Antixenosis, Anatomical and Biochemical Studies on some Egyptian Wheat Cultivars Infested with Bird Cherry-Oat Aphid (*Rhopalosiphum padi* L.) (Hemiptera: Aphididae)

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ABSTRACT

The bird cherry-oat aphid, (Rhopalosiphum padi L.) (Hemiptera: Aphididae), is one of the most important and serious polyphagous pest infesting cereals. Antixenosis test for twenty Egyptian wheat cultivars (Giza 148, Giza 152, Giza 156, Giza 168, Giza 171, Gimmeiza 7, Gimmiza 9, Gimmiza 10, Gimmiza 11, Gimmeiza 12, Misr 1, Misr 2, Sakha 8, Sakha 93, Sakha 94, Shandwel 1, Sids 1, Sids 12, Sids 13 and Sids 14) were evaluated against R. padi infestation after 24 hrs and 48 hrs of aphids release and when the plants aged 7 and 15 days. Giza 156 recorded highly preference for aphids after 24 and 48 hrs when the plants aged 7 days, while Sids 13 showed that the least preference to aphids. Giza 148 revealed that highly preference to aphids after 24 and 48 hrs when the plants aged 15 days, plus Giza 152 and Giza 156 had similar results after 48 hrs. Otherwise, Sakha 93 and Sakha 94 cleared that the least preference. Then ten of those cultivars were chosen (Giza 148, Giza 152, Giza 156, Gimmiza 11, Misr 1, Misr 2, Shandwel 1, Sids 1, Sids 12 and sids 13) to study their leaf morphological characters, anatomical structure and some biochemical studies were assayed. Giza 152 had the lowest chlorophyll a and total chlorophyll compared to other cultivars. Also, Giza 152 had the least thick for mesophyll thickness, lower epiderms thickness, blade thickness and the shortest main vascular bundle dimension length and midrib thickness length too. Moreover, Sids 13 showed that the highest number of trichomes density, the thickest thickness for mesophyll tissue, the thickest thickness for blade and the longest main vascular bundle dimension length. It could be concluded that existence of high level of antixenosis in Egyptian wheat cultivars may decrease the population density and preference of R. padi and also cause a suppression of cereal viruses (e.g. BYDV) and reduce the pesticides application to wheat fields.

Keywords: Bird cherry-oat aphid, Antixenosis, mesophyll tissue thickness, trichomes density, Chlorophyll a

INTRODUCTION

Aphids are the most widely distributed and seriously threat cereal crops (Yadev, 2003). Bird Cherryoat Aphid *Rhopalosiphum padi* (L.), is one of the most major economically important aphids on Egyptian wheat (Elenin *et al.*, 1989). In Egypt, wheat is an important staple food, with an annual consumption of about 12.4 million tons, of which about 9.4 million tons are grown locally on 3.4 million faddan in 2014/2015 about 90% of that is bread wheat and 10% is durum wheat (Rashed *et al.* 2016). This crop is normally planted in November and subsequently harvested in June.

Rhopalosiphum padi damages cereals by piercing and sucking the sap and depriving the plant of nutrients at their two leaf stage, which causes 40-60% yield loss. Additionally, it acts as the main vector of Barley yellow dwarf virus and it is also able to cause a yield loss of up to 85% in this way (Papp and Mesterhazy 1993). The bird cherry-oat aphid is an aphid that completes its life cycle on various hosts including the bird cherry (Prunus padus L.) as a primary host and cereal crops, particularly maize, barley, oats and wheat as the secondary hosts. In autumn, winter eggs are deposited on bird cherry. In spring, nearly two generations of fundatrigeniae are also generated on the plant. The parthenogenetic populations of this aphid are developed on the secondary hosts during late spring and summer (Blackman and Eastop 2000; Powell and Hardie 2001).

Susceptibility or resistance of plants is the result of series interactions between plants and insects, which influence the ultimate degree of establishment of insect populations on plants.

Unfavorable biophysical or biochemical plant characters interrupt one or more of insect responses, may inhabit the establishment of insect populations on a plant and render it resistant to infestation and injury (Akhtar et al., 2006a). The host plant quality is a crucial determinant of the fecundity of herbivorous insects that affects the fecundity of herbivorous insects at both the individual and the population scale (Awmack and Leather 2002). The low quality of plants can perform as a defense mechanism against herbivorous pests and cause a decline in their fecundity and an increase in developmental time (Legrand and Barbosa 2000). Various studies have been conducted to detect at least a relatively resistant cultivar for the development of control techniques of aphids and to decrease insecticide applications (Roberts and Foster 1983; Papp and Mesterhazy, 1993; Ozder, 2002 and Razmjou et al. 2006, 2009).

The objective of this study was to evaluate the antixenosis of Egyptian wheat cultivars against bird cherry oat aphid and know how is the biochemical and anatomical studies effect aphid preference and infestation.

MATERIALS AND METHODS

A. Greenhouse studies

1. Aphid Colony

The aphids used in this experiment were collected from Egyptian wheat cultivars planted under greenhouse conditions (Entomology and Plant Pathology Department, North Carolina state university, USA), and aphid species was identified by NCSU Plant Clinic. After that, colonies were established on seedlings of wheat in plastic framed cages ($134.1 \times 146.3 \times 152$ cm) in the greenhouse (conditions of $20 \pm 1^{\circ}$ C, $60 \pm 5\%$ RH and 14:10 L:D). The aphid population was reared for three generations before being



used in the experiment. To maintain the colony, 20–25 aphids were transferred weekly from an infested plant to a young plant.

2. Wheat cultivars

Twenty commercial Egyptian bread wheat cultivars were evaluated for their susceptibility to aphid infestation. The tested cultivars included the most recently registered bread wheat cultivars. Wheat seeds were obtained from Agriculture Research Center, Cairo, Egypt. The susceptibility of twenty wheat cultivars was evaluated to bird cherry oat aphid infestation; Giza 148, Giza 152, Giza 156, Giza 168, Giza 171, Gimmeiza 7, Gimmeiza 9, Gimmezia 10, Gimmezia 11, Gimmeiza 12, Misr 1, Misr 2, Sakha 08, Sakha 93, Sakha 94, Shandwel 1, Sids 1, Sids 12, Sids 13 and Sids 14 to determined their susceptibility or resistance (antixenosis test). The plants were grown under greenhouse conditions of $20 \pm 1^{\circ}$ C, $60 \pm 5\%$ RH and 14:10 L:D (Entomology Department, NCSU).

3. Antixenosis test

The non-preference of twenty wheat cultivars for bird cherry oat aphids were studied in a randomized complete block design in three replicates. Two seeds of each cultivar were sown in a circular pattern of 90 cm diameter plastic pots and when the seedlings reached to one week, they thinned to be one. After one week from sowing date and two weeks, one hundred of wingless R. padi were released in the center of pot on a white filter paper and pots were kept inside meshed cage to inhibit aphids from escaping. The number of settled aphids was counted after 24 hrs and 48 hrs. Three categories of preference were used to classify aphids; least preferred (LP) having least number of aphids, moderately preferred (MP) having moderate average number of aphids and highly preferred (HP) having highest number of aphids (Akhtar et al., 2006b).

B. Laboratory studies

Biochemical analysis and anatomical structure studies were implemented at Botany Department, Faculty of agriculture, Mansoura University, El Mansoura, Egypt. Ten Egyptian wheat cultivars (Giza 148, Giza 152, Giza 156, Gimmiza 11, Misr 1, Misr 2, Shandwel 1, Sids 1, Sids 12 and sids 13) were chosen to assay biochemical analysis, anatomical structures and trichomes density and length when the plants aged 15 days old.

1. Biochemical analysis

Chlorophyll determination

Fresh leaf samples (completely developed foliage leaf) of 0.05 g were extracted by methanol for 24 hrs at laboratory temperature after adding a trace from sodium carbonate (Robinson *et al.*, 1983), then Chlorophyll a, b and carotenoids were determined using Spectrophotometer (Spekol II) (at wave lengths 452, 650 and 665 nm). The quantities of total chlorophylls, chlorophyll a, b and carotenoids concentration (μ g/g) in leaves were determined by the equations proposed by (Mackiny, 1941).

2. Anatomical structure

After 15 days_from sowing date, small pieces (5 mm) from the midrib region from the middle of the first leaf. The samples were killed and fixed in formalin acetic acid alcohol (FAA) solution and dehydrated in series of ethanol (50%, 70%, 80%, 90% and 100%) and embedded in paraffin wax (52-54c melting point). Sections were

done at 15 µm thick using rotary microtome and double stained with Saffranin light green, cleared in clove oil and mounted in Canada balsam (Gerlach, 1977). The sections were examined microscopically and the following character were recorded; leaf blade thickness (mm), upper epidermis, lower epidermis and mesophyll thickness (mm). Dimensions of midrib and main vascular bundle (Length, width mm).

3. Morphological studies

Trichomes density and length

Trichomes desnity and length were measured for the bottom third of upper leaf surface when the plants aged 15 days old. Four replicates were sampled for each cultivar. Images of the Egyptian wheat cultivars were captured using a Dino-Lite AM4113ZT Polarizing Digital Microscope (AnMo Electronics Corporation, Hsinchu 300, Taiwan), and the provided Dino-Lite software were used to measure trichomes length (mm) and density in 1 mm².

4. Statistical analysis

The experiment was conducted in a randomized complete block design. Data obtained in the present study was subjected to an analysis of variance (ANOVA) with the honestly significant difference value, calculated as LSD (Post Hoc Test) Multiple Comparison Test at 0.05 Probability. Also, the correlation was carried out between number of aphid and wheat leaf morphological characters and biochemical analysis (IBM SPSS Statistics version 9.0) (1998).

RESULTS

1. Antixenosis test

Results indicated that after 24 hrs of aphid release and when the plants aged 7 days old, Giza 148, Giza 152, Shandwel 1, Sids 1, Sids 12 and Sids 13 were least preferred (LP) for aphids ranged between 1.0-2.7 (Table 1). Moderately Preferred (MP) cultivars were Giza 168, Giza 171, Gimmeiza 7, Gimmiza 9, Gimmiza 10, Gimmeiza 12, Misr 1, Misr 2, Sakha 8, Sakha 93 and Sakha 94 with the mean preference ranged between 3-5.7. Gimmiza 11 and Giza 156 were highly preferred (HP) by aphids with mean preference 8.3 and 9.0, respectively.

Otherwise, after 48 hrs of aphid infestation and when the plants were the same age, similar results were found for all cultivars except for Sids 1 and Sids 12 which showed that moderate preference for aphids with mean preference 6.0 and 6.7, respectively.

The experiment was repeated again when the plants aged 15 days old. Least preference to aphids was recorded for Gimmiza 10, Gimmiza 11, Misr 1, Sakha 93 and Sakha 94 after 24 hrs of aphid release and mean preference ranged between 0.3-2. Moderately preference was observed for Giza 152, Giza 156, Giza 168, Giza 171, Gimmeiza7, Gimmiza 9, Gimmeiza 12, Misr 2, Sakha 8, Shandwel 1, Sids 1, Sids 12, Sids 13 and Sids 14 with mean preference ranged between 3.3-6.7. Giza 148 recorded high preference for aphids reached to 7. Otherwise, the experiment evaluated after 48 hrs similar results were shown for least preference after 24 hrs, but 2 more cultivars showed the same trend Gimmeiza 7, Misr 2 (2.0 and 2.7 respectively). Similar result for Giza 148 which cleared that high preference for aphis plus that Giza 152 and Giza 156 had the same

preference (7.0 and 7.7 respectively). Moderately preference was recorded for Giza 168, Giza 171, Gimmiza 9, Gimmeiza12, Sakha 8, Shandwel 1, Sids 1,

Sids 12, Sids 13 and Sids 14 with mean preference ranged between 3.3-6.3.

Table 1. Antixenosis test for non preference of bird cherry oat aphid to twenty Egyptian wheat cultivars under greenhouse conditions

	Seedlings aged 7 days old				Seedlings aged 15 days old			
Wheat	After 24 hrs		After 48 hrs		After 24 hrs		After 48 hrs	
cultivars	Mean aphid	Preference	Mean aphid	Preference	Mean aphid	Preference	Mean aphid	Preference
	adults #	case	adults #	case	adults #	case	adults #	case
Giza 148	2.0	LP	1.7	LP	7.0	HP	7.3	HP
Giza 152	1.3	LP	1.0	LP	5.0	MP	7.0	HP
Giza 156	9.0	HP	7.0	HP	6.7	MP	7.7	HP
Giza-168	4.0	MP	4.0	MP	3.3	MP	4.3	MP
Giza-171	3.7	MP	3.7	MP	6.0	MP	5.0	MP
Gimmeiza-7	4.7	MP	5.0	MP	3.0	MP	2.0	LP
Gimmiza9	3.7	MP	4.0	MP	3.7	MP	3.3	MP
Gimmiza10	3.0	MP	4.0	MP	0.7	LP	1.7	LP
Gimmiza11	8.3	HP	7.7	HP	0.3	LP	1.0	LP
Gimmeiza-12	5.7	MP	5.7	MP	3.3	MP	3.7	MP
Misr1	5.0	MP	5.0	MP	2.0	LP	1.3	LP
Misr2	4.3	MP	6.0	MP	3.0	MP	2.7	LP
Sakha-08	4.3	MP	4.0	MP	4.7	MP	5.0	MP
Sakha93	3.7	MP	4.3	MP	1.0	LP	1.0	LP
Sakha94	4.0	MP	3.7	MP	0.3	LP	0.3	LP
Shandwel-1	1.7	LP	2.3	LP	3.3	MP	3.7	MP
Sids-1	2.7	LP	3.0	MP	6.0	MP	4.0	MP
Sids 12	2.3	LP	3.0	MP	5.3	MP	4.0	MP
Sids 13	1.0	LP	1.0	LP	6.7	MP	6.3	MP
Sids-14	3.3	MP	4.0	MP	4.3	MP	4.3	MP
IP-Lost pro	forred (< 3)		IP- Moderate	proforrod (3	6)	HP-	Highly profes	(7_0)

LP= Least preferred (< 3)

MP= Moderate preferred (3-6)

HP= Highly preferred (7-9)

2. Chlorophyll a, chlorophyll b, carotenoides and total chlorophyll determination

Chlorophyll a, chlorophyll b, carotenoids and total chlorophyll were assayed for ten Egyptian wheat cultivars (Giza 148, Giza 152, Giza 156, Gimmiza 11, Misr 1, Misr 2, Shandwel 1, Sids 1, Sids 12 and sids 13) when the plants aged 15 days old (Table 2). Giza 152 assured significantly lower total chlorophyll (1.15 μ g/g) than Gimmiza 11, Misr 1 and Sids 12 (1.82, 1.79 and 2.00 $\mu g/g$ respectively). Sids 12 had significantly higher total chlorophyll (2.00µg/g) than Sids 13 (1.35µg/g). No significant differences were found between other wheat cultivars (DF=9, F=1.66, P>0.05). Similar results were found for chlorophyll a as total chlorophyll. Otherwise, no significant differences were found between Egyptian wheat cultivars in chlorophyll b (DF=9, F=1.16, P>0.05). Also, similar results were found for carotenoids as total chlorophyll except for significant different between Sids 12 and Sids 13. Additionally, Sids 12 showed significantly higher carotenoids $(2.00 \ \mu g/g)$ than Shandwel 1 (1.37 μ g/g) and Sids 1 (1.38 μ g/g).

3. Anatomical structure of leaf blade seedling

Midrib tissue thickness length and width

The obtained results indicated that a significant difference between Egyptian wheat cultivars (DF=9, P=0.000, F= 14.26 for length and F=169.84 for width). Results showed that Misr 2 had significantly the longest heighest length for Midrib thickness (0.833 mm) compared to other Egyptian wheat cultivars ($P \le 0.05$) (Table 3). While no significant differences were found between Misr 2 and Giza 156, Misr 1 and Sids 13 (0.732, 0.797 and 0.821 mm, respectively) (P>0.05). Otherwise, Giza 152 had significantly the shortest length for Midrib thickness compared to all tested cultivars (0.356 mm) (P≤0.05). On the other hand, Giza 156 showed that significantly the highest width for Midrib thickness (0.619mm) (P<0.05) compared to other tested cultivars. No significant differences were found between Giza 152, Gimmiza 11, Misr 1, Misr 2, Shandwel 1, Sids 1 and Sids 12 (P>0.05), where all of these cultivars recorded low Midrib thickness width ranged between 0.265-0.288 mm.

Main vascular bundle dimension length and width

There were a significant differences between Egyptian wheat cultivars (DF=9, P=0.000, F= 9.9for length and F=6.6 for width).Sids 13 had significantly the longest length for main vascular bundle dimension (0.348 mm) (P<0.01) compared to other cultivars, but not significant with Misr 2 and Shandwel 1 (0.306 and 0.310 mm respectively) (P>0.05) (Table 3). While, Giza 152 had the shortest length for main vascular bundle dimension (0.189 mm) (P<0.05), but not different with Giza 148, Giza 156 and Sids 1 (0.199, 0.222, 0.228 mm, respectively). On the other hand, Giza 148 had the

highest width for main vascular bundle dimension (0.285mm). While, Sids 1 recorded the least width (0.189mm), but not different with Giza 152, Gimmiza 7, Misr 1, Misr 2, Shandwel 1 and Sids 12 ranged between 0.200-0.214mm

Blade thickness

Significant differences were found between

cultivars for Blade thickness (DF=9, F=28.44, P<0.01). Sids 13 had the thickest blade (0.620mm) (P<0.05) compared to tested cultivars except Misr 1, Misr 2, Gimmiza 7 and Sids 12 ranged between 0.569-0.607mm (P>0.05). Otherwise, Giza 152 had the least thickness for blade (0.276mm) (P<0.01) (Table 3).

Table 2. Mean \pm SE of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids content (μ g/g) for ten Egyptian wheat cultivars

Wheat cultivars	Total Chlorophyll (µg/g)	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Carotenoids (µg/g)
Giza 148	1.62±0.15	1.26 ± 0.06	0.36±0.09	0.45 ± 0.04
Giza 152	1.15±0.11	0.90±0.11	0.25 ± 0.001	0.33±0.02
Giza 156	1.43±0.24	1.12±0.17	0.30 ± 0.07	0.43 ± 0.05
Gimmiza11	1.82±0.42	1.44±0.33	0.38±0.10	0.49 ± 0.08
Misr1	1.79±0.27	1.42±0.22	0.37±0.06	0.48 ± 0.06
Misr2	1.43±0.15	1.14 ± 0.12	0.29±0.03	0.40±0.03
Shandwel 1	1.37±0.09	1.11±0.08	0.27±0.01	0.38 ± 0.02
Sids-1	1.38 ± 0.001	1.11±0.01	0.27±0.01	0.39±0.01
Sids 12	2.00±0.20	1.59 ± 0.11	0.41±0.03	0.53 ± 0.03
Sids 13	1.35±0.20	1.09±0.12	0.26±0.03	0.38±0.04

Upper epiderm thickness

Upper epiderm thickness results showed that a significant differences between cultivars (DF=9, F=7.98, P<0.01). Shandwel 1 was significantly the thickest thickness for upper epiderm (0.064mm) (P<0.01) (Table 3). While, Gimmiza 7 had the least thickness (0.029mm), but no different with Giza 148, Giza 152, Sids 1 and Sids 12 ranged between 0.031-0.036 mm (P>0.05).

Lower epiderm thickness

No significant differences were recorded between cultivars for this character (DF=9, F=1.83, P>0.05). Shandwel 1 had significantly a thicker thickness for lower epiderm (0.052mm) compared to Giza 152 (0.034mm) (Table 3). While Giza 152 had the least thickness for lower epiderm (0.034mm) compared to tested cultivars (P<0.05) except Sids 12, Giza 148 and Gimmiza 11 and (0.041, 0.042 and 0.043 mm, respectively) (P>0.05).

Fable 3. Mean ±SE of leaf blad	e tissues	dimensions	for ten	Egyptian	wheat cultidars
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Wheat cultivars	Midrib thickness		Main vascular bundle dimensions		Blade	Upper epidermis	Lower epidermis	Mesophyll
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	(mm)	thickness (mm)	thickness (mm)	(mm)
Giza 148	0.476 ± 0.002	0.509 ± 0.002	$0.199 {\pm} 0.001$	0.285 ± 0.002	0.385 ± 0.004	0.032 ± 0.001	0.042±0.003	0.326±0.018
Giza 152	0.356 ± 0.005	0.285 ± 0.010	$0.189 {\pm} 0.006$	0.213 ± 0.006	0.276 ± 0.010	$0.031 {\pm} 0.001$	0.034 ± 0.001	0.185 ± 0.014
Giza 156	0.732 ± 0.015	$0.619 {\pm} 0.007$	0.222 ± 0.002	0.250 ± 0.004	0.391 ± 0.002	$0.041 {\pm} 0.003$	0.047 ± 0.002	0.299 ± 0.004
Gimmiza 11	0.602 ± 0.028	$0.273 {\pm} 0.001$	$0.240 {\pm} 0.005$	0.202 ± 0.001	0.417 ± 0.012	$0.029{\pm}0.001$	0.043±0.002	0.310±0.014
Misr1	$0.797 {\pm} 0.025$	0.281 ± 0.004	$0.256 {\pm} 0.004$	0.200 ± 0.003	0.569 ± 0.025	0.043 ± 0.001	0.045 ± 0.001	0.414 ± 0.009
Misr2	$0.833 {\pm} 0.002$	0.288 ± 0.003	$0.306 {\pm} 0.006$	0.214 ± 0.002	0.582 ± 0.008	$0.041 {\pm} 0.002$	0.048 ± 0.001	0.478±0.010
Shandwel 1	0.710 ± 0.006	0.285 ± 0.002	0.310 ± 0.016	0.206 ± 0.004	0.585 ± 0.004	0.064 ± 0.002	0.052±0.003	0.477±0.012
Sids 1	$0.653 {\pm} 0.023$	0.265 ± 0.009	$0.228 {\pm} 0.004$	0.189 ± 0.014	0.506 ± 0.018	0.036 ± 0.002	0.045 ± 0.003	0.375 ± 0.011
Sids 12	0.703 ± 0.020	0.273 ± 0.002	$0.250 {\pm} 0.005$	0.203 ± 0.003	0.607 ± 0.008	0.034 ± 0.002	0.041 ± 0.002	0.504 ± 0.007
Sids 13	$0.821 {\pm} 0.048$	0.476 ± 0.006	$0.348 {\pm} 0.020$	0.248 ± 0.011	$0.620{\pm}0.011$	0.045 ± 0.002	0.048 ± 0.002	0.516±0.005

Mesophyll tissue thickness

Significant differences were found cultivars in Mesophyll thickness (DF=9, F=27.76, P<0.01). Sids 13 had the most thickness for mesophyll (0.516mm) compared to other cultivars (P<0.01), but not significant with Shandwel 1, Misr 2 and sids 12 (0.477, 0.478 and 0.505mm) (P>0.05). While, Giza 152 had the least thickness for mesophyll (0.185mm) than other cultivars (P<0.01) (Table 3).

Trichomes length and density

Trichomes length and density were measured for ten Egyptian wheat cultivars from bottom third of upper

leaf surface (Figure 1). Results showed that there is a significant difference between cultivars in both trichomes length and density (DF=9, P<0.01, F=48.07 for length, F=195.90 for density). Giza 156 had the highest trichomes length (0.263mm) (P<0.01). While, Sids 12 had the lowest trichome length (0.057mm) compared to other cultivars (P<0.01), but not significantly different with, Misr 2, Sids 1 and Misr 1 (0.084, 0.085 and 0.086mm, respectively) (P>0.05). Furthermore, Sids 13 revealed that the highest trichomes density (70 trichomes/mm²) compared to tested cultivars (P<0.01) except Giza 152 (68 trichomes/mm²) (P>0.05).

Misr 1 had the lowest number of trichomes (19 trichomes/mm²) (P<0.01).





Figure 1. Leaf trichomes density in mm² and length (mm) measured for ten Egyptian wheat cultivars.

4. Relationship between number of bird cherry oat aphid and measured parameters in the Egyptian wheat cultivars leaves

The correlation coefficients between number of aphids and selected leaf morphological and biochemical characters are presented in (Table 4). The number of aphids was significant positively associated with midrib tissue thickness width (mm) (r=0.464**), main vascular bundle dimension width (mm) (r=0.448*) and trichomes (1mm^2) (r=0.396*). Negative significant density correlations were found between number of aphids and Chlorophyll B $(\mu g/g)$ (r=-0.456*). The association between number of aphids and blade thickness (mm), upper Epidermis thickness (mm), lower Epidermis thickness (mm), mesophyll thickness (mm), midrib thickness length (mm), main vascular bundle dimension length (mm), trichomes length (mm), total chlorophyll, chlorophyll a and carotenoids were not significant.

Table
4. Correlation between measured parameters for Egyptian wheat cultivars and number of *R. padi*

Measured parameters	r value	P value
Blade thickness (mm)	-0.259 ns	P>0.05
Upper Epidermis thickness (mm)	0.141 ns	P>0.05
Lower Epidermis thickness (mm)	-0.207 ns	P>0.05
Mesophyll thickness (mm)	-0.146 ns	P>0.05
Midrib length (mm)	-0.206 ns	P>0.05
Midrib width (mm)	0.464**	P=0.01
Main vascular bundle length (mm)	-0.158 ns	P>0.05
Main vascular bundle width (mm)	0.448*	P=0.01
Trichomes length (mm)	0.342 ns	P>0.05
Trichomes density (1mm^2)	0.396*	P<0.05
Total Chlorophyll	-0.342 ns	P>0.05
Chlorophyll A	-0.299 ns	P>0.05
Chlorophyll B	-0.456*	P<0.05
Carotenoids	-0.356 ns	P>0.05

*Significant at 0.05 Probability level ** Significant at 0.01 Probability level ns is not significant

DISCUSSION

The aphid problem can be tackled with the application of commonly used insecticides but the drawback lies with their indiscriminate used which results in the problem of health hazards, environmental pollution and development of resistance in insects. Therefore, advisable to screen out wheat cultivars possessing resistance against aphids. Preference experiment is a choice test based on physical and chemical differences between tested cultivars. Our result for aphid preference experiment showed that Giza 152 showed highly preference to aphid, where in the same time we found that Giza 152 had a lower content of chlorophyll a and total chlorophyll. Also, Giza 152 showed the least thickness for mesophyll tissue, lower epiderm and blade thickness and shortest length for midrib thickness and main vascular bundle dimensions. In contrast, we found that Sids 13 showed least preference for aphid, also had the highest density of trichomes, highest thickness for blade and mesophyll tissue and longest length for main vascular bundle dimension. So, this could be interpreted that Sids 13 had antixenosis against aphid. While, because Giza 152 is highly preferred for aphid, therefore according to that preference there is a shortage in chlorophyll a and total chlorophyll content. Our results in agreement with (Burd and Elliott 1996, Rafi et al. 1996) who mentioned that Russian wheat aphid, Diuraphis noxia feeding results in destruction of plant chloroplasts that ultimately leads to reduced chlorophyll levels and photosynthetic activity. This decline in chlorophyll indicated that aphid feeding was adversely affecting the plant and directly impacting chlorophyll content (Heng-Moss et al., 2003). Also, mentioned that aphids feeding mainly on phloem tissue, D. noxia elicits a change in the pH either in the luminal side of the thylakoid membrane (is a membrane-bound compartment inside chloroplasts) avoiding the formation

of zeaxanthin, or in the stromal side where the regeneration of violaxanthin takes place.

Aphid subsists primarily on the fluid contents of the plant cells. Its principal feeding sites are the sieve elements, which are reached either via stomata or by puncturing through epidermal and mesophyll tissue. The route of penetration of the mesophyll of wheat was proposed to be entirely intercellular (Fouché, 1983; Fouché et al., 1984). When the aphid stylet reaches the vascular bundles, penetration begins in sequence with the bundle sheath, the vascular parenchyma, xylem elements and sieve element companion cell (SE-CC) complex (Evert et al., 1973; Fouché et al., 1984; Matsiliza and Botha, 2002). Based on aphid feeding mechanism and damage, whenever less thickness for mesophyll tissue, blade thickness and lower epiderm and less length for midrib thickness and main vascular bundle dimensions would be helpful and facilitation for aphid feeding and achieving damage to wheat leaves.

A correlation between number of aphid and trichomes density showed a positive association. So, trichomes are an important factor could obtain wheat cultivars character of resistance. Our result in agreement with Bahlmann et al. (2003) who examined the leaf trichome on Russian wheat aphid susceptible wheat cultivar and resistant cultivar. They reported the significantly greater trichome density in resistance wheat. Also, leaf trichomes density and position may act as a physical obstracle to the Russian wheat aphid feeding (Patil et al., 2016). Furthermore, our results showed there is a negative correlation between number of aphids and chlorophyll b. This is in harmony with Patil et al., 2016 who mentioned that the aphid infestation causes severe distortion of leaves and inflorescence and can significantly decrease the yield through direct feeding. Also, the production of chlorophyll (green colour) is prevented by the attack of aphid resulting in curling of leaves and delayed head emergence causing improper maturity of grains.

CONCLUSION

Using resistant wheat cultivars in culture control as a part of integrated pest management will help to reduce or eliminate excessive use of pesticides. Also, information on genetically plant resistance to aphids could be used for development of resistant cultivars to aphids.

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REFERENCES

- Akhtar, N. and M. Yaqoob. 2006a. Patterns of Resistance to Schizaphis. graminum (Rondani) among rain fed national uniform wheat varieties. Pakistan J. Zool., 38: 153-157.
- Akhtar, N., Ehsan-Ul-Haq and M. Asif. 2006b. Categories of resistance in National Uniform Wheat Yield Trials (NUWYT) N against Aphid, *Schizaphis germanium* (Rondani), (Homoptera: Aphididae). Pakistan J. Zool., 38: 167-171.
- Awmack, C. S. and S. R. Leather .2002. Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology, 47: 817–844.
- Bahlmann, L., P. Govender, and A.M. Botha. 2003. Leaf epicuticular wax ultra-structure and trichomes present on Russian wheat aphid (*Diaraphis noxia*) resistant and susceptible leaves. A frican-Entomol., 11: 59-64.
- Blackman, R. L. and V. F. Eastop. 2000. Aphids on the World's Crop. An Identification and Information Guide. John Wiley Ltd., London.
- Burd, J. D. and N. C. Elliott. 1996. Changes in chlorophyll a fluorescence induction kinetics in cereals infested with Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 89: 1332-1337.
- Elenin, R. A., S. I. Bishara, M. A. Hariri, G. S. Youssef, I. A. Moneim, and R. H. Miller. 1989. Evaluating cereals for aphid resistance in Egypt. Journal of Arabic Plant Protection, 7:72-74.
- Evert, R.F., W. Eschrich, S.E. Eichhorn and S.T. Limbach.1973. Observation on penetration of barley leaves by the aphid Rhopalosiphum maidis (Fitch). Protoplasma, 77: 95–110.
- Fouché, A., 1983. Voedingskade veroorsaak deur die Russiese koringluis, *Diuraphis noxia*, op koring en verwante gasheerplante. MSc Thesis, University of Free state, South Africa.
- Fouché, A., R. L. Verhoeven, P.H. Hewitt, M. C. Walters, C.F. Kriel and J. DeJager. 1984. Russian aphid (*Diuraphis noxia*) feeding damage on wheat related cereals and a Bromus grass species. In: Walters, M.C. (Ed.), Progress in Russian wheat aphid (*Diuraphis noxia* Mordw.) research in the Republic of South Africa. Technical Communication, vol. 191. Department of Agriculture, Republic of South Africa, pp. 22–33.
- Gerlach, D. 1977. Botanische Mikrotechnik, ed. 2. Stuttgart.
- Heng-Moss, T. M., X. Ni. T. Macedo, J. P. Markwell, F. P. Baxendale, S. S. Quisenberry and V. Tolmay. 2003. Comparison of chlorophyll and carotenoid concentrations among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines. J. Econ. Entomol. 96(2): 475-481.
- Legrand A. and P. Barbosa. 2000. Pea aphid (Hom.: Aphididae) fecundity, rate of increase, and within plant distribution unaffected by plant morphology. Environmental Entomology, 29: 987–993.
- Mackiny, G. 1941. Absorption of light by chlorophyll solution. J. Biol. Chem., 140:315-322.

- Matsiliza, B. and C.E.J. Botha. 2002. Aphid (*Sitobion* yakini) investigation suggests thin-walled sieve tubes in barley (*Hordeum vulgare*) to be more functional than thick-walled sieve tubes. Physiologia Plantarum 115:137–143.
- Ozder, N. 2002. Development and fecundity of *Sitobion avenae* on some wheat cultivars under laboratory conditions. Phytoparasitica, 30: 434–436.
- Papp, M. and A. Mesterhazy. 1993. Resistance to bird cherry-oat aphid (*Rhopalosiphum padi* L.) in winter wheat varieties. Euphytica, 67: 49–57.
- Patil, S.D., A.P. Padhye and S. Katare.2016. Resistance sources for wheat aphid : An update. International Journal of Plant Protection. 9(2): 628-631.
- Powell, G. and J. Hardie. 2001. The chemical ecology of aphid host alternation: How do return migrants find the primary host plant? Applied Entomology and Zoology. 36: 259–267.
- Rafi, M. M., R. S. Zemetra, and S. S. Quisenberry. 1996. Interaction between Russian wheat aphid (Homoptera: Aphididae) and resistant and susceptible genotypes of wheat. J. Econ. Entomol. 89: 239-246.
- Rashed, M. A., A. H. Atta, T. M. Shehab El-Din, and A. M. Mostafa. 2016. Development of SSR & STS molecular markers associated with stem rust resistance in bread wheat (*Triticum aestivum* 1.). Egypt. J. Genet. Cytol. 45:261-278.

- Razmjou, J., H. Tavakkoli and A. Fallahi. 2009. Effect of soybean cultivar on life history parameters of *Tetranychus urticae* Koch (Acari: Tetranychidae). Journal of Pest Science, 82: 89–94.
- Razmjou, J., S. Moharramipour, Y. Fathipour and S. Z. Mirhoseini. 2006. Effect of cotton cultivar on performance of *Aphis gossypii* (Hom.: Aphididae) in Iran. Journal of Economic Entomology, 99: 1820–1825.
- Roberts, J.J. and J. E. Foster. 1983. Effect of leaf pubescence in wheat on the bird cherry oat aphid (Hom.: Aphididae). Journal of Economic Entomology, 76: 1320–1322.
- Robinson, S.P.; W. J. S. Downton, and J. A. Millhouse.1983. Photosynthesis and ioncontent of leaves and isolated chloroplasts of salt-stressed spinach. Plant Physiol., 73:238-242.
- SPSS (1998). SPSS for Windows 9.0. SPSS, Chicago, IL.
- Yadev, R. 2003. A combined source of resistance against com leaf aphid and yellow rust in barley. Int. J. Pest Managem, 49: 293-296.

دراسات مورفولوجية وتشريحية وبيوكيمائية علي بعض اصناف القمح المصرية المصابة بمن الشوفان مروة فاروق كامل علي¹ و الشيماءعبد الله ابو الخير² ¹ قسم وقاية النبات-كلية الزراعة- جامعة المنيا ² قسم النبات-كلية الزراعة- جامعة المنصورة