ALLELOPATHIC EFFECT OF ROOT EXUDATES FROM SOME SUNFLOWER CULTIVARS ON ACCOMPANYING WEEDS

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ABSTRACT

The study looked at what happened to the weeds growth in the field when different types of sunflowers released root exudates-grown in the summer of 2023. The goal was to determine if sunflower types' allelopathic potential affects weed growth and number. The results showed that after 30, 60, 90, and 120 days of cultivation, the Flamme cultivar did better than the Sakha, Aqmar, and Abba5 cultivars ~~in~~ reducing the number of weeds and their dry weight. The weed density dropped by 60%, 46%, 38%, and 35% compared to the control treatment, and the weeds' dry weight dropped by 61%, 66%, 62%, and 53% compared to the control treatment, in that order. The Sakha and Aqmar cultivars were next. The Abba5 cultivar recorded the lowest reduction in the number of weeds and their dry weights. We used the staircase experiment method to get rid of any competition when we tested the allelopathic potential of the fluids from the Flamme and Sakha cultivars. The Flamme cultivar's root exudates were found to be better at stopping the growth and dry weight of wild beet and purple panic weed than the Sakha cultivar's root exudates. The high-performance liquid chromatography test revealed that the Flamme and Sakha cultivars' root exudates had nine different compounds. The amounts of these phenolic compounds varied between the two studied cultivars. Most of the isolated compounds were found in higher amounts in the Flamme cultivar than in the Sakha cultivar. Most of the isolated compounds are known to stop plants, even weeds, from sprouting and growing naturally.

Keywords: phenolic compounds, Flammee cultivar, Sakha cultivar, secondary compounds.

التأثير الأليلوباثى لإفرازات جذور بعض أصناف زهرة الشمس على الادغال المرافقة له

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المستخلص

درس تأثير افرازات جذور اصناف من زهرة الشمس في الادغال المرافقة لنموها في الحقل في الموسم الصيفي لعام 2023، وذلك لاختبار فيما اذا كانت التغايرات الملحوظة في اعداد الادغال و نموها بين اصناف زهرة الشمس يعود سببها الى وجود اختلافات في القابلية الاليلوباثية لها .اظهرت النتائج ان الصنف فلامي قد تفوق على بقية الاصناف وهي سخا و اقمار واباء5 في خفضه لعدد الادغال المرافقة له و وزنها الجاف معنويا بعد 30 و 60 و 90 و 120 يوما من الزراعة ، اذ انخفضت كثافة الادغال بنسبة 60 و46 و38 و35% عن معاملة المقارنة على التتابع ، والوزن الجاف للأدغال بنسبة 61 و66 و62 و53% عن معاملة المقارنة بالتتابع يليه الصنفين سخا و اقمار ، اما الصنف اباء5 فقد سجل اقل نسبة خفض في اعداد الادغال و اوزانها الجافة . وعند اختبار القابلية الاليلوباثية لإفرازات الصنفين فلامي و سخا باستخدام تجربة تقنية الدرج stair-case experiment لأبعاد عامل التنافس تبين ان افرازات جذور الصنف فلامي لها القابلية على تثبيط النمو والوزن الجاف لدغلي السليجة والدهنان وبنسبة اكبر من تأثير افرازات جذور الصنف سخا . و بينت نتائج التحليل بجهاز الكروموتوكرافي السائل عالي الاداء وجود تسعة مركبا في افرازات جذور الصنفين فلامي وسخا و جميعها ذات طبيعة فينولية مع تباين نسب هذه المواد بين الصنفين المدروسين وان اغلب المركبات المعزولة كانت بنسب عالية في الصنف فلامي مقارنة بالصنف سخا ، واغلب المركبات المعزولة معروفة بقدرتها التثبيطية لانبات ونمو النباتات وبضمنها الادغال.

الكلمات المفتاحية : المركبات الفينولية ، الصنف فلامي ، الصنف سخا، المركبات الثانوية.

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INTRODUCTION

Allelopathy is defined as a biological and natural phenomenon that constitutes an important branch of chemical ecology. Higher plants communicate with each other physiologically and environmentally through the release of chemicals called allelochemicals. Many parts of plants, including roots, seeds, leaves, stems, and other parts, naturally contain these chemical compounds (1,10,12,26). Most ecologists agree that allelopathy is an ecological phenomenon in which plants gain a competitive advantage through biochemical pathways. This is because the compounds that plants, microorganisms, viruses, and fungi make can either help or hurt the growth and development of other organisms, including plants (5,27,30). Sunflower is a valuable agricultural product in Iraq utilized in various processing sectors. There is optimism by many scholars that dedicated to enhance its cultivation conditions (9,34,39). Sunflower crop is considered one of the crops with allelopathic effects and has a significant impact on subsequent crops and accompanying weeds (7,31,37). It contains high levels of phenolic and terpenoid compounds, with variation among its cultivars in allelopathic potential (3,4,8). The present study was conducted to evaluate 4 sunflower cultivars for their allelopathic effect against companion weeds and to test if their root exudates involve in their allelopathic inhibition.

MATERIALS AND METHODS

Field experiment

The experiment was conducted at the University of Baghdad to test the allelopathic potential of four sunflower cultivars, namely Flamme, Sakha, Aqmar, and Abba5 The field was prepared for cultivation by ploughing, leveling, adjusting and dividing into plots measuring 4 × 4 meters. On 30/2/2023, Seeds of sunflower cultivars were manually sowed in their respective plots, keeping a distance of 70 cm between rows and 30 cm between holes. Phosphate fertilizer was added in one batch at the time of planting at a rate of 240 kg/ha, and urea fertilizer (46% nitrogen) was added in three equal batches at a rate of 80 kg/ha at the time of planting, 6 weeks later (33).The plots were irrigated periodically as needed. Number of plants of each weed were counted at 30, 60,90 and 120 days by randomly selecting one square meter in the middle of experimental unit. At the physiological maturity of crop, plants of each weed were harvested and oven dry weight were taken at 75 C for 3 days .The experiment was conducted using a randomized complete block design (RCBD) with three replications.

Root exudates bioassay

This experiment was conducted to determine the allelopathic effect of root exudates and exclude the factor of competition using the stair – step system (6). The system comprises of two series one consists of plastic pot filled with purified sand and contained of test sunflower cultivar and alternated with plastic pots contained test. The other series (control) comprised of plastic pots each contained test weed and alternated with pot filled with purified sand only. Each series was watered with Hoagland solution present in a container placed on the upper shelf . The solution dripped from the lower hole in the container and passed through the pots until collect in a container placed in the lower shelf. All the pots contained 2kg of purified sand and each one has a hole to recycle the solution.. The collected solution of each series was used to refill the upper respective container manually After two months , weeds shoot and root were harvested using running water for roots separation from sand, kept in perforated paper bag and dried in the oven at 70C for two days. Oven dry weights of shoot and roots of the test weeds were recorded. The data were statistically analyzed according to a one-factor CRD design, and the means of the treatments were compared using the least significant difference (L.S.D) test at the 0.05% level, using the Genstat statistical software (36).

Root exudates collection and identification

The purpose of this experiment was to find out if the root exudates of Flamme and Sakha sunflower cultivars contain allelopathic compounds that inhibit weeds growth and if the variation in allelopathic potential is due to the differences in the amounts of these compounds. The collection of allelopathic compounds was adirect XAD4 resin method which is basically that of Kong et al (20).Seeds of sunflower cultivars Flamme and

Sakha were sown in plastic containers containing acid washed white sand, Ten days after planting, four seedlings from each cultivar were transferred to non-perforated plastic pots that contained mM Mes-Tris Buffer solution, (pH of 5.5), 0.5 mg of Caso4 and 50 grams of the industrial resin XAD-8 The pots were randomly distributed inside the incubator at a temperature of 25 ± 1ºC and 12 hours of light, and the pots were watered daily to compensate for the depletion of the solution. And at 14 days of cultivation, the roots were removed from the pots, and the solution and resin were transferred to separation columns (5 cm diameter × 20 cm length), where the nutrient solution was first discarded, and then the resin was washed with distilled water. The resin was extracted by adding 50 ml of pure methanol three times. the methanol was evaporated to dryness by rotary evaporator at 40°C. The dry extract was then dissolved in three millilitres of methanol and stored in the freezer until measurement.We conducted the experiment with three replicates for each treatment. The allyl phenolic compounds in the root exudates of sunflower were separated using High-Performance Liquid Chromatography (HPLC) using the following conditions (Table 1)

Table 1. Conditions for the separation and identification of allopathic compounds using HPLC

Parameter	Characteristic
Colum dimensions	50 length × 2.6 mm I.d
Diatomite	Supleco wax 10
Attenuation	0.01 ppm
Rate of recorder	10 mm/ minutes
Detector	SPD-2010 spectrophotometer at 254 nm
Volume injection sample	50 µ1
Type of column	ODS-C18
Mobile face	1% acetic acid in H_2O : acetonitrite 30:7 (v/v)
Temperature	35 C^o

RESULTS AND DISCUSSION

Effect of test sunflower cultivars on companion weeds

Table 2 revealed that broad-leaved weeds made up more of the total (53% vs. 47%). The primary weeds identified are rough pigweed, lamb's quarter, field bindweed, door weed, and wild beets, with less occurrence of blady weed and Jimson weed. Narrow-leaved weeds included rigid rye, Tatarian orache, purple panic weed, pale centaury, and Gohnson weed, comprising 46% of the total. The variation in weed types and their densities is influenced by location and environmental conditions.

Table 2.Types of weeds growing in the sunflower field for 2023 agricultural season

Local name	Scientific name	Family name	Type	Life cycle
Rough pigweed	*Amaranthus retroflexus* L.	Amaranthaceae	Broad leaves	Annual
Lambs quarter	*Chenopodium album* L.	Chenopodiacea	Broad leaves	Annual
Field bind weed	*Convolvulus arvensis* L.	Convolvulacea	Broad leaves	Biennial
Bermuda weed	*Cynodon dactyylon* L.	Poaceae	Narrow leaves	Biennial
Rigid rye weed	*Liolium rigidum* L.	Poaceae	Narrow leaves	Annual
Blady weed	*Imperata cylindrical* L.	Poaceae	Broad leaves	Biennial
Tatarian orache	*Atriplex patula* L.	Chenopodiacea	Narrow leaves	Biennial
Purple panic-weed	*Echinochloa colonum* L.	Poaceae	Narrow leaves	Annual
Pale centaury	*Centaurea pallescens* L.	Compositae	Narrow leaves	Annual
Door weed	*Polygonum aviculare* L.	Polygonaceae	Broad leaves	Annual
Jimson weed	*Jimson weed stramonum* L.	Solanaceae	Broad leaves	Biennial
Gohnson weed	*Sorghum halepense* L.	Poaceae	Narrow leaves	Biennial
Wild beets	*Beta vulgaris* L.	Chenopodiacea	Broad leaves	Annual

Effect of sunflower cultivars on density of companion weeds : The results indicated that sunflower cultivars significantly affected weed density during the growing season of the sunflower crop (Table3). Flamme showed more inhibitory to weeds than other cultivars at all sowing dates of crop. Abba5 Showed the least inhibitory effect to test weeds, particularly at 90 and 120 days after sowing.

Table 3. The effect of sunflower Cultivars on weed density during the crop growth stages

Cultivars	Weed density (plants/m²) after			
	30 days after sowing	60 days after sowing	90 days after sowing	120 days after sowing
Control	120.7	187.7	231.3	262.0
Aqmar	64.3	118.7	161.7	198.3
Sakha	58.7	111.0	152.0	187.7
Abba5	95.7	163.7	218.0	261.7
Flamme	47.7	101.3	142.7	168.3
L.S.D.≤ 0.05	11.62	17.31	20.37	22.39

Effect of sunflower cultivars on dry weight of companion weeds: Dry weight of weeds often indicates the strength of competition between weeds and crops in extracting growth requirements and reflects this competition in the ability to accumulate dry matter. The results in Table 4 showed significant differences among test sunflower Cultivars in their ability to reduce the dry weight of weeds. After 30 days of planting, the highest dry weight of weeds was recorded at 18.31 g/m², and the lowest inhibition rate was 27% for the Abaa5 cultivar, while the Flamme cultivar recorded the lowest dry weight of weeds at 10.04 g/m² and the highest inhibition rate at 61.1%. This was followed by the Sakha and Aamar cultivars with 12.42 and 14.14 g/m² and inhibition rates of 51% and 44%, respectively. As for the mulched treatment (without planting), it was recorded the highest dry weight of weeds, reached 25.74 g/m². However, after 60 days of planting, the Flamme cultivar continued to record the lowest dry weight of weeds among the other cultivars, reaching 15.7 g/m², followed by the Sakha, Aqmar, and Abba5 cultivars, which recorded dry weights of 20.8, 22.7, and 34.4 g/m², respectively. The inhibition rate in the dry weight of weeds was highest with the Flamme cultivar, reaching 66%, while the Abba5 cultivar recorded the lowest rate at 24%. The sunflower cultivars continued to exhibit the same behavior after 90 and 120 days of planting as in the previous periods (30 and 60 days). Despite the increase in the dry weight of weeds with all sunflower cultivars, the Flamme cultivar It continued to record the lowest dry weight of weeds, reaching 25.6 and 38.5 g/m² for the 90 and 120 days after planting periods, respectively, and the highest inhibition rates of dry weight of weeds, with rates of 62% and 53% consecutively. As for the two cultivars, Sakha and Aqmar, the dry weight of the accompanying weeds reached 31.8 and 36.3 g/m² after 90 days of planting, with inhibition rates of 53% and 46%, respectively. After 120 days, the rates were 48% and 38%, respectively. Meanwhile, the lowest inhibition rate in the dry weight of the weeds was for the cultivar Abaa5, which was 20%. This result is consistent with (35,38), which suggested that the reduction in the dry weight of the accompanying weeds with different crop cultivars may be one of the important criteria for the cultivar's competitiveness with the weeds and that this variation in weed growth inhibition may be due to the allelopathic potential of the root exudates. Root exudates may cause a decrease in the dry weight of the shrubs by inhibiting photosynthesis and energy production (2,16,18,22,29). Some allelopathic compounds also affect photophosphorylation and electron transporters by blocking the enzyme Phosphoribulokinase (PRK). This could cause the sugar concentration to drop, which would then cause the shrubs' dry weight to drop. It also stops the enzyme phosphoglycerate kinase (PGK) from working, which lowers the production of ATP and, in turn, the energy that the shrubs need to grow (24). Also, the inhibition of the enzyme NADP-MDH leads to a decrease in NADPH production, which in turn reduces the carbon fixation rate and the dry weight of the weeds (11,32).

Table 4. Effect of sunflower Cultivars at different growth stages on dry weight of weeds.

Sunflower cultivars	Dry weight of weeds (g/m²) after				Sunflower cultivars	Inhibition rate%			
	30 days after sowing	60 days after sowing	90 days after sowing	120 days after sowing		30 days after sowing	60 days after sowing	90 days after sowing	120 days after sowing
Control	25.74	46.1	67.7	81.8	Control	0.00	0.00	0.00	0.00
Aqmar	14.14	22.7	36.3	50.1	Aqmar	44.4	50.2	46	38.5
Sakha	12.42	20.8	31.8	42.4	Sakha	51.6	55	53	48
Abba5	18.31	34.4	50.5	64.9	Abba5	27.6	24.5	22.7	20.1
Flamme	10.04	15.7	25.6	38.5	Flamme	61.1	66.1	62.3	53
L.S.D. 0.05	5.01	7.61	8.94	9.92	L.S.D. 0.05	18.53	15.89	12.62	11.80

Effect of root exudates of selected sunflower cultivars on growth of weeds: Root exudates of the test cultivars drastically reduced roots, shoots and whole plant of the test weeds (Table 5). Apparently, Flamme is more inhibitory to roots, shoots and whole plant of the test weeds than the other cultivar. Root system is more sensitive to root exudates of the test cultivar than shoot system of both weeds. Flamme reduced root , shoot and whole plant of wiled beet by 55.83, 36.31 and 49.03% of control and inhibited purple panic weed by 56.86,52.33 and 54.75% of control, respectively. The results of this study showed that the root exudates of sunflowers are a key way mechanism for phytotoxic compounds to get out of the plant. These exudates had a lot of power to stop purple panic weed and wild beet plants from growing. This implies that the release of phytotoxic compounds from the sunflower crop into the environment occurs through root exudates. Continued secretion of these inhibitors may lead to their accumulation in relatively high amounts in the soil during the plant's life cycle. This effect is one of the main ways to get rid of weeds. It involves planting a certain cultivar that has a high allelopathic ability to kill or inhibit the weeds that are nearby through root exudates (15,17,37). The results of this experiment clearly support the hypothesis that was put forward in the field experiment. They show that allelopathy is a major factor in how root exudates can lower the density and dry weight of the weeds that are growing nearby (Tables 3 and 4).

Table 5. Effect of sunflower root exudates on the growth of the vegetative a nd root systems of the weeds Purple panic weed and Wild beets

Sunflower cultivars	dry weight of Wild beets			Sunflower cultivars	dry weight of Purple panic weed		
	shoot system	root systems	Whole plant		Shoot system	root systems	Whole plant
Control	191.01	102.11	293.12	Control	116.35	71.18	187.53
Sakha	145.68	82.41	228.09	Sakha	72.53	48.85	121.39
Flamme	84.36	65.03	149.40	Flamme	50.91	33.93	84.84
L.S.D. 0.05	5.48	2.91	5.15	L.S.D. 0.05	3.87	2.65	2.75

Isolation and identification of allelopathic compounds in sunflower cultivars: Using high-performance liquid chromatography, the Flamme and Sakha cultivars were found to have nine compounds. All of these compounds are phenolic (Table 6). These chemicals change how the body works by taking in nutrients, stopping plant cells from dividing, stopping photosynthesis and expansive growth, and also changing how plants make proteins and breathe, changing how permeable cell membranes are, and stopping enzymes from working. Typically, these substances exert their effects by secreting toxic gases like ethylene, aromatic acids like benzoic acid, and aldehydes like malic acid and citric acid. Additionally, they secrete coumarins, steroids, quinones, tannins, flavonoids, alkaloids, and terpenes. They can have a direct effect by changing things like hormone production, stomata opening and closing, cell membrane balance, and the production of photosynthetic pigments. They can also have an indirect effect by changing things like respiration, protein and haemoglobin production, and nitrogen fixation. Finally, they can have an indirect effect by changing the properties of the soil, its nutritional status, and the activity of microorganisms that live in the soil. Some of the other ways phenolic acids affect plants have to do with how they absorb elements and mineral nutrients in the early stages of growth (14,20,28). Allelopathic chemicals emitted by plants act as a defense system against microbial attacks or competition from other plants (13,21,40).The shikimic acid pathway makes allelopathic compounds, which are a type of phenolics and alkaloids that can hurt the growth and development of nearby plants in a number of ways, such as by stopping seeds from germinating. Some allelopathic compounds prevent or delay the germination of competitive plant seeds and affect root growth. Some of these compounds influence root elongation and branching, weakening neighbors plants. Enzyme inhibition: Certain allelopathic compounds can inhibit the activity of specific enzymes in neighboring plants, thereby hindering their ability to utilise nutrients (19,23,25).The ratios of these compounds were found to vary between the two studied cultivars. However, it is evident that the Flammy cultivar had higher ratios of the isolated compounds compared to the Sakha cultivar. The results indicate that the total phenolic concentration doubled in the Flammy cultivar compared to the Sakha cultivar. These results show that the field experiment had an allelopathic effect, and this variation fits with what was seen in the field and the experiment, which showed that the Flammy cultivar was better at stopping the growth and appearance of weeds that were growing nearby (Table 3).

Table 6. Isolation and identification of phenolic compounds in the root exudates of sunflower Cultivars

phenolic compounds	Concentration (micrograms/mL) Cultivars	
	Flamme	**Skha**
Chlorogenic acid	**63.4**	**70.5**
Neochlorogenic acid	**52.7**	**138.6**
Isochlorogenic acid	**1578.9**	**653**
Caffeoylquinic acid	**177.8**	**164.2**
Caffeic acid	**342.7**	**129.5**
Hydroxycinnamic acid	**71.8**	**53.6**
Ferulic acid	**67.4**	**22.8**
5 – O – feruloyl quanic acid	**140.3**	**112.5**
5 – O – P – coumaroyl quanic acid	**138.7**	**45.3**
Total	**2633.7**	**1390**

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