show that more concentrated extracts presented higher ability to inhibit the seed germination. The root size was not altered significantly ($p>0.05$) at any test conducted in relation to the control test.

Table 1 - Allelopathic activity of *pinhão* (*Araucaria angustifolia*) seed coat aqueous extracts against lettuce seeds.

	Control	Pinhão Seed Coat		
		10kg/m ³	5kg/m ³	2,5kg/m ³
Germination (%)	60.1±4.1 ^a	20.9±1.7 ^c	46.5±2.2 ^b	68.5±2.5 ^a
FC (%)	55.4±2.4 ^a	16.4±1.2 ^c	28.5±24 ^b	61.6±4.2 ^a
GSI	34.1±3.3 ^a	9.2±1.8 ^c	20.2±0.9 ^b	35.5±3.5 ^a
Root size (cm)	1.7±0.4 ^a	1.9±0.5 ^a	1.8±0.5 ^a	1.9±0.4 ^a

^{a,b,c,d} Different superscripts in the same column indicate statistical differences ($p<0.05$).
FC: First Count. GSI: Germination Seed Index

The results of the allelopathic activity of PNS aqueous extract are shown in Table 2. Aqueous extract at concentration of 10kg/m³ and 5kg/m³ significantly reduced ($p<0.05$) the germination rates and FC lettuce seeds compared to control. There was no significant difference ($p>0.05$) for both parameters when extracts were applied in concentrations of 10kg/m³ and 5kg/m³. The root size was not altered significantly ($p>0.05$) at any test conducted in relation to the control test.

Prado *et al.* (2014) observed that PNS infusion presented 590.78 mg of gallic equivalent per gram of dried residue of total polyphenols, 412.10 mg catechin equivalents per gram of waste of condensed tannins and compounds with high scavenging capacity of ABTS and DPPH radicals. The authors also verified that the polyphenolic profile was made up mainly of gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, epigallocatechin gallate, and epicatechin. Cladera-Oliveira (2008) observed that aqueous extract from PSC extract showed a concentration of total phenolic compounds of 20.70 mg of catechol equivalent per gram of residue and the capacity scavenging of ABTS radicals, indicating antioxidant capacity.

Table 2 - Allelopathic activity of pecan nutshell [*Carya illinoiensis* (Wangenh) C. Koch] seed coat aqueous extracts against lettuce seeds.

	Control	<i>Pecan nutshell</i>		
		10kg/m ³	5kg/m ³	2,5kg/m ³
Germination (%)	60.1±4.1 ^a	40.3±1.9 ^b	42.5±3.4 ^b	63.8±2.5 ^a
FC (%)	55.4±2.4 ^a	30.4±4.2 ^b	29.6±1.2 ^b	68.1±4.2 ^a
GSI	34.1±3.3 ^a	12.0±4.3 ^c	24.2±2.2 ^b	35.5±2.1 ^a
Root size (cm)	1.7±0.4 ^a	1.8±0.2 ^a	2.1±0.5 ^a	2.1±0.4 ^a

^{a,b,c} Different superscripts in the same column indicate statistical differences ($p<0.05$). FC: First Count. GSI: Germination Seed Index

There are numerous natural bioactive compounds with allelopathic properties. Phenolic acids are mentioned as being responsible for reduced absorption of micro- and macronutrients in several species. Ferulic acid can act in the inhibition of phosphate absorption while chlorogenic acid may alter the balance of nutrients in plants. Hydrolyzed and condensed tannins present the ability to inhibit the growth and seed germination, due to the formation of metal chelation implying on micronutrient transport (JORGE *et al.*, 2001; CARMO *et al.*, 2007; ALMEIDA *et al.*, 2008; SERRANO *et al.*, 2009). The allelopathic effects on the germination and root elongation are associated with strong inhibition of mitosis that occurs in the nucleus and mitochondria and the disruption of cellular structures. Likewise, seed germination is inhibited, since most of allelochemicals operates in oxidative stress, producing reactive oxygens that induce cellular degradation process by preventing germination, development and normal metabolic processes of the plant (SCALBERT, 1991; SOUZA *et al.*, 2005; ALMEIDA *et al.*, 2008; TIGRE *et al.*, 2012, 2015). According to Ferreira & Borghetti (2004), the allelopathic effect does not occur through the reduction of germinability (final percentage of germination), but the germination speed. In this sense, the polyphenolic profile and compounds with antioxidant activity in the PNS and PSC matrix may play important role on the inhibition of seed germination capacity of the aqueous extract tested.

Conclusion

Thus, the PNS and PSC aqueous extracts show up as interesting alternative to be used potentially as natural herbicide, since both residues showed the ability to inhibit lettuce seeds germination as well as the germination rate when used in concentrations of up to 5

kg/m³. It also important to point out that the extract preparation procedure is easily reproduced by small farmers, making the transfer of knowledge easier and may represent a way of solid waste management by industries and small farmers.

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