Allelopathy of Root exuded of Two Sunflower Cultivars (*Helianthus annuus* L.) on weed companion

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الجهد الايلوباثي لإفرازات جذرية لصنفين من زهرة الشمس ضد نمو الادغال المرافقة لمحصول زهرة الشمس

المستخلص

تم أجراء تجربتين حقلتين خلال العام 2016/2015 في محافظة واسط /العراق ,لدراسة تأثير الافرازات الجذرية لصنفين من زهرة الشمس واللذان ادخلا الى العراق ضد نمو الادغال المرافقة لمحصول زهرة الشمس .بينت النتائج ان الصنفين اثرا معنويا بتثبيط العدد الكلي والوزن الجاف للأدغال وكان التأثير معتمدا على نوع صنف زهرة الشمس .اسكروا كان اكثر الأصناف تأثيرا بالنسبة لاختزال عدد الادغال مقارنة بمعاملة السيطرة حيث بلغ نسبة الاختزال 61.0% بالنسبة لعدد الادغال ونسبة تثبيط الوزن الجاف 68.6% مع معاملة السيطرة بينما صنف افلامي كان اقل معنويا لجهد الاليلوباثي حيث بلغ نسبة التثبيط 24% لعدد الادغال ونسبة 35.4% بنسبة لوزنها الجاف مقارنة مع معاملة السيطرة .

اكدت نتائج التجربة اللاحقة انه الإفرازات الجذرية لصنف اسكروا كان اكثر تثبيط لنمو الادغال مقارنة مع صنف افلامي معطيا دليلا واضحا على ان صنف اسكروا يملك اعلى جهد اليلوباثي. بينت نتائج التحليل المختبري وجود المركبات الفينولية بالافرازات الجذرية لكلا الصنفين .حيث بلغت اعلاها بصنف اسكرو0.403 ملغم /غم تربة مقارنة مع صنف افلامي والتي بلغت 0.220 ملغم/غم تربة .أوضحت نتائج التحليل الكروموتوغرافي بوجود اكثر من مركب فينولي بالإفرازات الجذرية منها protocatechuic, , p-coumaric acid. و ferulic, , syringic, vanillic وان تركيزها يختلف تبعا للصنف المدروس .حيث أوضحت ان زمن الترحيل يختلف تبعا لنوع المركب المتواجد داخل العين

Abstract

Two field experiments were conducted during 2015-2016 in Wasit province, Iraq to test the allelopathic potential of sunflower (*Helianthus annuus* .L). Two genotypes introduced to Iraq to screened allelopathic of root exuded against weeds in sunflower field. Two sunflower genotypes significantly inhibited total number and biomass of companion weeds and the inhibition is genotype dependent. Genotypes tested Asgria was the most allelopathic cultivars with the reduction in total weed number by 68.6% of control and weed biomass by 61.0% of the

control respectively. Flamme was the less allelopathic genotypes comparted Asgria Genotypes , which inhibition in total weed number by 24% and weed biomass by 35.4% respectively of the control respectively.

A staircase experiment indicated that root exudates of the Asgria cultivar suppressed weeds more than Flamme giving additional evidence for the superiority of the Asgria cultivar in its allelopathic weed suppression. Chemical analysis indicated the presence of phenols compound in root exudates of both cultivars with a significantly higher amount in the Asgria cultivar (0.403 mg/g) compared to cultivar Flamme(0.220 mg/g) and that total phenolic started to increase at 28 day then declined at end season.

Chemical analysis on these phenolics by HPLC indicated the presence of several allelochemicals, viz, protocatechuic, , vanillic, syringic, , ferulic and p-coumaric acid.Chromatographic analyses indicated the presence phytotoxins in the root exudes of the tested sunflower genotypes. All the isolated compounds appeared to have different retention times and were identified as phenolic compounds

Introduction

Weeds represent a global agronomic problem that reduces the productivity of cultivated crops. Weeds compete with cultivated crops for the available moisture, nutrients and light. Consequently, weeds significantly reduce either crop yield or quality. Control of weeds is essential to maintaining the production of economic crops. Agriculture worldwide is using about 3 million tons of herbicides per year, and herbicide-resistant weeds have become much prolific, which has further expanded the use of herbicides [1]. The overuse of agrochemicals has caused environmental pollution, weed tolerance and human health concerns. To solve these problems, it is necessary to develop sustainable weed management systems that may reduce herbicide dependency, inexpensive, easy to use and helpful in maintaining the ecosystem stability [2].

Allelopathy play a major role in nature ecosystems by determining vegetational patterning, plant dominance, plant succession and plant biodiversity, preventing seed decay and causing seed dormancy [3]. Also allelopathy has a significant role in agricultural ecosystems. It plays a significant role in weed-crop, crop-weed, crop-crop, forestry and nutrient cycling [4].

Several genotypes of sunflower have been introduced to Iraq for cultivation. Preliminary field observations revealed that growth and population of companion weeds were variable among the stands of selected genotypes. Also, differential growth and population variation were observed on weeds grown in the field after sunflower harvest. This suggests that allelopathy could be the mechanism responsible for the reduction of weeds growth and population and the differences among stands could be due to differences in the allelopathic potential of the test cultivars. Therefore, it was contemplated in the present studies to screen two sunflower genotypes for their allelopathic ability to control growth and population of companion weeds in order to determine the highly allelopathic genotypes and Evaluate the effect of allelopathic on companion weed in population and growth.

Materials

Site description

The proposed study was conducted at Research station of State Board of Agricultural Extension, Ministry of Agriculture, Waset Province, Iraq. The soil of experimental site was calcareous clay loam . Organic carbon, pH and EC were 0.8%, 7.7 and 7.4 dS m^{-1}, respectively. Average annual rainfall is less than 50 mm and day/night temperatures during the growing season were 30-40/15-30 $^{\circ}$C.

Seeds procurements

Seeds of sunflower cv Asagri and Flamme cv. were obtained from Department of Crop Production, College of Agriculture, Baghdad University.

Weeds identification:

Weeds grown in companion with sunflower were surveyed and their identification was performed by the help of Wasit university Herbarium and specialists of State Board of Agriculture Extension at Waist Province.

Effects of sunflower genotypes on companion weed.

Experiment was conducted during summer 2015 in a field located in Wasit province 180 km south to Baghdad. Field plots (2.5m x 2.5m) were made randomly in field heavily infested with weeds. The plots were plowed by spade to the depth of 30 cm and received Nitrogen as NP (46%N) at 240 $kgha^{-1}$ (50% before planting and 50% after two weeks of

planting) and P as triple superphosphate (46% P_2O_5) at 240 kg ha^{-1} at planting time [5] . Seeds of two sunflower genotypes (Asagri and Flamme) were sown manually on their respective plots in rows with a distance of 25 cm between seeds and 50cm between rows .The plots were selected in randomized complete block design (RCBD) with three replications. At the end of crop maturity, the plants of each sunflower genotype were harvested. Number of each weed species was calculated, after which time, the above ground total weed biomass was harvested, air-dried for several days under sun light during July of 2015 and weighed using digital balance .

Bioassay of root exudates by stair case technique

This experiment was carried out under greenhouse conditions to test the differential allelopathic potential of the test sunflower cultivars against weeds and eliminate the competition for light, moisture and minerals between sunflower plants and weeds. The assay was made using a stair case device described by[6] with little modifications. Seeds of sunflower cultivars and the weed of *Echinochloa colonum*, one of the companion weeds found in sunflower field were planted separately in trays filled with acid washed sand (4% HCl) to prepare seedlings for the assay device. Two uniform seedlings of Asagri and Flamme cultivars and *Echinochloa colonum* were transplanted in their respective plastic pots each contained 0.5 kg acid washed sand. Treatment series consisted of pots of sunflower cultivars alternated with pots of *Echinochloa colonum* the stair case system while a control series consisted of pots of *Echinochloa colonum* alternated with pots containing acid washed sand only. Complete nutrient solution [7], was stored at reservoirs fixed at the top of each series and allowed to pass the pots of each series by dripping and finally accumulated at the bottom reservoirs of each series, where it was recycled every day .The solution was changed *Echinochloa colonum* plants were allowed to grow for 30 days, then harvested, separated to roots and shoot and compared on the basis of oven dried weight at 70 °C for 3 days. All treatments were replicated three times and were arranged in a randomized design

The data on weed and crop attributes were statistically analyzed using Fischer's analysis of variance technique and mean values were separated using least significant difference (LSD) test at $P \leq 0.05$ [8].

Determination of total phenolics in sunflower residues amended soil.

Folin-Denis was used for phenolics analysis[9] and ferulic acid was used as standard since it is an allelopathic agent present in sunflower plant [10]. Soil samples were taken from soil of plots amended with all treatments at a depth of 30 cm at 1, 4, and 6 weeks after sowing (WAS). The soils were mixed thoroughly and allowed to dry at room temperature for 3 days. Samples of 250 g dry soil were extracted separately in 250 ml of distilled water by shaking for 24 h at 200 rpm [11]. Soil suspensions were filtered through Whatman No. 2 filter paper under vacuum. Folin-Denis (0.5 ml) and Na_2CO_3 (one ml) were added to one ml of soil water extract and left to stand for 30 minutes. Absorbance was determined at 750 nm on a spectrophotometer [12]. The total phenolic content was obtained by standard curve using different concentrations of ferulic acid.

Separation, identification and quantification of phytotoxins from sunflower

Water extract of the sunflower of high allelopathic cultivars (Asgria and Flamme) were prepared [13] with some modification. One gram of soil of test genotypes were soaked in 100 ml hot distilled water acidified with one milliliter of acetic acid. The mixture was heated gently, mixed thoroughly by ultrasonic apparatus to exclude air bubbles from the residues and allowed to stand for 4 hrs. The mixture of each sample was filtered by filter paper under vacuum condition and kept in refrigerator until use.

For identification, 50 µl of the extract of each sample was injected in High Performance Liquid Chromatogram (HPLC Shimadzu-C-6A) using procedure outlined by Hartley and Buchan . The peaks were detected by UV detector. Standards of suspected phytotoxins were run similarly for identification and quantification.The analysis was carried out in the laboratories of Ministry of Science and Technology. Concentration of each isolated compound was determined by the following equation:

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{Concentration of standard} \times \text{Dilution factor}$$

Result

Allelopathic potential of sunflower genotypes against number of companion weed. Field observations showed that 60% of weeds species grown in field were broad leaf *viz*. *Beta vulgaris* L, *Malva rotundifolia* L., *Ducus carota*, Plantago ovata., and the remain was grass weeds, namely *Avena fatua* L., *Lolium temulentum* L. and *Phalaris minor* L. (Table 1).The effect of test sunflower genotypes appeared to be non-selective. genotypes such as Asagri showed higher inhibitory effect on narrow and broad leaves weeds while others such as Flamme does not have inhibitory effects on particular weed species. No attempts were made to find out the reason; however, it could be attributed to the fact that the root exudates contain several inhibitory compounds of different mechanisms of effect. Therefore, it would be fruitful to investigate the inhibitory effect of the root exudates compounds separately or in combination to know if the compounds have selective effects on weeds.

The results also showed that weed species responded differently to allelopathic potential of the test sunflower genotypes. For example, Genotype Asagri appeared to be more inhibitory to all weeds except *Beta vulgaris.* In contrary, Flamme genotype exhibited higher ability to reduce the number of all test weeds except *Avena fatua,* L.*Plantago ovata* and *Beta vulgaris* which were slightly affected.

Table 1. Weed species grown companion with Sunflower field.

Common name	Scientific name	Family
	Broad leaf – weeds	
Common beet	*Beta vulgaris* L.	Chenopodiaceae
Mallow	*Malva rotundifolia* L.	Malvaceae
Wild carrot	*Ducus carota* L.	Umberlliferae
blond plantain	*Plantago ovata*	Plantaginaceae
	Narrow- leaf weeds	
Wild oat	*Avena fatua* L.	Poaceae
Rye grass	L. *Lolium temulentum*	Poaceae
Canary grass	*Phalaris minor* L.	Poaceae

Test of allelopathic potential of sunflower genotypes against growth of companion weed:

Results presented in table 2 indicated the presence of 7 weed species grown in the field of sunflower genotypes. However, the numbers of the recorded weed species are different between. The test sunflower genotypes. Asgria reduced the total number of weeds grown in the field by 68.6% of control where ether Flamme genotype slightly reduced the number of weed compared to the other genotypes causing a reduction of 24.0 % of control (Table 2),the inhibitory effect of the sunflower genotypes against the total number of weeds could be attributed to the allelopathy and competition exerted by sunflower genotypes[14]. However, the differential inhibition in weeds number among the test sunflower genotypes could be attributed mainly to the differences in the allelopathic potential of the test genotypes through root exudation because all plots of sunflower genotypes received equal agricultural managements in terms of water and fertilizer practices. The differences in allelopathic potential of several allelopathic crops other than sunflower has been reported and well documented by several workers . Alsaadawi *et al.* [15] found significant differences in allelopathic potential against weeds among the test sorghum genotypes. Reberg-Horton *et al.* [16] examined ten different cultivars of cerel rye and found significant differences among them in the amount of one common allelochemical (DIBOA). Dilday *et al.* [17] revealed that out of 16000 accessions screened for their allelopathic ability, many were found to suppress ducksalad (*Heteranthera limosa*) and redstem (*Amemenia coccinea* weeds drastically.

Results showed that the total above ground biomass of weeds were significantly reduced by all test genotypes of sunflower (Table 2). The magnitude of the reduction was genotype dependent. Asagri proved to be the inhibitory genotypes to weed biomass with a reduction up to 61.0%. Other genotypes showed a reduction ranged from 35.4% in Flamme compared with control. Measurements of weed dry weight is further confirmed the allelopathic potential of the test genotypes. In most cases, the reduction in total weeds dry weight appeared to be parallel with the reduction in weeds number, and the sunflower genotype (Asagri) which showed higher reduction in weeds number have also higher reduction potential in weed biomass.

Table 2. Allelopathic potential of sunflower genotypes on growth of companion weed under field conditions.

Genotypes	Dry weight of total weeds/plot (g) *	Reduction % control	total number weed m^2	Reduction % control
Asgria	99.3	61.0	32.7	68.6
Flamme	164.6	35.4	79.3	24.0
Control	254.7	……	104.3	……
LSD=0.05	19.66		7.21	

The suggests that the root exudes contain phytotoxic compounds which could be released in to the soil environment by the action of microorganism and affect on root . Rice [18] indicated that several species of soil microorganisms involve in the decomposition of plant residues and liberation of the allelopathic compounds. Chou and Lin [19] revealed that several microorganisms contribute in decomposition of rice residues in soil and liberated several allelopathic compounds including phenolics and short fatty acids. Blum [20] indicated that several species of microorganisms (*i.e*., algae, bacteria, actinomycetes, and fungi) in soil have a broad range of metabolic activities that can decompose organic matter and synthesis/release/loss of potential allelopathic agents.

Effect of root exudates of sunflower cultivars on growth of the test weed by stair case technique

Root exudates of both cultivars significantly inhibited root and shoot growth of *Echinochloa colonum* weed compared to the control (Table 3).With the superiority of Asagri cultivar over Flamme cultivar in the suppression of whole plant of the test weed by 68.9% in Asagri and 21.4% in flame compared with control. The result of staircase experiment supports the results of field experiment in that root exudates of both cultivars. significantly inhibited root and shoot growth of the test weed (Table 3), with the superiority of Asagri over Flamme cultivar in the suppression of root, shoots and whole plant of the test weed 75.8 % and 53.3% respectively. No attempts were made to identify the allelochemicals in root exudates.

Tawifiq and Alsaadawi [21] reported the presence of several allelochemicals of phenolics in nature such as neochlorogenic acid, 5 – *O* – P – Coumaroyl quanic acid, chlorogenic acid, neochlorogenic acid, caffeoylquinic acid and neochlorogenic acid acids. These phenolic acids are reported to interfere with several physiological processes including respiration, photosynthesis, ions uptake, hormones biosynthesis and cell division and others [22,23].

Table 3. Effect of root exudates of sunflower cultivars on growth of *Echinochloa colonum* weed.

Treatments	Dry weight (g)*		
	Shoots	Roots	Whole plant
Asgria	0.07	0.017	0.087
Flamme	0.15	0.070	0.22
Control	0.19	0.090	0.28
LSD ≤ 0.05	0.037	0.019	.....

*Average of 4 replicates

Determination of total phenolics compound in field of sunflower residues amended soil

All treatments including control showed no significant differences in total phenolics at the beginning of the experiment (Table 4). The release of total phenolics started after two weeks of agriculture , reached show that at 4-week and remained at higher concentration then decreased until

reach the lowest concentration at 6-week of decomposition. Soil from plots amended with sunflower root showed higher amount of total phenolics and the concentration increased with at 4-week after agriculture . weedy check showed lower concentration of total phenolics at all decomposition periods`

The presence and release of phytotoxins from the root exudes of plant incorporated into soil is further confirmed by phenolics dynamic determination in soil. The release of phenolics started after two weeks of agriculture, reached its at 4-week and remained at higher concentration then declined until reach the lowest concentration at 6-week of decomposition (Table 4). This suggests that phenolics released from sunflower root in soil are the main cause of suppressive activity of weeds. Meanwhile, these results give explanation for the poor growth of weeds observed during the first two months from cowpea sowing. Similar results were also reported when the residues of sorghum were added in broad bean field [24]. No attempt was made to isolate and identify the phenolic acids in the decomposed sunflower residues in soil; however, Al- Alsaadawi and Al- Temimi [25] and Sarbout., [26] were able to isolate and identify phenolic acids, namely Chlorogenic acid, isolchlogenic acid, caffeic acid, gallic acid, syrinigic acid, hydroxy benzoic acid, p- coumaric acid, ferullic acid, vanillic acid and Catechol from the soil containing sunflower residues and they reached their maximum beak at 4^{th} week and vanished at 2^{nd} month from incorporation of residues in to the field soil. These phytotoxins are reported to have inhibitory effects on several metabolic processes such as inhibition of chlorophyll biosynthesis [27,28], ions uptake [29,27], photosynthesis [30], inhibition of activity of plasma H^+-ATPase which leads to decreased ions and water absorption by guard cells of leaves and causing close of stomata [31], inhibition of photosystem II and thus decreases the production of ATP and $NADPH_2$ required for CO_2 fixation in dark reaction [32] , inhibition of oxidation phosphorylation [33], inhibition of activity of several enzymes involved in essential metabolic processes [34], interfering with hormones metabolism in plant. Inhibition of stomata opening [35]. Also phenolic acids are reported to reduce the number of mitochondria and disrupt the membranes surrounding nuclei, mitochondria and dictyosomes

[36]. Our results revealed that inhibition of ions uptake is one of the above mechanisms by which sunflower residues suppress the of test weeds.

Table 4. Concentration of total phenolics released from root exudes of sunflower at different periods of decomposition.

Treatments*	Total Phenol concentration**	
	28 day	52day
Weedy check (Control)	0.103	0.227
Asgria	0.403	0.107
Flamme	0.220	0.150
LSD ≤ 0.05	0.0869	0.0649

*Each number is an average of 4 replicates.
**Total phenolic acid is expressed in ferulic acid equivalents per gram of soil.

Table 5. Concentration of phytotoxins in the root exudes of test sunflower genotypes .

Phytotoxins	Concentration (ppm)					Total
	Sunflower genotypes					
	Protocatechuic	**Vanillic**	**syringic acid**	**p-coumaric acid**	**ferulic**	
Asgria	127.4	111.2	123.7	109.6	138	609.9
Flamme	147.9	60.0	83.7	77.6	55	424.3

Results of HPLC analyses indicated the presence of 5 phytotoxins in the soil root exudes of the test sunflower genotypes (table 5). All the isolated compounds appeared to have different retention times and identified as phenolic compounds .The profile of each compound appeared to be

different among the test genotype. The concentration of the isolated compounds was found in the following order: ferulic < protocatecheic acid < syringic acid < vanillic acid < p-coumaric acid in Asagri protocatecheic acid < syringic acid < p-coumaric acid < vanillic acid < ferulic in Flamme

The total concentration of phytotoxins appeared to be much higher in Asagri genotypes than in Flamme genotypes. The results of this study lead to the following conclusions the allelopathic potential is varied among the test sunflower genotypes and Concentration of the total phenolic phytotoxins isolated from the residues appeared to be responsible for the allelopathic potential of the test genotypes.

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