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Susceptibility of Some Ornamental Plants to Aphid Infestation and Effects on Plant Biochemical Components and Morphological Structures



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Ornamental plants, Aphid susceptibility, Superoxide dismutase, glutathione-Stransferase, Trichomes, Tannins **Abstract:** Aphid is a major insect pest attacking ornamental plants. It causes great damage by reducing the economic value of plants. The present research aims to determine the relationship between leaf biochemical components, morphological structures of plant leaf surface and the susceptibility of seven ornamental plants to aphid infestation. The highest recorded aphid infestation was observed on Tecoma stans during the 2017 and 2018 seasons followed by Rosa damascena and Jasminum grandiflorum. There was no recorded infestation on four inspected ornamental plants, Ruta graveolens, Schefflera actinophylla, Nerium oleander and Bougainvillea spectabilis. The activity of antioxidant enzymes plays a crucial role in the defense line when increasing the insect population density. The activity of glutathione-S-transferase and superoxide dismutase was determined. Biochemical components, e.g. tannins and phenols, vary among different plants and prevent or reduce insect infestation. Trichomes are considered as main morphological character in plant defense systems. These results enhanced the utilization of resistant ornamental plants to prevent aphid infestation. It is considered one of the integrated pest management programs, that achieves a healthy environment without more insecticidal application.

1 Introduction

Aphid is considered one of the major economic insect pests attacking ornamental plants that are used for medical purposes and decoration. Aphid population increases to reach high density in a short time when ecological factors are favorable for developing insects. Aphids feed extra plant sap by mouth parts more than their needs. Feeding behavior leads to the secretion of a lot of sugary substances on plant leaves known as honeydew. It could be described as a sticky substance that accumulates on the leaf surface and prevents plant breathing; it also affects the potential of the photosynthesis process. Aphid feeding also causes weakness in plants and may cause plant malformation (Mehrparvar et al

2008). It may stop the growth of leaf buds and prohibit plant flowering. Otherwise transmitting viruses by this economic insect pest (Khatab 2007).

Some biochemical plant components are considered the main factor in enhancing self-defense. That may protect plants from pest attacking, insect feeding and incomplete growth. Plants could produce toxic substances such as alkaloids and terpenoids that may kill the insect or prevent their development (Hanley et al 2007).

Specialized Reactive oxygen species (ROS) detoxifying enzymes, such as Glutathione *S*-transferase (GSTs) and Superoxide dismutase (SODs) play an important role in detoxifying the effect of biotic and abiotic stimulate which are considered the first line of ROS defense. Defense

enzymes can eliminate or protect the cell from the damage caused by ROS. That fostering plant protection against oxidative stress (Marrs et al 1995, Torres 2010). Plants may also protect themselves from different pests attacking by the presence of some morphological structures that may prevent insects from feeding or laying eggs (Rani and Jyothsna 2010).

Host plant resistance is one of the important programs for integrated pest management which is a safe alternative to pest controlling (Brzozowski and Mazourek 2020).

Therefore, the present research was carried out to estimate the biochemical components and morphological structure of the plant leaves and their relation to aphid infestation as a utilized method in integrated pest management.

2 Materials and Methods

2.1 Experimental area and design

Experiments were conducted at the experimental farm attached to Taif Governorate, Kingdom of Saudi Arabia. The experiments were carried out through the 2017 and 2018 successive seasons. To study the susceptibility of ornamental plants to Aphid infestation, seven plants were chosen, namely: Tecoma stans (F: Bignoniaceae), Bougainvillea spectabilis (F: Nyctaginaceae), Nerium oleander (F: Apocynaceae), Schefflera actinophylla Araliaceae), Rosa damascene (F: Rosaceae), Jasminum grandiflorum (F: Oleaceae), graveolens (F: Rutaceae). Seeds were sown on February 4th for the two tested seasons. The experimental area was planted in equal pots (of about 30*25 cm). Seven tested ornamentals were designed in complete randomized blocks. Pots of tested plants replicated three times. All experiments were free from chemical application during the experimental season.

2.2 Monitoring the seasonal fluctuation of aphids

To follow the seasonal fluctuation of aphids, weekly samples were started after 15 days of the sowing date and continued for 4 months. For recording the aphid's population density, samples of 10 plant leaves/ replicate (three replicates/cultivar) were chosen randomly and carefully examined early in the morning. Population density of aphids was determined by counting all individuals (nymphs and adults) and recorded by using 10X lenses in the experimental area.

2.3 Biochemical assessment of plant leaves

Samples of ten leaves of each tested plant were picked out during aphid population density and recorded the highest density during the season. Biochemical analysis was conducted at the Plant Protection Research Institute, Agricultural Research Center, Egypt. The plant samples were stored at 20°C, according to the described method by Ni et al (2001).

2.3.1 Nitrogen Determination

The nitrogen in protein is converted to ammonium sulfate by H₂SO₄ during digestion. This salt, on steam distillation, liberates ammonia which is collected in a boric acid solution that was titrated against standard acid till recorded violet color in the presence of phenolphthalene indicator (Moore and Stein1948).

2.3.2 Inorganic Phosphorus (P) and Potassium determination

Phosphorus was determined as described by Rockstein and Herron (1951). The phosphate ion was detected using a commercial kit of Quimica Clinica aplicada; phosphorous reacts with molybdate to produce phosphor-molybdate which was reduced to a molybdenum blue photometrically measured at 650 nm. Determination of Potassium was followed by the method described by Kelley (1946).

2.3.3 Antioxidant Defense Enzymes Activity

Plant leaves were ground with sodium phosphate buffer at pH 6.5 and centrifuged at 2000 rpm for 10 min. The supernatant was used to measure the activity of Antioxidant Defense Enzymes. The enzyme activity was expressed as units /g of fresh weight/hour. Glutathione S-transferase was estimated spectrophotometrically as described by Dean et al (1995), while Superoxide dismutase activity was assayed following the method of McCord and Fridovich (1969).

2.3.4 Proline

Determination of proline content in plant samples was followed by the described method by Boctor (1971).

2.3.5 Tannins

Leave tissues (50 mg) were mixed with 7.5 ml of H_2O at $100^{\circ}C$ for half an hour. The mixture was centrifuged at $10,000 \times g$ for 20 min. Estimating of tannins was described by Sadasivam and Manickam (1992).

2.3.6 Phenols

The leave sample was washed with distilled water and dried in an oven at 45°C for 4 days. The sample was crushed in an electric grinder into powder. Extraction was performed as described by Kâhkônen et al (1999).

2.3.7 Total proteins

Five hundred mg of the leaves sample was ground in 5 ml of 0.01 Mg phosphate buffer (pH 7). Total proteins were estimated as mentioned by the Bradford method (1976). The protein reagent was prepared by dissolving 100 mg of Coomassie Brilliant blue G-250 in 50 ml of 95% ethanol.

2.3.8 Total free amino acids

Ninhydrin reagents were used to estimate total amino acids according to the method described by Lee and Takahashi (1966), and Vartainan et al (1992). Plant Samples were grinding in ethanol (80%). The mixture was heated in a boiling water bath for 10 min and cooled in a tap water bath. It was centrifuged at 2000 rpm for ten minutes. The amount of total free amino acids was estimated by adding 1 ml of supernatant and 1.9 ml of ninhydrin. The mixture was boiled, and the bluish pink was read at 570 nm.

2.3.9 Total carbohydrates

Total carbohydrates were measured in the acid extract by the phenol-sulfuric acid reaction according to DuBois et al (1956) and Crompton and Birt (1967). A weight of 100 mg of the plant sample was added into a boiling tube then adding 10 ml of 2.5 N HCl. carbohydrates were hydrolyzed by keeping in a boiling water bath for three hours and were left to cool at room temperature. The absorbance of the characteristic yellow-orange color is measured at 490 nm against blank. Total carbohydrates are expressed as μg glucose/gm fresh weight.

2.4 Scanning electron microscope examination

The sample of leaves was immediately kept in glutaraldehyde solution (2%) at room temperature till preparation. Samples were washed several times with distilled water. Leaves were left at room temperature until dry. Pieces of leaves were cut with sizes 3 to 10 mm. Samples were coated with gold to allow expectation and fixed on an adhesive tab. The

methodology of scanning was described by Echlin (2009). Leaf samples were fixed with two sides of carbo tape. SEM Model Quanta 250 field emission gun (FEG) was used for inspection. Figures were captured at two magnification powers 500x and 1000x. The accelerating voltage of the figures was 30 KV and the resolution for Gun.1n. Scanning electron microscope was utilized at the Egyptian Mineral Resources Authority, central laboratories sector.

2.5 Statistical analysis

Data was analyzed by using the SAS program (SAS 2001). ANOVA test was conducted to evaluate the significance among the seven tested ornamental plants by using LSD at p < 0.05. The correlation coefficient (r) level was estimated to determine the relation between the chemical components and aphid infestation on the tested plants.

3 Results and Discussion

3.1 Population fluctuations of aphids on seven ornamental plants during the 2017 and 2018 seasons, susceptibility of ornamental plants to aphid infestations

Tabulated data in Table 1 showed distribution densities of aphid infestation in Taif Governorate on seven hosts of ornamental plants during the 2017 and 2018 seasons. The weekly inspection started on 19th February till the end of June. Data shows that there is a different appearance of aphid densities between three ornamental plants: Rosa damascena, Tecoma stans and Jasminum grandiflorum. No recorded infestation of aphids on four inspected ornamental plants, Ruta graveolens, Schefflera actinophylla, Nerium oleander and Bougainvillea spectabilis. Statistical analysis supported that there are significant differences between the three infested plants according to their susceptibility to infestation. Calculated F values 6.2 and 12.4, LSD 36.9 and 42.3 for the 2017 and 2018 seasons respectively. Obtained results were confirmed by the finding of Rashwan et al (2016) who recorded no aphid infestation on the four ornamental plants: B. spectabilis, N. oleander, S. actinophylla and R. graveolens during the 2015/2016 season. The most abundant density of aphids for all tested plants was noticed from 20th March to 5th June. Tecoma stans was the most susceptible one with mean numbers 144 and 145.8 individuals/leaflets in 2017 and 2018 respectively. R. damascena and J. grandiflorum were the lowest-infested plants in the two. inspected seasons. Seasonal mean numbers

Table 1. Weekly number of aphids of the seven tested ornamental plants during 2017 and 2018 seasons at Taif Governorate

	Mean number of aphids/leaflets													
Inspection date	Rosa damascena		Tecoma stans		Jasminum grandiflorum		Ruta graveolens		Schefflera actinophylla		Nerium oleander		Bougainvillea spectabilis	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
19th -Feb	10	0	4	12	2	9	0	0	0	0	0	0	0	0
29 th - Feb	18	0	14	19	7	6	0	0	0	0	0	0	0	0
6 th - Mar	8	7	102	38	12	8	0	0	0	0	0	0	0	0
13 th Mar	36	22	174	156	22	6	0	0	0	0	0	0	0	0
20th Mar	44	36	204	142	18	18	0	0	0	0	0	0	0	0
26 th Mar	32	39	150	189	22	16	0	0	0	0	0	0	0	0
3 rd Apr	16	41	248	220	18	12	0	0	0	0	0	0	0	0
10 th Apr	12	17	264	263	22	11	0	0	0	0	0	0	0	0
17 th Apr	24	16	398	349	29	10	0	0	0	0	0	0	0	0
24 th Apr	10	19	464	321	8	26	0	0	0	0	0	0	0	0
1st May	2	21	586	319	38	28	0	0	0	0	0	0	0	0
8 th May	22	15	180	356	44	35	0	0	0	0	0	0	0	0
15 th May	32	9	284	239	16	32	0	0	0	0	0	0	0	0
22 nd May	170	36	498	269	12	25	0	0	0	0	0	0	0	0
29 th May	66	96	144	415	39	29	0	0	0	0	0	0	0	0
5 th June	12	61	266	516	12	31	0	0	0	0	0	0	0	0
12 th June	2	81	26	158	5	18	0	0	0	0	0	0	0	0
19th June	4	26	26	38	0	12	0	0	0	0	0	0	0	0
26 th June	2	62	0	65	0	19	0	0	0	0	0	0	0	0
Mean	18.6b	21.5b	144a	145.8a	11.6c	12.5c	0	0	0	0	0	0	0	0

R. damascenae 18.6 and 21.5 were individuals/leaflets for 2017 and 2018, while J. grandiflora recorded 11.6 and 12.5 individuals/leaflets 2017 during and 2018, respectively. Therefore, the three infested ornamental plants could be arranged descending according to infestation susceptibility as Tecoma stans, Rosa damascena, and Jasminum grandiflorum.

The obtained results demonstrated the highest densities during the 2017 season, which may be due to environmental factors. Lebbal and Laamari (2015) showed variability of infestation degree by aphids on different plants between years.

Previous results are congruent with Abd El-Hadi (2002) who found that the highest population of *Aphis gossypii* on *Salivia splendens* flowers in Egypt in the third week of March. Also, Ahmed (1990) observed *A. gossypii* began to appear on some medicinal and ornamental plants around the 3rd week of June. Ullah et al (2014) in southern Punjab stated that aphid infestation started at the beginning of January and increased gradually to reach a peak at the end of March, while the population started to

decrease during June. Chapin et al (2001) recorded the highest population during December and January in Florida state. Infestation fluctuates according to weather factors which affect aphid development during different seasons and planting dates. Early planted crops recorded low densities (Shonga and Getu 2020). Patra et al (2012) reported the same results, recording less aphid infestation in the early planted season than late planted season depending on weather factor changes.

3.2 The relation between leaf biochemical components and aphid infestation

3.2.1 Effect of Nitrogen, Phosphorus and Potassium

Plant minerals play an important role in the growth of insects. They obtained needed minerals from plants by feeding. Nitrogen, phosphorus, and potassium are classified as primary macronutrients. Data obtained in **Table 2** showed a positive significance between the aphid population and the

nitrogen level. Nitrogen is considered a very important required mineral for the growth of insects (Rostami et al 2012).

Phosphorus and potassium levels are related to aphid infestation that may cause resistance or susceptibility of plants. Phosphorus contributes to decreasing the host's suitability to insect pests by changing secondary metabolites such as phenolics and terpenes. Increasing phenolics (tannin, lignin) act as antifeedant or toxic substances that negatively affect insect presence (Facknath and Lalliee 2005).

Increasing the Phosphorus treatment reduced the population of mustard aphids. Population decreased significantly with an increase in the rate of application, while increasing the phosphorous level led to an increase in the response of other insect pests such as *Empoasca* sp. and *Frankliniella occidentalis* (Bala et al 2018). They demonstrated also high levels of potassium help in plant resistance which has negative significant effects on the building up of aphid populations. High levels of potassium help in plant resistance enhance secondary compound metabolism and reduce the accumulation of carbohydrates that protect plants from pest attacks.

3.2.2 The activity of Antioxidant Defense Enzymes, Glutathione S-transferase (GST) and Superoxide dismutase activities (SODs)

Infestation by aphids causes increasing the activity of GSTs ad SODs in flag leaves in all the test ornamental plants as shown in **Table 2.** They recorded a highly significant correlation with aphid infestation where r values =0.8420 and 0.9332, respectively. GST activity was determined to be highly increased in infested leaf plants than in the uninfected leaf plant samples. The highly significant increase was observed in *Tecoma stans* followed by *Rosa damascena* and *Jasminum grandiflorum*. It recorded the lowest levels in the uninfected plants, *schefflera actinophylla*, *Nerium oleander* and *Bougainvillea spectabilis*.

SOD activity was observed with the highest activity also in the infested leaf samples of tested ornamental plants. Maximum SOD activity was observed in infested leaves *Tecoma stans* compared to un-infested leaves, *schefflera actinophylla*, *Nerium* oleander and *Bougainvillea spectabilis*. Antioxidant Defense Enzymes are considered the first line of defense protecting from the destructive activity that occurs by Reactive Oxygen Species (ROS).

Desingh et al (2007) supported that the increase in GST and SOD activities under salt stress conditions in cotton plants may be due to the feeding insect effect. The activity of antioxidant enzymes may help plants in facing environmental factor stresses (Mishra et al 2010). Simova-Stoilova et al (2009) recorded the high activity of SOD in the wheat plant because of environmental stress. The plant has enzymes that act as an antioxidant defense system including peroxidase, catalase, glutathione-S-transferase and superoxide dismutase enzymes; these enzymes protect the plant from oxidative damage (Jaleel et al 2008).

Antioxidant enzymes protect plants from the damage caused by free radicals before oxidizing cell components. It may disrupt the oxidizing process caused by free radicals. Glutathione S-transferase detoxifies the toxic substance by conjugation with glutathione which leads to reducing oxidative stress. Superoxide dismutase is responsible for eliminating the reactivity of superoxide anion to oxygen and then reducing the reactive species hydrogen peroxide.

3.2.7 Total carbohydrates

There is a negative correlation between the infestation level and the amount of total carbohydrates (r-value -0.5858). Zou and Cates (1994) added a 6% amount of galactose in an artificial diet to feed western spruce budworms. which interrupted larval growth and caused an incomplete stage. Rani and Jyothsna (2010) reported that the increased levels of carbohydrates in rice plants suggest their role in the plant's defense mechanism by inducing the signaling pathways. The obtained results were supported by the findings of Helmi and Rashwan (2015); they investigated the negative correlation between sap-sucking insect infestation and total soluble sugar content in tomato cultivars, where it recorded the highest levels in the resistant plants.

3.3 Effect of morphological features of ornamental plants on aphid infestation

The plant uses trichomes as a line defense against phloem feeders such as hemipters insects which are considered a vital role in plant protection (Wagner et al 2004). As shown in **Fig 1**, scanning electron microscope of seven ornamental plants, trichomes play an important role in aphid infestation. Three ornamental plants *Rosa damascena*, *Tecoma stans* and *Jasminum grandiflorum* showed no trichomes on their lower surface while *Ruta graveolens* and *Nerium oleander*.

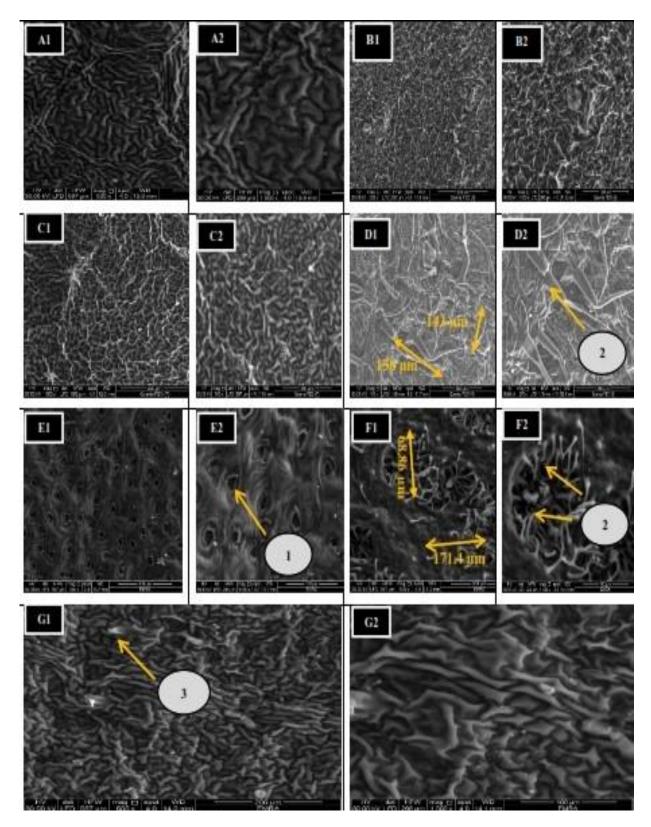


Fig 1. Scanning electron microscope of the lower surface of seven types of ornamental plants with two magnification powers (500x and 1000x). A: *Rosa damascena* B: *Tecoma stans* C: *Jasminum grandiflorum* D: *Ruta graveolens* E: *schefflera actinophylla* F: *Nerium oleander* G: *Bougainvillea spectabilis*.

1: Stomata opening, 2: non-glandular trichomes, 3: Glandular trichomes

Large intensity of trichomes were observed on the lower surface that interrupts biological activities and keeps plants free from infestation. These trichomes are varied in length and density. These structures may prevent insects from feeding on the sap plant, the leaf surface of a plant has a fundamental effect on the insect behavior. The finding of these results showed congruity with Amin et al (2017) who demonstrated a negative correlation between leaf trichomes of cotton verities and aphid abundance, while a non-significant correlation was recorded with the jassid population.

Schefflera actinophylla showed a high number of stomata. Nevertheless, Li et al (2011) and Coll and Hughes (2008) recorded high densities of aphid infestation on plants that have large numbers of stomata. Elevating a high amount of carbon dioxide might increase insect attacks on plants. The repellent effect of S. actinophylla may be due to the biochemical component of the plant leaf. Scanning electron microscope of Bougainvillea spectabilis showed glandular trichomes may play a defense effect against insect feeding. These results showed close conformity with Helmi and Mohamed (2016) who recorded a highly negative significant relationship between glandular trichomes and aphid infestation in tomato cultivars. It could be concluded that tomato plants have high densities of glandular and non-glandular trichomes on leaf surfaces that prevent attacking and feeding of many insect pests (Tian et al 2012). Price et al (2011) demonstrated that feeding by insects is interrupted because of long and dense soybean trichomes on the leaf surface. Morphological characters and biochemical components have an important role in plant resistance (Gameel 2014).

3.2.3 Proline

The obtained results in **Table 2**, demonstrated that increasing insect density on plants led to an increase of proline content. As shown, the highest amount of proline was observed in *Tecoma stans* which has the highest infestation. A highly positive correlation was observed between proline and infested plants, where the calculated value (r = 0.9530). Hayat Shamsul et al (2012) agreed with the finding, where they stated a positive correlation between accumulation of proline and plant stress. It may be suggested proline acts as an antioxidant against reactive oxygen species (Matysik et al 2002).

3.2.4 Tannins

The content of tannins increased in the un-infested leaf sample plants as shown in Table 2, where r value = -0.6672. The highest activity of tannins was recorded in un-infested leaf samples Increasing tannins content indicates their protective role as antioxidant defense against biotic and abiotic effects. Tannins can interact with proteins suggesting that tannins affect insect herbivores by inactivating insect enzymes as well as dietary proteins that might indicate the role of tannins in feeding (Robbins et al 1987). An increase in the amount of total tannins indicated that tannins might play a role in feeding inhibition (Grayer et al 1992). Bernays (1981) reported that accumulated tannins in leaves protect the plant from insect survival as they rapid proteins nonspecifically by covalent bonding or hydrogen bonding with proteins, which may reduce the mineralization of nitrogen or the digestion process.

3.2.5 Total phenols and Phenol oxidase

The highest amount of phenols and Phenol oxidase were found in the un-infested ornamental plant (schefflera actinophylla, Nerium oleander and Bougainvillea spectabilis). These results are in accordance with Perveen et al (2001), who demonstrated higher phenolic content in susceptible cotton plant varieties than resistant ones. Xu et al (2021) stated that phenols are secondary compounds in plants that enhance resistance to wheat against aphid attack. Resistance wheat cultivars synthesize higher enzymes and stimuli producing more tannins and phenols than susceptible ones.

The elevation of phenols could be explained as a mechanism of defense against insect feeding. Plant phenols produce toxic secondary metabolites which cause toxicity for herbivores (Helmi and Mohamed 2016), these phenolic compounds are known to inhibit insect development.

Phenol oxidations produce quinones that may inhibit the digesting protein in insects (Bhonwong et al 2009).

3.2.6 Total protein and total free amino acids

Results in **Table 2** indicated that total proteins and total free amino acids in leaves of the un-infested plants (*schefflera actinophylla*, *Nerium*) is lower than in the susceptible plant (*Tecoma stans*). When plants are exposed to ultra-stress, they may produce extra amounts of biochemical components and secondary

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Table 2. Biochemical analysis of some components of seven ornamental plants and its relation to aphid infestation

	Orname	Correlation (r)							
Biochemical components	Rosa damasc ena	Tecoma stans	Jasminu m randiflo rum	Ruta graveol ens	schefflera actinophyl la	Nerium oleander	Bougai nvillea spectab ilis		
Aphid population	20	144.9	12	0	0	0	0		
Nitrogen (ug/gm)	2098	1187.3	3984.3	2021.3	2144.6	2526.6	1756.6	0.4288	
Phosphorus (ug/gm)	225.3	200.3	451.6	354	427.6	386.6	262.3	-0.6309	
Potassium (uEq/gm)	52	26.4	1.6	44.9	29.2	50.9	49.2	-0.5455	
GST (mmolesub. conjugated/min/gm)	18.6	20.7	11.2	8.3	9.2	10.1	9.7	0.8420	
Superoxide dismutase (SOD	98	117.2	76	86	77.3	82.6	56.3	0.9332	
Proline ug proline/gm	291	634.3	198	90	156.3	100.3	45.6	0.9530	
Tannins ug tannic acid/gm	128	92.3	117.6	129.3	102.3	115.3	186.6	-0.6672	
Total phenols (ug GAE/gm	796	559.3	777.3	783	856.3	615	751.3	-0.6527	
Phenol oxidase (O.D. units/min/gm	5.1	3.2	3.2	5.8	13.8	13.2	25.2	- 0.5917	
Total proteins mg/gm	12.6	7.3	22.6	6.4	12.6	16.3	11.6	0.2794	
Free amino acids (ug D,L-alanine/gm)	511	942	430	850	1018.6	853	613.3	0.3136	
Total carbohydrates (mg/gm)	11.9	12.7	27.1	10.9	29.1	28.1	38.9	5858	

metabolites that affect insect activity such as feeding, oviposition, digestion and reproduction. These results are congruent with the findings of Rani and Pratyusha (2013) who recorded the highest level of proteins in the infested plant than un-infested plant. Mohamed and Abd-El Hameed (2014) concluded that increasing protein levels may be because of the defense against insect infestation. Amino acids are demonstrated as a product of metabolites that work as a defense line in infested plants (War et al 2012). Alkylation of amino acids may change the value of plant nutrition and prevent insect growth and development (Bhonwong et al 2009).

4 Conclusion

Seven ornamental plants showed different susceptibility to aphid infestation. Biochemical component activities varied between sensitive and resistant plants. Moreover, the morphological structure of plant leaves such as stomata, and glandular and non-glandular trichomes play an important role in plant sensitivity. Resistance plants showed higher densities of trichomes on leaf surfaces than in susceptible ones. Resistant plants could be recommended in integrated pest management programs to avoid using extra chemical insecticides.

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