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Garden pea allelopathy in non-harvest corn

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.), a member of the Poaceae family, is one of the most consumed cereals globally. In the 2022/23 harvest season, Brazil recorded a production of 127,767,000 tons (CONAB 2023). It is commonly cultivated in non-fermented systems (FUENTES-LLANILLO et al. 2021).

The no-till system requires permanent soil cover, minimization of soil harvesting, and planned crop rotation. This method has become essential for the sustainability of agroecosystems, especially annual crops, because the use of cover crops (CC) protects and helps to build the physical, chemical, and biological attributes of soil (SCAVO et al. 2022). These plants reduce erosion, improve soil structure, increase nutrient cycling, and levels of organic matter (NASCENTE & STONE 2018, CRESPO et al. 2023), and favor the positive biological activity of the soil (KIM et al. 2020).

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

When corn was preceded in three successive crops—garden pea, garden vetch (*Vicia sativa* L.), black oat (*Avena strigosa* Schreb.), oil radish (*Raphanus sativus* L.), and pea—the garden pea resulted in the highest dry mass production of corn in phosphorus- and potassium-deficient soil (MICHELON et al. 2019), demonstrating the rusticity of this Fabaceae. In addition, the species increased corn yield compared to salmon, indicating that garden pea may be a good option for the succession of crops before Poaceae.

However, it is common for farmers to select cover crops based on their planting time or seed availability without considering 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 successive crops (REGINATTO et al. 2020).

According to RICE (1984), allelopathy encompasses 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 subsequently cultivated plants, and their presence varies according to the decomposition of the tissue or the release time of the active plant (FORMIGHEIRI et al. 2018).

Therefore, this study identified and quantified allelochemical released by garden pea (*Pisum sativum* L. ssp. *arvense* (L.) Poir.) over time at different sowing densities and evaluated the allelopathic effects of these compounds on the emergence and growth of corn (*Zea mays* L.) in a controlled environment using a seeder system.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Southern Frontier, Laranjeiras do Sul, Paraná, Brazil, from September to November 2018, with an average temperature of 25 ± 2 ºC. Employed a completely random design (CRD) in a 4x3 factorial scheme with four densities of garden pea sowing (0; 27.5; 55; 82.5 kg ha^{-1}) (cv. IPR 83) and three periods of cover decomposition (7, 21, and 35 days), each with four replicas. An additional treatment, representing the initial soil, was used to analyze phenolic compounds to identify and quantify allelochemical present in the soil before the experiment. The pea was sown in 12-L pots (25 cm in height x 30 cm in top diameter x 22 cm in 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 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}			mol 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 fertilizer at a ratio of 4:1 (v/v). 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 and FRIGHETTO (2000), using soil samples collected at the beginning of the experiment.

In full bloom, the garden pea was cut close to the ground, and then the material was soaked in 5 cm particles. After 7, 21, and 35 days of decomposition, 25 corn (cv. IPR 164) seeds were placed in each pot for all treatments. The corn was cultivated for 30 days after sowing (DAS).

Phenolic compounds were identified and quantified (µg kg^{-1} of soil) when the pea was sown (initial) and after the corn. In *Z. mays*, the emergence (%), emergence speed index (ESI), chlorophyll (*a* and *b*), leaf surface area (cm3), and dry mass of the shoots (g) were evaluated.

The extraction of phenolic compounds from soil was adapted from the study of 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 sub-samples of 50 g. Then, 150 ml of PA methanol was added to each sub-muta, 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 the solvent was evaporated using a rotary evaporator at 40 °C under vacuum. The residue was dissolved in 2 mL of 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, gallic, vanillic, p-coumaric, trans-isoferulic acid, (-) resveratrol, myricetin, and quercetin.

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

Table 2. Elution of phenols used in 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 emergence of corn was evaluated based on the number of normal seedlings (≥2 cm, without damage, deformities, or signs of deterioration) on the 15th ^{day} after sowing. The emergency speed index (ESI) was performed simultaneously with the emergency 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 emergency speed index, E is the number of normal plants that emerged, and N is the time in days.

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

The aboveground parts of the corn plants were harvested near the surface of the substrate, placed in paper bags, and transported to the laboratory. The leaves were separated from the stem, and their area was measured using a leaf area meter (Bio Science CI 203). The upper part of the plant was then placed in a forced circulation oven at 62 ºC until a constant weight was reached to determine the dry mass.

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

RESULTS AND DISCUSSION

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

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

Seed density (kg ha^{-1})	Catechin	Epicatechin¹			Resveratrol¹		
	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	

Transformed data by √(x+1). The larger equal letters did not differ in the line (seeding density) and smaller 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 kg1 for catechin, epicatechin, and resveratrol, respectively.

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

The catechin concentration increased in the presence of corn in the absence of garden peas (Table 3). No difference was observed in catechin content between corn grown in garden pea straw or at different decomposition times. Previous studies have linked catechins to the presence of corn (LUZARDO-OCAMPO et al. 2017, AL-SAADAWI & AL-MALIKI 2019, ELSAYED et al. 2022), suggesting that the increase in catechism levels in the soil can be attributed to this species. Catechin is a flavonoid containing aromatic rings connected by three carbons to form a pyran ring (SILVA et al. 2015). In agricultural environments, catechin is associated with the control of weeds (GOMAA et al. 2014) and suppression of diseases (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 evaluation periods (Table 3). Epicatechin is an antioxidant flavonoid that is predominantly found in woody species (ZENG et al. 2008). Your production was 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 about its production by plants used as 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 decomposed for 7 days (Table 3). This suggests that the presence of resveratrol can be attributed to the cover crop, as it was not detected in the control (0 kg ha^{-1}) in all assessments. Resveratrol, a compound with two phenolic rings connected by an ethylene bridge, has been identified in more than 70 plant species and is known for its role in pathogen defense (SALEHI et al. 2018). The release of resveratrol from the decomposition of garden pea tissues has not been previously reported. In addition, higher concentrations of resveratrol were observed at higher densities of garden peas (55 and 82.5 kg ha-1) compared to the lower density (27.5 kg ha-1).

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

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

Table 4. Emergency and emergency speed index (ESI) of corn cv. IPR 164 after the management of garden pea (*Pisum sativum* ssp. *arvense* cv. IPR 83).

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

The same letters did not differ for the Tukey test ($p<0.05$).

The increased emergency and ESI observed in 21 days compared to 7 days can be attributed to the greater availability of nutrients 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 increased emergence of corn and ESI. However, no studies have supported this hypothesis. Generally, epicatechin is known for its antimicrobial properties (ALONSO-ESTEBAN et al. 2019, MARTINS et al. 2020) and role in the defense against herbivory (LI et al. 2021).

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

Table 5. The emergence of corn cv. IPR 164 after garden pea (*Pisum sativum* ssp. *arvense* cv. IPR 83) was cultivated at different densities.

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

The same letters did not differ for the Tukey test. ($p<0.05$).

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

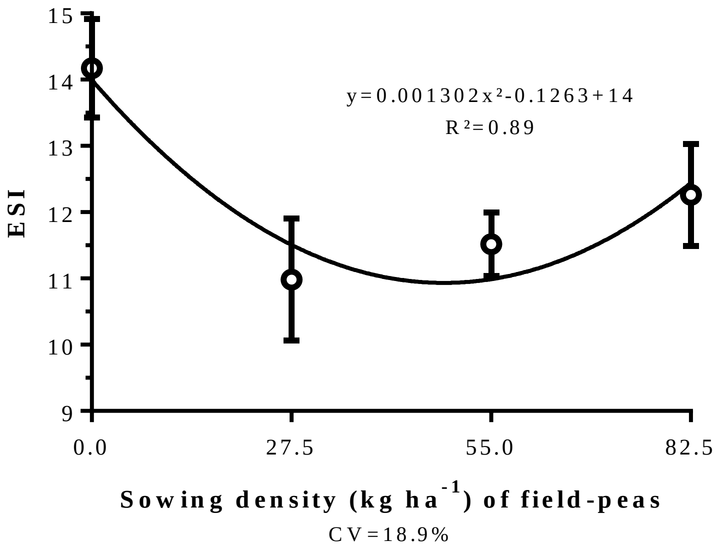

Figure 1. Emergency Speed Index (ESI) of corn cv. IPR 164 after garden pea (*Pisum sativum* ssp. *arvense* cv. IPR 83) was sown at different densities.

The levels of chlorophyll *a* and *b* in corn were higher in the presence of garden pea than in the control (Figure 2). This increase can 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). Once nitrogen is a key component of chlorophyll (TAIZ et al. 2017), its availability from decomposed cover crops likely results in high chlorophyll content in corn leaves.

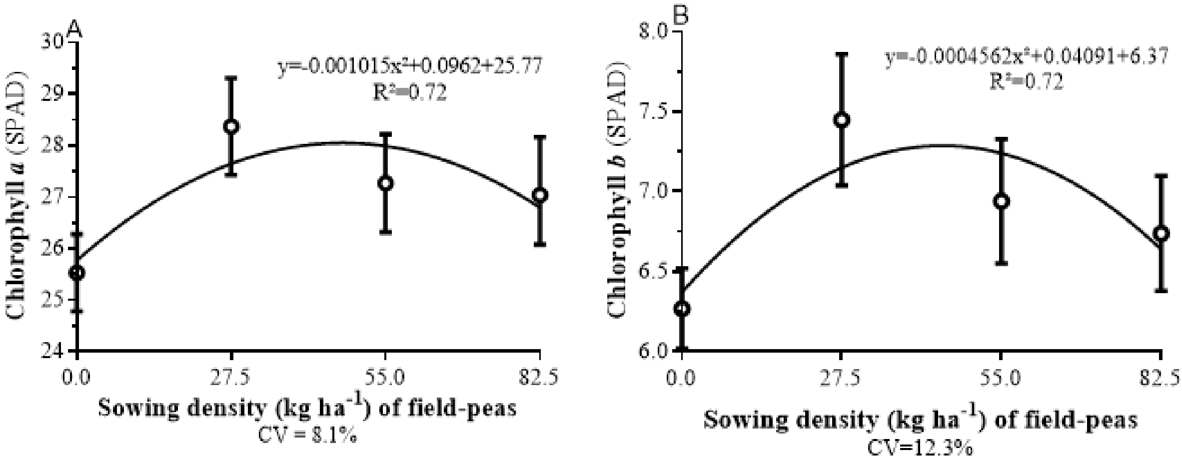

Figure 2. Chlorophyll levels *a* (A) and b (B) in corn cv. IPR 164 sheets after sowing garden pea (*Pisum sativum* ssp*. arvense* cv. IPR 83) at different densities.

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

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

Seeding density (kg ha^{-1})	Leaf area (cm^2)		
	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	

Larger equal letters did not differ between rows (seeding density) and columns (decomposition days) for the Tukey test. ($p<0.05$).

The corn sown 21 and 35 days after cutting the pea showed leaf areas similar to those of the control (Table 6). However, during these periods, the leaf area with a density of 82.5 kg ha^{-1} was lower than that during the 7-day period, probably due to the advanced decomposition of the cover crop.

The dry mass of corn was lower when sown 21 days after the field peas were cut compared to 7 and 35 days (Table 7). This reduction may be related to the increased immobilization of nitrogen by microbial activity, as garden pea straw is almost completely decomposed around this time (≈50 days) (KARKANIS et al. 2016). For 35 days, straw is mainly decomposed, and nitrogen is likely to be demobilized, allowing corn to grow.

Table 7. Dry mass of corn (cv. IPR 164) after several periods of decomposition 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

The equal numbers did not differ for the Tukey test (p<0.05).

CONCLUSION

The use of garden pea followed by corn in a no-harvest system increases the 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.

The levels of catechin nitrate increased with corn cultivation, regardless of the presence of garden peas. Epicatechin was detected in the soil after corn was sown 21 and 35 days after cutting garden peas. Resveratrol has been linked to the breakdown of cover crops, appearing after corn was sown seven days after garden peas were cut.

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

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