

Effect of Aphid infestation on the Biochemical Constituents of Mustard (*Brassica juncea*) plant

Pushpa Singh^{1*} and Vijay Kumar Sinhal²

¹Swami Shraddhanand College, Departemnt of Zoology, University of Delhi, Delhi-110007, India.

²Department of Botany, Maharaja Agrasen Mahavidyalaya, Bareilly, UP, India.

Article Info	Summary
Article History Received : 19-02-2011 Revised : 03-05-2011 Accepted : 07-05-2011 *Corresponding Author Tel : +91- 9718563301 Fax : +91-1127662203 Email: pushpa.ysingh@gmail.com ©ScholarJournals, SSR	Mainly two aphid species viz., <i>Lipaphis erysimi</i> (Kalt) and <i>Myzus persicae</i> (Sulzer) were recorded on mustard crop. The infestation of insect pests caused considerable damage to the individual parts (leaf, stem, inflorescence and seeds) of the crop plants and significantly reduced the nutritional constituents (lipid, carbohydrate, nitrogen and protein levels) of mustard plants at different growth stages. <i>Lipaphis erysimi</i> was found to be the most active pest of mustard. These aphids have appeared in the fields especially with the onset of flowering. The loss in biochemical nutritive of plant foliage due to aphid infestation would degrade the quality of the products made of from different parts of mustard crop. Key Words: Mustard crops, Aphid infestation, Biochemical analysis, Nutritional constituents

Introduction

The mustard is a multipurpose crop cultivated in different parts of world. Mustard is used in medicine, food and fodder. Mustard has considered a very nutritive fodder for cows and buffalos. Mustard is also used in traditional medicines for different kind of diseases in India. Among agricultural crops oilseeds are important in the national agricultural economy as they share about 13 % of the country's gross cropped area; contribute about 5 % of the gross national product and 11 % of value of agricultural products. India has the distinction of being one of the world's largest oilseed growers [1] (India 25.6 million hectares and world 125 hectares). Among oilseeds, rapeseeds and mustard are next to groundnut in both cropped area and production in the country and their corresponding share in oilseed production being about 32 % and 39 %. The mustard belongs to the family *Cruciferae* and has been placed in the genus *Brassica*. Indian mustard (*Brassica juncea*) commonly also known as 'raya', 'rai' or laha, is an important oil crop in India.

The production of agricultural crops per unit area is limited by many factors, such as, availability of arable land, farming practices, quality of seeds and damages caused by insect pest. Among various insect pests inflicting severe damages to crop plants aphids have acquired a significant majority particularly due to their polyphagous nature and special mode of reproduction. Mainly two aphid species viz., *L. erysimi* and *M. persicae* were recorded from the mustard plants in which *L. erysimi* was found to be the most active pest of mustard. These aphids have appeared in the fields especially with the onset of flowering. The total population of *L. erysimi* peaked in the month of February was found to be 1515 aphids per plant in the year 2002-03. The maximum aphid population and growth rate was recorded during the last fortnight of February [2,3]. Infestation of insect pests caused considerable damage to

the individual parts (leaf, stem, inflorescence and seeds) of the crop plants and it is expected that significantly reduced the nutritional constituents (lipid, carbohydrate, nitrogen and protein levels) of mustard plants at different growth stages.

Materials and Methods

Survey of aphid infestation on mustard plants was carried out at the Bilwa area near MJP Rohilkhand University of Bareilly, Uttar Pradesh (India) at fortnightly intervals to determine the effect of aphid infestations on mustard plants throughout the crop-season. The farmers were requested to follow the standard doses of N-fertilization (60 kg/ha of Urea) to be applied ½ of it at the time sowing and the remaining half at the time of first irrigation [4].

The Normal as well as infested plants was brought to the laboratory separately (leaf, stem, inflorescence and seeds) in the plastic bags and aphids were collected with a brush, forceps for their taxonomic identifications at different stages of the crop. The Plant parts were analyzed for the total nitrogen levels using Micro-Kjeldahl method [5] and the protein contents of different plant parts were determined by multiplying the contents of Kjeldahl nitrogen by a conversion factor calculated of 6.25 [6,7]. Carbohydrate contents of these plants were determined by the method of Morris [8] and Singh and Sinha [9], where as the lipid contents were estimated using Soxhlet Extraction procedure as described by Folch et. al., [10] and Singh and Sinha [9].

(i) Lipid

For the determination of lipids the dried (70± 1°C; 48h) and weighed samples (leaf, stem, inflorescence and seeds) of mustard plants were extracted in a Soxhlet lipid extractor using a solvent of chloroform AR (CDH, India) and methanol AR (CDH, India) (2:1v/v). For this purpose the weighed samples of the plant parts were kept inside weighed and marked pouches

of Whatman No.1 filter paper (Whatman, UK), which were stapled to prevent the loss of material during extraction. The extractions were carried out for 12 hrs after which the materials were dried and weighed again. The differences in the weight of the extracted samples were taken as the lipid content of the samples and expressed as the percentage of its dry weight [9,10].

Sample weight before extraction = W_1 Sample weight after extraction = W_2 , Lipid amount in the sample $W = W_1 - W_2$

$$\text{Lipid percentage} = \frac{W}{W_1} \times 100$$

(ii) Total carbohydrate

To determine the carbohydrate content of the dried ($70 \pm 1^\circ\text{C}$; 48h) and pulverised samples of mustard plants (0.1-0.04 gm) were homogenized in 2-5 ml of distilled water. 0.1ml aliquot of this homogenate was diluted to 1 ml by adding 0.9ml of distilled water. This 1 ml sample of the homogenate was treated with 4.0 ml of Drywood's Anthrone reagent [8,11,12]. The control sample comprised of 1 ml of double distilled water treated with 4 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 between the optical densities vis-e-vis the glucose concentrations as follows [13].

Regression equation: $y = B_0 + B_1 x$

Where, y = Concentration of glucose, B_0 = The y intercept, B_1 = Slope of the line, x = Optical density of glucose. Here, B_0 and B_1 have unique numerical values.

(iii) Total nitrogen (N_2)

The nitrogen contents of the dried ($70 \pm 1^\circ\text{C}$; 48 h) and pulverized samples of mustard plants (leaf, stem, inflorescence and seeds) were determined using the Micro-Kjeldahl's method [5]. Accordingly, 100 ml samples were digested in 5 ml of concentrated H_2SO_4 (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 upto 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 [13].

Regression equation: $y = B_0 + B_1 x$

Where, y = Concentration of nitrogen, B_0 = the y intercept, B_1 = Slope of the line, x = Optical density of nitrogen, Here, B_0 and B_1 have unique numerical values.

(iv) Protein contents

The protein contents of different plant parts were determined by multiplying the contents of Kjeldahl nitrogen by a conversion factor calculated of 6.25 [6,7].

(v) Statistical analyses

(a) **Arithmetic mean:** The individual data was transformed into arithmetic mean calculated as follows:

$$\text{Mean} = \bar{X} = \frac{\sum x_i}{N}$$

Where, Σ = Summation of all the individual observed values, x_i = observed data, N = Number of data

(b) **Standard error:** The standard error of mean (SE) were also determined using the following calculations:

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

$$\text{Standard Error (SE)} = \sqrt{\frac{[(x_2 - x^2) / n]}{n(n - 1)}}$$

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

(c) **Test of significance (T-test):** The significance of difference between the means was determined by carrying out the t-test. Accordingly, the values of 't' were determined as follows:

$$t = \frac{\text{Difference in two means}}{\sqrt{SE_1^2 + SE_2^2}}$$

Where, SE=standard error

Results

We surveyed the mustard crop at experimental site and identified the two aphid species namely *L. erysimi* and *M. persicae*. The population of *L. erysimi* was always higher than *M. persicae*. The highest population of aphids was found during 100 to 115 days old crop. The infestations of insect pests cause considerable damage to the individual parts of crop plants and significantly reduce the yield. The results on the effect of aphid infestation with respect to certain nutritional constituents (lipids, carbohydrates, nitrogen and protein levels) elaborated in different parts of mustard crop are presented below.

Biochemical analyses of different parts of mustard plants

(i) Leaf

The results presented in (Table-1 Fig-1) revealed that the levels of total lipid in the infested leaves of 'mustard plants' of Bilwa were significantly ($P < 0.05$) lower at the ages of 70 days (20.76%), 85 days (19.86%) and 100 days (18.53%) in comparison to those of uninfested ones. On the 115 days and 130 days of observations no significant difference was noticed between infested as well as uninfested leaves.

A similar trend of general decline in the levels of carbohydrates in the leaves was also recorded both in the infested and uninfested plants, but carbohydrate % in the infested leaves were significantly ($P < 0.05$) lower only on the 115th day (9.68 %) obviously on account of rapid population build-up of aphids.

The records of N-contents and protein levels in the leaves of plants also revealed that aphids significantly ($P < 0.05$) reduced

these nutrients as the crop matured with effect from its age of 70 days (N=3.95%, Protein=24.68% in uninfested plants and N=3.84%, Protein=23.99% in the infested plants) to the age of 130 days (N=2.82%, Protein=17.64% in uninfested plants and N=2.78, Protein=17.39% in the infested plants) suggesting that the infestation of aphids adversely effected the elaboration of proteins in the leaves of mustard plants. Normally also, the % of proteins declined from 24.68% at the age of 70 days (uninfested) to 17.64 % at the plant age of 130 days.

(ii) Stem

The observations on the stem of mustard plants (Table-2, Fig-2) revealed the lipid % of infested stem were significantly lower on the plant age of 85 days (15.73%) and 115 days (14.10%) in comparison to those of uninfested ones (16.85% and 17.01% respectively). On the other days (70 days, 100 days and 130 days) the lipid contents did not differ significantly.

The levels of carbohydrate (%) in the stem did not significantly differ in the control (12.00%) and the infested ones (11.89%) at the age of 70 days. On the subsequent days of observations, the carbohydrates content in the stem of infested plant were significantly lower ($P<0.05$) (9.87% at the age of 85 days, 9.45% at the age of 100 days, 8.40% at the age of 115 days and 8.11% at the age of 130 days) in comparison to those of uninfested plants. Generally, however, the levels of carbohydrate in uninfested stems declined from 12.00% (70-day-old plants) to 8.91% (130-day-old plants). A similar trend of decline was recorded in the infested stems also (11.89% on 70-days-old to 8.11% in 130-days-old plants).

The records of N-contents and protein levels in the stem of mustard plant revealed that infestation of aphids significantly reduced ($P<0.05$) these nutrients at the growth stages of 85 days (N=2.52% and Protein 15.77%) and 115 days (N=2.00% and Protein 12.54%) only. Normally also the % of nitrogen and protein declined from 2.84% and 17.74% at the age of 70 days (uninfested) to 1.91% and 12.05% respectively in infested plants at the age of 130 days.

(iii) Inflorescence

The results on quantification of lipids in the inflorescence of the mustard plants (Table-3, Fig-3) revealed that the levels of total lipid in the inflorescence of infested plants were significantly lower on the plant ages of 55 days (25.56%), 70 days (24.56%), 85 days (22.46%) and 100 days (20.60%) in comparison to those of the uninfested ones. It was also

apparent that the lipid % in the inflorescence declined more rapidly in the infested plants than those of uninfested ones, primarily on account of aphid infestation, which peaked in the 100th day of plant age.

A similar trend of general decline in the levels of carbohydrates in the inflorescence was also recorded both in the infested and uninfested plants, but carbohydrate % in the infested inflorescence was significantly lower ($P<0.05$) on 55 days (15.09%), 70 days (15.03%), 100 days (13.38%) and 115 days (12.50%) obviously on account of rapid population build-up of aphids.

The records of N-contents and protein levels in the inflorescence of mustard plants also revealed that infestation of aphids significantly reduced ($P<0.05$) these nutrients as the crop matured with effect from its age of 55 days (N=4.62%, Protein=29.00% in uninfested plants, and N=4.32%, Protein=27.07 % in infested plants) to the age of 115 days (N=3.22%, Protein=20.94% in uninfested plants and N=3.01%, Protein=18.81% in infested plants), suggesting that the infestation of aphids adversely effected the elaboration of protein in the inflorescence of mustard plants. Normally also, the % of proteins declined from 29.00% at the age of 55 days (uninfested) to 20.94 % (uninfested plants) at the plant age of 115 days.

(iv) Seeds

The results on analysis of lipids in the seeds of mustard plants (Table-4, Fig-4) revealed that the plant growth stage of 100 days no significant differences were noticed in the levels of lipids of seeds of infested and uninfested plants. On the subsequent days (115 days and 130 days) of observations, the total lipid contents of the infested seeds were significantly lower ($p<0.05$) (35.64% and 36.20% respectively) as compared to those of uninfested plants (37.85% and 36.96% respectively).

The levels of carbohydrates (%) in the seeds of mustard plants remained statistically identical at all the stages of plant growth although somewhat lower levels were recorded in case of 115-days and 130-days-old plants.

The observations on the total N-contents and protein levels revealed significant differences ($p<0.05$) between the seeds of uninfested (3.93% and 24.60% respectively) and infested (2.93% and 18.33% respectively) plants only at the growth stage of 115 days. Observations in case of seeds of 100-days and 130-days-old plants were statistically identical

Table 1: The level of biochemical constituents of mustard leaves from infested and uninfested plants

Age of plant	No. of aphids	Percent biochemical constituents (Mean $\pm$ SE)							
		Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
70-days	Nil (Control)	23.20 $\pm$ 0.23	5.57	13.58 $\pm$ 0.04	0.98	3.95 $\pm$ 0.03		24.68 $\pm$ 0.18	3.90
	22.00	20.76 $\pm$ 0.37*		13.31 $\pm$ 0.27		3.84 $\pm$ 0.02*	3.90	23.99 $\pm$ 0.12*	
85-days	Nil (Control)	20.92 $\pm$ 0.07	3.85	13.01 $\pm$ 0.04	2.75	3.79 $\pm$ 0.02	3.40	23.68 $\pm$ 0.13	3.39
	35.33	19.86 $\pm$ 0.59*		12.78 $\pm$ 0.07		3.62 $\pm$ 0.04*		22.66 $\pm$ 0.27*	
100-days	Nil (Control)	19.15 $\pm$ 0.07	2.97	11.00 $\pm$ 0.03	1.27	3.50 $\pm$ 0.01	2.00	21.87 $\pm$ 0.07	1.99
	55.00	18.53 $\pm$ 0.35*		10.88 $\pm$ 0.09		3.39 $\pm$ 0.05		21.20 $\pm$ 0.32	
115-days	Nil (Control)	18.08 $\pm$ 0.04	2.73	10.98 $\pm$ 0.06	15.30	3.38 $\pm$ 0.06	4.42	21.14 $\pm$ 0.37	3.50
	198.00	16.50 $\pm$ 0.58		9.68 $\pm$ 0.06*		2.96 $\pm$ 0.07*		17.62 $\pm$ 1.08*	
130-days	Nil (Control)	15.76 $\pm$ 0.15	0.93	9.55 $\pm$ 0.03	2.40	2.82 $\pm$ 0.02	0.86	17.64 $\pm$ 0.09	0.86
	15.33	15.62 $\pm$ 0.04		9.31 $\pm$ 0.09		2.78 $\pm$ 0.04		17.39 $\pm$ 0.27	

*Significant at 5% level, student t-test, table value of t at 5%=2.92

Table 2: The level of biochemical constituents of mustard stem from infested and uninfested plants

Age of plant	No. of aphids	Percent biochemical constituents (Mean $\pm$ SE)							
		Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
70-days	Nil								
	(Control)	17.26 $\pm$ 0.37	0.49	12.00 $\pm$ 0.06	1.06	2.84 $\pm$ 0.30	1.80	17.74 $\pm$ 0.79	1.79
85-days	26.66	17.08 $\pm$ 0.04		11.89 $\pm$ 0.29		2.75 $\pm$ 0.04		17.22 $\pm$ 0.22	
	Nil								
100-days	(Control)	16.85 $\pm$ 0.03	6.25	11.65 $\pm$ 0.03	22.10	3.02 $\pm$ 0.04	8.82	18.91 $\pm$ 0.28	8.09
	111.33	15.73 $\pm$ 0.18*		9.87 $\pm$ 0.07*		2.52 $\pm$ 0.04*		15.77 $\pm$ 0.27*	
115-days	Nil								
	(Control)	16.39 $\pm$ 0.02	2.77	10.52 $\pm$ 0.07	14.95	2.61 $\pm$ 0.02	2.44	16.35 $\pm$ 0.13	2.47
130-days	163.33	15.16 $\pm$ 0.44		9.45 $\pm$ 0.02*		2.40 $\pm$ 0.09		14.99 $\pm$ 0.54	
	Nil								
115-days	(Control)	17.01 $\pm$ 0.04	4.96	10.03 $\pm$ 0.03	38.98	2.27 $\pm$ 0.01	15.89	14.23 $\pm$ 0.05	24.14
	292.00	14.10 $\pm$ 0.59*		8.40 $\pm$ 0.02*		2.0 $\pm$ 0.008*		12.54 $\pm$ 0.05*	
130-days	Nil								
	(Control)	14.03 $\pm$ 0.03	1.84	8.91 $\pm$ 0.02	12.47	1.96 $\pm$ 0.02	0.70	12.27 $\pm$ 0.11	0.47
	70.00	13.50 $\pm$ 0.29		8.11 $\pm$ 0.06*		1.91 $\pm$ 0.07		12.05 $\pm$ 0.46	

*Significant at 5% level, student t-test, table value of 't' at 5%=2.92

Table 3: The level of biochemical constituents of mustard inflorescence from infested and un-infested plants

Age of plant	No. of aphids	Percent biochemical constituents (Mean $\pm$ SE)							
		Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
55-days	Nil								
	(Control)	29.56 $\pm$ 00.23	9.73	16.44 $\pm$ 00.03	24.51	4.62 $\pm$ 00.05		29.00 $\pm$ 0.38	5.06
70-days	114.33	25.56 $\pm$ 00.34*		15.09 $\pm$ 00.05*		4.32 $\pm$ 00.01*	6.04	27.07 $\pm$ 0.09*	
	Nil								
85-days	(Control)	26.77 $\pm$ 00.57	9.17	16.09 $\pm$ 00.10	9.51	4.39 $\pm$ 00.01	3.30	27.47 $\pm$ 0.09	3.31
	133.33	24.56 $\pm$ 00.23*		15.03 $\pm$ 00.04*		4.20 $\pm$ 00.06*		26.25 $\pm$ 0.36*	
100-days	Nil								
	(Control)	25.08 $\pm$ 00.04	6.92	15.02 $\pm$ 00.05	2.02	3.96 $\pm$ 00.008	15.63	24.76 $\pm$ 0.05	15.56
115-days	143.33	22.46 $\pm$ 00.38*		14.74 $\pm$ 00.13		3.61 $\pm$ 00.02*		22.56 $\pm$ 0.13*	
	Nil								
130-days	(Control)	23.09 $\pm$ 00.05	3.54	14.43 $\pm$ 00.09		3.56 $\pm$ 00.03	6.01	22.27 $\pm$ 0.21	5.99
	223.33	20.60 $\pm$ 00.70*		13.38 $\pm$ 00.03*	10.60	3.18 $\pm$ 00.05*		19.91 $\pm$ 0.32*	
115-days	Nil								
	(Control)	21.25 $\pm$ 00.14	2.67	13.15 $\pm$ 00.05	11.51	3.22 $\pm$ 00.04	5.01	20.94 $\pm$ 0.23	4.99
	152.33	19.76 $\pm$ 00.54		12.50 $\pm$ 00.31*		3.01 $\pm$ 00.02*		18.81 $\pm$ 0.13*	

*Significant at 5% level, student t-test, table value of 't' at 5%=2.92

Table 4: The level of biochemical constituents of mustard seeds from infested and un-infested plants

Age of plant	No. of aphids	Percent biochemical constituents (Mean $\pm$ SE)							
		Lipid	t-value	Carbohydrate	t-value	Nitrogen	t-value	Protein	t-value
100-days	Nil								
	(Control)	28.56 $\pm$ 0.25	2.19	16.25 $\pm$ 0.14	0.78	3.94 $\pm$ 0.05	2.12	24.62 $\pm$ 0.29	2.11
115-days	94.00	27.90 $\pm$ 0.16		16.12 $\pm$ 0.08		3.50 $\pm$ 0.20		21.87 $\pm$ 1.27	
	Nil								
130-days	(Control)	37.85 $\pm$ 0.49	3.34	29.98 $\pm$ 0.11	2.66	3.93 $\pm$ 0.04	8.60	24.60 $\pm$ 0.17	8.94
	173.32	35.64 $\pm$ 0.45*		28.74 $\pm$ 0.46		2.93 $\pm$ 0.11*		18.33 $\pm$ 0.68*	
130-days	Nil								
	(Control)	36.96 $\pm$ 0.03	18.08	25.14 $\pm$ 0.21	0.77	4.02 $\pm$ 0.04	2.56	25.17 $\pm$ 0.23	1.61
	30.00	36.20 $\pm$ 0.03*		24.96 $\pm$ 0.01		3.81 $\pm$ 0.07		23.83 $\pm$ 0.80	

*Significant at 5% level, student t-test, table value of 't' at 5%=2.92

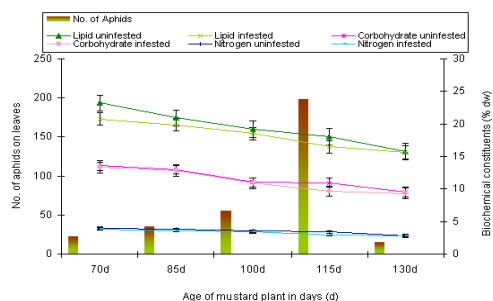


Fig. 1: Variation of biochemical constituents of mustard leaves as a function of age of plant and no. of aphids

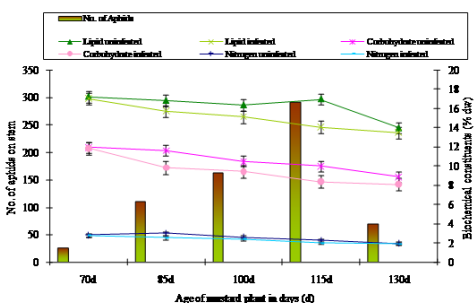


Fig. 2: Variation of biochemical constituents of mustard stem as a function of age of plant and number of aphids

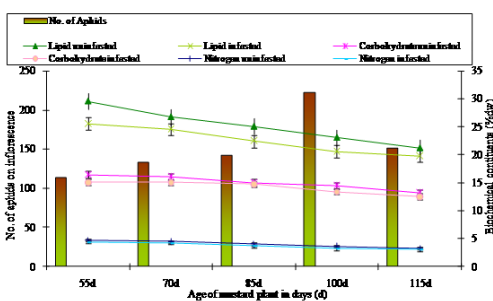


Fig. 3: Variation of biochemical constituents of mustard inflorescence as a function of age of plant and number of aphids

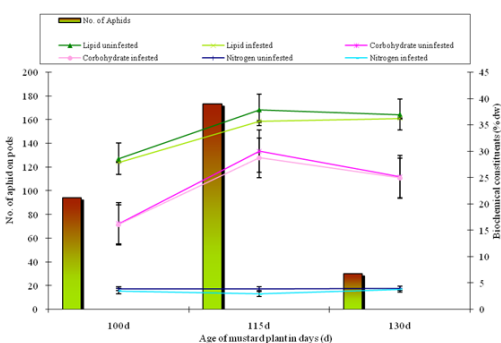


Fig. 4: Variation of biochemical constituents of mustard seeds as a function of age of plant and number of aphids.

Discussion

It was observed from the present study that the levels of biochemical constituents (lipids, carbohydrates, nitrogen and protein) should have some degree of negative co-relation with the extent of aphid infestation. The negative effect of glucosinolates on account of aphid appearance but positive co-relation on nitrogen content has been reported in literature [14, 15]. Any influence of aphids on the levels of nitrogen in mustard has not been observed earlier [16]. The significant inverse relation with the glucosinolates contents on leaves, stem, and inflorescence of Brassica genotypes has been reported, although reducing sugars, nitrogen and phenol contents had no relation with mustard aphid population [17]. However, Basky & Fónagy reported Individual kernel mass reduction is the major factor in yield loss caused by aphids. Aphid feeding did not change the protein structure of hard-kernel wheat [18].

Conclusions

We had carried out an extensive survey of mustard crop and reported two species of aphids viz., *L. erysimi* and *M. persicae*. The biochemical changes observed due to aphid infestation on different parts of mustard plants are given below:

- The most significant changes in lipid and protein levels in leaves were observed in 70-days-old crop and carbohydrate and nitrogen level changed significantly in 115-days-old crop.
- The most significant changes in biochemical constituents (lipid, carbohydrate, nitrogen and protein) of stem were observed in 85-days and 115-days-old crop.
- The most significant changes in the lipid and carbohydrate levels of mustard inflorescence were observed in the initial stage of plants on 55-days-old crop however nitrogen and protein levels were changed most significantly on 85-days-old crop.
- The level of lipid in the seeds of mustard were changed significantly on 130-day-old crop but the levels of carbohydrate, nitrogen and protein were changed significantly on 115-days-old crop.

These results are indicative of loss of biochemical nutritive in the mustard plants caused by aphid infestation. It would degrade the quality of the products made of from different parts of mustard crop.

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