



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



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Abstract: Aphid is a major insect pest attacking ornamental plants. It causes great damage by reducing the economic values of plants. The investigated research aims to determine the relation between leaf biochemical components, morphological structures of plant leaf surface and the susceptibility of seven ornamental plants to Aphid Infestation. Highest recorded aphid infestation was observed on *Tecoma stans* during 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*. Activity of antioxidant enzymes plays as defense line when increasing the insect population density. Biochemical components are varied among different plants that prevents or reduces insect infestation, as tannins, total phenol, and Phenol oxidase. Trichomes are considered as main morphological character in plant defense system. These results enhanced utilization resistant ornamental plants due to preventing aphid infestation. It is considered one of the integrated pest management programs, that achieves health environment without more insecticidal application.

1 Introduction

Aphid is considered one of the major economic insect pests attacking ornamental plants which is used in medical purposes and decoration. Aphid population could be increased to reach high density in a short time when ecological factors are favorable for developing insect. Aphids feed extra plant sap by mouth parts more than need. Feeding behavior led to secrete a lot of sugary substance on plant leaves known as honeydew. It could be describing as a sticky substance accumulate on leaf surface and prevent plant breathing, it also effects on the potential of photosynthesis process. Aphid feeding also causes weakness of plant and may cause plant malformation (Mehrparvar et al 2008). It may stop growth leaf buds and prohibit plant

flowering. Otherwise transmitting viruses by this economic insect pest (Khatib 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, flavonoids, terpenoids, nithocyanins, and phenol which may kill insect or prevent developing. (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 pest attacking by presence of some morphological structures that may prevent insect feeding or laying eggs (Rani and Jyothsna 2010).

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

Therefore, the present research was carried to estimate the biochemical components and morphological structure of the plant leaves and its 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 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* (F: Araliaceae), *Rosa damascene* (F: Rosaceae), *Jasminum grandiflorum* (F: Oleaceae), *Ruta 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 block. 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 seasonal fluctuation of aphids, weekly samples were started after 15 days of 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 recorded highest density during the season. Biochemical analysis was conducted in 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 sulphate by H₂SO₄ during digestion. This salt, on steam-distillation, liberates ammonia which is collected in boric acid solution. It titrated against standard acid till recorded violet color (Moore and Stein 1948).

2.3.2 Inorganic Phosphorus (P) and Potassium determination

Phosphorus determination could be determined as described by Rockstein and Herron (1951). The phosphate ion was detected using a commercial kit of Quimica Clinica aplicada phosphor reacts with molybdate to produce phosphor-molybdate. Finally, that reduced to a molybdenum blue which is 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 grinding 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 and was estimated spectrophotometrically as described by Dean et al (1995), while Superoxide dismutase activity 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 of Bocktor (1971).

2.3.5 Tannins

Leave tissues (50 mg) were mixed with 7.5 ml of H₂O at 100°C for half an hour. Mixture was centrifuged at 10,000 × g for twenty min. Estimating of tannins was described by Sadasivam and Manickam (1992).

2.3.6 Phenols

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

2.3.7 Total proteins

Five hundred mg of the leaves sample was grinding in 5 ml of 0.01 M phosphate buffer (pH 7). Total proteins were estimated as mentioned of Bradford (1976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50 ml 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 Takabashi (1966), Vartanian et al (1992). Plant Sample were grinding in ethanol (80%). The mixture was heated in a boiling water bath 10 min and cooled in a tap water bath. It centrifuged at 2000 rpm for ten minutes. Amount of total free amino acids was estimated by adding 1 ml of supernatant and 1.9 ml ninhydrin. Mixture was boiled, the bluish pink was read at 570 nm.

2.3.9 Total carbohydrates

Total carbohydrates were measured in acid extract by the phenol-sulfuric acid reaction according to (Dubois et al 1956, Crompton and Birt (1967). Weight of 100 mg of the plant sample was added into a boiling tube then adding 10 ml of 2.5 N HCL It hydrolyzed by keeping in a boiling water bath for three hours, it left for cooling at room temperature

The absorbance of characteristic yellow-orange color is measured at 490 nm against blank. Total carbohydrate is expressed as: µg glucose/gm fresh weight.

2.4 Scanning electron microscope examination

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 size 3 to 10 mm. Samples were coated with gold to allow expectation and fixed on adhesive tab. Methodology of scanning was described by (Echlin 2009). Leaf samples were fixed with two

side carbo tape. SEM Model Quanta 250 field emission gun (FEG) was used for inspection. Figures were captured at two magnification powers 500x and 1000x. Accelerating voltage of figures were 30 KV and resolution for Gun.1n. Scanning electron microscope were utilized at Egyptian mineral resources authority, central laboratories sector.

2.5 Statistical analysis

Data analyzed by using SAS program (SAS 2001). ANOVA test was conducted to evaluate the significance among the seven tested ornamental plants by using LSD at $p < 0.05$. 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 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 2017 and 2018 seasons. Weekly inspection started 19th February till the end of June. Data shown that there is 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 three infested plants according to their susceptibility to infestation. Calculated F values= 6.2 and 12.4, LSD = 36.9 and 42.3 for 2017 and 2018 seasons, respectively. Obtained results were confirmed with 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 2015/2016 season. The most abundant density of aphids for all tested plants was noticed during 20th March to 5th June. *Tecoma stans* was the most susceptible ones with mean number 144 and 145.8 individuals/leaflets in 2017 and 2018, respectively. *R. damascena* and *J. grandiflorum* were the lowest infested plants in two inspected seasons. Seasonal mean numbers of *R. damascenae* 18.6 and 21.5 individuals/leaflets for 2017 and 2018, while for *J. grandiflora* recorded 11.6 and 12.5 individuals/leaflets during 2017 and 2018, respectively. Therefore, the three

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

Inspection date	Mean number of aphids/leaflets													
	<i>Rosa damascena</i>		<i>Tecoma stans</i>		<i>Jasminum grandiflorum</i>		<i>Ruta graveolens</i>		<i>Schefflera actinophylla</i>		<i>Nerium oleander</i>		<i>Bougainvillea spectabilis</i>	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
19 th -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
20 th 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
1 st 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
19 th 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

infested ornamental plants could be arranged descending according to infestation susceptibility as *Tecoma stans*, *Rosa damascena*, and *Jasminum grandiflorum*.

Obtained results demonstrated highest densities during 2017 season, it may because of the 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 *Salvia splendens* flowers in Egypt at 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 in 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 highest population during December and January in Florida state. Infestation is fluctuated according to weather factors which effects on aphid development during different seasons and planting date. Early planted crops recorded low densities (Shonga and Getu 2020). Patra et al (2012) reported same results, that recorded less aphid infestation on 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 on the growth insects. They obtained needed minerals from plants by feeding. Nitrogen, phosphorus, and

potassium are classified as primary macronutrients. Data obtained in **Table 2** showed positive significant between the aphid population and the nitrogen level. Nitrogen is considered very important required mineral for the growth of insect (Rostami et al 2012).

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

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

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

Infestation by aphid causes increasing the activity of GSTs and SODs in flag leaves in all the test ornamental plants as showed in **Table 2**. They recorded 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 the uninfected leaf plant samples. Highly significant increase was observed in *Tecoma stans* followed by *Rosa damascena* and *Jasminum grandiflorum*. It recorded 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 defense protecting from the destructive activity occurred by Reactive Oxygen Species (ROS). Desingh and Kanagaraj (2007) supported the increasing in GST and SOD activities under salt stress condition in cotton plants may be due to 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 high activity of SOD in wheat plant because of environmental stress. Plant has enzymes act as antioxidant defense system including peroxidase, catalase, Glutathione S-transferase and superoxide dismutase enzymes, these enzymes protect plant from oxidative damage (Jaleel et al 2008).

Antioxidant enzymes are acting as destruction component for the damage caused by free radicals before oxidizing cell components. It may disrupt the oxidizing process caused by free radicals to reduce the damage as possible. Glutathione S-transferase detoxifying the toxic substance by conjugation with glutathione that led to reducing oxidative stress. Superoxide dismutase is responsible for inhibit the reactive of superoxide anion to oxygen then reducing reactive species Hydrogen peroxide.

3.2.7 Total carbohydrates

There is negative correlation between the infestation level and the amount of total carbohydrates where (r value = -0.5858). Zou and Cates (1994) added 6% mount of galactose in artificial diet to feed western spruce budworm, that interrupt larval growth and caused 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. Obtained results were enhanced with the finding of Helmi and Rashwan (2015), they investigated negative correlation between sap sucking insect infestation and total soluble sugars content in tomato cultivars, where it recorded highest levels in the resistant plants.

3.3 Effect of morphological features of ornamental plants on aphid infestation

Plant uses trichomes as a line defense against phloem feeders such as hemipters insects which considered a vital role in plan 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*. Large intensity

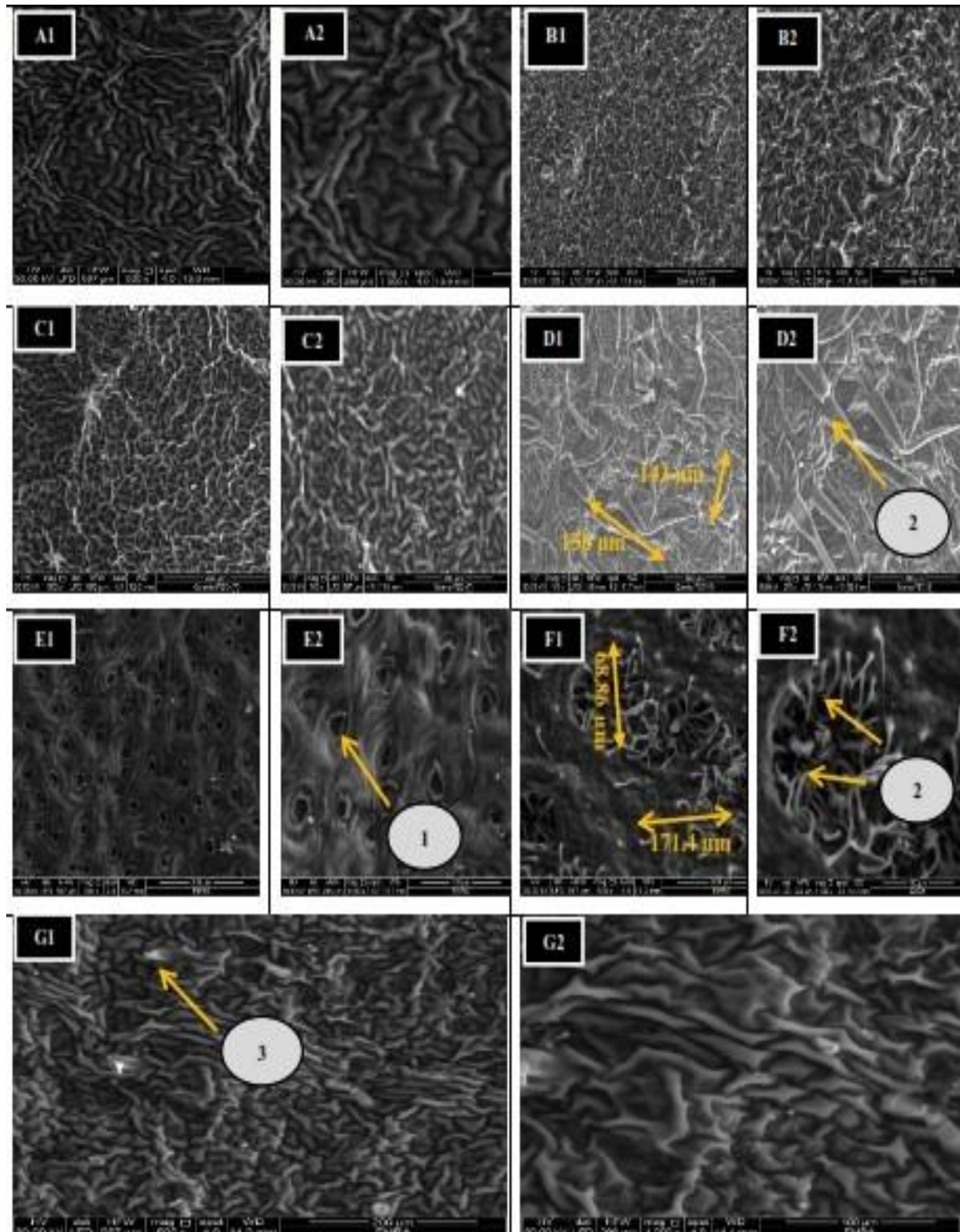


Fig 1. Scanning electron microscope of the lower surface of seven types of ornamental plants with two magnification powers (500x and 1000x). A: *Rosa damascena* B: *Tecoma stans* C: *Jasminum grandiflorum* D: *Ruta graveolens* E: *schefflera actinophylla* F: *Nerium oleander* G: *Bougainvillea spectabilis*. 1: Stomata opening, 2: non-glandular trichomes, 3: Glandular trichomes

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

Schefflera actinophylla showed high number of stomata. Nevertheless, Li et al (2011), Coll and Hughes (2008) recorded high densities of aphid infestation on plants that have large number of stomata. Elevating high amount of carbon dioxide might be increase insect attacking to plants. Repellent effective of *S. actinophylla* may be due to the biochemical component of plant leaf. Scanning electron microscope of *Bougainvillea spectabilis* showed glandular trichomes may play as defense effect against insect feeding. These results showed close conformity with Helmi and Mohamed (2016) who recorded highly negative significant relationship between glandular trichomes and aphid infestation on tomato cultivars. It could be conducted that tomato plants have high densities of glandular and non-glandular trichomes on leaf surface 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 of soyabean 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 the **Table 2**, demonstrated that increasing insect density on plants led to increase of proline content. As shown, the highest amount of proline was observed in *Tecoma stans* which has the highest infestation. Highly positive correlation was observed between proline and infested plants, where the calculated value ($r = 0.9530$). Hayat et al (2012) agreed with the finding, where they stated positive correlation between accumulation of proline and plant stress. It may be suggested proline act as antioxidant Reactive Oxygen Species scavenger (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 have the capacity to interact with proteins suggesting that tannins affected insect herbivores by inactivating insect enzymes as well as dietary proteins that might be indicated the role of tannins as feeding (Robbins et al 1987). An increasing in the amount of total tannins indicated that tannins might play a role as feeding inhibition (Grayer et al 1992). Bernays (2008) reported that accumulate tannins in leaves protect plant from insect survival as they rapid proteins nonspecifically by covalent bonding or hydrogen bonding with proteins, that may reduce the mineralization of nitrogen or 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), they demonstrated higher phenolic content in susceptible cotton plant varieties than resistant ones. Xu et al (2021) stated that phenols are secondary compounds in plants enhance resistance 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 causes toxicity for herbivores (Helmi and Mohamed 2016), these phenolic compounds are known to inhibit insect development.

Phenol oxidations produce quinones that may inhibit digesting protein in insects (Bhonwonget 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 expose to ultra-stress, they may produce extra amounts of biochemical components and secondary

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

Biochemical components	Ornamental plants							Correlation (r)
	<i>Rosa damascena</i>	<i>Tecoma stans</i>	<i>Jasminum randiflorum</i>	<i>Ruta graveolens</i>	<i>schefflera actinophylla</i>	<i>Nerium oleander</i>	<i>Bougainvillea spectabilis</i>	
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 on insect activity such as feeding, oviposition, digestion, reproductive. These results are congruent with the finding 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) investigated that increasing protein level may because of defense after the infestation of stored insect. Amino acids are demonstrated as a product of metabolites that work as a defense line in infested plant (War et al 2012). Alkylation of amino acids may change the value of plant nutrition that prevent growth and development of insect (Bhonwong et al 2009).

4 Conclusion

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

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