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Article

PHENOLIC COMPOUNDS WITH ALLELOPATHIC POTENTIAL OF *Secale cereale* L. AND *Raphanus sativus* L. GROWN UNDER AN AGROECOLOGICAL NO-TILLAGE SYSTEM

*Compostos Fenólicos com Potencial Alelopático de **Secale cereale** e **Raphanus sativus** Cultivados em Sistema de Plantio Direto Agroecológico*

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ABSTRACT - The identification of compounds with allelopathic potential produced by cover crops can assist in selecting species for weed management purposes in no-tillage systems. This study aimed to identify the main phenolic compounds with allelopathic potential in the shoot of rye (*Secale cereale* L.) and oilseed radish (*Raphanus sativus* L.) cover crops, as well as evaluate whether the cultivation system and phenological stage may influence secondary metabolite production and weed emergence. Samples of the shoot of these cover crops were collected at 60, 80, and 100 days after sowing (DAS) and 15 and 30 days after lodging (DAL) under field conditions. Weed emergence was evaluated at 45, 75, and 100 DAL of cover crops. The main compounds in rye were 6-methoxy-2-benzoxazolinone (MBOA) and 2-benzoxazolinone (BOA) under monocropping and intercropping, while flavonoid quercetin was found in oilseed radish at all evaluated times. During the growing cycle, the highest contents of phenolic compounds were found at the elongation stage (60 DAS) of rye under monocropping and intercropping systems (9.33 and 8.22 mg g^{-1} DM, respectively) and at grain filling stage (100 DAS) for oilseed radish intercropped with rye and black oat (3.24 and 3.83 mg g^{-1} DM, respectively). No differences were found in the contents of the main compounds when the species was grown under monocropping or intercropping systems. A reduction in the contents of MBOA, BOA, and quercetin was observed after lodging. Weed dry matter production was lower at 45 DAL in all treatments with rye and oilseed radish residues when compared to the control. The intercropping of rye with oilseed radish is an alternative management for weed control in agroecological systems due to the physical barrier created by these species and the presence of phenolic compounds with allelopathic potential.

Keywords: cover crops, allelopathy, quercetin, BOA, MBOA.

RESUMO - A identificação de compostos com potencial alelopático produzidos pelas plantas de cobertura pode auxiliar na seleção de espécies para fins de manejo de plantas daninhas em sistema de plantio direto. O objetivo deste estudo foi identificar os principais compostos fenólicos com potencial alelopático na parte aérea das plantas de cobertura centeio (Secale cereale L.) e nabo forrageiro (Raphanus sativus L.), bem como avaliar se o sistema de cultivo e o estádio fenológico influenciam na produção desses metabólitos secundários e na emergência de plantas daninhas. Foram coletadas amostras da parte aérea das plantas de cobertura aos 60, 80 e 100 dias após a semeadura (DAS) e 15 e 30 dias após o acamamento delas (DAA), em um experimento de campo. Foi avaliada a emergência de plantas daninhas aos 45, 75 e 100 DAA das plantas de cobertura. No centeio, os compostos majoritários foram o 6-metoxi-2-benzoxazolinona (MBOA)

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e o 2-benzoxazolinona (BOA) em cultivo solteiro e consorciado, e no nabo-forrageiro foi o flavonoide quercetina, em todas as épocas avaliadas. Ao longo do ciclo de cultivo, os maiores conteúdos de compostos fenólicos foram encontrados no estádio de alongamento (60 DAS) para a espécie centeio cultivada solteira e consorciada (9,33 e 8,22 mg g^{-1} MS) e no estádio de enchimento de grãos (100 DAS) para o nabo forrageiro em sistema de cultivo consorciado com centeio e aveia (3,24 e 3,83 mg g^{-1} MS). Não houve diferença nos conteúdos dos principais compostos quando a espécie foi cultivada solteira ou consorciada. Após o acamamento, houve redução nos conteúdos de MBOA, BOA e quercetina. A produção de MS de plantas daninhas foi menor aos 45 DAA em todos os tratamentos com resíduos de centeio e nabo, quando comparados com a testemunha. O consórcio do centeio com o nabo forrageiro é uma alternativa de manejo para o controle das plantas daninhas em sistemas agroecológicos, tanto pela barreira física exercida por essas espécies como pela presença de compostos fenólicos com potencial alelopático.

Palavras-chave: plantas de cobertura, alelopatia, quercetina, BOA, MBOA.

INTRODUCTION

The no-tillage system based on the principles of agroecological management, with soil mobilization restricted to planting rows, use of cover crops, and other management practices, can contribute to weed control and herbicide use reduction (Altieri et al., 2011; Bittencourt et al., 2013).

Rye (*Secale cereale* L.) and oilseed radish (*Raphanus sativus* L.) have been used as cover crops in no-tillage systems to control weed emergence, with physical, chemical, and/or biological effects (Vargas, 2012; Bittencourt et al., 2013; Vilanova et al., 2014). Regarding the physical effect, the intercropping of rye with oilseed radish can generate a residue with a C/N ratio close to 13, allowing covering the soil surface over time and, consequently, gradually releasing nutrients into the soil (Crusciol et al., 2005; Oliveira et al., 2016). Concerning the chemical effect, cover plant biomass has several compounds with allelopathic action, especially those of phenolic nature, which are biosynthesized by plants from the malonic and shikimic acid pathways. These chemical compounds can be released into the environment in various forms, such as through leaching, volatilization, residue decomposition, and root exudation (Rice, 1984) and perform several ecological functions, such as protection against herbivorous and pathogens (Zasada et al., 2005; Meyer et al., 2009) or even a reduction in the growth and establishment of other plants (Taiz and Zeiger, 2009; Hagemann et al., 2010; Inderjit et al., 2011).

Plants release secondary metabolites into the environment through decomposition of residues deposited on the soil surface, and this process may vary according to species, soil type, and phenological stage (Meyer et al., 2009; Rueda-Ayala et al., 2015; Sangeetha and Baskar, 2015; Sampaio et al., 2016; Tanwir et al., 2017). For instance, rye exudes several compounds with allelopathic action during its phytomass decomposition, mainly hydroxamic acids such as DIBOA (2,4-dihydroxy-1,4-benzoxazolinone-3), DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazolinone-3), and products of their degradation, decarboxylated into the form of BOA (2-benzoxazolinone) and MBOA (6-methoxy-2,3-benzoxazolinone), respectively (Copaja et al., 2006; Hanhineva et al., 2014; Tanwir et al., 2017). These hydroxamic acids are recognized by inhibiting the germination of weeds or crops and by protecting against bacteria, fungi, and insects (Hanhineva et al., 2014; Tanwir et al., 2017). Benzoxazolinones act on the activity of the main enzymes of the phenylpropanoid pathway, such as phenylalanine ammonia lyase (PAL) and peroxidase (POD), interfering with the biosynthesis of phenolic compounds directly involved in the general mechanisms of plant protection (Taiz and Zeiger, 2009).

On the other hand, studies on oilseed radish phytochemistry are scarce, especially those with emphasis on the determination of compounds with allelopathic potential. Most of them have investigated the total phenolic content, targeting the interest of the food industry (Sangthong et al., 2017; Maldini et al., 2017). However, species of the genus *Brassica*, such as oilseed radish, usually have the potential to control plant emergence (Boydston and Hang, 1995). Among the compounds with allelopathic potential already found in oilseed radish are glucosinolates and caffeic, p-coumaric, syringic, ferulic, and sinapic phenolic acids (Rehman et al., 2013;

Papetti et al., 2014). Another class of compounds present at high concentrations in the epidermis of oilseed radish leaves is the flavonoid, especially quercetin, which protects against UV rays, pigmentation, disease resistance, and inhibition of germination and plant growth (Parvez et al., 2004; Pereira et al., 2009).

Although the use of cover crops to control weeds is a well-established practice in agroecological systems, studies on compounds with allelopathic potential, phenological stage, and cultivation systems (monocropping or intercropping) in which their highest production occur are still scarce. Studies on the use of plants to phytotoxic potential (Hagemann et al., 2010; Bittencourt et al., 2013; Silva et al., 2014; Gomes et al., 2017) may subsidize the development of new strategies to control weeds (Soltys et al., 2013; Miri and Armin, 2013; Saif et al., 2016) and manage cover crops, allowing the reduction of the use of herbicides and a less harmful agriculture to the environment.

Thus, this study aimed to identify the main phenolic compounds with allelopathic potential in the shoot of the cover crops *S. cereale* and *R. sativus*, as well as evaluate whether the cultivation system (monocropping or intercropping) and phenological stage of plants cultivated under an agroecological no-tillage system may influence secondary metabolite production and weed emergence.

MATERIAL AND METHODS

Experimental design

The experiment in the field was installed in an area with a 20-year history of onion cultivation under a conventional tillage system (plowing and harrowing) until 1996. Then, a minimum cultivation system with rotation of onion and cover crops such as black oat (*Avena strigosa* Schreb), velvet bean (*Mucuna aterrima* Piper and Tracy), millet (*Pennisetum glaucum* L.), brown hemp (*Brown hemp juncea* L.), and vetch (*Vicia sativa* L.) was installed in the area from 1996 to 2009. The experiment with onion under no-tillage system has been used since then. Weeds were desiccated using herbicide in April 2009 at the time of experiment set up. At that time, soil presented the following characteristics at a depth of 0-10 cm: clay of 380 g kg^{-1}; organic matter of 40 g kg^{-1}; pH in water of 6.2; available P of 26.6 mg dm^{-3} and exchangeable K of 145.2 mg dm^{-3} (extracted by Mehlich-1); exchangeable Al of 0.0 $cmol_c$ kg^{-1}, exchangeable Ca of 7.2 $cmol_c$ kg^{-1}, and exchangeable Mg of 3.4 $cmol_c$ kg^{-1} (extracted by 1 mol L^{-1} KCl); cation exchange capacity (CEC) of 14.3 $cmol_c$ kg^{-1}; and $CEC_{pH7.0}$ saturation by bases (V) of 76%.

The experiment was located at the EPAGRI Experimental Station, in Ituporanga, in the Upper Itajaí Valley region, Santa Catarina, Brazil (27°24’52" S and 49°36’9" W, with an altitude of 475 m). The regional climate, according to Köppen classification, is a humid mesothermal subtropical climate (Cfa). The average values of temperature and precipitation during the experimental period and year in which the species were collected and presented in Figure 1. The soil is classified as a “Cambissolo Húmico” according to the Brazilian System of Soil Classification (Embrapa, 2013) and as an Inceptisol, according to the Soil Taxonomy System (Soil Survey Staff, 2006).

The experimental design was a randomized block design with three replications. Each experimental unit had 5 × 5 m, totaling 25 m^2. Treatments were installed under field conditions and consisted of (i) rye (120 kg ha^{-1} of seeds) (R), (ii) oilseed radish (20 kg ha^{-1} of seeds) (FR), (iii) oilseed radish (10 kg ha^{-1} of seeds) + rye (60 kg ha^{-1} of seeds) (FR + R), and (iv) weeds (without cover crops). Winter species were broadcasted sown and then a seed drill machine was passed twice on the area to promote seed incorporation into the soil. No fertilization, irrigation, or management practices were carried out during the cycle of cover crops. The amount of seeds per hectare was calculated based on the highest values recommended by Monegat (1991) plus 50% to ensure seed germination and higher dry matter formation.

All winter species had been lodged using a knife roller in July of every year since the experiment was set up. Then, 96 kg P_2O_5 ha^{-1} as the natural phosphate of Gafsa and 175 kg P_2O_5 ha^{-1}, 125 kg K_2O ha^{-1}, and 100 kg N ha^{-1} as poultry litter were applied half at the time of planting the onion seedlings and the remaining at 45 days after planting. No natural phosphate was applied from the 2011 season because P contents were interpreted as very high.

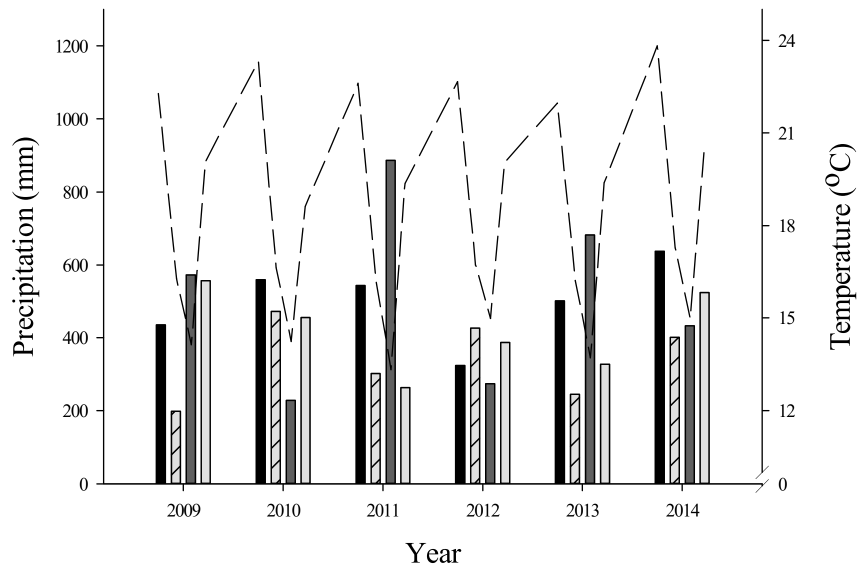

Figure 1 - Precipitation and mean temperature in the experimental area during the season from 2009 to 2014.

Collection of weeds

Three fixed subplots (0.5 × 0.5 m) were installed in 2014 at each plot to evaluate weed emergence at 45, 75, and 100 days after lodging of cover crops (DAL) in all treatments, totaling 0.75 m^2. Plants that emerged within the subplots had their vegetative parts cut close to the soil, considering all the phenological stages. The green weed masses were stored, dried in a forced air ventilation oven at 65 °C until constant weight, and weighed to quantify dry matter (DM) production, expressed in tons per hectare (Mg ha^{-1}).

Collection of cover crops

In 2014, three subsamples were randomly collected from each plot to compose a single composite sample. Each species was collected and evaluated separately, including the intercropping system. Samples were lyophilized (EDWARDS Micro-Modulyo) until total moisture removal (-54 °C), ground in a mill (0.42 mm), and stored at -20 °C for further analysis. Rye and oilseed radish under monocropping and intercropping systems were harvested at 60, 80, and 100 days after sowing (DAS) and 15 and 30 DAL. The phenological stages of cover crops at each collection period were determined according to the scale of Large (1954) and Embrapa Wheat (Embrapa, 2011) (Table 1).

Table 1 - Phenological stages of plant cover species at 60, 80, 100 days after sowing and 15 and 30 days after lodging in 2014

Cover crop	Days after sowing			Days after lodging	
	60	80	100	15	30
Rye	Elongation (8-9)*	Heading (10.1)**	Flowering (10.5)***	Senescence	
Oilseed radish	Vegetative development (2)	Flowering (3)	Grain filling (3)	Senescence	

* Ligule of the last visible leaf; ** first ears newly visible; *** all ears out of sheaths.

Preparation of extracts

The shoot of cover crops was macerated with 80% (v/v) methanol (1:50, w/v), followed by stirring for two hours and vacuum filtration. The filtrate was centrifuged at 4,000 rpm for 15 min, and the supernatant was collected and analyzed by high-performance liquid chromatography. A pre-treatment by solid phase extraction with 500 mg/6 mL SPE C18/18 cartridge was performed for extracts of *R. sativus*. The cartridge was filled with 1 mL MeOH, followed by 1 mL Milli-Q water. Subsequently, 1 mL of the sample was added to the cartridge, and the free fraction was collected for further analysis.

Three independent extractions were performed per sample. Samples of the shoot of cover crops at 100 DAS and 15 DAL were also characterized for lignin and cellulose contents, according to the procedure described by Aber and Martin (1999) (Table 2).

Table 2 - Chemical and biochemical characteristics of cover crops at 100 days after sowing and 15 days after lodging in 2014

Plant component	Rye	Oilseed radish	Oilseed radish + black oat	Oilseed radish + rye
	100 days after sowing			
Lignin (%)[1]	9.99	6.57	7.67	9.42
Cellulose (%)	30.11	46.00	42.96	44.63
	15 days after lodging			
Lignin (%)[1]	8.70	4.15	6.12	8.26
Cellulose (%)	15.25	25.18	25.09	21.37

[1] According to the methodology proposed by Aber and Martin (1999).

Analysis of phenolic profile by high-performance liquid chromatography (HPLC)

Aliquots (60 µL) of methanolic extracts were injected into a liquid chromatograph (Thermo Fisher Scientific, Dionex UltiMate 3000). Elution was performed at 30 ^{o}C in at a flow of 1 mL minute^{-1} on a reverse phase C18 column (Acclaimtm 120, 5 nm C18, 4.6 × 250 mm, Thermo Fisher Scientific) and pre-column (Acclaimtm, 5 nm, 4.6 × 10 mm), operating at 240, 260, 280, and 320 nm. Elution consisted of a gradient of solutions A (methanol) and B (Milli-Q water/pH 2.3) in the proportion of 15% solution A and 85% solution B (5 min), 15 to 100% solution A (5 to 45 min) and 85 to 0% solution B (40 to 60 min).

Compounds of interest, such as quercetin and trans-cinnamic, ferulic, p-coumaric, sinapic, caffeic, vanillic, gallic, syringic, p-hydroxybenzoic, 2-benzoxazolinone (BOA), and 6-methoxy-2-benzoxazolinone (MBOA) acids, were identified by comparing retention times and maximum λ values with standard compounds (Sigma-Aldrich) obtained under the same experimental conditions.

The area under the chromatogram at retention times was used for quantifying the identified compounds. The quantification of phenolic acids was carried out using the external standard curve of gallic acid ($y = 1.0301x$, $r^2 = 0.99$), MBOA ($y = 0.0976x$, $r^2 = 0.96$), BOA ($y = 0.3166x$, $r^2 = 0.99$), and quercetin ($y = 0.08671x$, $r^2 = 0.98$), based on the area of peaks of interest. The values corresponded to the mean of two injections per sample.

Statistical analysis

The data of the mean content of phenolic compounds detected by HPLC and weed dry matter production were tested for normality using the Kolmogorov-Smirnov method, transformed using square root (x) and submitted to analysis of variance. The means were compared by the Scott-Knott test at 5% probability when the effects were significant.

RESULTS AND DISCUSSION

The contents of phenolic compounds were similar when the species was grown under monocropping or intercropping systems. After lodging, the species had a reduction in the content of phenolic compounds (Tables 3, 4, and 5). Eight phenolic compounds were identified in samples of rye under monocropping and intercropping: trans-cinnamic, gallic, p-hydroxybenzoic, syringic, p-coumaric, sinapic, BOA, and MBOA acids (Table 3). All of these compounds were detected at 60, 80, and 100 DAS.

Table 3 - Content of phenolic compounds (mg g^{-1} dry matter) with allelopathic potential detected in extracts of the shoot of rye monocropping and intercropped with oilseed radish collected in the field at 60, 80, and 100 days after sowing (DAS) and 15 and 30 days after lodging (DAL) in 2014

Chemical compound	Retention time (min)	60 DAS	80 DAS	100 DAS	15 DAL	30 DAL
		(mg g^{-1} DM)				
Rye monocropping						
Trans-cinnamic acid	2.91	0.16	0.17	0.18	0.21	0.13
Gallic acid	6.12	0.56	0.85	0.38	nd	nd
P-hydroxybenzoic acid	15.72	0.30	0.20	0.12	nd	nd
Syringic acid	17.40	1.28	0.27	0.10	nd	nd
BOA	19.63	2.74	0.87	0.41	nd	nd
P-coumaric acid	20.65	0.25	0.12	0.07	nd	nd
Sinapic acid	21.70	1.77	0.32	0.22	nd	nd
MBOA	22.56	2.53	1.18	0.48	nd	nd
Rye intercropped with oilseed radish						
Trans-cinnamic acid	2.85	0.20	0.10	0.15	0.11	0.09
Gallic acid	6.20	0.53	0.17	nd	nd	nd
P-hydroxybenzoic acid	15.52	0.40	0.48	0.03	nd	nd
Syringic acid	17.70	0.83	0.35	0.13	nd	nd
BOA	19.62	2.58	1.57	0.25	nd	nd
P-coumaric acid	20.65	0.27	0.14	0.07	nd	nd
Sinapic acid	21.70	1.90	0.81	0.16	nd	nd
MBOA	22.53	2.47	1.05	0.63	nd	nd

nd = not detected; * mean of triplicates.

The relative abundance (%) of each peak, i.e., the ratio between the peak area and the total area of all peaks recorded in the chromatogram, showed that the contents of these compounds represented, on average, 63% of the total area of the chromatogram in extracts of rye under monocropping and intercropping systems at 60 DAS, 40% at 80 DAS, and 27% at 100 DAS. The major phenolics in rye were BOA and MBOA, with contents up to 17 times higher than the other compounds (Table 3).

The highest contents of BOA and MBOA were found when plants were at the elongation stage (60 DAS) under monocropping (2.74 and 2.53 mg g^{-1} DM) and intercropping systems (2.58 and 247 mg g^{-1} DM) (Table 3). Tanwir et al. (2017) also observed that the expression levels of the gene linked to BOA were higher during rye germination, as well as the contents (8.5 µmol g^{-1} DM) when compared to the seedling development period (4.5 µmol g^{-1} DM). The highest BOA production at the early stages of rye development found in the present study and previously described in the literature (Tanwir et al., 2017) suggests that the species has a higher allelopathic potential at this stage.

The presence of these compounds has been studied for a long time due to their allelopathic potential. The phytotoxic effects of DIMBOA, DIBOA, and their main degradation products (BOA and MBOA) have been described in the literature (Sicker and Schultz, 2002; Fomsgaard et al., 2004; Finney et al., 2005; Copaja et al., 2006; Alves et al., 2007; Tanwir et al., 2017). These allelopathic compounds act on plants, blocking the shikimic acid pathway in their secondary metabolism, causing their death or interfering with germination (Weidner et al., 2000; Taiz and Zeiger, 2009).

In an experiment carried out in a laboratory under controlled environment, Macias et al. (2005) observed that DIBOA and DIMBOA, obtained from natural grass sources by isolation in compound degradation studies, were phytotoxic to the roots of different target species, such as gardencress pepperweed (*Lepidium sativum*), lettuce (*Lactuca sativa*), and tomato (*Lycopersicon esculentum*). After characterizing allelochemicals and evaluating the allelopathic potential and use of different planting densities of rye, Souza and Furtado (2002) observed that densities of

Table 4 - Content of phenolic compounds (mg g^{-1} dry matter) with allelopathic potential detected in extracts of the shoot of oilseed radish monocropping and intercropped with rye and black oat collected in the field at 60, 80, and 100 days after sowing (DAS) and 15 days after lodging (DAL) in 2014

Chemical compound	Retention time	Oilseed radish monocropping			
		60 DAS	80 DAS	100 DAS	15 DAL
	(min)	(mg g^{-1} DM)			
Trans-cinnamic acid	2.89	0.13	0.51	0.14	0.26
P-hydroxybenzoic acid	15.31	0.02	nd	0.04	nd
Vanillic acid	16.84	0.02	nd	0.13	nd
Syringic acid	17.69	0.04	0.01	0.12	0.08
P-coumaric acid	20.82	0.42	0.06	0.03	0.09
Ferulic acid	21.01	0.11	0.27	0.34	0.07
Sinapic acid	21.39	0.32	0.35	0.55	0.15
Quercetin	24.34	1.49	1.46	1.75	0.08
		Oilseed radish intercropped with rye			
Trans-cinnamic acid	2.94	0.37	0.35	0.11	0.14
P-hydroxybenzoic acid	15.17	nd	0.02	0.01	nd
Vanillic acid	16.86	0.02	0.06	0.08	nd
Syringic acid	17.79	nd	0.18	0.19	nd
P-coumaric acid	20.65	0.03	0.12	0.08	0.08
Ferulic acid	21.08	0.20	0.19	0.35	0.13
Sinapic acid	21.49	0.30	0.27	0.66	0.30
Quercetin	24.35	1.16	1.44	1.75	nd
		Oilseed radish intercropped with black oat			
Trans-cinnamic acid	2.93	0.54	0.14	0.13	0.59
P-hydroxybenzoic acid	15.27	0.03	0.04	0.08	nd
Vanillic acid	16.80	0.01	0.13	0.13	nd
Syringic acid	17.80	0.01	0.12	0.13	nd
P-coumaric acid	20.71	0.19	0.04	0.15	0.12
Ferulic acid	21.00	0.38	0.26	0.49	nd
Sinapic acid	21.39	0.59	0.52	0.79	0.19
Quercetin	24.33	1.60	1.55	1.94	nd

nd = not detected; * mean of triplicates.

Table 5 - Mean content of phenolic compounds identified and detected by high-performance liquid chromatography on the shoot of cover crop in an agroecological no-tillage system at 60, 80, and 100 days after sowing (DAS) and 15 and 30 days after lodging (DAL) in 2014

Treatment	Phenolic compounds (mg g^{-1} DM)					CV (%)
	60 DAS	80 DAS	100 DAS	15 DAL	30 DAL	
Rye	9.33 aA	3.21 bB	1.62 cC	0.21 cD	0.13 aD	4.3
Rye intercropped with oilseed radish	8.82 aA	4.24 aB	1.43 cC	0.11 dD	0.09 bD	7.2
Oilseed radish	2.55 cA	2.95 bA	2.81 bA	0.73 bC	-	8.0
Oilseed radish intercropped with rye	2.09 cC	2.63 bB	3.24 bA	0.62 bD	-	5.6
Oilseed radish intercropped with black oat	3.35 bA	2.83 bA	3.83 aA	1.03 aB	-	6.1
CV (%)	5.4	4.4	5.5	4.9	16.1	

Means followed by the same lowercase letter between treatments and the same uppercase letter between times do not differ from each other by the Scott-Knott test ($\alpha = 0.05$).

50 and 100 kg seeds ha^{-1} inhibited the development and vigor of lettuce plants. Similar effects were observed when plants were sprayed with a commercial standard of BOA and atrazine. According to the authors, although the results were worse when compared to those of atrazine, the pure compound BOA and coexistence with rye plants caused symptoms similar to those of the herbicide such as yellowing between leaf ribs, which progressed to chlorosis and necrosis.

The content of the other phenolic compounds (trans-cinnamic, gallic, p-hydroxybenzoic, syringic, p-coumaric, and sinapic acids) ranged from 0.16 to 1.90 mg g^{-1} DM (Table 3). These acids are commonly associated with plant defense (Taiz and Zeiger, 2009), acting as precursors to a series of natural polymers, which protect against ultraviolet light, and defense against herbivores and pathogens, besides being involved in plant growth both suppressing and stimulating it. Photosynthesis and respiratory activity are inhibited by vanillic, ferulic, coumaric, and caffeic acids (Barkosky et al., 2000; González-Bernardo et al., 2003). Andreasen et al. (2000) quantified the content of phenolic acids from grains of 17 rye varieties and found concentrations of 70 to 140 µg g^{-1} DM of sinapic acid, 40 to 70 µg g^{-1} DM of p-coumaric acid, and concentrations below to 20 µg g^{-1} DM of caffeic and p-hydroxybenzoic acids. These same acids were detected in the present study but at concentrations up to 25 times higher. It may be attributed to the extraction method, solvents, and plant part used, which differed from the experiment carried out by Andreasen et al. (2000).

Phenolic profiles of rye under monocropping and intercropped with oilseed radish were very similar, mainly for compounds with allelopathic potential (BOA and MBOA), showing little or no effect of the type of cultivation on their chemical composition (Table 3). Overall, the contents of phenolic compounds were reduced throughout the growing cycle, with the highest contents found at 60 DAS, with values of 9.33 and 8.82 mg g^{-1} DM when grown under monocropping and intercropped with oilseed radish, respectively (Table 5). The total phenolic contents were reduced by 60 to 80% when rye was grown under monocropping system and 50 to 85% when intercropped with oilseed radish at 80 and 100 DAS. Weidner et al. (2000) verified that the content of total phenolic compounds varied according to the phenological stage of rye, with the highest content (44.24 µg g^{-1} dry weight) at 22 days after flowering, decreasing at the end of grain maturation stage (6.5 µg g^{-1} dry weight) at 57 days after flowering.

After plant lodging, only the trans-cinnamic acid was detected in the extracts, and its content increased mainly at 15 DAL when compared to the other evaluation periods (Table 3). Cinnamic acid is a precursor of lignins, being synthesized by the shikimic acid pathway by the enzyme phenylalanine ammonium lyase (PAL) (Peres, 2004). In fact, higher lignin contents were found at both 100 DAS and 15 DAL. These values were 10 and 8.7% in rye and 6.6 and 4.2% in oilseed radish, respectively (Table 2). Oliveira et al. (2016) also observed a similar result when characterizing the same species in the same experimental area. Thus, higher contents of cinnamic acid in rye are probably associated with the lignification process that occurs after plant lodging. The species had already started their decomposition process at 15 DAL. Therefore, the content of phenolic compounds at 60, 80, and 100 DAS and other nutrients in the plant tissue of the studied species may have been released into the environment by several ways, such as leaching, volatilization, and dry matter decomposition by soil microorganisms (Crusciol et al., 2005).

Eight compounds with allelopathic potential were identified in extracts of oilseed radish under monocropping and intercropped with rye and black oat (Table 4), which include the chemical compounds identified in the rye, except gallic acid, BOA, and MBOA, plus vanillic and ferulic acids and the flavonoid quercetin. The compounds MBOA and BOA are not characteristic of this species, being major in the Poaceae species (Hanhineva et al., 2014; Tanwir et al., 2017).

The flavonoid quercetin was the chemical compound with the highest content in all samples of oilseed radish under monocropping and intercropping systems, with mean values of 1.40, 1.48, and 1.81 mg g^{-1} DM at 60, 80, and 100 DAS, respectively (Table 4). The presence of quercetin in extracts of oilseed radish was already expected because the genus *Raphanus* is known for flavonoid production (Motta et al., 2009; Sangthong et al., 2017). Previous studies have already found quercetin in pods of this species (Papetti et al., 2014; Sangthong et al., 2017). Flavonoids are compounds mainly involved in signaling between plants and other organisms, in addition to presenting an allelopathic effect (Peres, 2004). Parvez et al. (2004) evaluated the effect of quercetin and its seven derivatives on the growth of *Arabidopsis thaliana* and *Neurospora crassa* and observed that all the substances inhibited bud growth of *A. thaliana* and the conidial germination of *N. crassa*.

Regarding the phenological stages, the highest phenolic contents were observed at 100 DAS, when oilseed radish was at the grain filling stage, mainly when intercropped with rye (3.24 mg g^{-1} DM) and black oat (3.83 mg g^{-1} DM) (Table 5). In general, the eight chemical compounds detected at 60 DAS in the samples of oilseed radish intercropped with rye and black oat were also found at 80 and 100 DAS and, in large part, their contents increased throughout the crop cycle, except for trans-cinnamic and p-coumaric acids (Table 4). After plant lodging (15 DAL), the highest phenolic contents were observed in oilseed radish under monocropping and intercropping systems when compared to rye (Table 5) mainly because trans-cinnamic, p-coumaric, syringic, vanillic, ferulic, and sinapic acids and quercetin were still in the extracts (Table 4).

Oilseed radish showed lower mean phenolic contents when compared to rye. These contents were, on average, 3 and 1.5 times lower at 60 and 80 DAS, respectively (Table 5). Oilseed radish Ohas a lower C/N ratio and less fibrous material when compared to rye, thus presenting a faster release of nutrients and probably compounds in the plant tissue (Oliveira et al., 2016). Silva (2014) worked with a dry extract from leaves of *R. sativus* and found contents of up to 47.02 mg gallic acid equivalents (GAE) g^{-1} DM of total phenolics. Moreover, Beegamashi (2010) quantified the total phenolic content of an aqueous extract of residues of leaves and stems of oilseed radish and found, on average, 17.7 mg GAE mL^{-1} and 8.68 mg GAE mL^{-1} for the hydroethanolic extract. These values were higher when compared to those obtained in the present study. The phytotoxicity of chemicals with allelopathic potential may be influenced by soil type, plant nutrition, pH, climate factors, microbiota, and phenological stage of plants (Monquero et al., 2009; Inderjit and Weiner, 2001). Some of these factors were the same for both evaluated species, but their phenological stages and soil microbiota can be influenced the differentiated production of compounds (Weidner et al., 2000; Bertin et al., 2003). According to Czelusniak et al. (2012), the production of secondary metabolites can vary according to plant development, including leaf development, the emergence of new organs, and biochemical, physiological, ecological, and evolutionary processes.

In general, dry matter production of weeds that emerged under residues of cover crops in the experiment installed in the field had the lowest values in the first evaluation periods (45 DAL) when compared to the control (without cover crops) (Table 6). It may be due to the higher presence of dry matter of cover crops on the soil surface since this material is degraded over time (Martins et al., 2014; Oliveira et al., 2016), exposing the soil and favoring weed growth. Also, phenolic compounds produced by rye and oilseed radish throughout the growing cycle may have been released into the soil because these compounds were reduced when the plants were lodged on the soil surface (Table 5), which may also have contributed to a reduction in weed emergence (Table 6).

Table 6 **-** Weed dry matter production under residue of rye, oilseed radish, oilseed radish intercropped with rye, and oilseed radish intercropped with black oat at 45, 75, and 100 days after lodging of cover crops – 2014

Treatment	Days after lodging			CV (%)
	45	75	100	
	($Mg\ ha^{-1}$)			
Weed (without cover)	1.51 aA	1.49 aA	1.89 aA	17.0
Rye	0.85 aB	1.41 aA	1.34 bA	25.4
Oilseed radish	1.00 aB	1.38 aA	1.55 bA	8.6
Oilseed radish intercropped with rye	1.08 aB	1.39 aA	1.61 bA	11.9
Oilseed radish intercropped with black oat	1.20 aB	0.97 aB	1.87 aA	21.3
CV (%)	20.8	22.1	11.1	

Means followed by the same lowercase letter between treatments and the same uppercase letter between times do not differ from each other by the Scott-Knott test ($\alpha = 0.05$).

Studies performed by Gatiboni et al. (2009) in Chapecó, SC, showed that rye had 60% of the initial amounts of DM on the soil at 88 DAL. These authors also estimated that 50% of DM of rye remained on the soil surface at 109 DAL. Oliveira et al. (2016) evaluated the decomposition of cover crops in an agroecological no-tillage system with onion in the same experiment of the present study and observed that rye residues remained longer on the soil surface. The dry mass of rye could be found on the soil surface even at 90 DAL, but for only 30 days in the treatment with weeds.

Therefore, besides the presence of plant material under the surface, the production of compounds with allelopathic potential by these species may also have contributed to reducing the incidence of weeds in all the evaluated periods (Table 6).

Overall, the intercropping of rye with oilseed radish is an alternative management for weed control in agroecological systems due to the physical barrier created by these species and presence of phenolic compounds, which are recognized by their allelopathic potential (Souza and Furtado, 2002; Yamauti and Alves, 2012; Tanwir et al., 2017).

During the growing cycle, the highest contents of phenolic compounds were found at the elongation stage (60 DAS) of rye grown under monocropping and intercropping systems (9.33 and 8.22 mg g^{-1} DM, respectively) and at grain filling stage (100 DAS) for oilseed radish intercropped with rye and black oat (3.24 and 3.83 mg g^{-1} DM, respectively). Therefore, it is possible to manage these species in the field by maintaining oilseed radish sowing in April and delaying rye sowing or carrying out it in two stages: one in April and the remaining 40 days later. Thus, both species may have higher phenolic content with allelopathic potential when lodged.

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