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Allelopathy of garden pea on corn in no-tillage system

Alelopatia da ervilha-forrageira no milho em sistema de plantio direto

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ABSTRACT

The cover crop selection for the no-tillage system generally does not consider the possible allelopathic effects between species. This study identified and quantified the allelochemicals released by garden pea (*Pisum sativum* L. ssp*. arvense* (L.) Poir.) as a cover crop, at different sowing densities (0; 27.5; 55; 82.5 kg ha^{-1}) and decomposition times (7, 21 and 35 days) before sowing corn (*Zea mays*). Soil allelochemicals were identified and quantified using high-performance liquid chromatography (HPLC). The variables emergence, emergence speed index (ESI), chlorophyll *a* and *b*, leaf area, and dry mass were assessed in corn aboveground. There was an increase in catechin with corn cultivation. Epicatechin was identified after corn was sown 21 and 35 days after cutting the garden pea. Resveratrol was associated with the decomposition of the cover crop and was identified when the corn was sown seven days after it was cut. Emergence, ESI, and leaf area were higher in corn sown 21 and 35 days after cutting the garden pea, a period in which epicatechin was found. The use of garden pea increased corn chlorophyll *a* and *b*. Dry mass production was higher in corn sown seven and 35 days after cutting the garden pea. Garden pea followed by corn in a no-tillage system increases the soil levels of catechin, epicatechin, and resveratrol. The use of garden pea increases chlorophyll levels in corn compared to the control (without the cover crop) and increases the leaf area of the corn when sown seven days after cutting the *P. sativum* (82.5 kg ha^{-1}).

KEYWORDS: Allelochemicals; Cover crops; *Pisum sativum L. ssp. arvense; Zea mays L.*

RESUMO

A seleção das espécies de cobertura para compor o sistema de plantio direto geralmente não considera os possíveis efeitos alelopáticos entre as espécies. Neste trabalho, foi identificado e quantificado os aleloquímicos liberados pela ervilha-forrageira (*Pisum sativum* L. ssp. *arvense* (L.) Poir.) como planta de cobertura, em diferentes densidades de semeadura (0; 27,5; 55; 82,5 kg ha^{-1}) e tempos de decomposição (7, 21 e 35 dias) antes de semeadura do milho (*Zea mays*). Os aleloquímicos do solo foram identificados e quantificados por cromatografia liquida de alta eficiência (HPLC). No milho se avaliou a emergência, índice de velocidade de emergência (IVE), clorofila *a* e *b*, área foliar e massa seca de parte aérea. Houve incremento de catequina com o cultivo do milho. A epicatequina foi identificada após o cultivo do milho semeado 21 e 35 dias após o corte da ervilha-forrageira. O resveratrol foi associado a decomposição da espécie de cobertura e identificado após o cultivo do milho semeado sete dias após o corte da mesma. A emergência, IVE e área foliar foram superiores no milho semeado 21 e 35 dias após o corte da ervilha-forrageira, período em que foi encontrado a epicatequina. O uso da ervilha-forrageira aumentou os teores de clorofila *a* e *b* do milho. A produção de massa seca foi superior no milho semeado sete e 35 dias após o corte da ervilha-forrageira. A sucessão de milho após a ervilha-forrageira em sistema de plantio direto aumenta os níveis de catequina, epicatequina e resveratrol no solo. A utilização de ervilha forrageira aumenta os teores de clorofila no milho em relação ao controle (sem a presença do cultivo de cobertura) e aumenta a área foliar do milho quando semeado sete dias após o corte de *P. sativum* (82,5 kg ha^{-1}).

PALAVRAS-CHAVE: Aleloquímicos; Cultivos de cobertura; *Pisum sativum* L. ssp*. arvense; Zea mays* L.

INTRODUCTION

Corn (*Zea mays* L.), from the Poaceae family, is one of the most widely consumed cereals globally. In the 2022/23 harvest season, Brazil set a record with a production of 127,767,000 tons (CONAB 2023). It is commonly grown using no-till systems (FUENTES-LLANILLO et al. 2021).

The no-till system involves adopting permanent soil cover, minimal soil tillage, and planned crop rotation. This method has become essential for the sustainability of the agroecosystem, especially in annual crops, since using soil cover crops (CC) protects and helps build the soil's physical, chemical, and biological attributes (SCAVO et al. 2022). These plants reduce erosion, benefit soil structure, increase nutrient cycling and organic matter levels (NASCENTE & STONE 2018, CRESPO et al. 2023), and favor positive soil biological activity (KIM et al. 2020).

Among the species used as cover crops, those from the Fabaceae family, such as garden pea (*Pisum sativum* L. ssp. *arvense* (L.) Poir.), are notable for their biomass production and their ability to fix atmospheric nitrogen through symbiosis with nitrifying bacteria (KOCIRA et al. 2020). Garden pea is fast-growing, precocious, uniform, and hardy (TOMM et al. 2002). It produces up to six tons of aboveground dry biomass per hectare (DORN et al. 2015) and incorporates up to 180 kg ha^{-1} of nitrogen (AITA et al. 2001, DONEDA et al. 2012).

When preceding corn in three successive crops with garden pea, garden vetch (*Vicia sativa* L.), black oats (*Avena strigosa* Schreb.), oilseed-radish (*Raphanus sativus* L.), and fallow, garden pea resulted in the highest corn dry mass production in a phosphorus and potassium deficient soil (MICHELON et al. 2019), demonstrating the rusticity of this Fabaceae. In addition, the species increased the yield of corn compared to fallow, which indicates that the garden pea can be a good option for crop succession preceding Poaceae crops.

However, it is common for farmers to select cover crops based on their planting time or seed availability without taking into account the possible allelopathic effect caused by root exudation (REGINATTO et al. 2023) and biomass degradation (GIOVANETTI et al. 2019, KOEHLER-COLE et al. 2020) on the emergence, growth, and development of crops in succession (REGINATTO et al. 2020).

According to RICE (1984), allelopathy comprises any effect, beneficial or harmful, of one plant on another or even on microorganisms through the release of chemical compounds (allelochemicals) produced by the plant's secondary metabolism into the environment. These compounds can directly interfere with the growth of later cultivated plants, and their presence varies according to the tissue decomposition or active plant release time (FORMIGHEIRI et al. 2018).

This study, therefore, identified and quantified allelochemicals released by garden pea (*Pisum sativum* L. ssp. *arvense* (L.) Poir.) over time at different sowing densities and assessed the allelopathic effect of these compounds on the emergence and growth of corn (*Zea mays* L.) in a no-tillage system-controlled environment.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Fronteira Sul, in Laranjeiras do Sul, Paraná, Brazil, from September to November 2018, with an average temperature of 25 ± 2 °C. It employed a completely randomized design (CRD) in a 4x3 factorial scheme, with four sowing densities of garden pea (0; 27.5; 55; 82.5 kg ha^{-1}) (cv. IPR 83) and three cover crop decomposition periods (7, 21, and 35 days), each with four replications. An additional treatment, representing the initial soil, was used for analyzing phenolic compounds to identify and quantify allelochemicals present in the soil before the experiment. The garden pea was sown in 12-L pots (25 cm height x 30 cm top diameter x 22 cm bottom diameter), filled with a substrate consisting of a 1:1 (v/v) mixture of soil (Table 1) and sand.

Table 1. Physical-chemical characterization of the soil used in the pot substrate.

pH	OM	P	K	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	V	Clay
$CaCl_2$	g dm^{-3}	mg dm^{-3}	cmol dm^{-3}						%	%
4,2	38,1	2,7	0,05	1,2	1,0	8,57	2,48	11,05	22,4	49

pH: hydrogen potential; OM: organic matter; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation.

The substrate was fertilized for corn cultivation (SBCS 2019) with calcitic limestone, boiler ash, and a mixture of cattle and poultry manure in a 4:1 (v/v) ratio. Irrigation was performed manually every two days until the soil reached field capacity. The volume of water applied was based on the guidelines of MONTEIRO & FRIGHETTO (2000), using soil samples collected at the beginning of the experiment.

At full bloom, the garden pea was cut close to the ground and then the material was ground into five-centimeter particles. After 7, 21, and 35 days of decomposition, 25 corn (cv. IPR 164) seeds were placed on each vase in all treatments. Corn was grown for 30 days after sowing (DAS).

Phenolic compounds were identified and quantified (μg kg^{-1} of soil) when the garden pea was sown (initial) and after the corn. In *Z. mays*, emergence (%), emergence speed index (ESI), chlorophyll (*a* and *b*), leaf area (cm^3), and shoot dry mass (g) were assessed.

The extraction of phenolic compounds from the soil was adapted from BITTENCOURT et al. (2018). A 0.15 kg sample of soil was dried in a forced-circulation oven at 40 °C until a constant mass was obtained, ground with a pestle, sieved to 2 mm, and stored at -20 °C. At the time of extraction, each sample was divided into three 50 g sub-samples. 150 mL of PA methyl alcohol was added to each sub-sample, and they were homogenized at 25 °C and 200 rpm for 48 h in an orbital shaker. After this procedure, the samples were centrifuged (1,200 g for 15 min at 25 °C). The supernatant was filtered through filter paper (25 μm), and then the solvent was evaporated using a rotary evaporator at 40 °C under vacuum. The residue was dissolved in 2 mL of methyl alcohol PA. The final extracts were filtered through a PTFE syringe filter (0.22 μm) and stored in a vial.

The phenols were identified and quantified using standard solutions of (+) catechin, (-) epicatechin, caffeic acid, gallic acid, vanillic acid, p-coumaric acid, trans-iso-ferulic acid, (-) resveratrol, myricetin, and quercetin.

The analysis was conducted on a Shimadzu UFLC liquid chromatograph equipped with a diode array detector and an NST-C18 column, 25 cm long, 4.6 mm in diameter, with particles 5 μm in diameter and a temperature of 40 °C. Using an automatic sampler, 1 μL of the extracts were injected into the column at a mobile phase flow rate of 1.2 mL min^{-1}. The gradient of mobile phase B was 99.9% methanol and 0.1% formic acid, while 99.9% water and 0.1% formic acid were mobile phase A (Table 2).

Table 2. Elution of phenols used for High-performance liquid chromatography (HPLC).

Stage	Time (min)	Concentration of mobile phase B (%)
1	0,01	14
2	16	55
3	16,01	100
4	17	100
5	17,01	14
6	20	14

The corn emergence was assessed based on the number of normal seedlings (≥2 cm, without damage, deformations, or signs of deterioration) on the 15th day after sowing. The emergence speed index (ESI) was carried out concurrently with the emergence test and calculated according to MAGUIRE 1962 (Equation 1):

$$ESI= (E_1/N_1) + (E_2/N_2) + (E_3/N_3) + ... + (En/Nn) \quad \text{(Equation 1)}$$

Where ESI is the emergence speed index, E is the number of normal plants that emerged, and N is the time in days.

Thirty days after sowing the corn, measurements of the other variables were taken from 10 random plants per pot. Chlorophyll *a* and *b* levels were determined using the Chlorophyllog® equipment, with the analysis focused on the leaf closest to the apex that was fully expanded (ALVES & MONTAGNER 2016).

The aboveground portion of the corn plants was harvested close to the substrate surface, placed in paper bags, and transported to the laboratory. The leaves were separated from the stalk, and their area was measured using a leaf area meter (Bio Science CI 203). The plant aboveground part was then taken to a forced circulation oven at 62 °C until a constant weight was achieved to determine dry mass.

The data was subjected to the Shapiro-Wilk test for normality and Bartlett's test for homogeneity of variance. The variables of the allelochemicals were transformed by √(x+1). Once normality and homogeneity had been verified, the data was submitted to a two-factor analysis of variance (p≤0.05) and fitted to regression models, except the quantification of allelochemicals, emergence at different sowing densities, and the variables significant only for the time factor, which has three levels. These were submitted to the Tukey test (p≤0.05) in the Sisvar 5.8 software (FERREIRA 2011).

RESULTS AND DISCUSSION

The allelochemicals catechin and epicatechin were identified in the soil following sequential cultivation of garden pea and corn at densities of 27.5, 55, and 82.5 kg ha^{-1} as well as in soil without cover crops (0 kg ha^{-1}). Resveratrol was detected in soil where corn was sown seven days after harvesting the garden pea (Table 3).

Table 3. Quantities of phenolic compounds in the substrate after garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83) sown at different densities and corn cv. IPR 164 in succession.

Sowing density (kg ha^{-1})	Catechin	Epicatechin[1]			Resveratrol[1]		
	µg kg^{-1}						
	-	7	21	35	7	21	35
	Decomposition days						
Initial soil	50.6 B	0 Aa	0 Ba	0 Ba	0 Ca	0 Aa	0 Aa
0	88.9 A	0 Ab	38.5 Aa	38.7 Aa	0 Ca	0 Aa	0 Aa
27.5	77.2 A	0 Ab	41.0 Aa	37.6 Aa	3.3 Ba	0 Ab	0 Ab
55	93.6 A	0 Ab	38.5 Aa	41.4 Aa	4.4 Aa	0 Ab	0 Ab
82.5	79.8 A	0 Ab	43.8 Aa	45.2 Aa	4.1 Aa	0 Ab	0 Ab
CV (%)	21.1		9.3			3.8	

[1]Data transformed by √(x+1). Equal uppercase letters do not differ in the row (sowing density) and lowercase letters in the columns (decomposition days) for the Tukey test in each allelochemical (p<0.05). The minimum detection levels were 5, 7 and 2 µg kg-1 for catechin, epicatechin and resveratrol, respectively.

The initial soil (before sowing the garden pea) presented only catechin (Table 3). This behavior is expected since allelochemicals can be found in natural environments and released by plants and microorganisms (FAVARETTO et al. 2018). This allelochemical has already been observed in other studies carried out in Brazil (KREMER & BEN-HAMMOUDA et al. 2009, ARAUJO et al. 2018, FAVARETTO et al. 2018).

The concentration of catechin increased with the cultivation of corn in the absence of garden pea (Table 3). No difference was observed in catechin levels between corn grown on garden pea straw or across the different decomposition times. Previous studies have linked catechin to the presence of corn (LUZARDO-OCAMPO et al. 2017, AL-SAADAWI & AL-MALIKI 2019, ELSAYED et al. 2022), suggesting that the increase in cateching levels in the soil is attributed to this species. Catechin is a flavonoid with aromatic rings connected by three carbons to form a pyran ring (SILVA et al. 2015). In agricultural environments, catechin is associated with weed control (GOMAA et al. 2014) and disease suppression (BAIS et al. 2010).

Epicatechin was detected in treatments with corn sown after 21 and 35 days, regardless of the presence of garden pea, and remained consistent across different densities and periods evaluated (Table 3). Epicatechin is an antioxidant flavonoid predominantly found in woody species (ZENG et al. 2008). Its production has been documented in corn (LU et al. 2023), as well as in perennial Fabaceae (WINK 2013) and medicinal plants (OBISTIOIU et al. 2021), but there is limited information on its production by plants used as soil cover crops. In this study, the presence of garden pea did not affect the production of epicatechin, supporting the conclusion that the observed epicatechin originated from corn cultivation.

Resveratrol was detected in treatments where corn was sown after garden pea were decomposed for seven days (Table 3). This suggests that the presence of resveratrol was attributable to the cover crop, as it was not detected in the control (0 kg ha^{-1}) across all evaluations. Resveratrol, a compound with two phenolic rings connected by an ethylene bridge, has been identified in over 70 plant species and is known for its role in pathogen defense (SALEHI et al. 2018). The release of resveratrol from decomposing garden pea tissues had not been previously reported. Additionally, higher concentrations of resveratrol were observed at the greater garden pea densities (55 and 82.5 kg ha^{-1}) compared to the lowest density (27.5 kg ha^{-1}).

Resveratrol was only detected during the first evaluation period (7 days) (Table 3). This observation is likely due to its rapid volatilization (ABO-KADOUM et al. 2022) and possible degradation by biological activity (KOSTINA-BEDNARZ et al. 2023), as this period coincided with the highest presence of straw.

Corn emergence and the emergence speed index (ESI) were influenced by both the decomposition times and sowing densities of the garden pea. Specifically, these variables were higher when corn was sown after 21 days of garden pea decomposition (Table 4).

Table 4. Emergence and emergence speed index (ESI) of corn cv. IPR 164 following management of garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83).

Decomposition days	Emergence (%)	ESI
7	60 B	10.6 B
21	69 A	12.8 A
35	67 AB	13.3 A
CV (%)	16.6	18.9

Equal letters do not differ for Tukey test ($p<0.05$).

The increased emergence and ESI observed at 21 days compared to seven days, may be attributed to greater nutrient availability and reduced physical barriers as the pea plants decompose (NEVINS et al. 2020). During this period, epicatechin was detected (Table 3), which may have contributed to the enhanced corn emergence and ESI. However, there are currently no studies supporting this hypothesis. Generally, epicatechin is known for its antimicrobial properties (ALONSO-ESTEBAN et al. 2019, MARTINS et al. 2020) and its role in defense against herbivory (LI et al. 2021).

The sowing density of 27.5 kg ha^{-1} reduced corn emergence by 18% compared to the control (without garden pea) (Table 5), although it did not differ from the other sowing densities. While cover crops can enhance soil physical, chemical, and biological properties, they may also create a physical barrier that can impede seedling emergence, which helps explain the observed results.

Table 5. Emergence of corn cv. IPR 164 sown after garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83) sown at different densities.

Sowing density (kg ha^{-1})	Emergence (%)
0	72 A
27.5	59 B
55.0	66 AB
82.5	64 AB
CV (%)	16.6

Equal letters do not differ for Tukey's test. ($p<0.05$).

The emergence speed index (ESI) of corn behaved showed a similar pattern to emergence, being lower at sowing densities of 27.5, 55 and 82.5 kg ha^{-1} compared to the control (0 kg ha^{-1}) (Figure 1). The ESI is a key indicator of seed vigor (EGLI & RUCKER 2012). The observed reduction in ESI at the higher densities may be attributed to the physical barrier created by the cover crops (HAO et al. 2023). Additionally, the reduction at the lowest density (27.5 kg ha^{-1}) might be related to nitrogen immobilization by microbial activity.

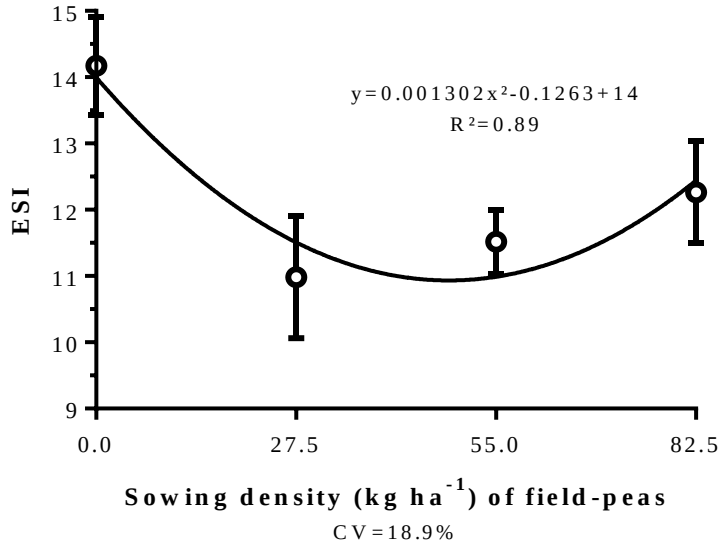

Figure 1. Emergence speed index (ESI) of corn cv. IPR 164 after garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83) sown at different densities.

Chlorophyll *a* and *b* levels in corn were higher in the presence of garden pea compared to the control (Figure 2). This increase may be attributed to the decomposition of the cover crop, which has a low C:N ratio (14:1) and contributes up to 180 kg ha^{-1} of nitrogen (DONEDA et al. 2012). Since nitrogen is a key component of chlorophyll (TAIZ et al. 2017), its availability from the decomposed cover crop likely resulted in elevated chlorophyll content in the corn leaves.

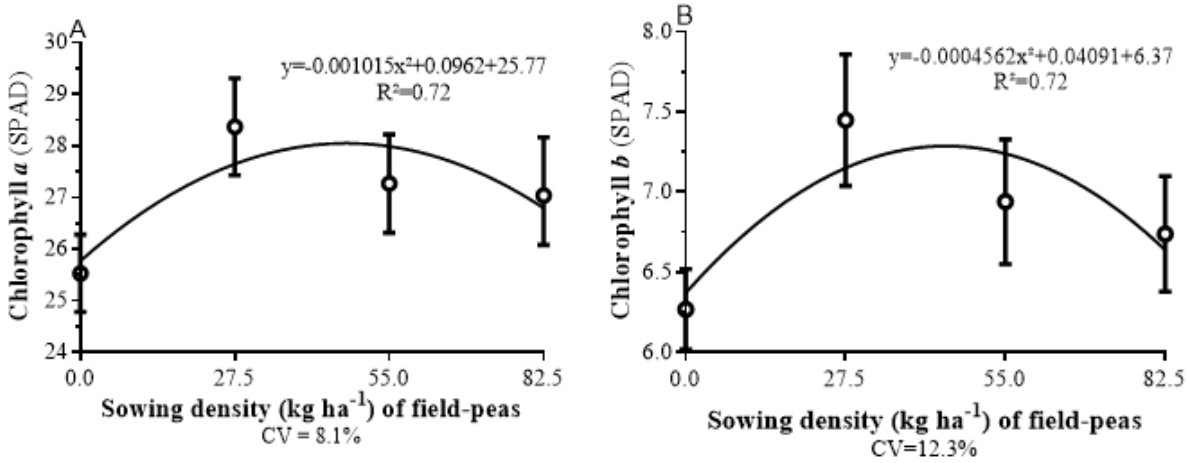

Figure 2. Chlorophyll *a* (A) and chlorophyll *b* (B) levels in corn cv. IPR 164 leaves following sowing of garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83) at different densities.

Leaf area was higher in corn grown after seven days of decomposition of garden pea sown at 82.5 kg ha^{-1} compared to other densities at the same period (Table 6). Garden pea decompose rapidly due to their low C:N ratio, releasing a substantial amount of nutrients, particularly nitrogen (DONEDA et al. 2012). At higher sowing densities, the intense microbial activity likely did not immobilize this nitrogen, making it available for assimilation by the subsequently sown corn plants (GRDC 2017).

Table 6. Leaf area of corn cv. IPR 164 after different decomposition times of garden pea (*Pisum sativum* ssp. *arvense* cv. IPR 83) sown at different densities.

Sowing density (kg ha^{-1})	Leaf area (cm²)		
	7	21	35
	Decomposition days		
0	200.03 Ba	164.22 Aa	152.06 Aa
27.5	158.90 Ba	183.34 Aa	157.17 Aa
55	198.85 Ba	152.42 Aa	200.84 Aa
82.5	273.38 Aa	172.85 Ab	196.71 Ab
CV (%)		19.6	

Equal uppercase letters do not differ in the row (sowing density) and lowercase letters in the columns (decomposition days) for Tukey test. ($p<0.05$).

Corn sown 21 and 35 days after cutting the garden pea showed similar leaf areas to the control (Table 6). However, during these periods, the leaf area at a density of 82.5 kg ha^{-1} was lower compared to the seven-day period, likely due to the advanced decomposition of the cover crop.

Corn dry mass was lower when sown 21 days after cutting the field peas compared to seven and 35 days (Table 7). This reduction may be related to increased nitrogen immobilization by microbial activity, as the garden pea straw is nearly fully decomposed around this time (≈50 days) (KARKANIS et al. 2016). By 35 days, the straw is mostly decomposed, and nitrogen is likely demobilized, becoming available for corn growth.

Table 7. Dry mass of corn cv. IPR 164 following various decomposition periods of garden pea (*Pisum sativum* ssp. *arvense* cv. IPR 83).

Decomposition days	Dry mass (g)
7	0.7446 A
21	0.6332 B
35	0.8508 A
CV (%)	18.6

Equal letters do not differ for Tukey test ($p<0.05$).

CONCLUSION

The use of garden pea followed by corn in a no-tillage system enhances soil levels of catechin, epicatechin, and resveratrol, with concentrations ranging from 50.6 to 93.6, 37.6 to 45.2, and 3.3 to 4.4 µg kg^{-1}, respectively.

Catechin levels increased with corn cultivation, regardless of garden pea presence. Epicatechin was detected in soil after corn was sown 21 and 35 days following garden pea cutting. Resveratrol was linked to the decomposition of the cover crop, appearing after corn was sown seven days post-field garden pea cutting.

Garden pea also improved corn leaf chlorophyll content and increased leaf area, particularly when sown at a density of 82.5 kg ha^{-1} seven days after garden pea cutting, compared to the control (0 kg ha^{-1}) without cover crop presence.

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