PLANT RESISTANCE TO THE SAFFLOWER APHID, *UROLEUCON CARTHAMI* (THEOBALD) (HOMOPTERA: APHIDIDAE) IN SAFFLOWER GENOTYPES

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ABSTRACT: The safflower aphid, *Uroleucon carthami* (Theobald) (Homoptera: Aphididae), is an important pest afflicting safflower in most safflower growing countries in the world. Plant infestation with aphids can reduce plant growth and thus reduce fecundity and crop production. Deliniating the categories of resistance in safflower genotypes under field conditions could be helpful in management of this pest. Antixenosis is defined as a resistance mechanism affecting pest establishment on their host plants. In this research, antixenosis mechanism was evaluated in eight safflower genotypes namely, Padideh, Sina, Zarghan, Sofeh, Goldasht, Golmehr, Esfahan and Varamin at College of Agriculture, Urmia University, during 2016-2017, to identify antixenotic resistance against safflower aphid, *U. carthami*. Choice tests were conducted at 25 ± 1 ºC, 60 ± 5% RH and a photoperiod of 16:8 (L: D) h. After introduction of apterous adult aphids to test arena, the number of aphids on each entry was counted at 2, 4, 6 and 24 hours of release. Significant differences were found between genotypes for Total phenolic content, NPK essential elements, leaf thickness and leaf trichome density. It was found that the most antixenosis effect was observed on 'Sina'. Increase in antixenosis correlated with increase in leaf trichomes. Antixenosis can be important mode of resistance by reducing host selection and delaying aphid colonization. The identification of antixenotic resistance in several genotypes provides additional options for management of this pest. Moreover, the factors associated with this mode of resistance can be effectively used in an integrated pest management of the safflower aphid.

KEY WORDS: *Carthamus tinctorius*, Safflower aphid, Plant resistance, Morphological characteristics, Pest management

Safflower (*Carthamus tinctorius* L.) is an ancient crop of the family Compositae or Asteraceae, originated in the near east and has been grown for centuries in China, India and North Africa (Vargas et al., 2008). It is a multi-purpose species with many traditional uses (Hallman, 2008). In Iran, safflower cultivations is being done for centuries for its orange red and yellow dye (Carthamine) extracted from the florets were once used to color food and clothing and for its oil, rich in poly unsaturated fatty acids which are considered to reduce blood cholesterol and good for heart patients (Sabzalian et al., 2008). There are several causes for low productivity in Gachsaran, among them biotic factors play key role (Akashe et al., 2012; Saeidi et al., 2016).

Among the insect pests that attack safflower the aphid, *Uroleucon carthami* (Theobald) is considered as a major pest causing severe losses to the crop throughout the world. Safflower aphid, *U. carthami* is one of the most destructive pests (Akashe et al., 1999; Jadhav et al., 2012; Akashe & Sawant, 2012), which alone causes 35-72 percent yield loss, during heavy infestation period (Anon, 2007; Bade & Kadam, 2001). Saeidi et al. (2012) recorded 46.2 percent yield loss of safflower in Gachsaran. Seed and oil content losses due to this pest to the extent of 20 to 80 percent have been reported from different parts of country (Bhumaneshvar & Thondarya, 1979; Shetgar & Tahir, 1992; Vijay, 2002; Saeidi et

al., 2015). The aphids not only reduce yields of seed and oil content but also attack petals lowering the quality of the value added product of this part of the plant (Saeidi et al., 2015).

The widespread use of insecticides to control this pest and selection pressure has resulted in developing resistance to the insecticides (Sykes, 1977). The development of insecticide resistant biotypes and other harmful effects of chemical control methods to the environment require alternative control strategies (Margaritopoulos et al., 2007). Using resistant genotypes is one of the alternative control methods for this aphid. Several factors in safflower plants may contribute to its resistance to aphids, such as the existence of resistance factors on the plant surface (Gibson, 1971; Alvarez et al., 2006) or at the mesophyll/phloem tissues. In addition, the age and different parts of the plant and can influence the aphid population on plant (Eigenbrode et al., 2002; Alvarez et al., 2006, 2007).

Plant resistance also varies with nutritional quality of phloem sap (primary plant metabolites) or on the amount and nature of secondary metabolites (Gibson & Pickett 1983, Ave & Tingey 1986, Karley et al., 2002). So far, resistances of several wild safflowers and their related accessions and also some commercial genotypes have been assessed to *U. carthami* and some of them have shown various degree of resistance to the aphid, (Alvarez et al., 2006; Leroux et al., 2007, 2008).

The use of resistant genotypes in safflower fields will help to reduce aphid damage and enhance production yield, what is valuable to develop a successful integrated pest management (IPM) programme for the safflower aphid. The objective of this study was to determine the resistance mechanism of eight commercial safflower genotypes in Iran and to measure the biological parameters of the safflower aphid on these genotypes to select the most resistant genotype.

MATERIALS AND METHODS

Plant Materials

In this experiment eight safflower genotypes including Padideh, Sina, Zarghan, Sofeh, Goldasht, Golmehr, Esfehan and Varamin were tested for antixenosis. Seeds of native genotypes were obtained from Seed and Plant Improvement Institute, Karaj, Iran. The seeds of genotypes were sown in polyvinyl chloride pots (27 × 27cm) filled with fertilized field soil. The potted plants were then transferred to a screen-house condition at 25 ± 1 ºC, 60 ± 10% RH and a photoperiod of 16:8 h (L: D).

Aphid colony

The rearing of *U. carthami* was started from virginoparous apterous females collected in summer 2016 from a safflower field in Gachsaran, Iran. Aphid colonies were maintained on *C. tinctorius* var. Sina (Compositae) in a climatic room at 20 ± 2ºC and a photoperiod of 16:8h (L: D). To maintain the colony, every 15 days some aphids from the infested plants were transferred to a new young safflower plant of Sina genotype.

Antixenosis experiment

The eight safflower genotypes were planted in the perimeter of plastic basins (50 cm diameter × 15 cm height), which were filled with suitable field soil and maintained in the growth chamber at 20 ± 2ºC, 65 ± 5% RH and a photoperiod of 16:8h (L:D). These plants were used in the experiment at the 3-4 leaf stage of development. The plastic basins were surrounded by clear cylindrical plastics covered with muslin (50 meshes) for ventilation. This experiment was conducted in five replicates in a randomized design. For each replicate 120 viviparous

apterous adults were randomly selected from the colony and released in the centre of each plastic basin on the soil surface to choose the plants. After 2h, 4h, 6h and 24h, the number of aphids on each plant was counted and recorded (Laamari et al., 2008).

Choice tests

One detached leaf from fifth or sixth leaf of each genotype was used for this test. The leaves were arranged in a circular arena in a completely randomized design with 10 replicates for each accession of each test. Eighty apterous adult of aphids released on a filter paper (8cm diameter) were placed at the center of the circle. Dishes were closed using a net to prevent aphids from escaping and placed in a climate room. The number of aphids on each leaf discs was counted after 2, 4, 6 and 24 hours.

Trichome density measurement

To estimate leaf trichome density, in the laboratory we counted the numbers of trichomes density/cm^2 of leaf was carried out by using one cm^2 stopper cutter/borer to punch in a fixed area at one side of the midrib and the stopper was used for tracing on the leaf then within the one cm^2 the number of trichomes were counted. The process of counting trichomes was done under the microscope with the aid of 10x lens and objective on microscope 10/0.25-160/0.17 Kyowa optical Co. Ltd. Japan. Ten trichomes were selected for size measurement from the midrib of the central portion of the leaf blade. Size of trichomes was measured on Microscope (Nikon Alphaphot, Ys, Japan) by ocular micrometer in micron on 5x eyepiece and objective then converted in (mm) millimeter.

Leaf thickness

A digital micrometer was used to measure thickness of the leaves, took care to ensure a constant pressure by using the instrument's ratchet clutch and the leaflet mid and lateral ribs were avoided in measurements (White & Montes, 2005).

Essential elements (NPK)

The amount of nitrogen (N), potassium (K) and phosphorus (P) were measured according to methods of Kjeldahl (1883), Olsen (1954) and Jackson (2005), respectively. These tests were done in faculty of Agriculture, Shiraz University, Iran.

Determination of total phenolic content

Plant samples were washed with distilled water and air dried and then cut into small bits. Small plant bits were refluxed for 30 minutes in 25 ml of 80 percent alcohol on hot water bath. Supernatant extract was decanted into another flask and the residue was again re-extracted with small quantity of hot ethanol. Both the extracts were pooled and filtered through What man number 1 filter paper (Mahadevan, 1965; Jayapal & Mahadevan, 1968). The final volume was maintained at 25 ml. This extract was directly used to estimate total phenol. The alcohol part of the extract was evaporated and the aqueous fraction was analyzed. Total phenols were estimated from the various extracts using Folin-ciocalteau reagent.

Data analysis

Antixenosis data were square root transformed to standardize the variance before analysis. Data of antixenosis, Total phenolic content, NPK essential elements, leaf thickness and leaf trichome density of the safflower aphid were evaluated using the analysis of variance (ANOVA) using the MINITAB-13.1 statistical software (Minitab lnc. 1994 Philadelphia, PA) and comparisons among means were carried out by using the LSD test at $\alpha = 0.05$.

RESULTS

According to the choice test two hours after releasing aphids, the number of aphids on Padideh, Sina, Zarghan and Sofeh was lower than on the other genotypes ($F_{7, 72}$ = 12.436, P < 0.05). The most antixenosis effect after 4 hours was recorded for Sina and Zarghan ($F_{7, 72}$ = 35.764, P < 0.05). The number of aphids at third time evaluation ranged from 8.80 aphids on Sina to 12.40 aphids on Varamin (F7, 72 = 12.04, P < 0.05). After 24 hours, the least number of aphids settled on Sina ($F_{7, 72}$ = 14.245, P < 0.05) (Table 1). The greatest differences were detected at 6 h after safflower aphid introduction. According to the results of repeated measures design, there was significant difference in number of aphids on the genotypes (Table 2). And the most overall antixenotic effect to safflower aphid was observed in 'Sina', whereas Varamin and Golmehr exhibited little or no antixenosis (Table 1).

The amount of measured plant factors are summarized and illustrated in Table 3. There was no significant different among the genotypes with respect to leaf thickness ($F_{7, 16}$ = 1.620, P = 0.314) and NPK contents ($F_{7, 16}$ = 2.468, P = 0.265 for N; $F_{7, 16}$ = 1.620, P = 0.452 for P and $F_{7, 16}$ = 1.332, P = 0.520 for K) but significant differences in the leaf trichome density and phenolic content were observed. The highest trichome density and total phenolic content were recorded for Sofeh. On the basis of Pearson correlation coefficient, there was a negative correlation between leaf trichome density and number of aphids. But there was no relationship between the number of aphids and leaf thickness, total phenolic content, and NPK (Table 4).

DISCUSSION

Plant species are different with respect to their suitability as hosts for different insects when their performance and preference are measured on these plants (Storer & van Emden, 1995; Frei et al., 2003). Meanwhile, different genotypes of a plant species differ in chemical and morphological characteristics which influence their suitability as hosts (Ave & Tingey, 1986). Therefore, assessing the resistance of different genotypes to the pests with respect to the plants differences can provide valuable information on their suitability or unsuitability to the insects.

The current study revealed that there were significant differences in the safflower aphid performance among the eight safflower genotypes tested. We tested safflower genotypes for antixenosis to *U. carthami* by assessing feeding deterrence and aphid settling in choice test. The genotypes with lowest number of aphids on them have the highest antixenosis resistance. Therefore, in our study the most antixenotic effect belonged to Sina.

Several mechanisms such as morphological characteristics and quality of the host plant could be responsible for the variation in aphid's performance on different genotyps. Host plant quality is an important factor that is responsible for the antibiotic resistance of plants, as host plant suitability is affected by the level of amino acids or nitrogen in the phloem sap and the secondary metabolites that influence aphids' performance (Gibson & Pickett, 1983; Ave & Tingey, 1986; Dixon, 1998; Cisneros & Godfrey, 2001; Karley et al., 2002). In this study, antixenosis in the safflower genotypes was positively correlated with morphological features. At different test times there were aphid density fluctuations on some genotypes. One reason for such fluctuations may be diurnal changes in the phloem sap composition (van Emden & Harrington, 2007; Winter et al., 1992); changes in concentration of some amino acids and sugars may cause

aphids to stop feeding and to pull out their stylets as shown in *Nasonovia ribisnigri* (Mosley) on lettuce (*Lactuca sativa*) and *Aphis fabae* Scopoli on beans (Van Helden et al., 1993). Another reason may be an increase in mobility of individuals in dense colony by tactile disturbance from other members as in colonies of *Drepanosiphum platanoidis* (Schrank) (Dixon, 2012) or attributed to volatile semiochemicals as in colonies of *Rhopalosiphum padi* (L.), (Quiroz et al., 1997). The allelochemicals can be as stimulant or deterrent for the aphids (Smith, 2005).

The antixenosis was positively correlated with leaf trichome density. The role of leaf trichomes is generally water control and resistance against herbivory in some plants (Gonzales et al., 2008). The simple trichomes of these genotypes probably act as mechanical barriers that hinder insect movement and/or feeding (Le Roux et al., 2008; Levin, 1973; Smith, 2005).

Plant acceptance is a critical phase for aphid colonization and population establishment (Le Rouxet et al., 2008). Antixenosis can deter aphids, reduce colonization and keep the size of population under economically injurious levels (Hesler & Tharp, 2005; Hesler & Dashiell, 2011). Deterrence form settling on host plants may cause aphid to continue searching. Aphids maybe exhausted after long time searching or be preyed before finding a suitable host plant for feeding and reproduction (Hesler & Dashiell, 2011). Aphids initially invade crops in low numbers, and then populations increase gradually to reach damaging levels. For these pests, low-to-moderate levels of antixenosis and antibiosis can be effective (Hesler & Tharp, 2005). So, we have focused on evaluation of antixenosis in safflower against *U. carthami*. Such findings in combination with information on other resistance mechanisms (Saeidi et al., 2015) can be helpful in IPM programs of safflower.

CONCLUSION

As a result, the characterization and use of resistant genotypes can be an effective strategy to aid in the control of the population level of insect pests and in reducing the use of chemical treatments in the crop. Besides, it can be integrated with biological control and any other control strategy devoted to IPM programs. Therefore, with respect to our findings, antixenotic effect was observed in the Sina genotype and this genotype can be used as a moderately resistant genotype in IPM of the safflower aphid.

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Table 1. Mean (± SE) number of safflower aphids on eight safflower genotypes in several sampling times.

Genotypes	Number of aphids per leaf disc (± S.E)				
	2h	4h	6h	24h	Mean
Padideh	9.65 ± 0.522abc	10.36 ± 0.763abc	10.30 ± 0.667bc	9.50 ± 0.453cd	9.952 ±0.283cd
Sina	9.25 ± 0.564c	8.12 ± 0.482c	8.80 ± 0.533b	8.40 ± 0.582d	8.642 ± 0.281d
Zarghan	9.55 ± 0.376bc	8.66 ± 0.423bc	10.80 ± 0.573ab	10.50 ± 0.687abcd	9.877 ±0.282cd
Sofeh	10.15 ± 0.668abc	10.85 ± 0.567ab	11.20 ± 0.814b	10.10 ± 0.605bcd	10.575 ± 0.284bc
Goldasht	11.15 ± 0.668abc	12.84 ± 0.706a	10.10 ± 0.482ab	12.30 ± 0.423ab	11.597 ± 0.283ab
Golmehr	12.55 ± 0.838a	12.25 ± 0.616a	10.20 ± 0.712ab	13.20 ± 0.712a	12.05 ± 0.284a
Esfehan	10.65 ± 0.928abc	11.00 ± 0.471ab	10.30 ± 0.633ab	10.10 ± 0.900bcd	10.512 ± 0.283bc
Varamin	12.45 ± 0.432ab	11.76 ± 0.490a	12.40 ± 0.236a	11.30 ± 0.473abc	11.977 ± 0.284a

* Means in a column followed by the same letters are not significantly different (LSD test at 5% significance level).

Table 2. Repeated measures variance analysis of genotype effects on aphids density in choice test.

Source of Variations	SS	df	Mean of Square	F	*P*
Genotypes	465.318	7	63.654	18.002	0.01
Error	278.416	72	5.670		

Table 3. Means (± SE) of some measured features of safflower genotypes.

Genotypes	N%	P%	K%	TPC (ppm)	Thickness(mm)	Trichome density(mm)
Padideh	3.240 ± 0.122	0.470 ±.012	3.825 ± .190	779.795± 63.275a	0.468±.045	40.123±2.511abc
Sina	4.065 ± 0.123	0.460 ± .043	3.225 ± .432	679.125±11.241ab	0.473±.040	45.765±2.123ab
Zarghan	3.223 ± 0.410	0.520 ± .022	4.590 ± .300	519.684±31.655bc	0.484±.062	38.187±3.044bcd
Sofeh	3.250 ± 0.456	0.485 ± .164	5.125 ± .377	880.898±39.23a	0.555±.041	47.244±4.222a
Goldasht	3.468 ± 0.311	0.455 ±.017	3.315 ± .180	311.396±55.186d	0.532±.019	30.411±3.066d
Golmehr	3.789 ± 0.225	0.340 ± .067	3.175 ± .400	258.451±32.540d	0.485±.055	30.333±1.542d
Esfahan	3.856 ± 0.076	0.295 ± .077	4.675 ± .500	398.543±61.321bc	0.451±.067	31.255±1.235d
Varamin	3.765 ± 0.345	0.370 ± .062	4.075 ± 1.70	333.528±42.486bc	0.476±.024	32.561±1.127cd
F(df =7,16)	2.468	1.620	1.332	39.551	1.620	14.677
	0.265	0.452	0.520	< 0.05	0.314	< 0.05

Abbreviations: N: nitrogen; P: Phosphor; K: potassium and TPC: total phenol content
1 Means in a column followed by the same letters are not significantly different (LSD test at 5% significance level).

Table 4. Pearson correlation coefficient (r) between number of aphids *Uroleucon carthami* and some plant factors which may have role in antixenosis of safflower genotypes to safflower aphid.

	n	p	k	TPC	Thickness	Trichome density
Number of Aphids	-0.410	-0.525	-0.211	-0.780	0.075	-0.864*

Abbreviations: N: nitrogen; P: Phosphor; K: potassium and TPC: total phenol content.
*: Significant $p < 0.05$.