

# Experimental Investigation on Nutritional Variation in Plant Foliage of Rose (*Rosa damascene*): Effect of Pest Infestation

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**Abstract-** The results on the biochemical constituents of rose inflorescence revealed that the levels of lipid, carbohydrate, nitrogen and protein were significantly lower ( $P < 0.05$ ) in infested parts when aphid population were maximum as compared to uninfested ones in most of the cases. The rose plants were mainly infested with two aphid species viz., *Macrosiphum rosae* (Linnaeus) and *Macrosiphum rosaeiformis* (Das). The infestation of insect pests caused considerable damage to the individual parts (leaf, stem and inflorescence) of the rose plants and significantly reduced the nutritional constituents at different growth stages. *Macrosiphum rosae* was found to be the most active pest of rose. These aphids have appeared in the fields especially with the onset of flowering. The loss in biochemical nutritives of plant foliage due to aphid infestation would degrade the quality of the products mainly made from the inflorescence of rose plants. This study reports results obtained from different localities, which may have almost similar ecological and environmental conditions.

**Index Terms-** Aphid infestation, Biochemical analysis, Nutritional constituents, Rose plantations, Traditional herbal plant

## I. INTRODUCTION

The rose plants have been cultivated for thousands of years in middle east and during the Roman era. The fossils of rose of 35 millions years to oligocene epoch were reported by Cox (1999). The *Rosa* genus is endemic to temperate regions of the northern hemisphere, including North America, Europe, Asia and the Middle East, reported by Phillips and Rix (1988). The genus *Rosa* is comprised of hundreds of species of prickly shrubs which may also have a climbing or trailing habit. The Roses are used for beauty and decoration of garden, extraction of attar for making fragrant mixtures. But main use of roses is in cut flower industry and land scaping where it is mainly use in production as trade, described by Datta (1997).

More than 400 volatile biochemical compounds have been identified in the floral scent of various rose cultivars. The damask rose (*R. damascena*) is the most important species used to produce rose water, other essential oils etc. in the perfume industry, reported by Lavid et al. (2002). Similarly, different rose species are grown for perfume purposes like *Rosa damascena*, *Rosa barboniana* etc. The compounds are generally classified into five groups based on their functions: hydrocarbons, alcohols, esters, aromatic esters and others. It has also medicinal value and

used mainly for different stomach disorders. This herb is used to stimulate the liver, increase the appetite and improve the blood circulation. The rose hips long valued for food, richest natural source of Vitamin C and are now pressed commercially to give rose hip syrup. Tannin contents of rose hips are mildly diuretic and reduce thirst and alleviate gastric inflammation. The dried hips of the wild rose are especially high in vitamin C, having three times that of citrus fruits, and have long been used to prevent scurvy. Rose hip herbal tea is full of nutrients that we need to maintain optimum health. It strengthens the immune system and can help prevent colds and flu due to high vitamin C content, studied by Ziegler et al. (1986). Rose's ability to firm boggy or damaged tissues, reduce inflammation and lessen bacterial proliferation while encouraging the growth of healthy tissue makes it ideal in the treatment of many microbial infections, reported by Jacoby and Wokes (1944). The Chinese use the flowers as energy stimulant and blood tonic to relieve stagnant liver energies. They are also used for digestive irregularities or with motherwort for heavy menstruation. Rose petal herbal tea is given for mastitis and breast pain, menstrual problems and to soothe a restless fetus. Medicinally, it is an important nervine used for depression and anxiety. The Damask rose blooms for only a couple of weeks, during which time the petals are collected and steam distilled to produce true Bulgarian rose oil used in about 96% of all women's perfumes. Rose water can be used as eyewash and as a mouthwash. It can also be used to treat acne and irritated skin. It is good for aging skin. Add the herb to creams, lotions, moisturizers, massage oils, after shaves, salves, balms and antiseptic sprays. Mahmood et al. (1996) and Basim & Basim (2003) reported anti HIV properties of the rose oil and can stop and kill some strains of Xanthomonas. The studies have been conducted simultaneously to breed cultivars with a longer flowering period and improved quality and quantity rose oil. Therefore, there are many challenges and threats to rose plants from the pest and insects. Teulon et al. (1999) reported reduction in the medicinal value of the plant and damage its generations and economic loss to growers due to infestation of insects and by infestations of aphids during springs and summer season. The insect pests come and go, but various aphid species are present on roses from spring through fall, with some species spanning the entire growing period. Alford (1991) reported damage and tender unfolding leaves and buds by the clusters of aphid colonies. Aphid eggs live through the winter in protected nooks and crannies on the plant. In the spring, eggs hatch into females that are capable of reproducing without mating. They give birth to live female aphid

young that have the same capability. This process of asexual reproduction is called parthenogenesis. In the fall, triggered by the change in day length, winged sexual forms (males and females) are produced. They mate, and the females lay eggs for overwintering, reported by Dixon (1987) and Blackman & Eastop (2000).

Minks & Harrewijn (1989) reported that aphids feed on plant cell contents and sap by piercing the plant and sucking up the liquids. These colonies make for easy pickings by aphid predators and parasites. However, many of the natural enemies of aphids are more susceptible to chemical controls than are the aphids.

Due to infestation of insect the biochemical composition of plant may also get affected. Nine species of aphids are known to infest rose viz., *Acyrtosiphon (Rhodobium) porosum*, *Chaetosiphonchaetosiphon*, *Chaetosiphon (Pentatrichopus) tetrahoda*, *Macrosiphumeuphorbiae*, *Macrosiphumpachysiphon*, *Macrosiphumrosae*, *Macrosiphum (Sitobian) rosaeiformis*, *Matsumurajacapitophoroides* and *Myzaphisrosarum*. Three species viz., *Macrosiphumpachysiphon*, *Macrosiphum (Sitobian) rosaeiformis* (Das) and *Matsumurajacapitophoroides* are indigenous species whereas the rest may be looked upon as introduced ones, described by Remaudière & Remaudière (1997), Maelzer (1977), Chakrabarti and Ghosh (1970).

The present investigation has, therefore, been planned to elucidate the positive or negative effects of aphids on chemical composition of rose plants.

## II. MATERIALS AND METHODS

The widespread investigation of rose plants (*Rosa damascena*) generally grown in North India were carried out at three areas (Karamcharinagar, PhoolBagh and University garden) nearby Bareilly city at fortnightly intervals to determine the aphid infestation in relation to the manifestation of their ladybeetle predators throughout flowering season of rose plantations. The plants were grown with the standard doses of organic fertilizers to be applied at the time of planting and irrigation before flowering described by Kumar et al. (2002).

The Normal as well as infested plant parts was brought to the laboratory separately (leaf, shoot, and petals) in the plastic bags and aphids were collected with a brush, forceps for their taxonomic identifications at different stages of the crop using the Blackman et al. (1998)'s TAXAKEY, Aphids on the World's Crops and confirmed by Zoological Survey of India, Kolkata. The rose petals were analyzed for the nitrogen content using Micro-Kjeldahl method from AOAC (1950) and the corresponding protein contents were calculated by multiplying the total nitrogen content by 6.25, reported by Van Gelder (1981) and Ezeagu et al. (2002). The amount of carbohydrate in rose petals was determined using the method of Morris (1948) and Singh and Sinha (1977) whereas, the Soxhlet extraction procedure has been adopted to extract the lipid from rose petals. The amount of lipid is determined as defined by Folch et al. (1951) and Singh and Sinha (1977).

### Lipid

The rose petals were dried at 70 °C for 48 hours. The dried and weighed inflorescence of rose plants were extracted in a

Soxhlet lipid extractor with chloroform AR (CDH, India) and methanol AR (CDH, India) (2:1v/v) as a solvent. For this purpose, the weighed samples of the plant parts were kept inside weighed and marked pouches of Whatman filter paper (Whatman, UK), which were fastened to prevent the loss of material during the process. The extractions were carried out for 12 hours after which the materials were dried and weighed again. The differences in the mass of the extracted samples were taken as the lipid content of the samples and expressed as the percentage of its dry mass, described by Folch et al. (1951) and Singh and Sinha (1977).

Mass of rose petals before extraction =  $m_1$ , Mass of the rose petals after extraction =  $m_2$ , the mass of lipid in the rose petals,  $m = m_1 - m_2$

$$\text{Total lipid (\%)} = (m/m_1) \times 100 \dots\dots\dots(i)$$

### Total carbohydrate

To determine the carbohydrate content of the dried rose petals at 70°C for two days and pulverised rose petals (0.1-0.4 g) were homogenized in 2-5 ml of distilled water. The 0.1ml aliquot of this homogenate was diluted to 1.0 ml by adding 0.9 ml of distilled water. This 1.0 ml sample of the homogenate was treated with 4.0 ml of Drywood's Anthrone reagent, reported by Morris (1948), Fairbairn (1953), Singh et al. (1976). The control sample comprised of 1 ml of double distilled water treated with 4.0 ml of Anthrone reagent only. The optical densities of the sample were taken in a Spectronic 20 UV-Vis spectrophotometer at 620 nm and comparing them with those of known concentrations of glucose standard. For this purpose a regression equation was calculated to describe the relationship (ii) between the optical densities vis-e-vis the glucose concentrations as follows (Fig.1), reported by Campbell (1976).

$$\text{Regression equation: } y = P_1x + C_1 \dots\dots\dots(ii)$$

Where,  $x$  = Concentration of glucose,  $C_1$  = The  $y$  intercept,  $P_1$  = Slope of the line,  $y$  = Optical density of glucose. Here,  $C_1$  and  $P_1$  have unique numerical values calculated from Fig.1.

### Total nitrogen (N<sub>2</sub>)

The nitrogen contents of the dried and pulverized samples of rose inflorescences (at 70°C for 48 h) were determined using the Micro-Kjeldahl's method given in AOAC (1950). Accordingly, 100 mlsamples were digested in 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> (s. d. fine, India) in 30 ml Kjeldahl's flasks for a period of 12 h, until the turbidity of the sample disappeared. Thereafter, the reaction mixtures were diluted up to 100 ml using double-distilled water. 0.1 ml aliquots of this digested reaction mixture were mixed with 2.9 ml of double-distilled water and 1 ml of Nestlers reagent. The optical densities (OD) of the samples so obtained were recorded in a Spectronic 20 UV-Vis spectrophotometer at 440 nm using a reagent blank and ammonium sulphate solution (Spectrachem, India, 132 mg/100 ml) as a standard. For this purpose a regression equation was calculated to describe the relationship between the optical density vis-e-vis nitrogen concentrations (Fig-2), reported by Campbell (1976).

Regression equation:  $y = P_2x + C_2$  .....(iii)

Where,  $x$  = Concentration of nitrogen,  $C_2$  = the y intercept,  
 $P_2$ = Slope of the line,  $y$  = Optical density of nitrogen.  
Here,  $C_2$  and  $P_2$  have unique numerical values calculated from Fig.2.

**Protein contents**

The rose petals were analyzed for the nitrogen content using Micro-Kjeldahl method reported in AOAC (1950) and the corresponding protein contents were calculated by multiplying the total nitrogen content by 6.25, reported by Van Gelder (1981) and Ezeagu et al. (2002).

**Statistical analyses**

*Standard error:* The standard error (SE) of mean was determined using the following equations:

$$\text{Variance}(V) = \frac{[(x_2 - x^2) / n]}{(n - 1)} \dots\dots\dots\text{(iv)}$$

$$\text{Standard Error} = \sqrt{\frac{V}{n(n - 1)}} \dots\dots\dots\text{(v)}$$

Where,  $x$  = Sum of the observed values,  $x_2$  = Sum of squares of observed values,  $n$  = Number of replicates.

*Test of significance (T-test):* The significance of difference ( $\Delta D$ ) between the two means was determined using the t-test method. The values of  $t'$  were calculated using the equation written below:

$$t = \frac{\Delta D}{\sqrt{SE_1^2 + SE_2^2}} \dots\dots\dots\text{(vi)}$$

Where,  $SE_1$ =standard error of first mean, and  $SE_2$ = standard error of second mean.

**III. RESULTS AND DISCUSSION**

We surveyed the rose plantations on different experimental site and identified mainly two aphid species namely *Macrosiphumrosae* and *Macrosiphumrosaeiformis*. The aphids feeding resulted in wrinkled, downward-curling that eventually discolors and wilts the foliage. The population of *Macrosiphumrosae* was always higher than *Macrosiphumrosaeiformis*. The infestations of insect pests cause considerable damage to the individual parts of crop plants and significantly reduce quality as well as quantity of the yield. The results on the effect of aphid infestation with respect to certain nutritional constituents (lipids, carbohydrates, protein levels) elaborated in petals of rose plantations are presented below. The presented results are based on studies in three farm sites e.g. karamcharinagar, phoolbagh and university garden.

**Biochemical analyses of petals of rose at: Karamcharinagar**

The results on quantification of lipids in the inflorescence of rose plants (Table-1, Fig-3) cultivated at ‘Karamcharinagar’ revealed that its level were significantly lower ( $P < 0.05$ ) in the infested plants on the 45<sup>th</sup>-day (242.66 aphids, 5.55% lipid) and 60<sup>th</sup> day (236.33 aphids, 5.89 % lipid) in comparison to that of infested ones (6.05% and 5.89% on 45<sup>th</sup> and 60<sup>th</sup> day respectively). Carbohydrate levels (10.95% and 11.06%) were also significantly lower ( $P < 0.05$ ) as compared to those of uninfested ones (11.67 % and 12.04 %) on the same days. On the other stages of observations (15<sup>th</sup>-day, 30<sup>th</sup>-day, 75<sup>th</sup>-day and 90<sup>th</sup>-day) lipid and carbohydrate levels were not significantly affected by infestation of aphids.

Total N-contents and protein levels revealed a significant decline ( $P < 0.05$ ) in the infested inflorescence on the 30<sup>th</sup> (97.33 aphids, N=4.31% and Protein=26.97%), 45<sup>th</sup> (242.66 aphids, N=4.27% and protein=26.70%) and 60<sup>th</sup>-day (236.33 aphids, N=3.96% and protein=24.79%) as compared to those of uninfested ones (Table-1).

**PhoolBagh**

The results presented in Table-2, Fig-4 indicated that the levels of total lipid in the inflorescence of rose plants cultivated at ‘PhoolBagh’ were significantly lower ( $P < 0.05$ ) in the infested bunches only on the 60<sup>th</sup> day of observations ( 236.66 aphids, 7.38 % lipid) in comparison to those of uninfested ones (7.97%).

The levels of carbohydrates were also significantly lower on the 60<sup>th</sup> day (236.66 aphids, 12.02% lipid ) and 75<sup>th</sup>-days ( 184 aphids, 11.91% lipid) in infested bunches as compared to those of uninfested ones (12.92% and 12.36% respectively), obviously due to higher infestation of aphids (236.66 and 184 individuals/plant on 60<sup>th</sup> and the 75<sup>th</sup>-days respectively). At the initial (30<sup>th</sup>-day, 106.98 aphids) stage of observations no significant difference was noticed in the levels of lipid and carbohydrate.

The observations on the N-contents and protein levels in the inflorescence of rose plants (Table-2, fig-4) did not reveal any significant differences between uninfested and infested ones at the initial stage (30<sup>th</sup> day, 106.98 aphids). However, in the later stages as 45<sup>th</sup> day (155.32 aphids, 3.63 % nitrogen and 22.72% protein), 60<sup>th</sup> day (236.66 aphids, 3.51 % nitrogen and 21.93 % protein) and 75<sup>th</sup> day (184 aphids, 3.45 % nitrogen and 21.58 % protein) the N-contents and protein levels were significantly lower ( $P < 0.05$ ) in infested plants compared to that of uninfested ones.

**University gardens**

The results on quantification of lipids in inflorescence of rose plants (Table-3, Fig-5) cultivated at University garden revealed that the lipid % of infested inflorescence were significantly lower ( $P < 0.05$ ) only on the 45<sup>th</sup> (173.99 aphids, 5.07% lipid) and the 60<sup>th</sup>-days ( 272.99 aphids, 4.42% lipid) as compared to those of infested inflorescence (5.23% and 5.10% respectively). On the remaining days of observations (30<sup>th</sup>-day, 75<sup>th</sup>-day and 90<sup>th</sup>-day) no significant differences was noticed between infested as well as uninfested inflorescence. The levels of carbohydrate (%) in the

infested and uninfested inflorescence of rose plants were always statistically identical and were not affected by the aphids.

The records of N-contents and protein levels in the inflorescence revealed a significant difference ( $P < 0.05$ ) between infested ( $N = 4.03\%$ , protein =  $25.20\%$ ) and uninfested plants ( $N = 4.47\%$ , protein =  $27.95\%$ ) only on the 60<sup>th</sup>-day (272.99 aphids) of observations, due to higher population of aphids on rose inflorescence. On the remaining days (30<sup>th</sup>, 45<sup>th</sup>, 75<sup>th</sup> and the 90<sup>th</sup>-days) no significant difference in N-contents and proteins was noticed between infested and uninfested inflorescence of rose plants.

The levels of nutritional constituents have decreased in all the cases observed at the three locations with the increasing population of aphids. The negative effect of glucosinolates on account of aphid appearance but positive co-relation on nitrogen content has been reported in literature for mustard crop reported by Malik (1981), Gill and Bakhetia (1985), but no studies has been carried out for rose petals. Aphids drain rose sap through their sharp mouth. This sap is a source of carbohydrates, lipid and protein contributing to its medicinal value. The significant inverse relation with the glucosinolates contents in the inflorescence of roses has been observed.

#### IV. CONCLUSIONS

We had carried out an extensive survey of rose plants and reported mainly two species of aphids viz., *Macrosiphum rosae* and *Macrosiphum raeaeiformis*. The biochemical changes observed due to aphid infestation on different parts of rose plants are given below:

- (i) It would degrade the quality of the products made of from rose petals.
- (ii) The most significant changes in lipid, carbohydrate, nitrogen and protein levels in the inflorescence were observed in 45<sup>th</sup> day and 60<sup>th</sup>-day in February when the aphid population was maximum (242.66 and 236.33 aphids respectively) in Karamcharinagar area.
- (iii) In the PhoolBagh area all the four constituents (lipid, carbohydrate, nitrogen and proteins) of inflorescence were also significantly lower ( $P < 0.05$  %) on the 60<sup>th</sup> day when the aphid population (236.66 aphids) was maximum, which also shows a negative co-relation with aphid infestation.
- (iv) The levels of lipid, nitrogen and protein of infested rose inflorescence at University gardens were also statistically lower as compared to those of uninfested ones on the 60<sup>th</sup> day when the aphid population was also maximum (272.99 aphids) in February.

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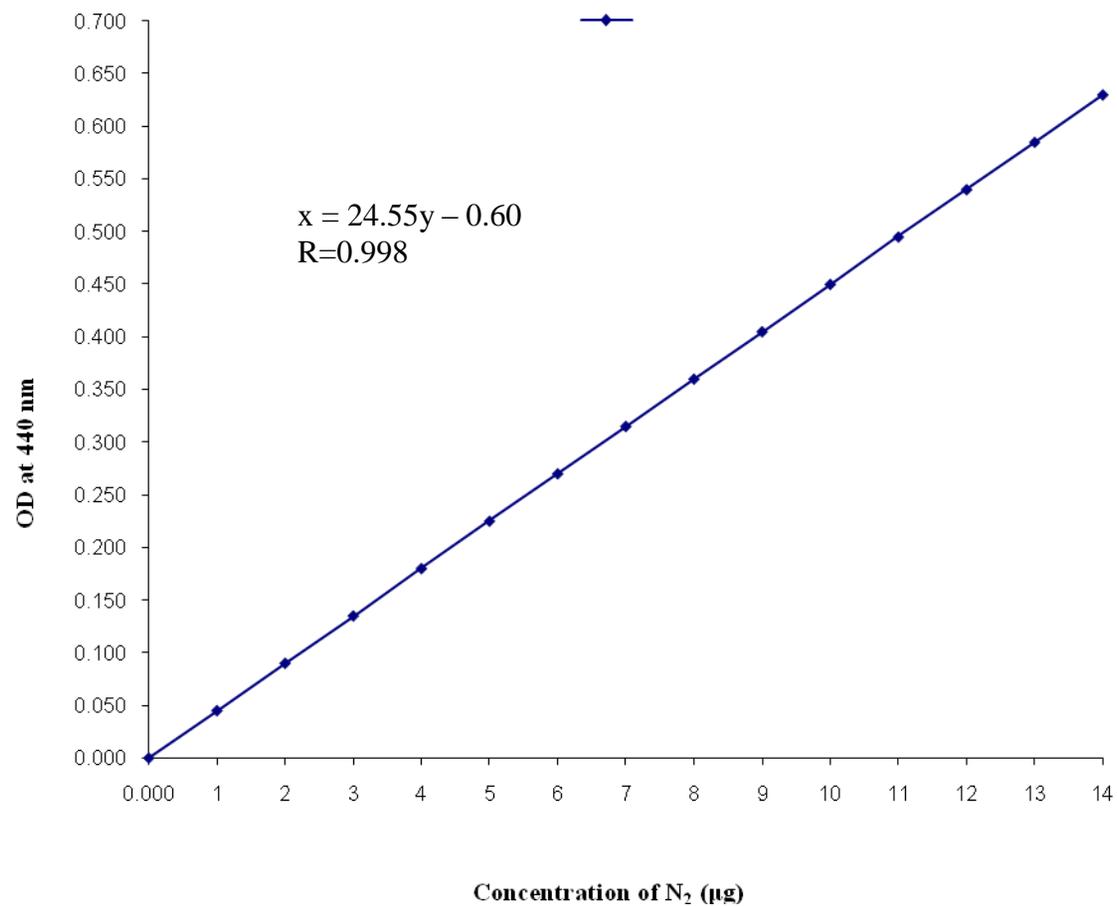
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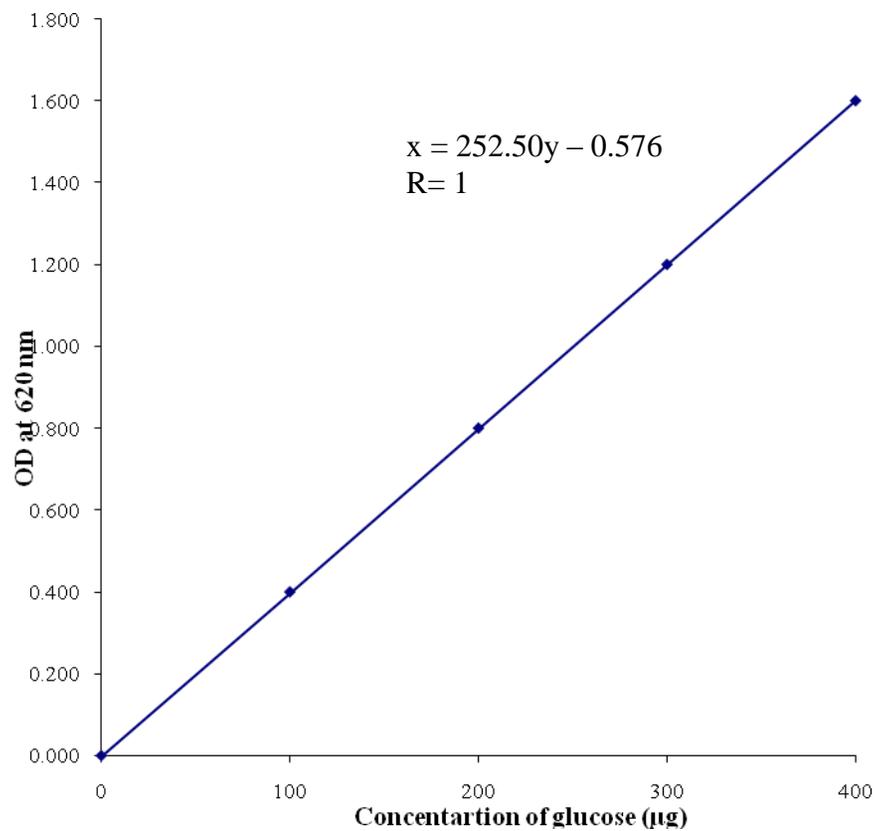
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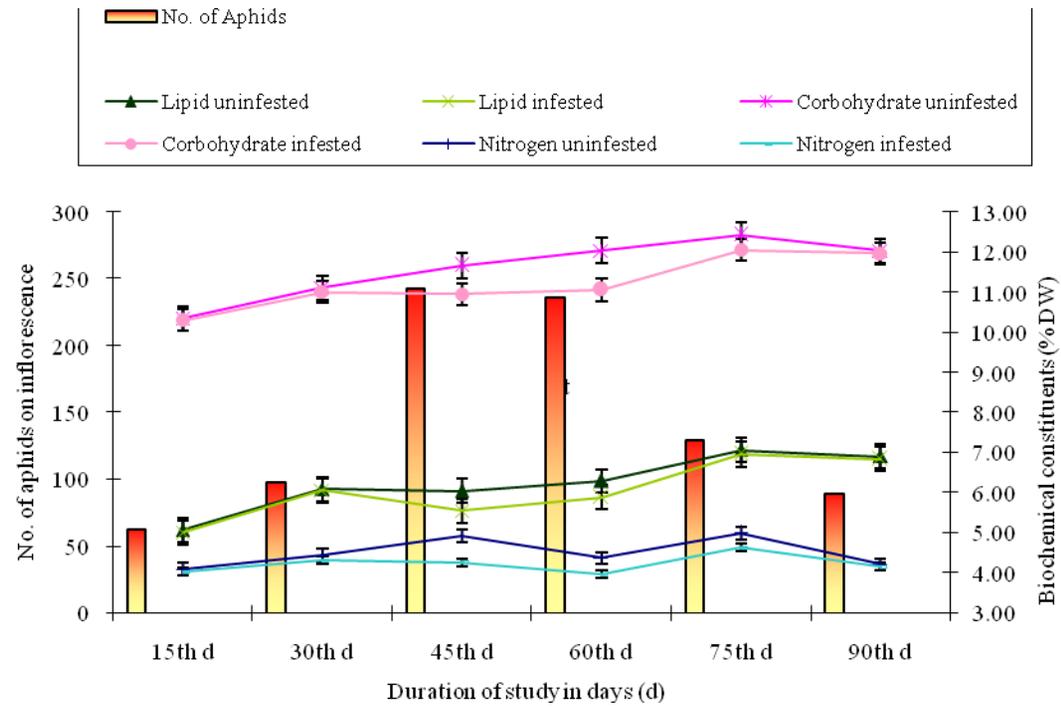
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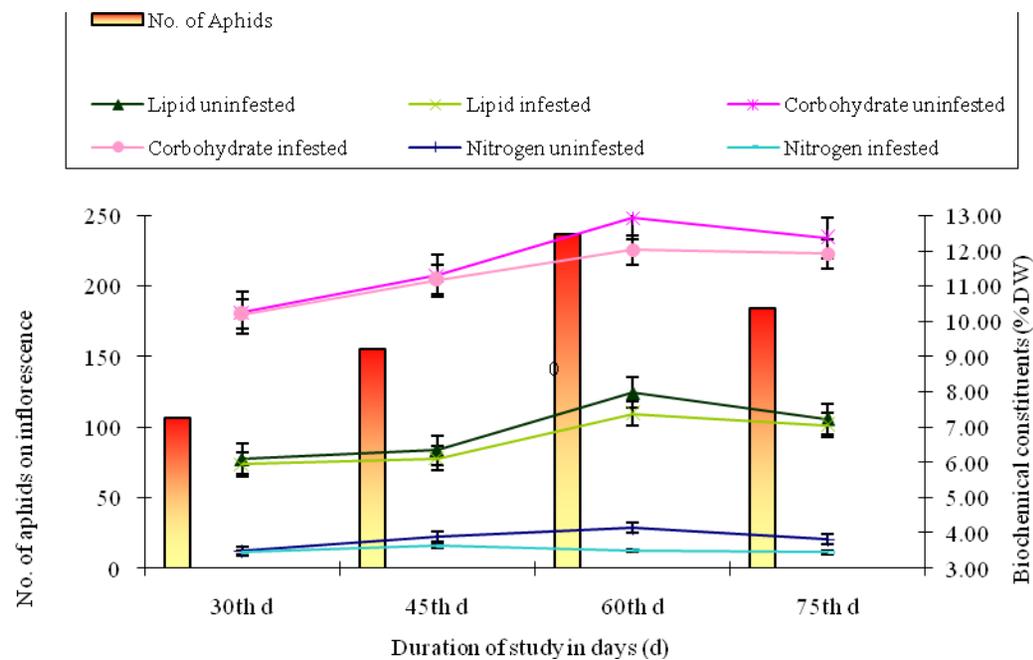
**Fig.1: The optical density (OD) versus concentration of nitrogen (corresponding protein) standard curve at wavelength 440 nm.**



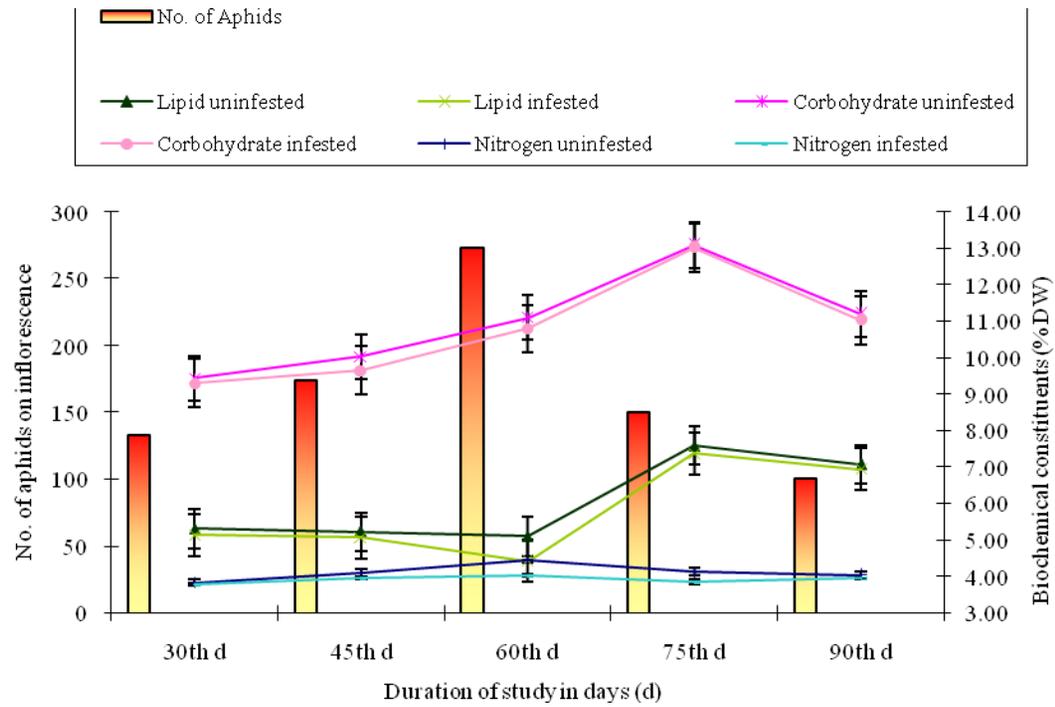
**Fig.2: The optical density (OD) versus concentration of glucose standard curve at wavelength 620 nm.**



**Fig.3: The plot of variation of the biochemical constituents i.e. lipid, carbohydrates, nitrogen and protein of rose inflorescence at Karamcharinagar with number of aphids versus relative duration of study**



**Fig.4: The plot of variation of the biochemical constituents i.e. lipid, carbohydrates, nitrogen and protein of rose inflorescence at PhoolBagh with number of aphids versus relative duration of study**



**Fig.5: The plot of variation of the biochemical constituents i.e. lipid, carbohydrates, nitrogen and protein of rose inflorescence at University Garden with number of aphids versus relative duration of study**

**Table-1: The percentage of lipid, carbohydrates, nitrogen and protein of rose inflorescence at Karamcharinagar with number of aphids and relative duration of study (\*Significant at 5% level, student t-test, table value of 't' at 5%=2.92.)**

S.No.	Duration of study	No. of aphids	Percent biochemical constituents (Mean ±SE)							
			Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
1	15 <sup>th</sup> -day	Nil (Control) 62.66	5.07±0.03	0.98	10.35 ±0.32	0.09	4.09 ±0.04	0.89	25.56 ±0.28	0.89
			5.01±0.04		10.31±0.32		4.03±0.04		25.20±0.27	
2	30 <sup>th</sup> -day	Nil (Control) 97.33	6.09±0.04	0.37	11.11±0.06	1.34	4.44±0.03	3.28	27.76±0.21	3.28
			6.06±0.04		11.01±0.04		4.31±0.01*		26.97±0.10*	
3	45 <sup>th</sup> -day	Nil (Control) 242.66	6.05±0.02	10.91	11.67±0.10	6.42	4.92±0.06	3.73	30.79±0.39	3.73
			5.55±0.03*		10.95±0.05*		4.27±0.16*		26.70±1.02*	
4	60 <sup>th</sup> -day	Nil (Control) 236.33	6.30±0.09	3.89	12.04±0.05	11.41	4.37±0.07	5.20	27.30±0.43	5.20
			5.89±0.04*		11.06±0.06*		3.96±0.03*		24.79±0.21*	
5	75 <sup>th</sup> -day	Nil (Control) 128.99	7.07±0.04	2.36	12.43±0.23	1.51	4.99±0.006	1.65	31.20±0.04	1.65
			6.96±0.03		12.06±0.06		4.63±0.21		29.06±1.26	
6	90 <sup>th</sup> -day	Nil (Control) 189.66	6.91±0.06	0.80	12.03±0.06	0.92	4.21±0.15	0.40	26.35±0.92	0.40
			6.84±0.07		11.97±0.01		4.15±0.03		25.97±0.18	

**Table-2: The percentage of lipid, carbohydrates, nitrogen and protein of rose inflorescence at PhoolBagh with number of aphids and relative duration of study (\*Significant at 5% level, student t-test, table value of 't' at 5%=2.92)**

S.No.	Duration of study	No. of Aphids	Percent biochemical constituents (Mean ±SE)							
			Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
1	30 <sup>th</sup> -day	Nil (Control) 106.98	6.09±0.05	2.56	10.25±0.14	0.27	3.49±0.006	0.77	21.83±0.04	0.78
			5.95±0.02		10.20±0.11		3.45±0.04		21.60±0.29	
2	45 <sup>th</sup> -day	Nil (Control) 155.32	6.34±0.08	2.08	11.30±0.19	0.43	3.89±0.06	3.45	24.30±0.37	3.46
			6.12±0.06		11.18±0.20		3.63±0.04*		22.72±0.25*	
3	60 <sup>th</sup> -day	Nil (Control) 236.66	7.97±0.01	9.62	12.92±0.04	11.09	4.15±0.08	7.43	25.97±0.50	7.45
			7.38±0.06*		12.02±0.06*		3.51±0.03*		21.93±0.20*	
4	75 <sup>th</sup> -day	Nil (Control) 184.00	7.24±0.11	1.57	12.36±0.13	3.09	3.82±0.03	8.30	23.89±0.21	8.32
			7.05±0.05		11.91±0.06*		3.45±0.02*		21.58±0.18*	

**Table-3: The percentage of lipid, carbohydrates, nitrogen and protein of rose inflorescence at University Garden with number of aphids and relative duration of study (\*Significant at 5% level, student t-test, table value of 't' at 5%=2.92)**

S.No.	Duration of study	No. of aphids	Percent biochemical constituents (Mean ±SE)							
			Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
1	30 <sup>th</sup> -day	Nil (Control) 132.66	5.31 ±0.09	1.85	9.44±0.03	1.83	3.84 ±0.02	1.22	24.01±0.13	1.21
			5.14±0.01		9.31±0.06		3.78±0.04		23.64±0.27	
2	45 <sup>th</sup> -day	Nil (Control) 173.99	5.23±0.02	3.93	10.03±0.06	2.15	4.09±0.05	2.52	25.60±0.31	2.53
			5.07±0.04*		9.66±0.16		3.95±0.03		24.68±0.18	
3	60 <sup>th</sup> -day	Nil (Control) 272.99	5.10±0.05	3.65	11.11±0.06	1.93	4.47±0.02	11.67	27.95±0.10	11.77
			4.42±0.17*		10.80±0.15		4.03±0.03*		25.20±0.20*	
4	75 <sup>th</sup> -day	Nil (Control) 150.66	7.59±0.21	0.69	13.09±0.04	1.07	4.13±0.07	2.77	25.85±0.45	2.78
			7.38±0.20		13.02±0.03		3.85±0.07		24.10±0.43	
5	90 <sup>th</sup> -day	Nil (Control) 100.33	7.08±0.04	1.18	11.21±0.14	1.06	4.04±0.04	1.82	25.25±0.25	1.83
			6.95±0.01		11.03±0.08		3.95±0.03		24.68±0.18	

