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Biochemical Constituent of *Brassica Juncea* Genotypes In Relation To Mustard Aphid (*Lipaphis Erysimi* Kalt.) Infestation

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Abstract
Selected thirty genotypes of *Brassica juncea* were evaluated for mustard aphid infestation on the basis of average number of aphids per plant and their bio-chemical analysis was carried out at flowering stage. Higher sinigrin content was obtained in genotype RH 7846 (73.49 µ mole/g DM) and lower in Kranti (31.02 µ mole/g DM). A highly significant and negative correlation was observed between sinigrin content and aphid infestation. Phenol content varied from 1.4 % (Varuna) to 2.44 % (RH 7846). The correlation coefficient between phenol content and aphid infestation index was negative and significant. The amount of waxes in leaves of *Brassica juncea* genotypes varied from 2.62 % (RB 50) to 5.22 % (RH 8701 & RLM 198). The correlation coefficient between waxes content in leaves of plant and mustard aphid infestation was negative and non-significant.

Keywords: Biochemical, Mustard Aphid, Infestation

Introduction
Among the biotic stresses, damage caused by aphids is a major constraint in the growth and productivity of mustard crop. Aphids (Sternorrhyncha: Aphididae) are exclusive phloem feeders distributed worldwide. They cause serious losses to cultivated plants, their economic impact is related to highly efficient colonization and settlement. All growth stages of the mustard crop are attacked by aphids but the greatest damage is done during the flowering and pod formation stages (Agarwal *et al*, 1996) [1]. Both the nymphs and adult aphids devitalize the crop by sucking the cell sap. Losses in yield of oilseed *Brassica* due to attack by *L. erysimi* have been reported several times and the mean loss in yield has been estimated to vary from 35.4 to 91.3% (Singh and Sachan, 1994) [24] under different agro climatic conditions and is about 54.2% on all India basis (Bakhetia and Sekhon, 1989) [3].
Host plant resistance to insect pests is a vital component of integrated pest management because of low impact on non target organisms and the environment. Painter (1951) [20] classified the cause of host plant resistance to herbivores as non-preference (renamed antixenosis by Kogan and Ortman, 1978) [16], antibiosis and tolerance. One of the most promising examples of plant-based resistance (antixenosis) is the reduced survival of pests on waxy plant species (Eigenbrode and Shelton, 1990; Jenks and Ashworth, 1999; Eigenbrode and Pillai, 1998) [8, 15, 7]. Moreover, plants contain hundreds of different chemicals compounds, some of these play significant roles in resistance against their herbivores. Phenolic compounds are part of the natural defense system against insects, fungi, viruses and bacteria and they can act as plant hormone controllers. Sinigrin is a naturally occurring glucosinolate which is found in plants of the family Brassicaceae, abundantly present in *B. juncea.* Sinigrin, in particular, is a glucosinolate of interest due to its ability to degrade to AITC (Allyl Isothiocynate) upon combining with water and myrosinase (Belliveau *et al,* 2010) [4].
The availability of resistant sources has been greatly emphasized all over the world as one of the most appropriate tool of the integrated pest management as it is easily to adopt, economical and safer than chemical pesticides. The importance of assessment of biochemical resistance can be well understood by looking into the fact that there is some genotypes are reported to differ significantly in their levels of resistance but none was found to be immune to aphids. Therefore, the aim of the present research was to study the defence mechanism of Indian mustard genotypes against mustard aphid.

Material and methods

Plant material
We carried out experiments on *B. juncea* plants during the flowering season, a time when aphids can be found in high numbers on the flowering stems. There is evidence that flowering stems have high levels of anti -herbivore protection, perhaps because damage to the reproductive parts of the plants may have fitness costs. Preference of *L. erysimi* to different *B. juncea* was studied in the experimental field of Plant Breeding and Genetics Division, Indian Agricultural Research Institute, New Delhi during 2008-09 and 2009-10. The seeds of Indian mustard genotypes were sown late in Plot size measured 4.5 x 2.5 m^2 with a row to row spacing of 45 cm for aphid infestation. After germination all the cultural practices were performed throughout the growing season; however insecticides were not sprayed in and around the experimental area.

Observation on Aphid Population
The observations on aphid population were recorded at weekly intervals starting with invasion of aphids (last week of January). Five plants from each plot, i.e. 15 plants of each genotype were selected at random and tagged for aphid counts throughout the crop season. The population was estimated by counting aphids on a measured part (10cm) of plant.

Sampling and analysis
Biochemical compounds were extracted from plants of genotypes of *B. juncea* at 80 days after sowing. The leaf samples of six competitive and randomly selected plants were collected from genotypes and brought in the laboratory under separates cover of polythene sheets. These samples were sun dried for 3 days and later on kept in an incubator at 70ºC for 3 days.
Later on the samples were ground and kept in polythene bags for further quality analysis. Total phenol (per cent), wax (per cent), and sinigrin (µ mole/gram dry matter) contents were estimated in leaf of the plant.

Estimation of Phenol
The estimation of phenol was done by the method of Swain and Hill (1959) [27].
To 1ml of sample, 0.5 ml Folin-Ciocalteau reagent was added and contents were shaken thoroughly. After 3 min. 1ml of saturated sodium carbonate solution was added. The volume was made to 10 ml with water and placed in dark for one hr. The absorbance was read at 725 nm and of total soluble phenolics in the extract was calculated from a standard curve prepared with gallic acid.

Estimation of sinigrin
The estimation of sinigrin was done by high performance liquid chromatography (HPLC) according to the 'NF EN ISO 9167-1' method as suggested by Haddad and Allaf (2007) [11].
Samples of 200 mg introduced in to 10ml tubes that were plugged into a water bath at 75^{0}C for 1 min. 2 ml of boiling methanol (70%) was added in tubes, kept for 1 hr in the water bath and shaken every 20 min. Then tubes were cooled at room temperature before being centrifuged at 960g for 3 min. the supernatant was poured into 5ml crystal tubes. Two ml of boiling methanol (70%) was added to the tubes containing the sediments (oil cakes) the procedure was repeated as before. Finally, the crystal tubes supernatants were adjusted with pure water and homogenized.

Purification and desulfatation of the extracts
One ml of extract was placed in an ion-exchange column containing 1 ml Sephadex DEAEA25 and allowed to run off. Then, 1ml of sodium acetate buffer (0.02M, pH4) was added twice and allowed to run off. 75 µl of purified sulfatese (E.C. 3.1.6.1, type H-1 from Helix pomatia) (Sigma) solution were then added and left overnight at room temperature. The desulfo glucosinolates obtained was eluted with 3 ml of water and analyzed by HPLC.

Chromatographic condition for sinigrin analysis
The quantification of sinigrin was done through HPLC analysis using a JASCO MD 1510 apparatus coupled with a DAD JASCO MD 1510 detector. A NUCLEOSIL C18 column was used and the analysis protocol was the following: volume 20µL; sample flow 1mL /min; mobile phase: methanol: phosphate buffer solution pH=7, (95:5); column temperature: 35°C; wavelength: 227 nm.
High resolution and good separation of a sinigrin peak (Fig. 1) obtained from this chromatographic system.

Estimation of Waxes by Colorimetric analysis
Leaves of similar age were taken and wax was extracted by method of Ebercon *et al.* (1977) [6] by colorimetric analysis.
The development of the method was based on the colour change produced due to the reaction of wax with acidic potassium bichromate. The reagent was prepared by mixing 40 ml deionized water with 20 g powered potassium bichromate. The resulting slurry was mixed vigorously with 1 liter concentrated sulfuric acid and heated (below boiling) until a clear solution was obtained. The individual sample consisted of 10 mustard leaves. Each sample was immersed in 15 ml redistilled chloroform for 15 sec. the extract was filtered and evaporated on a boiling water bath, until the smell of chloroform could not be detected. After adding 5 ml of reagent, samples were placed in boiling water for 30 min, after cooling; 12 ml of deionized water was added. Several min were allowed for colour development and cooling and then the optical density of the sample was read at 590 nm. N-tetracosane (C24 Akkane) was used as Standard wax solution because is found very similar to mustard wax.

Statistical Analysis
Correlation coefficient was analyzed by the statistical software 'OP STAT' between total phenol (per cent), wax (per cent), sinigrin (µ mole/gram dry matter) contents and average no. of aphids per plant of thirty genotypes of *B. juncea*.

Results and discussion

Screening of Indian mustard genotypes
The flowering stage of mustard was reported to be most vulnerable stage to *L. erysimi* (Jat *et al.,* 2006; Gupta & Rai, 2005) [14, 10]. The population attained its peak during 5th standard week. These results are similar to the results of Hasan *et al.* (2011) [12] that the incidence of *L. erysimi* appeared on mustard commenced from 2nd standard week and gradually reached its peak in 5th standard week, thereafter population started to decline significantly and reached its lowest.
The population of *L. erysimi* recorded in 5th standard week revealed that the minimum population observed on RH-7846 (15 aphids per 10cm twig) during 2008-2009 crop season whereas during 2009-2010, it was minimum on B-85 glossy (14 aphids per 10 cm twig) attributed the population

development of aphid to the different genotypes of Indian mustard. It was recorded highest on Varuna (170 &110 aphids per 10 cm twig) followed by Kranti (115 & 159 aphids per 10 cm twig) in 2008-09 & 2009-10 (Table1). It was concluded that the population of *L. erysimi* was high during the crop season 2009-2010 as compared to 2008-2009.

All screened genotypes were grouped on the basis of average number of aphids present on per 10 cm twig plant of each genotype. Different genotypes were categorized into three groups i.e. highly susceptible, susceptible and tolerant (Table-2).

The genotype harboring less than 50 aphids per 10 cm twig were allotted tolerant group and genotypes having aphid population ranging from 50 to100 (per 10 cm twig) as susceptible, while the rest having aphid population more than 100 aphids as highly susceptible. Based upon the above groupings 14 genotypes were found to be highly susceptible, 17 as susceptible and 8 as tolerant to aphid infestation.

Effect of Variation in biochemical constituent

It was observed by Singh & Sinhal (2011) [26] 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 has been reported by Malik (1981) [18], Gill and Bakhetia (1985) [9].

(i) Sinigrin (µ mole/g DM)

There were significant differences in sinigrin concentration in the leaf tissues among *B. juncea* genotypes (Table 3). Sinigrin varied from 31.02 µ mole/g DM (Kranti) to 73.49 µ mole/g DM (RH 7846) and was more than 60 µ mole/g DM in Pusa jagganath,

Purple Mutant, T-6342, B-85 glossy, CS-54, RH-8701 and had low incidence of mustard aphid per plant where as Krishna, RGN-73, Pusa Bold, Pusa Jaikisan, RH-30 and Kranti had low sinigrin content and high aphid population. Highly significant and negative correlation was observed between sinigrin and aphid infestation (Table-4).

Cartea *et al.* (2011) found that differences in glucosinolate concentrations among the genotypes affected the performance of the herbivores. Gill and Bakhetia, 1985 [9]; Ramdhari *et al.,* 1994 [21], Singh *et al.* 2000 [25], and Anon., 2007 [2] also observed that the genotypes rich in glucosinolate content harboured low aphid population. Rohilla *et al.* (1990) [22]. Screened Eighteen varieties in the field for size of aphid population and found late flowering varieties RL18 and RLM198 were resistant. RLM514, Vardan, RH819 and RH7859 were tolerant.

Work carried out on sinigrin glucosinolate in *B. nigra* shows that generalist and specialist herbivores exhibit different responses to sinigrin, and suggests that heritable variation in sinigrin concentration can be maintained by differential attack from specialists and generalists (Lankau 2007) [17]. James *et al.* (2009) [13] observed that Aphid colony sizes were significantly smaller on plants producing sinigrin, compared with plants producing alternative aliphatic glucosinolates.

The effect of glucosinolate structural variation on six herbivore species was investigated by Newton *et al.* (2009) [19] across two ecological scales. Within populations, *B. brassicae* exhibited a negative response to sinigrin which varied with time of year. *P. brassicae* and snails exhibited a positive response to sinigrin in June, but the direction of the responses of these herbivores changed with time of year. *B. brassicae* and snails showed a consistent negative correlation with sinigrin at both scales, suggesting that their preference for plant metabolic phenotypes may simply scale up to population level patterns, or vice versa.

(ii) Phenol %

Phenol varied from the 1.40 % (Varuna) to 2.44 % (RH-7846). The genotype having phenol content more than 1.7 % in case of RW-2-2, RW white glossy, RC-199,Purple Mutant and B-85 glossy and lower in RH-30,Rohini and Pusa Jaikisan indicating that genotypes having lower phenol content with high aphid population. The correlation coefficient between phenol content and mustard aphid infestation was found negative and significant (Table-4).

These findings are in close conformity with Sachan and Sachan (1991) [23] who reported that phenol contents were higher at 80 days in RW 15-6, RW 2-2 and B 85 (14.67, 13.83 and 12.67 mg/g, respectively) and these varieties were resistant as compared to varieties having low phenol contents. According to Singh *et al.* (2000) [25] PM, T-27 and RK-9501 were less infested by aphid due to higher content of total phenol, flavonoidsand glucosinolates. At Ludhiana, higher amount of total phenols, ortho-dihydroxyphenols, flavonols and glucosinolates were found responsible for low aphid infestation in purple mutant, DLSC-2 and T-27 (Anon. 2007) [2].

(iii) Waxes %

The amount of waxes in leaves of *Brassica* genotypes varied from 2.62 % (RB 50) to 5.22 % (RH 8701& RLM 198), showing that the genotypes having high waxes in leaves i.e. T-6342 (4.97 %), Purple Mutant (5.1 %), and RL-18 (4.94 %) had low infestation of mustard aphid except, Pusa Bold, RH-8701 and Krishna in which comparatively high amount of waxes (4.99 %,5.12%,4.24% respectively) were present but also harboured more infestation. No concrete conclusion could be drawn based on the waxes content in leaves and aphid incidence over a genotype. The correlation coefficients between waxes content in leaves of plant and mustard aphid infestation were negative and non-significant (Table-4).

Table 1: Screening of *B. juncea* genotypes for resistance against mustard aphid at full flowering stage during 2008-09 & 2009-10

Code	Genotype	Mean number of aphids/ plant* (2008-09)	Mean number of aphids/plant* (2009-10)	Avg. Mean number of aphids/ plant*
G1	RLM-514	72	56	64
G2	Vardan	90	70	80
G3	Varuna	170	110	140
G4	RW-2-2	54	34	44
G5	RLM-198	76	102	89
G6	RW white glossy	22	38	30
G7	T-6342	15	29	22
G8	Kranti	115	159	137
G9	RK-9501	58	82	70

G10	RL-18	45	95	70
G11	B-85 glossy	22	14	18
G12	PCR-7	50	122	86
G13	Rohini	72	88	80
G14	RH-30	94	130	112
G15	Pusa Jaikisan	50	74	62
G16	RH-8812	73	87	80
G17	PM	23	17	20
G18	Pusa Bold	69	57	63
G19	RGN-73	92	100	96
G20	RB-50	90	114	102
G21	RN-393	59	62	61
G22	NPJ-93	40	92	66
G23	Krishna	97	143	120
G24	CS-54	47	73	60
G25	RH-819	36	62	49
G26	RC-199	35	65	50
G27	BEC-144	57	75	66
G28	Pusa Jagganath	43	65	54
G29	RH-7846	15	31	23
G30	RH-8701	42	66	59

*Based on 10cm top twig each from 10 plants

Table 2: Grouping of *B. juncea* genotypes on the basis of relative response against mustard aphid incidence at full flowering stage during 2009-09 &2009-10.

Avg. Mean number of aphids/ plant*	Reaction	Number of genotypes	Genotypes
0.0-50	T	8	RW white glossy, T-6342, B-85 glossy, PM, RC-199, RH-7846, RW-2-2, RH-819
50-100	S	17	RGN-73, RN-393, NPJ-93, Pusa Jaikisan, RH-8812, Pusa Bold, RK-9501, RL-18, PCR-7, Rohini, RLM-198, RLM-514, RH-8701, Vardan, CS-54, BEC-144, Pusa Jagganath
100-150	HS	14	Varuna, Kranti, RH-30, Krishna, RB-50

*Based on 10cm top twig each from 10 plants

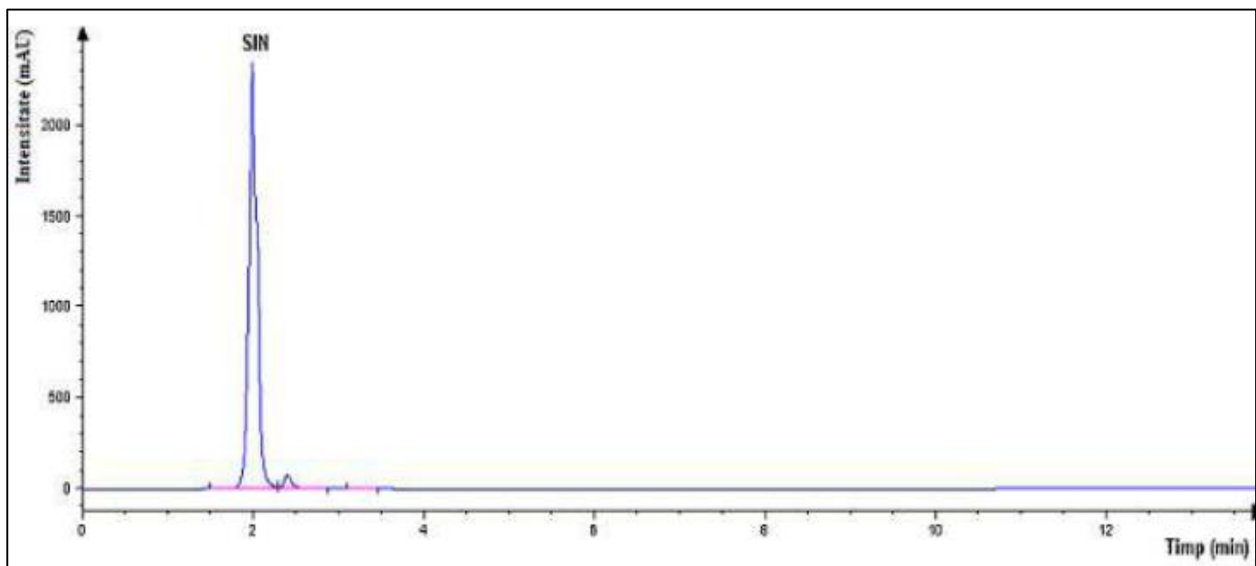

Fig 1: Chromatogram HPLC of sinigrin from Indian mustard

Table 3: Bio-chemical constituents in relation to *L. erysimi* infestation in leaves of *B. juncea* genotypes

Genotypes	Sinigrin µmol/gm DM	Phenol %	Wax %	Avg. Mean number of aphids/ plant*
G1	49.1	1.62	4.11	64
G2	36.2	1.42	3.22	80
G3	38.3	1.4	4.24	140
G4	42	1.7	2.96	44
G5	49.1	1.67	5.12	89
G6	50.2	1.82	4.5	30
G7	64.6	1.39	4.97	22
G8	31.02	1.49	4.02	137
G9	36.36	1.52	3.97	70
G10	50.4	1.62	4.94	70
G11	68.37	1.79	3.9	18

G12	49.1	1.45	4.2	86
G13	48.6	1.41	3.99	80
G14	37.1	1.52	4.05	112
G15	38.2	1.42	3.92	62
G16	42.96	1.61	4.21	80
G17	70.02	1.7	5.1	20
G18	46.6	1.66	4.99	63
G19	43.1	1.48	2.91	96
G20	49.1	1.59	2.62	102
G21	44.2	1.52	3.21	61
G22	59.66	1.61	4.01	66
G23	39.3	1.41	4.24	120
G24	69.1	1.68	3.22	60
G25	40.9	1.63	3.39	49
G26	58.96	1.74	3.22	50
G27	50.1	1.43	2.96	66
G28	70	1.48	3.21	54
G29	73.49	2.44	4.01	23
G30	69	1.56	5.12	59

*Based on 10cm top twig each from 10 plants

Table 4: Correlation coefficient between total phenol, wax, sinigrin content and average no. of aphids/plant of *B. juncea*.

	Sinigrin µmol/gm DM	Phenol %	Wax %	Avg. Mean number of aphids/ plant*
Sinigrin µmol/gmDM				
Phenol %	0.454^{*}			
Wax %	-0.036^{NS}	0.260^{NS}		
Avg. Mean number of aphids/ plant*	-0.529^{**}	-0.498^{**}	-0.203^{NS}	

*Based on 10cm top twig each from 10 plants
** Significant at 5%

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