Research Article

Physiological and biochemical effects of *Lantana camara* L. allelochemicals on the seed germination of *Avena sativa* L.[1]

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ABSTRACT

Lantana camara (L.) is considered one of the main weeds of agricultural crops, and allelochemicals produced by this species may be a source for the development of natural herbicides. In this study, phytotoxic effects of *L. camara* aqueous extracts on seed germination and seedling growth of *Avena sativa* (L.) were assessed. The experimental design was completely randomized, in a 2 x 5 factorial scheme, with treatments resulting from two assays, testing five concentrations of the extract (0 %, 2.5 %, 5.0 %, 7.5 % and 10 %; vv^{-1}). During the seed germination, physiological (*e.g.*, seed imbibition, germination and vigor) and biochemical (*e.g.*, catalase, ascorbate peroxidase, proline, phenols, malondialdehyde and hydrogen peroxide) proprieties were evaluated. The leaf extracts of *L. camara* affected the response variables on *A. sativa* by reducing the germination speed index (phases I and II) and seed viability (phase III of germination), thus causing a solute leakage and increasing the production of reactive oxygen forms and, finally, lipid peroxidation. Extract concentrations above 2.5 % inhibit the growth of epicotyls. Therefore, the studied allelochemicals showed potential to be exploited in the development of natural herbicides.

KEYWORDS: Antioxidant system, bioactive compounds, seed physiology, phytotoxicity.

RESUMO

Efeitos fisiológicos e bioquímicos de aleloquímicos de *Lantana camara* L. na germinação de sementes de *Avena sativa* L.

Lantana camara (L.) é considerada uma das principais plantas invasoras de culturas agrícolas, e aleloquímicos produzidos por essa espécie podem ser fonte para o desenvolvimento de herbicidas naturais. Avaliaram-se efeitos fitotóxicos de extratos aquosos de *L. camara* sobre a germinação de sementes e o crescimento de plântulas de *Avena sativa* (L.). O delineamento experimental foi completamente randomizado, em esquema fatorial 2 x 5, com tratamentos resultantes de dois ensaios, testando-se cinco concentrações do extrato (0 %, 2,5 %, 5,0 %, 7,5 % e 10 %; vv^{-1}). Durante a germinação das sementes, foram avaliadas propriedades fisiológicas (*e.g.*, embebição, germinação e vigor de sementes) e bioquímicas (*e.g.*, catalase, ascorbato peroxidase, prolina, fenóis, malondialdeído e peróxido de hidrogênio). Os extratos foliares de *L. camara* afetaram as variáveis respostas em *A. sativa*, reduzindo o índice de velocidade de germinação (fases I e II) e a viabilidade das sementes (fase III da germinação), provocando, então, vazamento de soluto e aumento da produção de formas reativas de oxigênio e, finalmente, peroxidação lipídica. Concentrações de extrato acima de 2,5 % inibem o crescimento de epicótilos. Portanto, os aleloquímicos estudados mostraram potencial para exploração no desenvolvimento de herbicidas naturais.

PALAVRAS-CHAVE: Sistema antioxidante, compostos bioativos, fisiologia de sementes, fitotoxicidade.

INTRODUCTION

Allelochemicals are mainly secondary metabolites produced by plants or with microbial origin, which influence the growth or development of biological systems (IAS 1996).

Lantana camara (L.), as a perennial aromatic shrub of the Verbenaceae family, is considered worldwide as a major invasive plant (Chengxu et al. 2011).

Among the metabolites found in *L. camara*, 1,8-cineol, β-pinene, dipentene, lantadene A and lantadene D have been cited as important intermediate sources for herbicide development (Kegge & Pierik 2010, Mishra 2015, Latif et al. 2017, Gindri et al. 2020).

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Allelopathic compounds (*e.g.*, phenolics, terpenoids, alkaloids and their derivatives) are reported to inhibit seed germination and reduce the germination speed index and seedling growth (Abu-Romman et al. 2010, Hussain & Reigosa 2011). Such phytotoxity effects occur because of their action on cellular degradation processes through the production of reactive oxygen species, resulting in cellular oxidative stress (Bogatek & Gniazdowska 2007, Qian et al. 2009).

The mechanisms that define how the allelochemicals of *L. camara* act have not been described yet. The response to alelochemicals leads to the induction of adaptive changes in the biochemical and gene expression of plants, such as induction of the enzymatic and non-enzymatic antioxidant defense system. The ability of the cell to cope with the oxidative stress produced by alellochemicals will largely depend on the endogenous free radical scavenging capacity of the species (Mittler 2017).

In general, abiotic and biotic stresses will induce perturbations in the fruit cellular homeostasis, which will then result in an increased generation of reactive oxygen species. When the free radicals scavenging capacity exceeds the endogenous scavenging, reactive oxygen species interact with sensors that will initiate cascade reactions and also up-regulation of transcription factors (Toivonen & Hodges 2011), resulting in oxidative stress.

Studies on enzymatic activity, free radical content, cell membrane system integrity and other tests routinely used for seed vigor and germination may be important tools in elucidating the action mechanisms of allelopathic compounds. Moreover, despite the extensive literature about the effects of *L. camara* allelochemicals, studies regarding such effects on *Avena sativa* are still scarce. In this research, it was hypothesized that *L. camara* extracts affect physiological and biochemical proprieties of *A. sativa* seed germination and, therefore, have a potential to be used in the development of natural herbicides. This study aimed to evaluate such effects.

MATERIAL AND METHODS

Commercial seeds of *A. sativa* were obtained from the seed laboratory of the Universidade do Estado de Santa Catarina and stored in a dry and cold chamber (50 ± 5 % relative humidity and 8 ± 2 ºC), until analysis, and *L. camara* leaves were collected from wild plants in the city of Lages, Santa Catarina state, Brazil. Leaves were then washed using distilled water and dried in an oven with air circulation at 40 ºC, for 72 h. After drying, the leaves were shredded and stored in a dry and cold chamber (50 ± 5 % relative humidity and 8 ± 2 ºC), until analysis. The extracts were prepared by mixing 10 g of leaf powder in 90 mL of distilled water (25 ºC, for 24 h). The mixture was filtered and then centrifuged at 3,600 rpm, for 10 min. The resulting supernatant (crude extract) was diluted in distilled water to obtain the different tested extract concentrations: 0 %, 2.5 %, 5.0 %, 7.5 % and 10 % (vv^{-1}).

For the viability and vigor tests, four replications of 25 seeds were sown in acrylic boxes (12 cm x 12 cm), on three sheets of germitest paper moistened 2.5 times the weight of the dry paper with water and extracts in the different concentrations. The boxes were transferred to a biochemical oxygen demand (BOD) chamber at 25 ºC and 12-h photoperiod.

The samples for seed imbibition assessment were prepared in the same way; but, after the seed preparation at each different time (0, 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 h), two samples of 25 seeds were kiln dried (105 ºC, for 24 h) to determine the moisture. The imbibition curve was obtained by using the difference of humidity between the times.

For seed germination, after preparation, the seeds remained in water (control) or in each one of the *L. camara* extract concentrations, until seven days after sowing. Also, in the beginning of the germination phase III (root protrusion), a part of the seeds previously exposed only to water at the germination phases I and II were transferred to boxes containing the extract at different concentrations; and those previously exposed to the extract were transferred to boxes with water. The results were expressed as percentage of normal seedlings.

The germination speed index (GSI) was evaluated by counting, every 12 h of the assay, the total number of germinated seeds (root protrusion of 2 mm), until a constant number of germinated seeds. Thus, it was calculated according to following equation: $GSI\ (\%) = \sum[(G_{S1}/C_1) + (G_{S2}/C_2) + \ldots + (G_{Sn}/C_n)] \times 100$, where: C_1, C_2 … C_n represent the first, second and n^{th} seed counts, and G_{S1}, G_{S2} … G_{Sn} are the respective numbers of germinated seeds at these counts (Souza-Filho et al. 2010).

To evaluate the vigor by electrical conductivity, four replications of 50 seeds were previously

weighed and soaked (1 h) in the different extract concentrations (0 %, 2.5 %, 5.0 %, 7.5 % and 10 %). After imbibition, the seeds were washed in distilled water, transferred to a cylindrical flat bottom beaker containing distilled water (50 mL) and stored in a BOD chamber (20 ºC/24 h). The electrical conductivity was evaluated after 24 h, with results expressed as μS cm^{-1} g^{-1} of seeds (Vieira & Carvalho 1994).

In the evaluation of vigor by initial seedling growth, after preparation, the hypocotyl and root length, as well as the presence of secondary roots, were then measured. The results were expressed as growth inhibition rate and presence of secondary roots rate (Nakagawa 1999).

The biochemical properties measured in this study were contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), enzymatic activities of catalase and ascorbate peroxidase, as well as proline and total phenolic compounds contents.

For the MDA and H_2O_2 determination, seed samples (200 mg) were macerated in liquid nitrogen and homogenized in 2 mL of trichloroacetic acid (TCA) 0.1 %. The homogenate was centrifuged at 3,600 rpm, for 15 min, and the supernatant recovered. For MDA, the assay mixture consisted of 20 % of TCA and 0.5 % of thiobarbituric acid. The reaction was performed by mixing 250 μL of supernatant and 1 mL of the mixture, followed by incubation in water bath (95 ºC) for 30 min. Then, the reaction was stopped on ice (10 min). The samples were left to stand at room temperature for 10 min, and absorbance was measured using a spectrophotometer at 600 nm and 535 nm. The results were expressed as μmol g^{-1} of fresh mass (Heath & Packer 1968).

The reaction mixture for H_2O_2 consisted of 200 μL of supernatant, 800 μL of potassium iodide and 200 μL of phosphate buffer (0.1 M; pH 7.5), followed by incubation on ice during 1 h. Then, the samples were left to stand at room temperature for 5 min and absorbance readings were recorded using a spectrophotometer at 390 nm, with results expressed as nmol g^{-1} of fresh mass (Alexieva et al. 2001). Both assays were performed with three replicates.

To enzyme extraction and protein determination, fresh samples collected at 24, 48, 72 and 120 h of germination were macerated in a mortar using liquid nitrogen, and 1 g of sample was homogenized with 1.8 mL of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM of ethylenediaminetetraacetic acid (EDTA) and 20 % of polyvinylpolypyrrolidone (PVPP). The extract was centrifuged at 13,000 g/20 min (4 ºC) and the supernatant was used to determine the enzymatic activities (Bradford 1976).

The catalase activity (CAT - EC 1.11.1.6) was assayed spectrophotometrically at 25 ºC, in a reaction mixture containing 1 mL of 100 mM potassium phosphate buffer (pH 7.5) plus 2.5 μL of H_2O_2 (25 % solution), prepared immediately before its use. The reaction was started by the addition of 25 μL of plant extract, and the catalase activity was monitored by H_2O_2 degradation at 240 nm, over a 1-min period (Azevedo et al. 1988).

The ascorbate peroxidase activity (APX - EC 1.11.1.11) was assayed by monitoring the oxidation rate of ascorbate during 2 min at 290 nm ($\varepsilon = 2.80 \times 103$ M^{-1} cm^{-1}). The reaction mixture consisted of 13 mL of 100 mM potassium phosphate buffer (pH 7.0), 2 mL of 0.5 mM ascorbic acid and 2 mL of 1 mM EDTA. The reaction was started by adding 1,780 μL of the reaction medium, 200 μL of 0.1 mM H_2O_2 and 20 μL of the enzyme extract. The results, such as for the catalase, were expressed as μmol min^{-1} mg $prot^{-1}$ (Nakano & Asada 1981).

To quantify the proline content, seed samples (0.3 mg) were macerated in liquid nitrogen and homogenized in 6 mL of sulfosalicylic acid (3 %). The homogenate was centrifuged at 3,600 rpm for 30 min and the supernatant was used. Proline was determined by mixing 2 mL of the supernatant, 2 mL of an acidic ninhydrin solution and 2 mL of glacial acetic acid. The reaction mixture was incubated in water bath (100 ºC, for 60 min) and left to stand on ice for 10 min. After that, 2 mL of toluene were added and the mixture rested for 20 min at room temperature, for phase separation. The upper phase was collected and absorbance readings were performed spectrophotometrically at 520 nm. The results were expressed as μmol g^{-1} of fresh mass (Bates et al. 1973).

The total phenolic compounds were similarly quantified from the seed samples (0.3 mg), also macerated in liquid nitrogen, homogenized in 3 mL of acidified methanol (1 mL of hydrochloric acid + 100 mL of methanol at 80 %) and centrifuged (3,600 rpm, for 30 min). Thus, the supernatant (100 μL) was mixed with 700 μL of Folin-Ciocalteau (0.25 M) and incubated in the dark for 3 min. Then, 700 μL of Na_2CO_3 (0.2 M) were added and incubated in the dark for 30 min. The absorbance readings

were carried out spectrophotometrically at 725 nm. The results were expressed as mg GAE (gallic acid equivalent) g^{-1} of fresh mass (Folin & Ciocalteu 1927).

The data for all variables were summarized, subjected to normality (Shapiro-Wilk) and homogeneity (Levene) tests, and analyses of variance (Anova) were conducted. Two independent analyses were performed, because the statistical results showed no significant differences between the experiments. The results are presented as mean and standard deviation of all replicates, and the response variables were submitted to regression analysis. The best models displayed in graphics were selected according to the higher R-squared and lower Akaike Information Criterium (AIC). All statistical analyses were performed by the R software (R Core Team 2019).

RESULTS AND DISCUSSION

The reduction of water potential during seed germination may result in lower germination rates, or even its inhibition (Cardoso 2004). Under full water availability, the imbibition of *A. sativa* seeds presents a three-phase curve. In the phase I, the water content in the seed increases rapidly, what is followed by a stabilization in the phase II, until the protrusion of the radicle, thus starting the phase III, with another increase in water content due to the growth of the embryo (Bewley et al. 2013).

The three-phase seed germination pattern was observed for all treatments in the imbibition curve, with a rapid absorption of water up to 9 h, a phase of low water absorption between 9 h and 36 h, and a recovery in the water absorption rate after 48 h. The results indicate that the osmotic potential of aqueous solutions of *L. camara* leaves did not interfere in the water absorption of the seeds.

The germination of *A. sativa* seeds, expressed as percentage of normal seedlings, was not affected by the aqueous extract concentrations of *L. camara* leaves, when exposed to the phases I and II of germination (Figure 1A). However, the germination of the seeds, when they were exposed to the extract in the phase III, was significantly affected at all concentrations of the extract (Figure 1B). In the phase III, the seed germination from 78 ± 5 % in the control treatment was reduced to 54 % ± 1, 43 % ± 12, 14 % ± 8 and 6 % ± 6, respectively, at aqueous extract concentrations of 2.5 %, 5 %, 7.5 % and 10 % (vv^{-1}). Also, when *A. sativa* seeds were exposed to the extract during all the germination phases (I, II and III), there was a reduction of the percentage of normal seedlings for all extract concentrations (Figure 1C). In these treatments, because of the longer exposure time to the extract (from phase I to III), there was a greater damage to seed germination. The seed germination was completely inhibited at the aqueous extract concentration of 10 % (vv^{-1}).

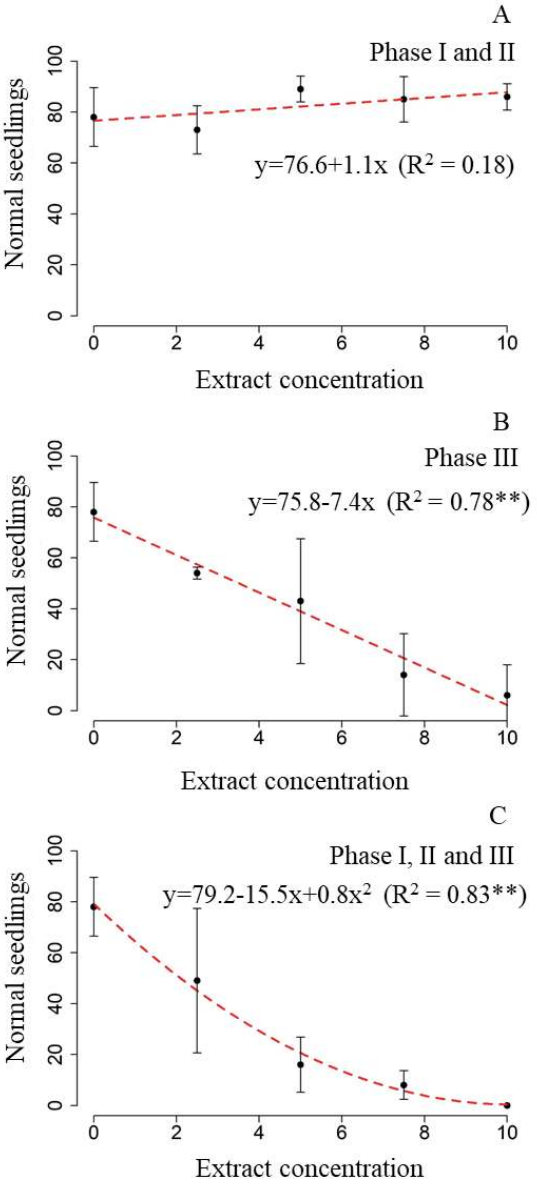

Figure 1. Regressions for the percentage of normal seedlings, when *Avena sativa* seeds were exposed to the *Lantana camara* extract, at different concentrations (%; vv^{-1}), in the germination phases I and II (A), III (B) or during the three phases (C). The bars for observed points are the mean standard deviations.

According to Kupidlowska et al. (1994), the phyto-chemical coumarin found in *L. camara* leaves by Yi et al. (2006) causes necrosis and inhibition of root growth in seedlings. Atrophied and necrotic roots were the main anomalies found in seeds exposed to these phytochemicals, although the phytotoxic damages of the extract to the seed metabolism were not sufficient to promote the death of the seedlings.

DNA synthesis, mitotic divisions and mobilization of reserve substances occur after the embryo growth begins, in the phase III of germination (Cardoso 2004, Bewley et al. 2013). The results indicate that the phytochemicals of *L. camara* present in the extract interfere in these metabolic processes.

The aqueous extract of *L. camara* caused a reduction in the germination speed index at all tested concentrations (Figure 2A). The germination speed index values were reduced from 13.8 % ± 0.3 to 11.7 % ± 0.8, 10.5 % ± 0.5, 9.17 % ± 0.3 and 8.4 % ± 0.2, respectively, at the concentrations of 2.5 %, 5 %, 7.5 % and 10 %. The delay in the radicle emission may be explained by the interference in the growth of the allelochemicals present in the extract, such as reactivation of mitochondrial Krebs cycle enzymes and oxidative phosphorylation, as well as protein synthesis from substrates (enzymes, mRNA, etc.), that occur during the germination phases I and II (Cardoso 2004, Bewley et al. 2013).

Allelochemicals may rapidly depolarize the cell membrane, increasing the permeability and inducing lipid peroxidation (Yu et al. 2003). Previous reports have shown that the increase of electrolyte flow may be one of the consequences of membrane integrity damage (Poonpaiboonpipat et al. 2013). A significant seed solute leakage was observed when exposed to the extract. The solute leakage increased with the increase of extract concentration (Figure 2B); hence, it may be stated that there is damage to the repair system of cell membranes during seed imbibition, caused by the allelochemicals present in the extract. Aumonde et al. (2013) also reported a higher release of lettuce seed electrolytes with an increase in the concentration of the aqueous extract of *Philodendron bipinnatifidum*.

The exposure of the aqueous extract of *L. camara* during seed germination at the phases I and II did not affect the epicotyl and root development of the *A. sativa* seedlings. The seeds submitted to the extract during the phase III of germination had their growth negatively affected. The extracts reduced the epicotyl growth by 57 %, 66 %, 84 % and 90 % (Figure 3A) and root growth by 28 %, 43 %, 57 % and 62 % (Figure 3B), respectively, at concentrations of 2.5 %, 5 %, 7.5 % and 10 %. The exposure of the seeds to the extract during all phases of seed germination (I, II and III) caused the greatest damage to the epicotyl and root growth, reaching 99 % of inhibition. The reductions reached 57 %, 81 %, 89 % and 99 % for epicotyls (Figure 3C) and 41 %, 58 %, 74 % and 99 % for roots (Figure 3D), respectively, at concentrations of 2.5 %, 5 %, 7.5 % and 10 %.

Seedling growth is known to depend on DNA synthesis, mitotic divisions and seed reserve mobilization (Cardoso 2004, Bewley et al. 2013). Mitotic divisions can be reduced by the action of allelochemicals (Ladhari et al. 2014), compromising the normal plant development. Romagni et al. (2000) reported that the compound 1,8-cineol is present as a mitotic index and root growth reducer. The phytochemical 1,8-cineol is present in *L. camara*

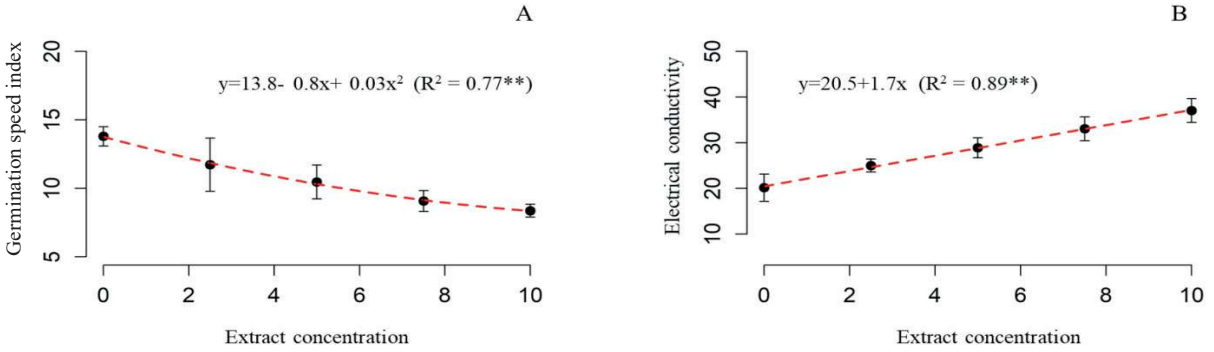

Figure 2. Regressions for the germination speed index (A; %) and electrical conductivity (B; µS cm^{-1} g^{-1}) of *Avena sativa* seeds, as a function of *Lantana camara* extract at different concentrations (%; vv^{-1}). The bars for observed points are the mean standard deviations.

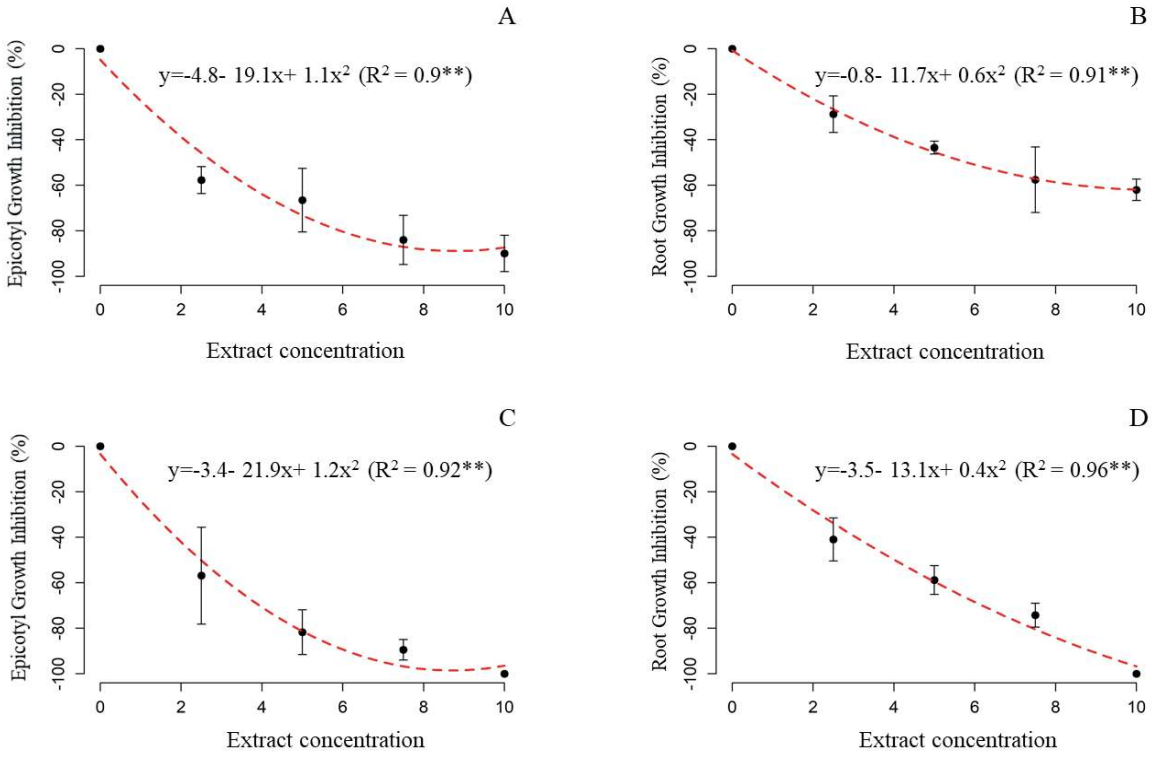

Figure 3. Regressions for the epicotyl and root growth inhibition at the phase III (A-B) and phases I, II and III (C-D) of germination of *Avena sativa* seeds, as a function of *Lantana camara* extract at different concentrations (%; vv^{-1}). The bars for observed points are the mean standard deviations.

extracts (Singh et al. 2012) and may be involved in the phytotoxic action of the aqueous extract of *L. camara* leaves in *A. sativa* seedlings. Kenany & Darier (2013) did not identify the compounds involved; however, they reported that the extract of *L. camara* leaves is able to inhibit the root and stem elongation during the seed germination of *Phalaris minor* and *Sorghum bicolor*.

The malondialdehyde (MDA) levels, at 24 and 48 h of germination, were not affected by the exposure to the allelochemicals in the extract concentration of 5 % (vv^{-1}). After 48 h of germination, the MDA levels remained stable in the control; however, there was an increase in the MDA levels at 72 h of germination of the seeds exposed to phytochemicals (3.0 ± 0.1 μmol g^{-1}), and they were also high at 120 h of germination (2.7 ± 0.1 μmol g^{-1}) (Figure 4A). When the seeds were removed from the water and exposed to the extract within 48 h of germination, there was a large increase in the MDA levels at 72 h of germination (3.6 ± 0.1 μmol g^{-1}), indicating a phytotoxic action. Conversely, the seeds removed from the extract within 48 h of germination had reduced MDA levels by 72 h of germination (1.8 ± 0.2 μmol g^{-1}). The phase III of germination is more sensitive to allelochemicals, as observed by the higher levels of oxidative stress markers. Increases in the MDA levels related to phytochemical stress are also reported in lettuce (Ladhari et al. 2014), soybean (Haddadchi & Gerivani 2009), mustard (Oracz et al. 2007), maize, pea and radish (Gmerek & Politycka 2011). Gmerek & Politycka (2011) attribute the lipid peroxidation to the phytochemicals ferulic and p-coumaric acids present in *L. camara* extracts (Yi et al. 2006).

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. The MDA content is commonly known as a marker of oxidative stress (Gawel et al. 2004). Kurek et al. (2019) reported that the genomic integrity considerably affects the seed viability and vigor. Quinones and phenols (groups of allelochemical compounds) are thought to have a toxic effect, because of their ability to form semiquinone radicals that donate electrons to molecular oxygen, forming reactive oxygen species (Hammond-Kosak & Jones 1996).

The H_2O_2 levels increased throughout the period of seed germination; however, there were

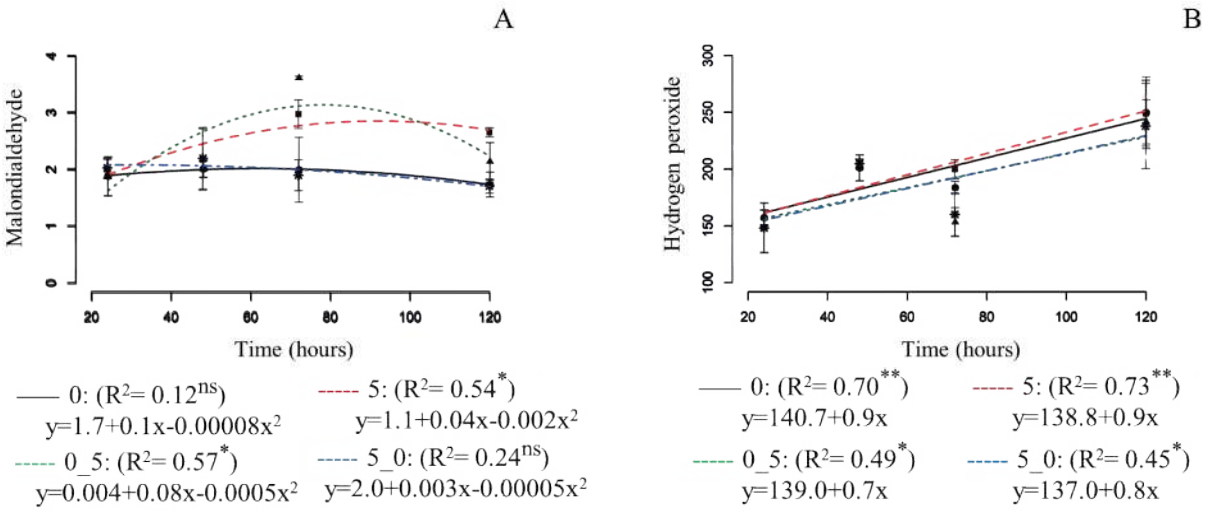

Figure 4. Regressions for malondialdehyde (A; µmol g^{-1} of fresh mass) and hydrogen peroxide (B; nmol g^{-1}) contents, during the germination period (in hours) of *Avena sativa* seeds, when kept in water (0) or exposed to *Lantana camara* extract at the concentration of 5 % (vv^{-1}) (5), and with induction (0_5) or removal (5_0) of the phytotoxic stress. The bars for observed points are the mean standard deviations.

no differences between the control and the seeds exposed to the extract (Figure 4B). Contrary to our results, allelopathic metabolites increase the H_2O_2 levels in some plant species, such as *Sinapis alba* (Oracz et al. 2007) and *Chlorella vulgaris* (Qian et al. 2009). The non-increase of H_2O_2 levels may be related to an efficient antioxidant defense system of *A. sativa*.

Lower levels of catalase activity, between 48 and 120 h of germination, were observed in the seeds exposed to the extract (5 %; vv^{-1}), when compared to the control (0 %; vv^{-1}) (Figure 5A). The reduction in the levels of catalase enzyme in the seeds exposed to the extract is possibly related to the action of the enzyme in neutralizing reactive oxygen species, accumulated by the phytotoxic effect of the extract.

When the seeds were removed from the water (0 %; vv^{-1}) and exposed to the phytotoxic extract at the concentration of 5 % (vv^{-1}) (treatment denoted as "0_5"), a high increase in the catalase activity

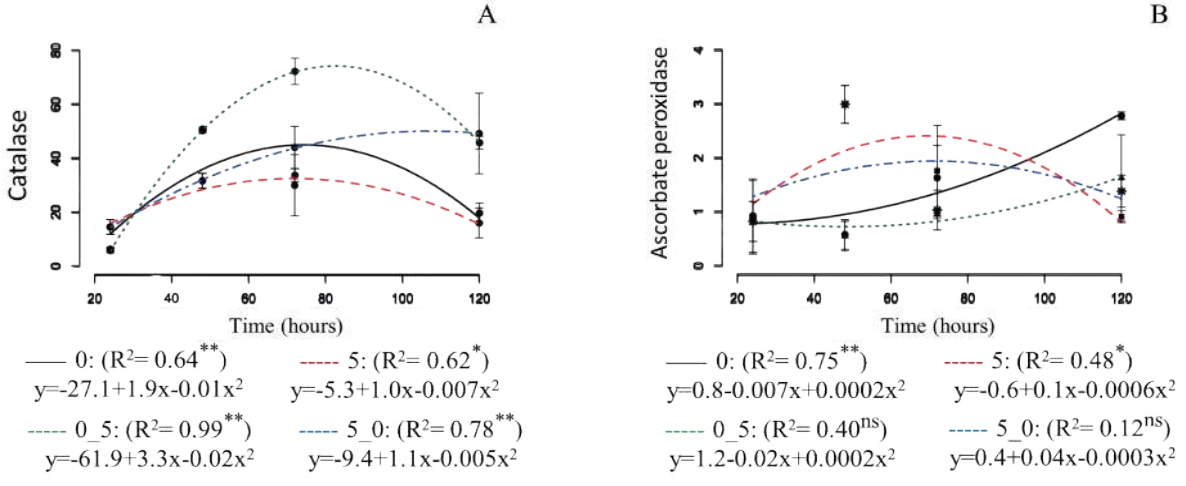

Figure 5. Regressions for the enzymatic activities (µmol min^{-1} mg prot^{-1}) of catalase (A) and ascorbate peroxidase (B), during the germination period (in hours) of *Avena sativa* seeds, when kept in water (0) or exposed to *Lantana camara* extract at the concentration of 5 % (vv^{-1}) (5), and with induction (0_5) or removal (5_0) of the phytotoxic stress. The bars for observed points are the mean standard deviations.

was observed, with a subsequent rapid reduction (Figure 5A). This indicates a metabolic response of reactive oxygen species for neutralization. In seeds removed from the phytotoxic stress (extract at the concentration of 5 %) to water, here denoted as "5_0", an increase in the catalase activity was also observed, although less than for the treatment "0_5", signaling, in this case, the interruption of the phytotoxic action.

Oracz et al. (2007) reported increases in the catalase activity in *Sinapis alba* L. seeds subjected to *Helianthus annuus* L. extract up to eight days of germination, while Pandey et al. (2005) in *Chara zeylanica* and Lin et al. (2000) in *Echinochloa crus-galli* found a reduction in the catalase activity in plants subjected to allelopathic extract. Such differences may be explained by plant-specific mechanisms to scavenge the production of reactive oxygen species.

The activity of ascorbate peroxidase in seeds under normal germination conditions (0 %; vv^{-1}) remains stable during the phases I and II (up to 48 h), gradually increasing during the seedling growth and development (Figure 5B). However, in seeds exposed to allelochemicals (5 %; vv^{-1}), the levels of ascorbate peroxidase increase rapidly during the germination phases I and II (up to 48 h) and decrease rapidly during the seedling growth and development phase (after 48 h).

The increase and reduction in the levels of the ascorbate peroxidase enzyme, in seeds exposed to the extract, are possibly related to the metabolic response of production and action of the enzyme to neutralize the reactive oxygen species, accumulated by the phytotoxic effect of the extract. The treatments "0_5" and "5_0" did not present significant changes in the ascorbate peroxidase activity.

The results corroborate those by Pandey et al. (2005), who reported a reduced ascorbate peroxidase activity in *C. zeylanica* plants. Muscolo et al. (2001) reported that phenolic compounds such as vanillic, p-coumaric and p-hydroxybenzoic acids are able to inhibit the enzymatic activity in seedlings. These substances are present in leaves of *L. camara* (Yi et al. 2006, Mishra 2015).

It was observed that, during the phases I and II of germination (up to 48 h), all treatments showed a rapid increase in the proline levels (Figure 6A). During the seedling growth and development (phase III), there was a reduction in the level of proline in the control seedlings (without stress). However, it is observed that the proline level remained high in the seedlings subjected to phytochemical stress. Higher levels of proline, during the phase III of germination, were also observed in the treatments that suffered stress, either only during the phase III (treatment "0_5") or just before it (treatment "5_0") (Figure 6A).

According to Singh & Rao (2003), proline protects proteins from denaturing, what may explain the elevation and maintenance of high levels in stressed seedlings, as an attempt to defend seedlings against allelochemical damage. An increase in the proline content was also recorded in *Triticum*

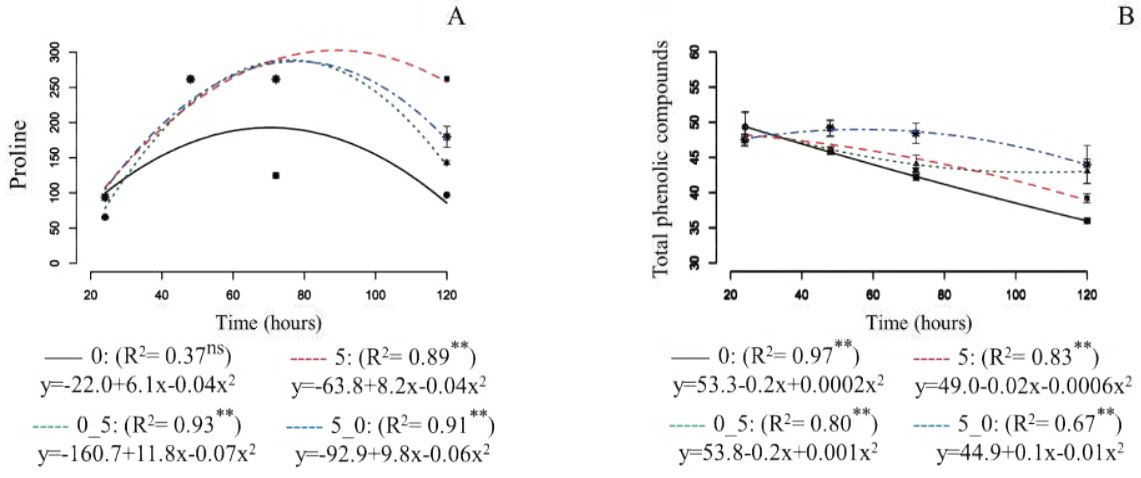

Figure 6. Regression for the contents of proline (A; µmol g^{-1} of fresh mass) and total phenolic compounds [B; mg GAE (gallic acid equivalent] g^{-1} of fresh mass), during the germination period (in hours) of *Avena sativa* seeds, when kept in water (0) or exposed to *Lantana camara* extract at the concentration of 5 % (vv^{-1}) (5), and with induction (0_5) or removal (5_0) of the phytotoxic stress. The bars for observed points are the mean standard deviations.

turgidum and *Pisum sativum*, as a response to phytochemical coumarin (Abenavoli et al. 2006); *Tagetes erecta* under treatment with *Jatropha curcas* extract (Wang et al. 2009); and *Triticum aestivum* leaves with aqueous extract of *Zea mays* leaves (Ibrahim et al. 2013). After that, Ladhari et al. (2014) also reported an accumulation of proline in lettuce leaves, in response to the methanolic extract of *Cleome arabica*; however, this accumulation was reduced in the roots.

The levels of total phenolic compounds were reduced throughout the germination of seeds in all treatments; however, the levels remained higher than for the control in the treatments exposed to allelochemicals (Figure 6B). Djanaguiraman et al. (2005) reported that allelochemicals increase the phenolic content in *Sorghum bicolor* and *Phaseoulus vulgaris*. Ladhari et al. (2014) stated that allelochemicals increase the phenolic content in lettuce roots, but reduce it in lettuce leaves; while Pandey et al. (2005) reported a reduction in the phenolic content of *C. zeylanica* exposed to allelochemicals.

CONCLUSION

Concentrations of the aqueous extract of *Lantana camara* leaves above 2.5 % (vv^{-1}) impair the physiological development of *Avena sativa* seeds during germination. The extract affects the phases I and II of germination, damaging cellular membranes and delaying the radicle emission, and, at the phase III, it causes abnormalities in the seedling development. In addition, this phytotoxic effect triggers the enzymatic and non-enzymatic antioxidant metabolism of *A. sativa* seeds during germination. Hence, this study corroborates the potential of *L. camara* allelochemicals for the development of natural herbicides.

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