ISOLATION AND IDENTIFICATION OF ALLELOPATHIC COMPOUNDS FROM THE RESIDUES OF SOME SUNFLOWER CULTIVARS USING HPLC TECHNOLOGY

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ABSTRACT

This research was conducted to detect some of the allelopathic chemical class in plant residues of nine cultivars of sunflower, including Zahret Al-Iraq, Luleo, Turkie, Al-Isaqi 1, Al-Isaqi 2, Tarzan, Akmar, Shumoos, and Sin Al-Theeb, using appropriate chemical reagents. Some of these chemical were furthered analysed for their content of allelopathic comopounds using High-Performance Liquid Chromatography (HPLC). Results revealed the presence of glycosides, alkaloids, tannins, terpenes, steroids and flavonoids the plant residues of the test sunflower cultivars. Concentrations of glycosides, tannins, alkaloids, and flavonoids were measured, were found to be highter in Shumoos and Tarzan 1018 cultivar residues. The analysis with the High-Performance Liquid Chromatography (HPLC) indicated the presence of several allelopathic compounds of phenolic in nature in the different sunflower cultivar residues, namely Rutin, Vanillin, Resorcinol, and Quercetin.

Keywords: secondary metabolites, phenolic acids, shumoos cultivar, tarzan 1018 cultivar.

عزل وتشخيص المركبات الأليلوباثية في بقايا أصناف زهرة الشمس باستخدام تقنية HPLC

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المستخلص

تم اجراء هذا البحث بهدف الكشف عن بعض المركبات الكيميائية الاليلوباثية في البقايا النباتية لتسعة اصناف من زهرة الشمس وهي زهرة العراق، Luleo ، تركي، الاسحاقي 1، الاسحاقي 2، طرزان، اقمار شموس، سن الذيب. وذلك بأستعمال الكواشف الكيميائية المناسبة، وقد تم تحليل بعض هذه المركبات الكيميائية لمعرفة محتواها من المركبات الاليلوباثية باستخدام جهاز الفصل الكروموتوغرافي السائل عالي الاداء HPLC وقد اظهرت النتائج وجود الكلايكوسيدات، القلويدات، التانينات، التربينات، الستيرويدات، الفلافونيدات في بقايا اصناف زهرة الشمس موضوع البحث، وتم قياس تركيز كل من الكلايكوسيدات والقلويدات والفلافونيدات اذ بينت النتائج ان اعلى تراكيز لها كانت في بقايا الصنف شموس في حين كانت اقل التراكيز مع مخلفات الصنف طرزان 1018، كما اظهرت نتائج التحليل بجهاز الكروموتوغرافي السائل عالي الاداء HPLC وجود عدد من المركبات الاليلوباثية الفينولية في بقايا اصناف زهرة الشمس المختلفة وهي Rutin , Vanillin , Resorcinol , Quercetin .

الكلمات المفتاحية: المركبات الثانوية، الفينول الكلي، صنف شموس، صنف طرزان1018.

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INTRODUCTION

Allelopathy is known as a complex environmental phenomenon that involves biochemical interactions between plants and including microorganisms. This phenomenon occurs as a result of the release of some chemical compounds into the surrounding environment in various ways . These compounds are products of the plants secondary metabolic processes such as alkaloids, phenols, tannins, coumarins, flavonoids, and others. They are naturally produced within the plant as secondary metabolites (3,4,16). They stored inside the cell vacuole or released into the external environment to affect plants and other organisms. Alternatively, they are produced incidentally as a result of mechanical or physiological damage such as diseases, insects, and other environmental stress. The types of these compounds vary according to the plant species, its growth stage and the plant part in which they are found, and their concentrations increase or decrease under the influence of various environmental and biological factors (15). allelopathic crops produce plant toxins that inhibit the germination of the crop's own seeds or the crops that follow it in the crop rotation cycle (21) through the decomposition of residues of those crops in the soil, volatilization, root exudates, rainwater leaching, and not all of these methods are necessary in allelopathic plants. The scientific progress in studying the allelopathy phenomenon has led to the discovery of its active role in both natural and agricultural ecosystems. In the natural ecosystem, this phenomenon plays a determining vegetation cover patterns. As for the agricultural system, studies and research have shown that allelopathy has a role in influencing crops in subsequent crop cycles, crops on weeds, weeds on crops, weeds on weeds, crops and weeds on microorganisms (14). Research related to allelopathy in the past was limited to discovering allelopathic effects and their impacts on some plant species in natural ecosystems. However, scientific progress in studying this phenomenon has revealed new perspectives, including the possibility of utilizing it in agricultural cycles as alternatives to environmentally harmful chemical pesticides, as part of integrated pest management as a pathogen inhibitors (22,18) . There are many crops, including sunflower, with high inhibitory potential that weaken the growth of certain accompanying weeds in the field directly through root secretions or indirectly by leaving residues of allelopathic crops on the soil surface, especially in no-till farming systems, besides being an organic material that improves soil properties, prevents erosion, and preserves soil temperature (7) there are many allelopathic crops that can affect subsequent crops in the agricultural cycle through the residues they leave in the soil (25). These effects can be direct or indirect through inhibiting the nitrogen-fixing microorganisms that the plant uses for growth . Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in Iraq and the world, cultivated for its seeds which contain a high oil percentage reaching up to 50% in some cultivars. These oils are of high quality and used in human food, soap production, and other applications (17). It has been observed that after harvesting sunflower crops and planting wheat and barley crops using the remaining residues, there is a noticeable decrease in the growth of weeds accompanying these crops, along with an improvement in the productivity of these crops. Due to the known variation in allelopathic activity among sunflower genotypes on one hand, and the entry of many sunflower cultivars into Iraq on the other hand, which necessitates the analysis and determination of their allelopathic compounds for the purpose of benefiting from this information and utilizing genotypes with high allelopathic activity to control the growth of weeds accompanying the growth of subsequent crops. The research aims to identify the effective compounds in the plant residues of nine sunflower cultivars using appropriate chemical reagents, separating and estimating the levels of some of them, as well as isolating and diagnosing the allelopathic compounds using a high-performance liquid chromatography. The reason for screening this number of cultivars within the same species is attributed to the genetic variations among these cultivars.

MATERIALS AND METHODS

The experiments were conducted in the laboratories of the College of Science/University of Baghdad determine some active compounds and identify The secondary metabolites in the plant residues of nine cultivars of sunflower (Zahret Al-Iraq, Luleo, Turkie, Al-Isaqi 1, Al-Isaqi 2, Tarzan, Akmar , Shumoos, and Sin Al-Theeb) The research included the following steps:

1. Detection of some active compounds in the plant residues of sunflower cultivars:

A- Detection of flavonoids and glycosides according to Alshaker (5)

B- Detection of alkaloids, terpenes and steroids according to Abbas *et al* (1)

C- Detection of tannins: followed method by Atanassova *et al* (6)

D- Detection of resins: method adopted by Shihata (24).

2. Determination of the proportions of some active compounds in the plant residues of nine cultivars of sunflower crops:

A. Estimation of the Glycosides: Glycosides were extracted and separated from the plant residues of various sunflower cultivars according to the method of (2). the percentage of Glycosides was calculated on a dry weight basis, obtaining Glycosides in a pure, crystalline solid form.

B. Estimation of Alkaloids: Alkaloids were measured according to the method described by Abbas *et al* (1)

C. Estimation of the Percentage of Tannins: Tannins were estimated according the method of (5), and the percentage of tannins was calculated a dry weight basis using the following equation:

$$\textbf{tannins \%} = \frac{\textbf{weight the remaining sediment before burning} - \textbf{weight the remaining sediment after burning}}{\textbf{weihgt of sample}} \times 100$$

D. Estimation of Flavonoids: The percentage of flavonoids was measured and expressed as a percentage based on dry weight as mentioned by Bohm *et al* (9).

3. Identification of Phenolic Compounds in the residues

Alcoholic extracts Of the residues were prepared according the method (13). Twelve suspected standard compounds obtained from the private sector were prepared. and 5 microliters of each compound were injected into the HPLC system using the supelcosil TM Column L C18 separation column with dimensions of 15cm×4.65mm. The amount of phenolic compounds was estimated using the following equation:

$$\textbf{Quantity of separated materials} = \frac{\textbf{Area under the curve of the sample} \times \textbf{standard compound concetration}}{\textbf{Area under the standard compound curve} \times \textbf{sample volume (ml)} \times \textbf{sampleweight (g)}}$$

RESULTS AND DISCUSSION

Preliminary detection of secondary chemical compounds in plant residues of various sunflower cultivars showed the presence of glycosides, alkaloids, flavonoids, tannins Table 1. The results showed a positive detection of active chemical compounds in all replicates tested in the plant residues of the test sunflower cultivars. These results may be considered as an indicator of the plants biological activity, especially since these compounds participate in many biological activities that play a significant role in the growth and reproduction of plants, as well as their importance in defending the plant against environmental stresses such as temperature drops, exposure to ultraviolet radiation, nutrient deficiencies, bacterial, fungal, and insect attacks, thus serving as natural resistance factors for the plant by making cell walls impermeable to water and gases, responsible for providing the plant with the characteristic of rigidity (23). The effect can be either direct, such as influencing cellular structures or various physiological processes like protein synthesis, hormone production, cell membrane permeability, opening and closing of stomata, synthesis of plant pigments, photosynthesis, respiration, legume hemoglobin formation, nitrogen fixation, ion absorption, tissue blockage, water-plant relationship, and genetic material. Indirect effects include changes in soil properties, nutritional status, and the activity of microorganisms growing and spreading in the soil (26).The results in Table 1 revealed that the glycosides, tannins, alkaloids, and flavonoids recorded the highest amounts, reaching 1.32%, 5.11%, 2.25%, and 14.37%, respectively, in the residues of the Shumoos cultivar. These compounds have considerable effect on plant physiological processes as well

as on germination and growth of plant. Glycosides are important active substances in the plant's defense and metabolic system, helping them complete their life cycle by protecting them from biotic stresses, including defending against bacteria, viruses, fungi, nematodes, herbivores, and others. Non-biotic stresses involve reducing the harmful effects of light, heat, salinity, and drought. Plant alkaloids play a significant biological and physiological role during various plant life cycles, as alkaloids are known to be toxic compounds. Their presence in plants protects them from herbivorous insects and animals. Additionally, alkaloids act as growth regulators for the plant, Alkaloids also serve as reservoirs for elements needed by the plant in its different growth stages, with nitrogen being one of the most important elements (10). Flavonoids have various functions and roles in plants, including protection against UV radiation and oxidation, defense against pathogens, control of growth-regulating hormones like auxins, importance in coloring flowers and fruits, and antifungal, antimicrobial, and insecticidal effects (11). Tannins are found concentrated in specific plant parts like leaves, stems, bark, and unripe fruits, disappearing as the fruits ripen. This disappearance is attributed to tannins being a source of energy consumed by the plant in metabolic processes, decreasing in quantity to be exhausted during ripening. The remaining tannins transform into acids, giving the fruits their acidic taste. Tannins also protect plants from harmful insects and fungi, preserving plant life during growth, with their concentration increasing in dead plant parts to shield the plant from biological infestations (12). The residues of Sin Al-Theeb cultivar recorded the highest concentrations of Glycosides 1.1% and flavonoids 13.84%, reaching . The residues of the Tarzan cultivar recorded the lowest concentration of the secondary compounds studied Table 1. This variation in the concentration of effective secondary compounds in the plant residues of different cultivars of sunflower crop reflects on the effectiveness of these compounds (inhibitory effect). Studies have indicated that allelopathic compounds present in plant residues and added to the soil are released into the environment through leaching (soluble) or undergo degradation by the influence of microorganisms in the soil. These compounds may undergo chemical transformations as they suffer oxidation, degradation, and polymerization that alter their nature, concentration, and some of these compounds may bind to soil elements such as clay or humus, which may increase soil fertility. Additionally, some compounds like phenolic acids can accumulate in the soil to reach toxic levels or inhibitory effects. The allelopathic compounds released into the soil may undergo biochemical transformations by microorganisms, leading to their conversion into simpler or more complex compounds, reflecting their allelopathic effect on germination, growth, and physiological processes associated with the plant (27).

Table 1. Percentage (%) of Some Secondary Compounds in Sunflower Cultivars residues

Residues	Glycosides	Tannins	Alkaloids	Flavonoids
Zahret Al-Iraq	0.61	4.22	1.68	13.28
Luleo	0.38	3.12	0.59	8.45
Turkie	0.74	4.64	1.48	12.86
Al-Isaqi 1	0.54	2.89	1.12	9.72
Al-Isaqi 2	0.31	3.87	0.72	11.93
Tarzan	0.25	2.13	0.38	7.12
Akmar	0.82	5.03	2.11	13.52
Shumoos	1.32	5.11	2.25	14.37
Sin Al-Theeb	1.11	4.83	1.83	13.83

Identification of phenolic compounds in plant residues of sunflower cultivars: The results in Table 2 show the presence of several phenolic compounds known for their high allelopathic ability. The highest percentage of identified phenolic compounds was recorded in the residues of the cultivars Shumoos, Akmar, and Sin Al-Theeb, including Rutin, Vanillin, Resorcinol, and Quercetin. However, it decreased in the residues of the Tarzan cultivar, as indicated by the results of the analysis using the high-performance liquid chromatography device, with the percentages of the compounds being: Vanillin at 2.41%,

Quercetin at 2.52%, Rutin at 2.11%, and Resorcinol at 1.88%. It is also noted that the compound P-Hydroxy benzoic acid recorded its highest percentage in the residues of the Shumoos cultivar at 2.82%, followed by the Sin Al-Theeb residues at 2.65%, while the lowest percentage was with the residues of the Turkiee cultivar at 1.89%, with no percentage recorded for this phenolic acid in the residues of the Luleo, Al-Isaqi 1, Al-Isaqi 2, and Tarzan cultivars. Additionally, varying proportions of Caffeic acid and Hydroquinone were recorded in some sunflower cultivar residues compared to others. This variation in the presence of phenolic compounds and their proportions among different sunflower cultivars is attributed to the genetic diversity among the studied cultivars. Furthermore, the presence of the compound Rutin and the compound Hydroquinone in the residues of sunflower cultivars, which affect the balance of hormones such as auxin and gibberellin, and that the natural growth of cells requires the regulation of the levels of these hormones (8) , As allelopathic compounds affect the balance of some hormones like auxin hormone, which leads to a disturbance in its level affecting the growth of secondary roots (19). Studies have also shown that caffeic acid compound inhibits root growth, reduces the activity of the enzyme phenylalanine ammonia lyase, the content of the peroxidase enzyme, and increases the number of lignin units in roots exposed to caffeic acid, increasing the cell wall's rigidity and preventing root growth (20).

Table 2. Distribution of Identified Phenolic Compounds and Retention Time Rt Values Identified by HPLC Technique for Alcoholic Extracts of Sunflower Cultivars residues

	Compounds	Rt	Zahret Al-Iraq	Luleo	Turkie	Al-Isaqi 1	Al-Isaqi 2	Tarzan	Akmar	Shumoos	Sin Al-Theeb
1	P-Hydroxy benzoic acid	2.89	2.11	-	1.89	-	-	-	2.45	2.82	2.65
2	Rutin	3.16	3.09	2.52	2.52	2.24	2.78	2.11	3.08	3.29	3.10
3	Vanillin	2.89	3.16	2.19	2.79	2.15	2.91	2.44	3.16	3.19	3.11
4	Caffiec acid	1.71	-	1.85	1.54	-	-	-	-	1.58	-
5	Hydroquinone	2.67	1.85	-	1.89	-	-	-	2.13	-	2.71
6	Catichol	2.92	-	-	-	-	-	-	-	-	-
7	Benzoic acid	2.88	-	-	-	-	-	-	-	-	-
8	Resorcinol	3.11	2.71	2.89	2.97	2.66	2.24	1.88	2.98	3.39	3.21
9	Gallic acid	2.98	-	-	-	-	-	-	-	-	-
10	Quercetin	3.45	2.97	2.71	2.93	2.77	2.61	2.52	3.44	3.57	3.25
11	Salicylic acid	2.84	-	-	-	-	-	-	-	-	-

Quantitative estimation of phenolic compounds separated from plant residues of different sunflower cultivar: The results in Table 3 showed significant differences in the concentration of chemical compounds isolated from plant residues of nine different sunflower cultivars. The highest concentrations of Rutin, Vanillin, Resorcinol, Quercetin, and P-Hydroxy benzoic acid compounds were obtained by separating phenolic compounds from the residues of the Shumoos cultivar using HPLC registered, reaching 27.5, 28.2, 27.7, 28.4, 21.3, and (micrograms/gram) respectively. Following, the residues of the Akmar cultivar had the highest concentration of Vanillin and Quercetin compounds at 26.9 and 27.5 (micrograms/gram) respectively. As for the residues of the Sin Al-Theeb cultivar, they followed the Shumoos residues in recording the highest concentrations of Rutin and Resorcinol compounds, reaching 26.1 and 27.5 (micrograms/gram) while registering the highest concentration of Hydroquinone compound at 18.4 (micrograms/gram). The residues of the Luleo cultivar recorded the highest concentration of Caffeic acid compound at 13.8 (micrograms/gram). The effectiveness of plant residues and their water extracts is attributed to the allelopathic compounds released from them due to the process of plant residue decomposition and washing. This has been more precisely confirmed in the current study through the detection, diagnosis, and measurement of some chemical compounds (secondary metabolites) from residues of different sunflower cultivars.

Table 3. phenolic acids concentration (mg/g) in ethanol extract of sunflower cultivars on measured by HPLC

	Compounds	Zahret Al-Iraq	Luleo	Turkie	Al-Isaqi 1	Al-Isaqi 2	Tarzan	Akmar	Shumoos	Sin Al-Theeb
1	P-Hydroxy benzoic acid	16.8	-	14.4	-	-	-	18.5	21.3	19.8
2	Rutin	25.2	23.6	23.1	22.5	24.3	21.8	25.1	27.5	26.1
3	Vanillin	25.5	23.2	23.9	23.0	24.3	23.3	26.9	28.2	24.9
4	Caffiec acid	-	13.8	10.2	-	-	-	-	12.5	-
5	Hydroquinone	16.3	-	16.2	-	-	-	17.5	-	18.4
6	Catichol									
7	Benzoic acid	-	-	-	-	-	-	-	-	-
8	Resorcinol	25.8	26.1	26.5	25.5	24.7	23.7	27.2	27.7	27.5
9	Gallic acid	-	-	-	-	-	-	-	-	-
10	Quercetin	26.3	22.6	25.1	23.8	22.2	21.9	27.5	28.4	27.1
11	Salicylic acid	-	-	-	-	-	-	-	-	-

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