AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Aqueous extract of *Brugmansiasuaveolens* leaves and physiological performance of lettuce seeds

[1]Manoela Andrade Monteiro, [1]Guilherme Menezes Salau, [1]Felipe Santos Zulli, [1]Andréa BiccaNoguez Martins, [1]Vinicius Jardel Szareski, [1]Ivan Ricardo Carvalho, [3]Maicon Nardino, [2]Francine Lautenchleger, [1]Gustavo Henrique Demari, [4]Velci Queiróz de Souza, [1]Tiago Pedó, [1]Emanuela Garbin Martinazzo e [1]Tiago Zanatta Aumonde

[1]DepartmentoofPlant Science, AgronomySchool Eliseu Maciel, Federal Universityof Pelotas (UFPel), Campus Capão do Leão, Rio Grande do Sul, Brazil
[2]DepartmentAgronomyFederal Universityof Santa Maria (UFSM), Campus Frederico Westphalen, Rio Grande do Sul, Brazil
[3]DepartamentofMathematicsandStatistics, Federal Universityof Pelotas (UFPel), Campus Capão do Leão, Rio Grande do Sul, Brazil
[4]FederalUniversityof Pampa (UNIPAMPA), Dom Pedrito, Rio Grande do Sul, Brazil

Address For Correspondence:
Manoela Andrade Monteiro, Federal University of Pelotas, Capão do Leão, Brazil.
E-mail: manu_agro@hotmail.com.

ARTICLE INFO

Article history:
Received 18 December 2016
Accepted 16 February 2017
Available online 25 February 2017

Keywords:
initial growth, esterase, germination, Lactuca sativa (L.), vigor.

ABSTRACT

The release of chemicals produced by plants can affect the development of other species directly or indirectly. The objective of this work was to evaluate the physiological performance of lettuce seeds exposed to concentrations of aqueous extract of angel trumpet leaves (*Brugmansiasuaveolens* Willd.). The treatments consisted of concentrations of 0; 6; 12; 18 and 24% of the extract. Germination, first count, germination speed index, shoot length and primary root length, total dry mass of seedlings, expression of the isoenzymes esterase and acid phosphatase were evaluated. There was a linear reduction of germination and germination speed index. The length of the primary root was reduced up to the highest concentration of the extract. The alleles of the isoenzymes esterase and acid phosphatase were present at the concentrations of 18 and 24% of the aqueous extract. The aqueous extract of angel trumpet leaves adversely affects the physiological performance of lettuce seeds.

INTRODUCTION

The plants release, from the secondary metabolism, toxic products that can prevent the germination or the development of other plants. The study of allelopathic substances can contribute to the knowledge of interspecific relationships between plants (Maraschin-Silva &Aqüila 2006). These substances belong to different classes, such as phenols, terpenes, alkaloids, polyacetylenes, fatty acids and peptides (Periotto *et al*., 2004). The presence of phenolic compounds, coumarins, terpenoids, flavonoids, alkaloids, glycosides, tannins and quinones may trigger beneficial or harmful effects to other species (Souza *et al*., 2005).

The species *Brugmansiasuaveolens* (Willd.) Bercht. & J. Presl. belongs to the Solanaceae family, is popularly known as angel trumpet, and is considered a toxic plant with scopolamine, atropine and hyoscyamine in the constitution of its tissues (Oliveira *et al*., 2003, Alves 2003). Due to this and its toxicity, the extract of this species can negatively influence the physiological performance of seeds.

The toxic or allelopathic effect of plant extracts can be determined from target species, known as indicator plants, such as lettuce. In order to be indicated as a test plant, the species must exhibit rapid, uniform germination and a sensitivity to the allelochemical even at low concentrations (Silva *et al*., 2009).

ToCite ThisArticle: Manoela Andrade Monteiro, Guilherme Menezes Salau, Felipe Santos Zulli, Andréa BiccaNoguez Martins, Vinicius Jardel Szareski, Ivan Ricardo Carvalho, Maicon Nardino, Francine Lautenchleger, Gustavo Henrique Demari, Velci Queiróz de Souza, Tiago Pedó, Emanuela Garbin Martinazzoe Tiago Zanatta Aumonde., Aqueous extract of *Brugmansiasuaveolens*leaves and physiological performance of lettuce seeds. ***Aust. J. Basic & Appl. Sci.,*** *11(2): 1-5, 2017*

Carvalho&Fontanétti (2002), evaluating the allelopathic potential of jack bean (*Canavaliaensiformis*) and mucunapruriens (*Stizolobiumaterrimum*), verified that these species have an allelopathic effect on the germination of lettuce seeds. Tokura&Nóbrega (2002) observed that wild radish and rapeseed positively affect the root length of soybean seedlings as well as the dry matter mass. Neves (2005), when assessing the allelopathic potential of canola, verified that both the germination and the primary root of the seedlings are reduced with an increase of the extract concentration.

Germination consists of the process of resumption of embryo growth and, according to Mano (2006), the germination test can be used to evaluate the allelopathic effect. The isoenzymes are highly influenced by the environment (Malone *et al*., 2007), being important to evaluate the physiological performance of seeds in different environment conditions (Henning *et al*. 2010, Veiga *et al*. 2010).

The objective of this work was to evaluate the physiological performance of lettuce seeds exposed to concentrations of the aqueous extract of angel trumpet leaves.

MATERIALS AND METHODS

The work was carried out in the Didactic Laboratory of Seed Analysis of the Department of Plant Breeding - Postgraduate Program in Seed Science and Technology of the Federal University of Pelotas.

To obtain the aqueous extract of angel trumpet leaves (*B. suaveolens* (Willd.), the leaves were dehydrated in a forced air circulation oven at a temperature of 40°C until a constant mass was obtained. Subsequently, 25g of dry matter leaves were added to 120 ml of boiled distilled water at a temperature of 70°C. The vessel was closed and left to rest for 24 hours and after the aqueous extract was subjected to simple filtration, the extract being considered to be 100% concentration.

The concentrations of 0; 6; 12; 18 and 24% of the extract, remained within the range of pH and osmotic potential not harmful to germination. To evaluate the physiological performance of lettuce seeds, submitted to the different concentrations of the aqueous extract of angel trumpet leaves, the following evaluations were performed:

1- Germination test: conducted in four replicates with four 50-seed sub-samples, seeded in gerbox boxes, on two sheets of blotter paper moistened at 2,5 times the dry paper mass, with the different concentrations of extract. The gerbox boxes were transferred to the BOD germination chamber at a temperature of 25 ºC and 12 hours of photoperiod. The evaluations were performed seven days after sowing and the results expressed as percentage of normal seedlings (Brazil, 2009).

2- First germination count: performed in conjunction with the germination test, three days after sowing, as indicated by the Rules for Testing Seeds. The results were expressed as percentage of normal seedlings (Brazil, 2009).

3- Germination speed index: obtained from daily germinated seeds counts (minimum radius protrusion from 3 to 4 mm), the counts were performed until the constant number of germinated seeds was obtained. The rate of germination was obtained according to the recommendation of Nakagawa, 1994.

4- Shoot and primary root length of seedlings: four replications were used for this evaluation with four subsamples of 10 seedlings, obtained at the end of the germination test. The length of the aerial part was determined from the distance between the insertion of the basal portion of the primary root to the apex of the aerial part, while the length of the primary root would be measured by the distance between the apical and basal part of the primary root. The results were expressed in millimeters per seedling (mm seedling^{-1}).

5- Total dry mass: obtained from four subsamples of 10 seedlings at the end of the germination test. The seedlings were conditioned in brown paper envelopes and dried in a forced ventilation oven at a temperature of 70°C until constant mass. The results were expressed in milligrams per seedling (mg seedling^{-1}).

6- Isoenzyme expression: the expression of the esterase and acid phosphatase isoenzymes were determined by the vertical electrophoresis system in polyacrylamide gel (Malone *et al*., 2007). For this, the seedlings collected at the end of the germination test were macerated separately in porcelain grains in an ice bath. Next, 200 mg of the macerate from each sample were transferred to microcentrifuge tubes, plus 0,2M Lithium Borate extractor at pH 8.3 + Tris Citrate + 0,2M at pH 8.3 + 0.15% of 2-mercaptoethanol in the ratio 1:2 (m/v). The electrophoresis was performed on polyacrylamide gels at 7%, applying 20 µL of each sample. The coloring systems used were those described by Scandálios (1969) and Alfenas (1998). The interpretation of the results was by visual analysis of the gels, presence or absence and intensity of expression of the bands.

The experimental design was randomized blocks with five treatments and four replicates. Data were submitted to analysis of variance and when significant at 5% probability, the results were expressed by polynomial regression.

RESULTS AND DISCUSSION

There was no significant difference in total dry mass of seedlings when submitted to lettuce seeds at different concentrations of the aqueous extract of angel trumpet leaves.

The germination and the first count were reduced with the increase of the extract concentration, adjusting to the linear model with $R^2 \geq 0.82$ (Fig. 1A). From the 6% concentration of the extract, there was a reduction on these processes related to the resumption of embryo growth, however, it was observed a more pronounced reduction considering the concentration 0%, compared to the concentration of 24% of the extract, being the percentage difference between both of 9 and 13% for germination and the first count, respectively.

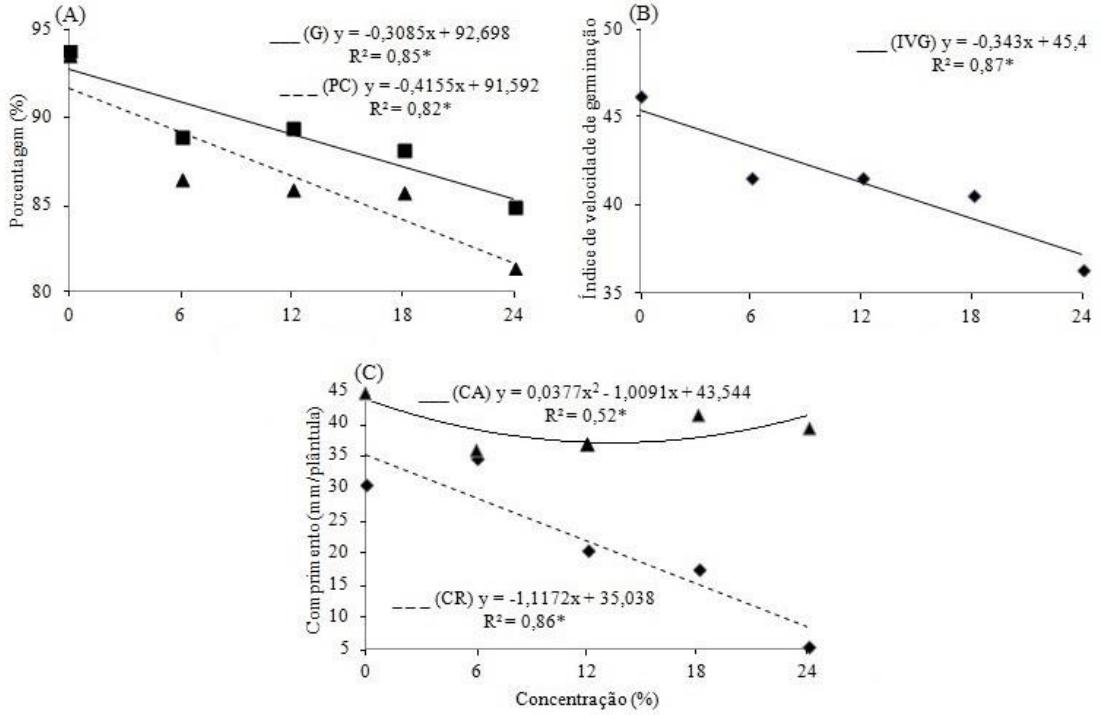

Fig. 1: Germination and first germination count (A), germination speed index (B) and shoot and primary root lenght of lettuce seedlings (C) under the influence of different concentrations of the aqueous nngel trumpet extract (*B. Suaveolens*) (Willd.).

The increase in the concentration of the extract and the consequent reduction of the germination can be related to the damage of the integrity and the alteration of the permeability of the cellular membranes (Omezzine *et al*., 2014), affecting the germination and mainly the vigor of the seeds, as verified from the results of the first germination count test.

The germination speed index presented linear reduction until the highest concentration of the extract (Fig. 1B). There was reduction of the germination speed index in the order of 22% in seeds of the concentration of 24% of the extract compared to the concentration of 0% of the extract.

The reduction of the germination speed index refers to the reduction of the number of seeds germinated per day, consequently, the reduction of vigor. The reduction of the vigor of the seeds exposed to the extract may be related to the damage of different cellular structures, involved in the physiological and biochemical processes related to hydrolysis and translocation of reserves (Kaur *et al*., 2010). According to Ferreira &Borghetti (2004), the allelopathic effect is more pronounced on germination speed compared to seed germination.

The length of aerial part and of primary root were altered by the action of the aqueous extract of angel trumpet leaves (Fig. 1C). The increase in the concentration of the extract reduced the length of the lettuce root until the highest concentration, while the shoot length had a minimum point at 6% of the extract concentration, increasing to a concentration of 24%. Alves *et al*. (2003) report that the aqueous extract of *Solanumcrinitinum*seeds (Lam.) negatively affects the growth of lettuce seedlings.

Allelopathic compounds may impair the metabolism of seeds and seedlings (Albuquerque *et al*., 2011). Scognamiglio *et al*. (2014) report that the aerial part is less affected than the roots and according to Omezzine *et al*. (2014), these responses are associated with detrimental action of the allelochemicals on plant growth and development. According to Goetze&Thomé (2004), extracts of fresh eucalyptus leaves (*Eucalyptus grandis*) reduce the growth of the root of lettuce seedlings.

The isoenzymatic profile of the esterase presented two alleles (*EST* 1 and *EST* 2) at the highest concentrations of the extract (Fig. 2A). The expression and intensity of the alleles varied in relation to the different concentrations of allelopathic angel trumpet extract and there was a greater intensity in the *EST2* allele at the doses of 18 and 24% of the extract.

This isoenzyme is involved in ester hydrolysis and plays an important role in lipid metabolism (Santos *et al*., 2001), and the increase in band intensity in the lower osmotic potentials may be associated with a possible increase in peroxidation of lipids, causing damage to cell membranes and affecting the expression of seed vigor. Reduction of seed vigor was verified at the first count and germination speed index (Fig. 1A and Fig. 1B).

Acid phosphatase showed similarity in the expression of bands between the different concentrations (Fig. 2B). However, with the increase of the concentration of the extract, there was greater intensity of the bands in the seedlings under the concentrations of 18 and 24%. Acid phosphatase participates in ester hydrolysis and can act on the cell membranes system (Santos *et al*., 2005), impairing the performance of seedlings.

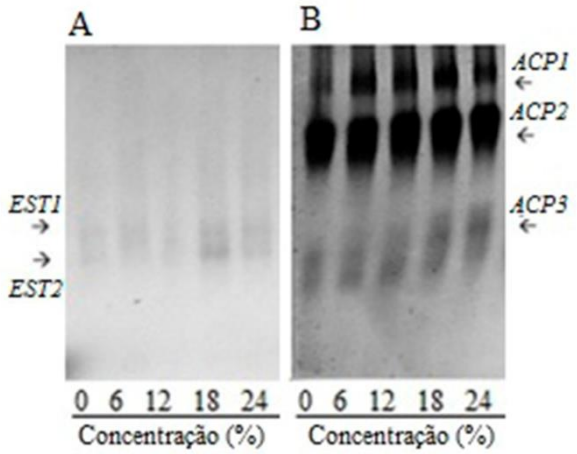

Fig. 2: Electrophoretic profile for the isoenzymatic system of esterase (A) and acid phosphatase (B) determined in lettuce (*L. sativa* L.) seedlings submitted to different concentrations of the angel trumpet extract (*B. suaveolens* (Willd.)).

The aqueous extract of angel trumpet showed an allelopathic effect and caused a reduction in the germination percentage and the vigor of the lettuce seeds (Fig. 1). In addition, it negatively influenced the growth of the primary root, having the inhibitory effect, if intensified with the increase of the concentration of the extract. In this sense, according to Ferreira and Borguetti (2004), some allelopathicsubstances can reduce germination. Tokura&Nóbrega (2005) found that aqueous extracts of wheat plants, black oat, pearl millet, wild radish and rapeseed have an allelopathic effect on maize seedlings, affecting the root and shoot growth of the seedlings.

Conclusion:

The aqueous angel trumpet extract (*B. suaveolens*) negatively affects the physiological performance of lettuce seeds;

The concentrations of 18 and 24% reduce germination and the germination speed index;

Primary root growth is reduced by about 82% in seedlings under the influence of 24% concentration compared to those at zero concentration.

The angel trumpet extract provides an increase in the intensity of isoenzyme esterase bands at concentrations of 18 and 24%.

REFERENCES

Alfenas, A.C., 1998. Eletroforese de isoenzimas e proteínas afins. Viçosa: UFV, p: 574.

Alves, C.C.F.,J.M.Alves,T.M.S.SILVA, M.G.CARVALHO, J.JACOB NETO, 2003. Atividade alelopática de alcaloides glicosilados de *Solanumcrinitum*Lam**.** Forest and Environment, 10(1): 93-97.

Alves, M.N., 2003. Alocação de alcalóidestropânicos em Brugmansiasuaveolens (Solanaceae). 92 f. Tese apresentada ao Instituto de Biologia para a obtenção do título de doutor em Biologia Vegetal. Universidade Estadual de Campinas. UNICAMP.

BRASIL, 2009. Regras para análise de sementes. Secretaria de Defesa Agropecuária. Brasília, DF: Ministério da Agricultura, Pecuária e Abastecimento/ACS. p: 399.

Carvalho, G.J.,A.Fontanétti,C.T.Cançado,2002. Potencial alelopático do feijão de porco (*Canavalia ensiformes*) e da mucuna preta (*Stilozobiumaterrimum*) no controle da tiririca (*Cypeursrotundus*). Ciência Agrotécnica, 26(3): 647-651.

Ferreira, A.G.,F.Borghetti, 2004. Germinação: do básico ao aplicado. Porto Alegre: Artmed. p: 520.

Goetze, M., G.C.H.Thomé, 2004. Efeito alelopático de extratos de *Nicotinianatabacum*e *Eucalyptusgrandis*sobre a germinação de três espécies de hortaliças. Revista Brasileira de Agrociência,10(1): 43-50.

Henning, F.A., L.M.Mertz,E.A.Jacob Junior, R.D.Machado,G.Fiss,P.D.Zimmer,2010. Composição química e mobilização de reservas em sementes de soja de alto e baixo vigor. Bragantia, 69(3): 727-734.

Imolesi, A.S., E.V.R.V.Pinho, R.G.V.Pinho,M.G.G.C.Vieira,R.S.B.Corrêa,2001. Efeito da adubação nitrogenada em características morfo-agronômicas e nos padrões eletroforéticos de proteínas e isoenzimas de sementes de milho. *Revista* Brasileira de Sementes,23(1): 17-25.

Kaur, S.,H.P.Singh,S.Mittal,D.R.Batish,R.K.Kohli,2010. Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide.Industrial Crops and Products,32(1): 54-61.

Malone, G., P.D.Zimmer, G.E.Meneghello, M.A.Castro, S.T.PESKE,2007. Expressão diferencial de isoenzimas durante o processo de germinação de sementes de arroz em grandes profundidades de semeadura. Revista Brasileira de Sementes, 29(1): 61-67.

Mano, A.R.O., 2006. Efeito alelopático do extrato aquoso de sementes de cumaru (*Amburana cearensis* S.) sobre a germinação de sementes, desenvolvimento e crescimento de plântulas de alface, picão-preto e carrapicho. 102 f. Dissertação (Mestrado). Fortaleza – Ceará.

Maraschin-Silva, F., M.E.A.Aqüila,2006. Potencial alelopático de espécies nativas na germinação e crescimento inicial de *Lactuca sativa* L. (Asteraceae). Acta Botanica Brasilica,20(1): 61-9.

Nakagawa, J., 1994. Testes e vigor baseados na avaliação de plântulas. *In*: VIEIRA, R. D.; CARVALHO, N. M. (Ed.). Testes de vigor em sementes. Jaboticabal: FUNEP, pp: 49-85.

Neves, R., 2005. Potencial alelopático da cultura de canola (*Brassicanapus*L. var. oleifera) na supressão de picão-preto (*Bidens* sp.) e soja. 77 f. Dissertação (Mestrado em Produção Vegetal) - Universidade de Passo Fundo, Passo Fundo.

Oliveira, R.B.de., A.S.Pires de Godoy, F.B.Costa,2003. *In:* PLANTAS TÓXICAS.Conhecimento e prevenção de acidentes. Ed. Holos, pp: 34-37.

Omezzine, F., A.Ladhari,R.Haouala, 2014. Physiological and biochemical mechanisms of allelochemicals in aqueous extracts of diploid and mixoploid *Trigonellafoenum-graecum*L. South African Journal of Botany,93:167-178.

Periotto, F., S.C.G.A.Perez, M.I.S.Lima,2004. Efeito alelopático de *Andirahumilis*Mart. Benth na germinação e no crescimento de *Lactuca sativa* L. e *Raphanussativus*L. Acta Botanica Brasilica,18(3): 425-30.

Santos, C.M.R., N.L.Menezes, F.A.Villela,2005. Modificações fisiológicas e bioquímicas em sementes de feijão no armazenamento. Revista Brasileira de Sementes,27(1): 104-114.

Scandálios, J.G., 1969. Genetic control of multiple molecular forms of enzymes in plants: revisão. Biochemical Genetics,3(1): 37-79.

Schopfer, P.,C.Plachy, G.Frahry, 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide & hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid.Plant Physiology, 125: 1591-1602.<http://dx.doi.org/10.1104/pp.125.4.1591>

Scognamiglio, M.,V.Fiumano,B.D'abrosca,A.Esposito,Y.H.Choi,R.Verpoorte, A.Fiorentino, 2014. Chemical interactions between plants in Mediterranean vegetation: The influence of selected plant extracts on *Aegilopsgeniculata* metabolome. Phytochemistry, 106:69-85. <dói: 10.1016/j.phytochem.2014.07.006>

Silva, H.L.,H.L.Silva, M.M.Trezzi, J.A.Marchese, G.Buzzello, E.MiottoJr., F.Patel, F.Debastiani, J.Fiorese,2009. Determinação de espécie indicadora e comparação de genótipos de girassol quanto ao potencial alelopático.Planta daninha, 27(4): 655-663.

Souza, S.A.M., L.V.Cattela, D.P.Vargas, C.F.B.Piana,V.L.Bobrowski, B.H.G.Rocha,2005. Atividade alelopática e citotóxica do extrato aquoso de espinheira-santa (*Maytenusilicifolia* Mart. ExReiss.). Ciências Biológicas e da Saúde, 11(3): 7-14.

Tokura, L.K., L.H.P.Nóbrega,2002. Potencial alelopático de coberturas de inverno no desenvolvimento de plântulas de soja. Revista Varia Scientia,02(2): 19-26.

Tokura, L.K., L.H.P.Nóbrega,2005. Potencial alelopático de cultivos de cobertura vegetal no desenvolvimento de plântulas de milho. Acta ScientiarumAgronomy, 27(2): 287-292.

Veiga A.D., É.V.R.V.Pinho, A.D.Veiga, P.H.A.R.Pereira, K.C.Oliveira, R.G.V.Pinho,2010. Influência do potássio e da calagem na composição química, qualidade fisiológica e na atividade enzimática de sementes de soja. Ciência e Agrotecnologia, 34(4): 953-960.