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Allelopathic potential from cover crops aqueous extract on weeds and maize

Potencial alelopático do extrato aquoso de plantas de adubação verde sobre plantas daninhas e milho

Potencial alelopático del extracto acuoso de abonos verdes sobre malezas y maíz

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Abstract

Cover crops are used as a strategy to recover degraded soils, improving their physical, chemical and biological characteristics, as well as assisting in the control of weeds through allelochemical compounds released by the shoot degradation process. This work aimed to evaluate the phytotoxicity of the aqueous extract of the cover crops *Raphanus sativus* L., *Avena strigosa* (Schreb) and *Vicia villosa* R. on the germination and initial growth of *Zea mays*, *Amaranthus spinosus* L. and *Ipomoea grandifolia* (Dammer) O'Donnell, as well as to identify and quantify the phenolic compounds present in the extract. Straws from the dried and crushed cover crops were used. The extracts were obtained by mixing 10% *R. sativus* and *A. strigosa*, and 12% *V. villosa* in distilled water (m/v). DIC was used, with four replications, in a 3x4 factorial scheme, with 3 cover crops (*R. sativus*, *A. strigosa* and *V. villosa*) and 4 concentrations (0%, 25%, 50% and 75%). The variables analyzed were: germination, germination speed index (GSI), seedling growth, root protrusion (RP), root protrusion speed index (RPSI) and dry mass of the root and shoot. Higher concentrations of the extracts affected the initial development of maize and weeds. The phenolic compounds identified in greater quantity were benzoic acid, p-coumaric acid, followed by ferulic acid and p-hydroxybenzoic acid. It is suggested that studies of the same nature be carried out in the field, since the interactions between soil microorganisms, climatic conditions can interfere with the results.

Keywords: Phytotoxicity; Phenolic compounds; Allelopathy; Agroecology.

Resumo

Plantas de adubação verde (PAV) são utilizadas na estratégia de recuperar os solos degradados, melhorando suas características físicas, químicas e biológicas, além de auxiliar no controle de plantas espontâneas através de compostos aleloquímicos liberados pelo processo de degradação da parte aérea. Este trabalho objetivou avaliar a fitotoxicidade do extrato aquoso das plantas de adubação verde (PAV) *Raphanus sativus* L., *Avena strigosa* (Schreb) e *Vicia villosa* R. sobre a germinação e o crescimento inicial de *Zea mays*, *Amaranthus spinosus* L. e *Ipomoea grandifolia* (Dammer) O'Donnell, bem como identificar e quantificar os compostos fenólicos presentes no extrato. Foram utilizadas palhadas das plantas de adubação verde secas e trituradas. Os extratos foram obtidos misturando 10% de *R. sativus* e *A. strigosa*, e 12% de *V. villosa* em água destilada (m/v). Foi utilizado DIC, com quatro repetições, em esquema fatorial 3x4, sendo 3 PAV (*R. sativus*, *A. strigosa e V. villosa*) e 4 concentrações (0%, 25%, 50% e 75%). As variáveis analisadas foram: germinação, índice de velocidade de germinação (IVG), crescimento de plântula, protrusão radicular (PR), índice de velocidade de protrusão radicular (IVPR) e Massa

Seca da raiz e da parte aérea. Maiores concentrações dos extratos afetaram o desenvolvimento inicial do milho e das plantas daninhas. Os compostos fenólicos identificados em maior quantidade foram ácido benzoico, ácido p-cumarico, seguidos pelo ácido ferúlico e ácido p-hidroxibenzócio. Sugere-se que estudos da mesma natureza sejam realizados a campo, visto que as interações entre os microrganismos do solo, as condições climáticas podem interferir nos resultados.

Palavras-chave: Fitotoxicidade; Compostos fenólicos; Alelopatia; Agroecologia.

Resumen

Los abonos verdes se utilizan en la estrategia de recuperación de suelos degradados, mejorando sus características físicas, químicas y biológicas, así como ayudando en el control de malezas a través de compuestos aleloquímicos liberados por el proceso de degradación de la parte aérea. Este trabajo tuvo como objetivo evaluar la fitotoxicidad del extracto acuoso de los abonos verdes *Raphanus sativus* L., *Avena strigosa* (Schreb) y *Vicia villosa* R. sobre la germinación y crecimiento inicial de *Zea mays*, *Amaranthus spinosus* L. e *Ipomoea grandifolia.* (Dammer) O'Donnell, además de identificar y cuantificar los compuestos fenólicos presentes en el extracto. Se utilizaron pajitas de las plantas de abono verde secas y trituradas. Los extractos se obtuvieron mezclando 10% de *R. sativus* y *A. strigosa* y 12% de *V. villosa* en agua destilada (m/v). Se utilizó DIC, con cuatro repeticiones, en un esquema factorial 3x4, con 3 abonos verdes (*R. sativus, A. strigosa* y *V. villosa*) y 4 concentraciones (0%, 25%, 50% y 75%). Las variables analizadas fueron: germinación, índice de velocidad de germinación (IVG), crecimiento de plántula, protrusión de raíz (PR), índice de velocidad de protrusión de raíz (IVPR) y masa seca de la raíz y parte aérea. Las concentraciones más altas de los extractos afectaron el desarrollo inicial del maíz y las malezas. Los compuestos fenólicos identificados en mayor cantidad fueron ácido benzoico, ácido p-cumárico, seguido del ácido ferúlico y ácido p-hidroxibenzoico. Se sugiere que se realicen estudios de la misma naturaleza en campo, ya que las interacciones entre microorganismos del suelo, condiciones climáticas pueden interferir en los resultados.

Palabras clave: Fitotoxicidad; Compuestos fenólicos; Alelopatía; Agroecología.

1. Introduction

The use of cover crops is an agricultural practice that consists in the employment of plant species, usually legumes (Fabaceae), grasses (Poaceae) and some species of Cruciferae and compositae, either in rotation or in consortium with the agricultural crops of interest (Wutke

et al. 2014). The adoption of such practice is due to the countless benefits originated to the soil and the commercial crops and it has been used in different countries as an alternative for the diversification and improvement of the productive potential of agricultural areas (Calegari 2014).

Adopting cover crops recovers soils that are degraded due to cultivation, improves naturally poor soils and preserves those that are already productive. This occurs as a result of the increase in organic matter, decrease in erosion indices, decompaction and increase in water retention in the soil, nutrient cycling and intensification of the biological activity in the soil (Lafay 2016, Bruno et al. 2017, Costa 1993).

This practice also contributes to the decrease in weed infestations, for both the physical impediment generated by the straw layer formed on the soil, and the release of chemical substances (Correia & Durigan 2006) by the plants, defined by Molisch (1938), as allelopathy. The latter is characterized by the ability the plants have to produce substances that once released in the environment influence favorably or unfavorably the development of other organisms.

The sustainable management of weeds constitutes one of the greatest bottlenecks of the agricultural crops, either perennial or annual, mainly in organic production systems, where there are few alternatives to the chemical management employed in the conventional agriculture. As pointed out by Bàrberi (2002), weed control in agroecosystems has become one of the main obstacles to the transition from the conventional to the organic production system.

Since 2008, Brazil occupies the first position in the list of agrochemical consumers (Carneiro 2015). While the agrochemical global commerce grew 93% in the last ten years, the Brazilian market grew 190%, and the category with the highest percentage of commercialization is the herbicides, with 45% of the total commercialized (ANVISA 2012, Carneiro 2015).

One way of reducing the herbicide load employed in the agriculture is the adoption of agroecological techniques such as the no tillage system (NTS). However, although the NTS has been widely employed in the last few decades, there is little information on the existing relations between the plants used as cover crops, the weed and the plants of economic interest sowed in succession.

For this reason, the aim of this study was to identify the compounds with allelochemical potential present in the shoot aqueous extract from cover crops and evaluate the phytotoxic effect of this aqueous extract on the germination and development of weeds and the maize.

2. Methodology

The biotests were carried out at the laboratories of Vegetable Physiology, Germination and Growth of Seedlings, Organic Chemistry and the Analysis Center of the Federal University of Fronteira Sul, Campus Laranjeiras do Sul, during the period from March 2017 to March 2018.

The seeds of the cover crops plants Vicia villosa R. (hairy vetch), Raphanus sativus L. (radish) and Avena strigosa (Schreb) (black oat) were purchased in the local commerce. The Amaranthus spinosus L. (spiny amaranth) seeds, Ipomoea grandifolia (Dammer) O'Donnell (morning glory), were collected in June 2017 after reaching physiological maturity, from infested areas in the municipalities of São Miguel do Oeste – SC (26°40'47.1"S 53°31'21.5"W) and Marmeleiro – PR (26°13'28.0"S 53°08'09.6"W), respectively. The Zea mays L. seeds used were conventional hybrid maize cv. 22D11 from the company SEMPRE Sementes.

The sample seeds of spiny amaranth and morning glory were cleaned with a seed blower to remove impurities and damaged seeds. The seed quality tests of the cover crops, weeds and maize were carried out obtaining moist content through the oven standard method at 105 °C and germination rate through the germination standard test, described in the Rules for Seed Analyses (Brasil 2009).

The quantification and identification of compounds with allelochemical potential present in the aqueous extracts of the shoot of the cover crops V. villosa, R sativus and A strigosa were carried out and the phytotoxicity of these extracts on the germination and initial developement of A. spinosus, I. grandifolia and the Z. mays crop was investigated.

Solid-liquid extraction

The solid-liquid extraction was performed following the methodology used by Bittencourt (2017). The hairy vetch, radish and black oat plants were collected in their blossoming peak, placed in kraft paper bags and dried in oven with forced air circulation at 40 ºC up to constant mass. After that, they were ground in a Willey mill with a 10mesh sieve and stored in glass flasks protected with aluminum paper and sealed with plastic film.

The aqueous extracts were obtained by mixing the ground dry matter with distilled water in a homogeneous mixture at the proportion 12% (mass/volume) hairy vetch and 10% black oat and radish. The mixtures were kept in agitation for two hours in a refrigerated incubator with a "Shaker" orbital agitator, brand Fortinox, model STAR FT 38 at 230 rpm and 40 ºC.

Next, they were filtered in surgical gauze to remove the excess of ground material and

centrifuged at 4000 rpm for 10 minutes at room temperature ~ 24 ºC in a Sigma centrifuge, model 3-16 KL. The supernatant was filtered in filter paper (25 µm pores) and stored in refrigerator at 5°C.

Gross extract

The gross extract of the hairy vetch, radish and black oat plants was obtained following Bittencourt et al. (2018) with modifications, that is, 50 mL aqueous extract added to 150 mL acetone. The mixture was agitated for 12 hours in a magnetic agitator, brand IKA®, model C-MAG HS7, at ~20 ºC to remove nonpolar compounds such as lipids and proteins from the aqueous extract. The solution was filtered in filter paper (25 µm pores) and the acetone removed in a Biothec rotating evaporator, model BT351, at 40 ºC.

The resulting material was washed three times with 100 mL hexane, in a separation funnel to remove the nonpolar compounds of difficult separation that had not been removed by the acetone, and the hexane fraction was discarded. The extract was washed again, three times, with 100 mL ethyl ether, and the ethereal phase was used.

The ether was removed using a rotating evaporator at 40 ºC and the resulting fraction was considered the gross extract, which was used to quantify total phenols and to identify the compounds. The final amounts of gross extract from radish, black oat and hairy vetch were 7.04, 5.73 and 23.54 g, respectively.

Total phenol quantification

The total phenol quantification was carried out according to Bittencourt (2018) and López & Juan (2013), adapted, and the gross extract samples were transferred to cuvettes and the reading was performed using a Thermo Scientific spectrophotometer, model Evolution 201, with light beam in the 760 nm band. The cuvette solution composed of 50 µL extract, 600 µL Na2CO3 (7.5%, m/v solution), 750 µL distilled water and 200 µL Folin Ciocalteau reagent. The mixture was incubated in water bath for 10 minutes at 50 ºC with the addition of 1 mL distilled water. The total phenol quantification was carried out in relation to µg mL-1 galic acid, used to calibrate the equipment reading.

The standard curve was obtained in the concentrations 0, 10, 20, 30 and 40 µg mL-1 galic acid, which were transferred to test tubes with the addition of 600 µL Na2CO3 (7,5%, m/v solution), 700 µL distilled water and 200 µL Folin Ciocalteau reagent.

Identification of phenolic compounds

The identification of phenolic compounds followed a methodology adapted from Dias (2010), being performed by analyzing the extracts in gas chromatographer with mass detector, Shimadzu, model GCMS-QP2010 Ultra. The equipment was adjusted with an injector at 300 ºC in the splitless mode and interface at 280 ºC with a 260 ºC ion source. The oven temperature program with the initial temperature of 80 ºC was kept for a minute, increased up to 250 ºC at a 15 ºC per minute rate, kept for another minute, increased up to 300 ºC at a 4 ºC per minute rate, and kept at this temperature for another five minutes, totaling 30.83 minute analysis time. The mass spectrometer was adjusted to scan masses between 50 and 800 ua.

The sample derivatization was carried out by drying 0.25 mL gross extract in a vial with nitrogen constant flow. To the solid, 30 µL pyridine (C5H5N) and 70 µL N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA-C8H18F3NOSi2) were added. The mixture was heated at 70 ºC in sand bath for 70 minutes. From the resulting solution, 2 µL were manually injected in the chromatographer. The identification of compounds was obtained through the comparison of the mass spectrum with data from the libraries NIST08, NIST08s, NIST11 and NIST11s.

Shoot aqueous extract Biotest

These biotests employed the aqueous extracts from the shoot solid-liquid extraction of the plants hairy vetch, radish and black oat as the germitest paper humidifying mean, and the paper was humidified 2.5 times its mass in the concentrations 25, 50 and 75% (v/v) diluted in water and distilled water was used as a witness.

The following variables were determined: germination, germination speed index, seedlings growth and dry matter for the maize; germination, germination speed index and seedling growth for A. spinosus; while for I. grandifolia, the variables evaluated were root protrusion, root protrusion speed index and seedling growth.

The germination tests were carried out with 4 replications of 50 seeds for maize and 4 replications of 25 seeds for A. spinosus and I. grandifolia. Each species remained in the germinator for the time recommended by the Rules for Seed Analysis (Brasil 2009) and at the end of the test, the number of normal and abnormal seedlings and dead and dormant seeds were calculated for the maize, while for A. spinosus and I. grandifolia, the seeds with root protrusion were calculated.

The germination speed index (GSI) was carried out along with the germination test. To evaluate the maize and A. spinosus GSI, the number of normal seedlings was recorded daily from the appearance of the first normal seedling until the number of seedlings became constant. for I. grandifolia, the seeds with root protrusion were recorded. The GSI was calculated by the addition of the number of normal seedlings recorded every day, divided by the number of days required for the seedling formation, using the formula proposed by Maguire (1962) as a reference:

$$GSI = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn);$$

where: GSI: Germination speed index; G1, G2, Gn: number of normal seedlings recorded in the first count, second count and last count; N1, N2, Nn: number of days from the sowing to the first, second and last count.

For I. grandifolia, the germination and germination speed index tests were substituted by the root protrusion tests and the root protrusion speed index (RPSI), since this species weakened quite fast, making the analysis of normal seedlings more difficult.

The seedling growth test was carried out with 5 replications of 20 seeds for each species, which remained in the germinator by the period recommended in the Rules for Seed Analysis (Brasil, 2009). At the end of the period, all crops had the shoot and root system length measured with a digital caliper.

Experimental Design and Statistical Analyses

A completely randomized experimental design in a 3x4 factorial scheme was used, with 3 cover crops (R. sativus, A. strigose and V. villosa) and 4 concentrations (0%, 25%, 50% and 75%). For I. grandifolia, only the hairy vetch and radish allelopathic potential was evaluated.

The data was submitted to hypothesis tests by employing the variance analysis ($p \leq 0.05$), and whenever significant, the means of the treatments were compared using the Tukey test ($p \leq 0.05$) or the regression curve was carried out by employing the statistical program Sisvar 5.6 (Ferreira 2014).

3. Results and discussion

Total Phenol Quantification

The shoot aqueous extracts from cover crops presented the following concentrations

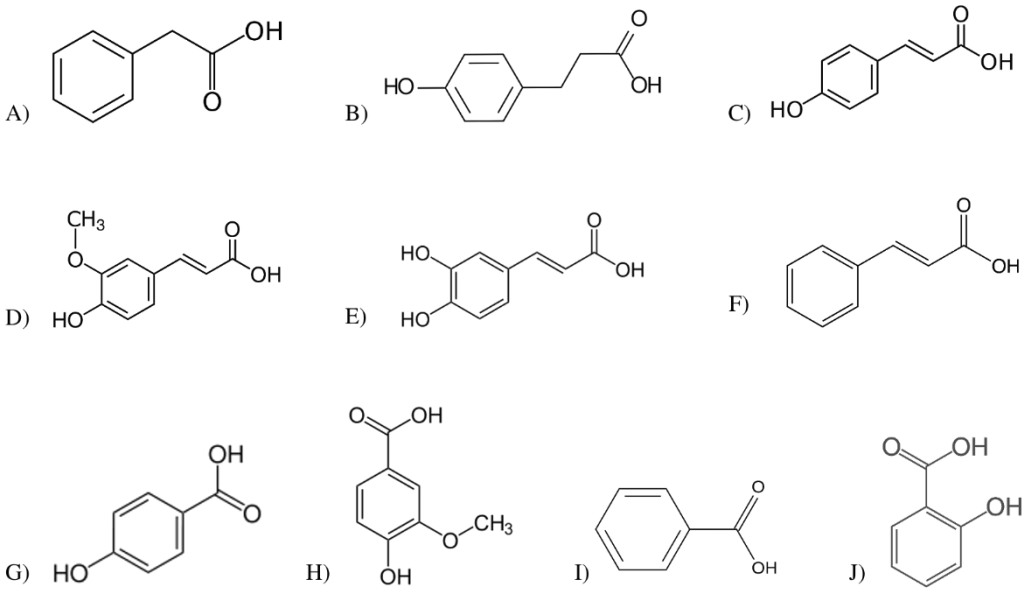
regarding total phenols per plant dry mass: 0.76 mg g-1 for the radish, 0.4 mg g-1 for the black oat and 1.23 mg g-1 for the hairy vetch. Higher values were found by Souza et al. (2017), who reported 8.19 mg g-1 for the dry mass of black oat cut at 80 days and 15.97 mg g-1 for the dry mass of radish cut at 60 days. Such variation in the research results might be ascribed to several factors that interfere in the concentration of phenols produced by the plants such as: weather conditions, nutritional status and the plant development stage (Pires & Oliveira, 2011; Farooq et al. 2020). In addition, the methods to extract allelopathic compounds from plants are varied, which might result in different research results.

According to Calegari (2006), the yield of cover crops dry matter might vary between 3,000 and 9,000 kg ha-1 for radish, 2,000 and 11,000 kg ha-1 for black oat and 3,000 and 5,000 kg ha-1 for hairy vetch. Through the quantification of the total phenols present in the shoot of cover crops, the release of total phenols can be estimated through the degradation of the shoot phytomass of radish, black oat and hairy vetch as 2.30 to 6.75; 0.81 to 4.44; and 3.97 to 6.62 kg ha-1, respectively.

Identification of phenolic compounds

The chromatographic analysis identified phenolic compounds in the extract from the shoot dry mass of cover crops whose structural formulas are shown in Figure 1.

Figure 1. Structural formula of phenolic compounds identified in the shoot dry mass of cover crops. A. Phenylacetic acid; B. Propionic 3(4-hydroxyphenil) Acid; C. p-Coumaric Acid; D. Ferulic acid; E. Caffeic acid; F. Cinnamic acid; G. p-hydroxybenzoic acid; H. Vanillic acid; I. Benzoic acid; J. Salicylic acid.



Source: Libraries NIST08, NIST08s, NIST11 and NIST11s, adapted.

The compounds shown in Figure 1 were the ones that showed the highest amounts in the spectrophotometric evaluation. Although other elements were found, they were not cited because they are not directly linked to secondary compounds and do not have a citation in the literature as allelopathic.

The phenolic compounds identified in the radish were: benzoic acid, salicylic acid, p-coumaric acid, ferulic acid and caffeic acid. In the black oat, the following compounds were found: benzoic acid, salicylic acid, p-coumaric acid, ferulic acid, cinnamic acid, p-hydroxybenzoic acid and vanillic acid, while the hairy vetch extract contained benzoic acid, p-coumaric acid, p-hydroxybenzoic acid, phenylacetic acid and propionic 3(4-hydroxyphenil) acid (Table 1).

Table 1. Presence of phenolic compounds found in the shoot dry mass aqueous extract from radish (Raphanus sativus), black oat (A. strigosa) and hairy vetch (V. villosa). *Presence of compounds in the samples under analysis, and - Absence of compounds in the samples under analysis.

Phenolic compounds	Radish	Black oat	Hairy vetch.
Benzoic acid	*	*	*
Salicylic acid	*	*	-
p-coumaric acid	*	*	*
Ferulic acid	*	*	-
Caffeic acid	*	-	-
Cinnamic acid	-	*	-
p-hydroxybenzoic acid	-	*	*
Vanillic acid	-	*	-
Phenylacetic acid	-	-	*
Propionic 3(4-hydroxyphenil) acid	-	-	*

Source: Authors.

Some compounds might interact with other elements or microorganisms present in the soil, transform and increase their toxicity. They might also turn into less severe compounds such as the ferulic acid, which is transformed by fungi into caffeic or vanillic acid and those, in turn, can be transformed in protocatechuic acid. In the latter, a rupture occurs in the benzenic ring which is then decomposed into CO2 and water (Blum 2004).

Great part of the phenolic compounds found present specific actions in the plant growth and development, interfering with the phytohormone activity, mineral absorption, plant water balance as well as the stomatal function, photosynthesis, breathing, organic synthesis of certain compounds and carbon flow (Einhellig 2004). The action of the benzoic and cinnamic acids might be linked to decrease in nutrient absorption due to cell membrane integrity damage caused by a reduction in the sulfhydryl groups followed by lipid peroxidation (Baziramakenga et al. 1995).

The ferulic acid is a compound that is related to nitrate and ammonia absorption and affects negatively the capture of ions and the water-plant relations (Bergmark et al. 1992, Booker et al. 1992). The caffeic, ferulic, p-coumaric, p-hydroxybenzoic and vanillic acids are linked to the reduction in water conductivity and nutrient absorption by the plant root, and some secondary effects such as photosynthesis low rate, carbon allocation to the roots, increase in the abscisic acid levels, reduced transpiration and leaf expansion rates might be observed (Blum 1994, Rice 1984, Siqueira et al. 1991).

Blum (1996) pointed out that phenolic compounds present a wide range of

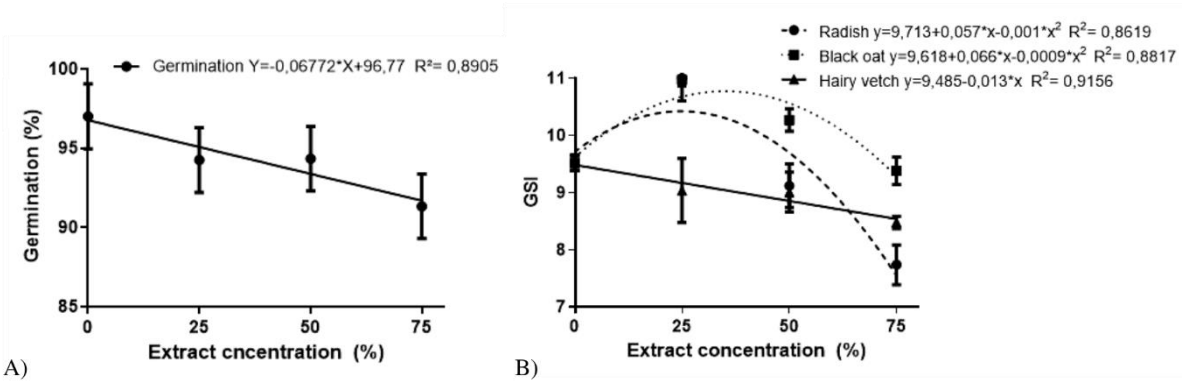
phytotoxicity, however, they all present the same apparent action mode. The primary effect of these compounds on sensitive species seems to be a reduction in the water conductivity and nutrient absorption by the roots, thus interfering in the plant growth.

Shoot aqueous extract biotest

Maize

The concentrations of the aqueous extract from cover crops influenced the variables in different ways. The 75% concentration altered the germination of maize seeds only in the highest concentration of extracts (Figure 1A), however, there was no differentiation between the cover crops. The continuous line presents the germination general rate with the different extracts in a linear model, where Y represents the germination of maize seeds (%) in the different concentrations (%) X. Likewise, Faria et al., (2009) did not observe differences in the maize germination when using Pennisetum americanum and Stizolobium aterrimum extracts.

Figure 2. Effect of the concentrations of the aqueous extract from cover crops on the maize germination and GSI (Zea mays cv. PRE 22D11).



Source: Authors.

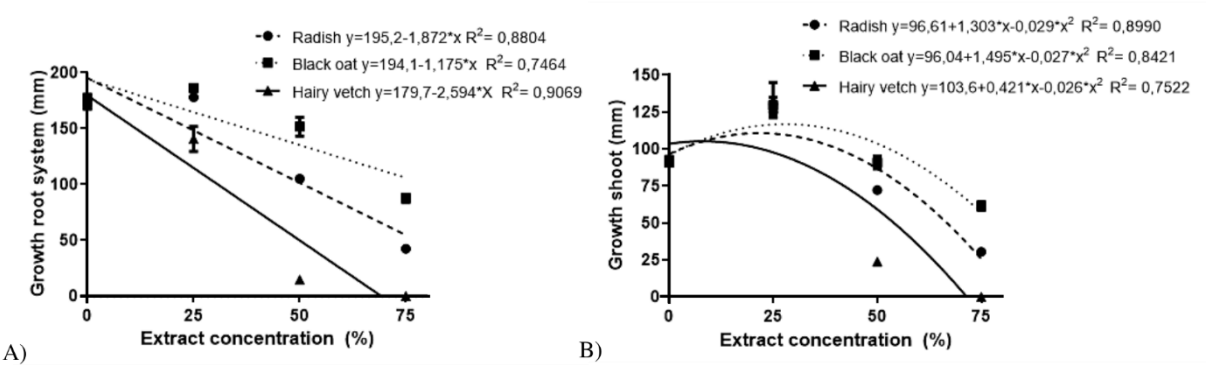
The increase in the concentration of the hairy vetch aqueous extract up to 50 % did not hamper the maize germination speed index (Figure 2B). On the other hand, in the highest concentration of the radish aqueous extract, this parameter reduced significantly. The black oat aqueous extract did not hamper the maize seed GSI, however, in the lowest concentration (25 %), similarly to what was observed for the radish, it accelerated the GSI. The continuous lines present a second order polynomial model for the radish and black oat, and a linear one for the hairy vetch, where Y represents the Germination Speed Index (GSI) according to the different extract concentrations (%), X. These results differ from those found by Bulegon et al.,

(2015) in which the black oat extracts reduced germination and the GSI of lettuce seeds.

The GSI stimulus in the lowest concentration might be explained by the hormesis theory, which is described by Calabrese & Baldwin (2002) as any process in which a cell, organism or group of organisms present a two-phase response when exposed to increasing amounts of a substance or condition, where the exposure to lower doses provoke a stimulant or beneficial response, while high doses cause inhibition or toxicity.

The maize shoot and root growth variables were affected by the aqueous extracts of the three species of cover crops. The root growth presented a decreasing linear behavior in response to the increase in the extract concentration, and the highest phytotoxicity was observed for the hairy vetch, followed by the radish and black oat, respectively. The latter, inhibited root growth significantly in the 50% and 75% concentrations (Figure 3A). Navas and Pereira (2016) also reported decrease in the root growth of lettuce seedlings when exposed to the radish aqueous extract.

Figure 3. Effect of the concentrations of aqueous extracts from cover crops on the maize seedling vigor (Zea mays cv. PRE 22D11).



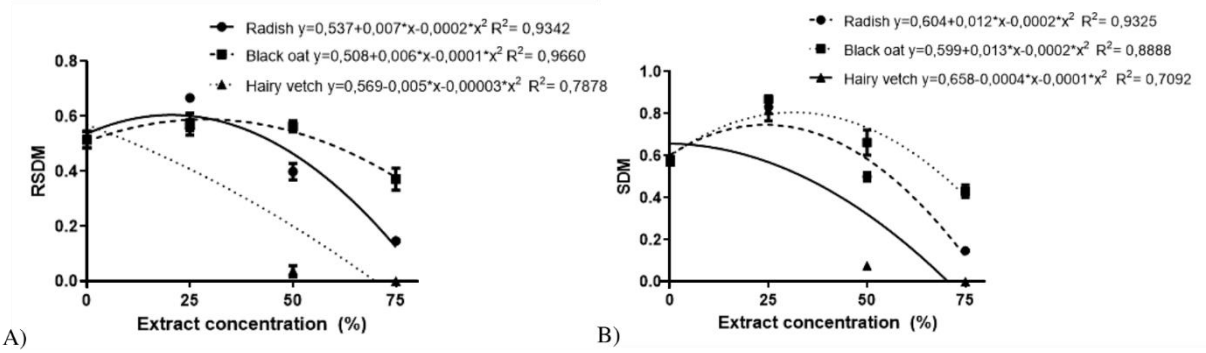
Source: Authors.

The reduction in the maize seedling root system might be related to the presence of phenolic compounds (Table 1), which are responsible for root growth and cell division inhibition such as the case of the cinnamic acid present in the black oat extract and the coumarins (Li et al. 2010).

The growth, the root system dry matter and shoot dry matter (Figure 3B and Figures 4A-B), differently from what was observed int he root system length, was better adjusted by the second order polynomial model, in which the 25% concentration promoted increase in growth, root system dry matter and shoot dry matter. From this concentration upwards, a decreasing trend was observed for both the growth and root system and shoot dry matter.

Similar to effect seen in root growth, higher phytotoxicity was promoted by the hairy vetch aqueous extract, followed by the radish and black oat, respectively.

Figure 4. Effect of the aqueous extracts from cover crops on the maize seedling dry matter (Zea mays cv. PRE 22D11) root system dry matter (RSDM) (A) and shoot dry matter (SDM) (B).



Source: Authors.

The stimulating effect in low concentrations and the toxic effect in higher concentrations of the shoot aqueous extract from cover crops on GSI, growth and shoot dry matter weight suggests the extract hormesis effect. Similar effect was found by Bernardes et al. (2020) when evaluating the potential allelopathic effect of the aqueous extract of the leaves of Mimosa ramosissima Benth, on the germination and initial growth of Panicum maximum cv. aruana and Amaranthus retroflexus L. where at 10% concentration there was a stimulatory effect and higher concentrations hove inhibition.

The results obtained in the phytotoxicity biotests in shoot aqueous extracts from cover crops suggest the existence of a relation between these results and the compounds found in the straw of cover crops.

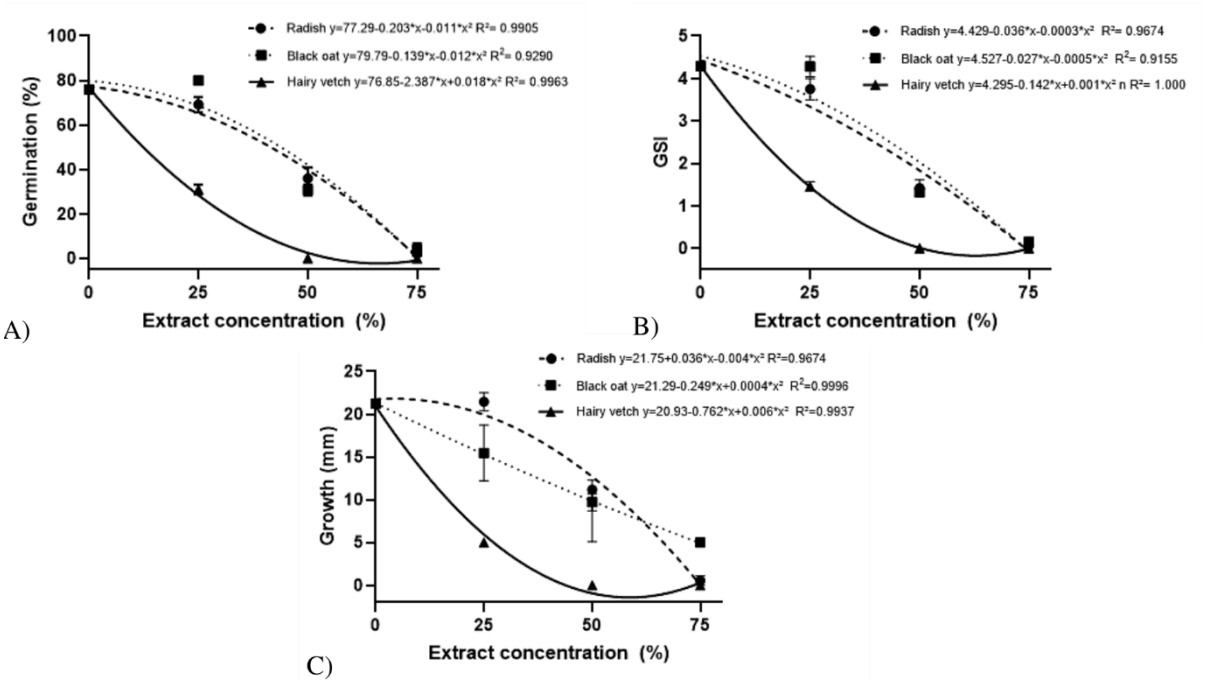
Amaranthus spinosus

The aqueous extract from cover crops reduced significantly the germination and vigor of A. spinosus seeds, presenting a decreasing linear behavior as a function of the increase in the extract concentration (Figures 5A-C). The hairy vetch aqueous extract was the most harmful to the physiological quality of the A. spinosus seeds, while the other extracts did not differ one from another. The hairy vetch extract reduced germination and GSI to zero in the 50% concentration, while the remaining cover crops extracts reduced germination and GSI in around 50%.

Similar results were found by Pires et al. (2001) when testing different concentrations

of the Leucaena leucocephala extract on the germination of Amaranthus hybridus seeds. Those authors verified sharp reduction in germination with the 25% concentration, which was reduced to 0% germination in the extract 50% concentration.

Figure 5. Effect of the concentration of the aqueous extract from cover crops during the A. spinosus initial development phase, germination (A), germination speed index (GSI) (B) and Growth (C).



Source: Authors.

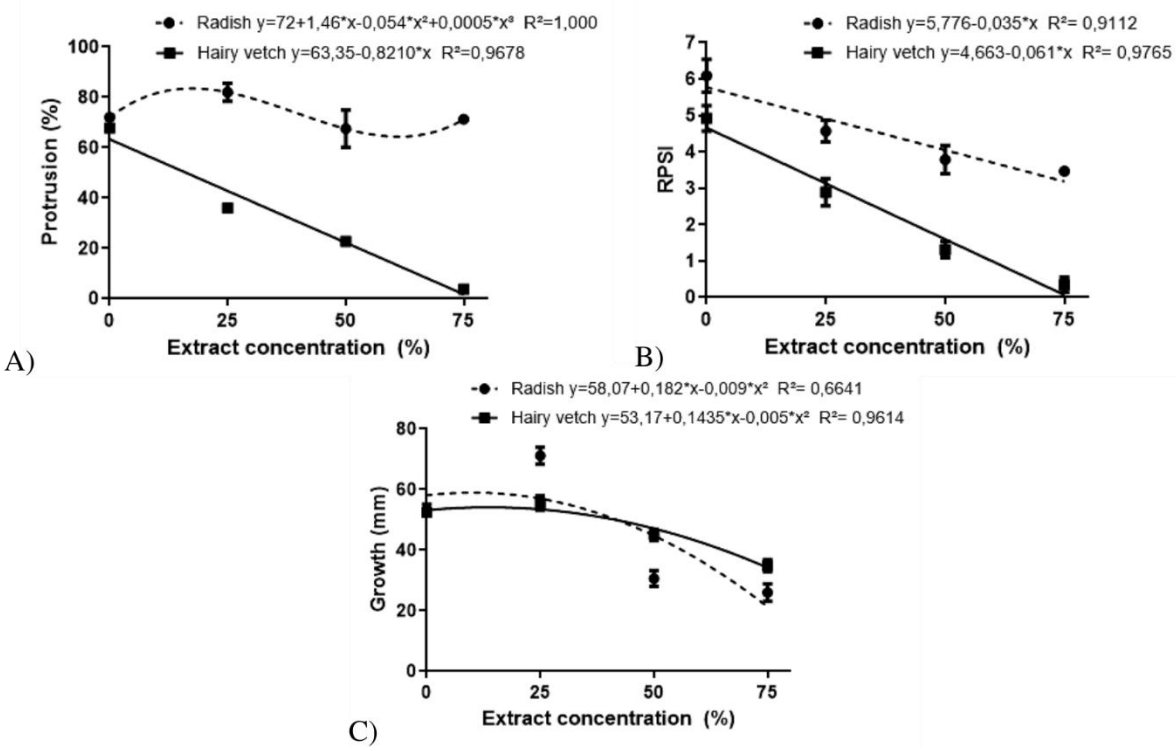
Although the extracts from cover crops interfered in the physiological quality of maize seeds, the A. spinosus seeds presented greater sensitivity to the extracts. Therefore, it seems that the use of cover crops, in addition to enabling some improvement to the soil physical and chemical conditions, also controls the incidence of weeds, due to both the physical limitation imposed to their germination and the action of the allelopathic compounds released by the decomposition of the straw from cover crops.

The decomposition of vegetable residues appears as one of the main sources of release of allelochemicals in the soil. After released in the environment, these chemical compounds need to be absorbed by the target plant, and from then on, they can impact the plant physiology, altering the division and cell stretching processes and modifying the cell ultrastructure and, with that, they also affect the vegetable community populational dynamics (Politycka 1999, Einhellig 2004, Sampietro et al. 2006).

Ipomoea grandifolia

The radish aqueous extract did not hamper the I. grandifolia root protrusion, however, the hairy vetch extract reduced its value to close to zero in the extract 75% concentration (Figure 6A), and a noticeable presence of fungi occurred. Similar values were obtained by Araújo (2010), who reported decrease in the I. grandifolia seed germination with the increase in the Crotalaria juncea extract concentration.

Figure 6. Effect of the concentrations of aqueous extracts from cover crops in the I. grandifolia initial development phase, protrusion (A), root protrusion speed index (RPSI) and growth (C).



Source: Authors.

Regarding RPSI, both aqueous extracts affected this parameter negatively. However, the hairy vetch extract was more phytotoxic than that of the radish (Figure 6B). The decrease in the I. grandifolia root protrusion in the presence of hairy vetch shoot extract is in agreement with the study carried out by Medeiros & Lucchesi (1993), where the aqueous extract 75% and 100% concentrations prevented the germination of lettuce seeds. The reduction in the weed seed germination speed (Figures 5B and 6B) might limit considerably their ability to survive, since the seeds that germinated more slowly might originate seedlings of reduced size (Figures 5C and 6C) and, as a consequence, with lower chances to compete for resources such as water,

light and mineral nutrients (Jefferson & Pennachio, 2005). The I. grandifolia seedling growth (Figure 6C) was affected by the increase in the concentration of the aqueous extract from the cover crops. The hairy vetch was the plant that most affected growth.

Almeida et al. (2008) pointed out that great part of allelochemicals act on the oxidative stress, producing oxygen reactive species, which might act directly or as markers of the cell degradation process, thus hampering germination and the development of seedlings, as well as physiological processes that are vital to the plants, which might explain the low germination in the extract high concentrations, since specific tests were not carried out to determine such influence.

4. Conclusions

The cover crops species that presented the highest concentration of total phenols was the hairy vetch, followed by the radish and the black oat.

The phenolic compounds that appeared most frequently in the shoot samples were: benzoic acid and p-coumaric acid, followed by the ferulic acid and the p-hydroxybenzoic acid.

The phytotoxicity of the aqueous extracts from cover crops depends on the concentration and the receiving species.

High concentrations of the extracts affect the maize and weed vigor.

Weeds tend to present higher sensitivity to the extracts from cover crops than the maize.

It is suggested that studies of the same nature be carried out in the field, since the interactions between soil microorganisms, climatic conditions can interfere with the results.

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