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Sahab Singh Pippal
Department of Entomology, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India

ML Sharma
Department of Entomology, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India

Sushma Tiwari
Department of Plant Molecular Biology and Biotechnology, RVSKVV, College of Agriculture, Gwalior, Madhya Pradesh, India

Neha Gupta
Department of Biotechnology, Jiwaji University, Gwalior, Madhya Pradesh, India

MK Tripathi
Department of Plant Molecular Biology and Biotechnology, RVSKVV, College of Agriculture, Gwalior, Madhya Pradesh, India

Corresponding Author:
Sahab Singh Pippal
Department of Entomology, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India

Impact of biochemical constituents of varieties against Mustard aphid (*Lipaphis erysimi* Kalt.) infestation

Sahab Singh Pippal, ML Sharma, Sushma Tiwari, Neha Gupta and MK Tripathi

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Abstract
Selected twenty varieties/genotypes of mustard were evaluated for mustard aphid infestation on the basis of average number of aphids per 10 cm apical top twig and their biochemical estimation for parameters phenol, wax, lipid, total sugar, reducing sugar, non-reducing sugar were carried out at flowering stage. Higher phenol content was obtained in genotypes 1.9% (Varuna) and lower in 1.1% (JM-2). A highly significant and negative correlation was observed between phenol content and aphid infestation. Wax content varied from 3.1% (JMWR 908-1) to 4.4% (Shradha). The correlation coefficient between wax content and aphid infestation was negative and non-significant. Lipid content varied from 2.3% (NRCHB-506) to 3.8% (Varuna, Vasundhra). The correlation coefficient between lipid content and mustard aphid infestation was negative and non-significant. Amount of total sugar content varied from 39.7 mg/g (Varuna) to 43.8mg/g (JM-2). The correlation coefficient between total sugar content and aphid infestation was positive and significant. Amount of reducing sugar content varied from 22.5mg/g (Pusa Jaikishan) to 29.9mg/g (JM-3). The correlation coefficient between reducing sugar content and aphid infestation was positive and significant. Amount of non-reducing sugar content varied from 13.1mg/g (NRCHB-506) to 20.1 mg/g (Pusa Jaikishan). The correlation coefficient between reducing sugar content and aphid infestation was negative and significant.

Keywords: Biochemical, mustard aphid, infestation

Introduction
Rapeseed-mustard is the third most important oilseed crop in the world. It contributes about 28.6% in the total oilseed production in India, whereas it is the 2nd most important edible oil seed after peanut sharing 27.8% in India's oilseed economy. In India, rapeseed-mustard occupy 5.99 million ha area with production and productivity of 6.31 million tones and 1053 kg/ha respectively (Kumar *et. al.*, 2018) [9]. The United States and China were the leading importing countries of mustard oil in the world, India was the 7th largest importing country in 2018-19. (Anonymous, 2018) [2]. Indian mustard *Brassica juncea* (L.) is primarily cultivated in Rajasthan, Haryana, MP, UP, and West Bengal (Anonymous, 2021) [1]. Varietal screening for aphid resistance and seed yield stability in aphid-infested and protected environments might assist in the identification of aphid-tolerant varieties (Dey *et al*., 2005) [5]. The loss of production in rapeseed mustard varies depending on the germplasm used and the agro ecological strategies used. (Ansari, *et al*., 2007) [3]. Field testing for aphid resistance and seed yield stability in aphid infested and protected environments would aid in the identification of aphid-resistant varieties. Develop a resistant variety/accession under these conditions, as well as discover the cause of resistance and improve your technique. As a result, the current study aimed to identify the most promising tolerant/resistant *Brassica spp*. accession/variety against *Lipaphis erysimi* Kalt. Plants produce specialized defense compounds that have anti-nutritional effects to overcome the pest attack (Stahl *et al*., 2018) [17]. Certain plant chemicals like constitutive and induced compounds regulate the plant-herbivore interaction (Holopainen and Blande 2013) [8]. However, little is known about the role of different biochemical compounds in *B. Juncea* to impart defense against *L. Erysimi*. Therefore, present studies were conducted to know the variation in development and survival of *L. Erysimi* on diverse *B. Juncea* varieties and understand the role and contribution of certain biochemical compounds in plant defense against mustard aphids.

Plant defense against aphids adversely affects the preference, development and survival resulting in increased plant fitness, which is function of initial selection process (Carrasco *et al*., 2015) [4].

Materials and Methods

Plant Material

We carried out experiments on mustard plants during the flowering season, a time when aphids can be found in high numbers on the apical top twigs. There is evidence that flowering top twigs 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 mustard was studied in the experimental field at Entomological research farm, College of agriculture, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior (MP) during 2019-20 and 2020-21. The seeds of mustard genotypes were sown late in Plot size measured 3.0 x 0.9m. With a row to row spacing of 30 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 number of aphid on 3 randomly selected plant as 10cm apical top twig/plant at weekly intervals starting from aphid infestation up to harvesting stage.

Biochemical estimation

The estimation of phenol was done by the method of Swain and Hill (1959) [18], leaves of similar age was taken, the wax was extracted by the method of Ebercon *et al.*, (1977) [7] by colorimetric analysis, lipid was extracted by the Soxhlet method, and total sugar as per the protocol of Dubois *et al.*, (1956) [6], Reducing sugar by DNS method.

Sampling

Biochemical compounds were extracted from plants of varieties of mustard at 80 days after sowing. The leaf samples of three competitive and randomly selected plants were collected from varieties 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 70 ºC for 3 days. Late on the samples were ground and kept in polythene bags for further quality analysis.

Estimation of Phenol

The estimation of phenol was done by the method of Swain and Hill (1959) [18]. Take 25 mg fresh random leaf samples from the field after 80 days after sowing and crush 25 mg leaf sample in 1ml (80% ethanol) in a mortar pestle until the leaf completely disappears and make a fine liquid solution. Pour the solution (leaf +ethanol) in 2 ml eppendrof tube and vortex for 10 minutes and again centrifuged at 10,000 rpm for 10 minutes then transfer the supernatant in eppendrof tube, supernatant in eppendrof tube in the oven at 60 ºC until gets dried overnight. After drying samples add 1ml distilled water and leave it until it gets dissolved. Now take 50 microliters from a flask with the help of a pipette in an eppendrof tube then add 50 microliters Folin-Ciocalteau Reagent. Add 200 microliters of 20% sodium carbonate and make up the volume up to 1ml with distilled water after boiling at water bath for 1 minute and then keep it at room temperature for 2 hours. At last take absorption at 650 nm in a spectrophotometer.

Estimation of waxes

Leaves of similar age were selected wax was extracted by method of Ebercon *et al.* (1977) [7] 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 gm powered potassium bichromate. The resulting slurry was mixed vigorously with 100 ml concentrated sulfuric acid and heated (below boiling) until a clear solution was obtained. The individual sample consisted of 1gm sample mustard leaves. Each sample was immersed in 0.75 ml redistilled chloroform for 10 minute, and after the samples solution in an eppendrof tube centrifuge at 10,000 rpm for 10 minutes. The extract was filtered and evaporated on a boiling water bath, until the smell of chloroform could not be detected. After adding 0.5 ml of reagent, samples were placed in boiling water for 30 min, after cooling; 1.2 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 in a spectrophotometer.

Estimation of lipid

Leaves of similar age were taken and lipid extracted by soxhlet lipid extractor. The weighted samples of the plant parts were kept inside weighted and marked pouches of filter paper, which were stapled to prevent the loss of material during extraction. Following this extraction step, in thimble is raised above the boiling solvent at 50 ºC to 60 ºC overnight in a soxhlet extractor using a heating mantle. Let the sample cool remove the solvent from the extractor in a rotatory evaporator. After calculating the amount lipid recovered and its percentage in the original sample given below.

Sample weight before extraction = W_1
Sample weight after extraction = W_2
Lipid amount in the sample $W= W_1-W_2$
Lipid percentage = $W/W_1 \times 100$

Estimation of total sugar

Leaves of similar age were taken and Total sugar was extracted by method of Dubois *et al.* (1956) [6]. Take 25 mg fresh random leaf samples from the field after 80 days after sowing and crush 25 mg leaf sample in 1ml (80% ethanol) in a mortar pestle until the leaf completely disappears and make a fine liquid solution. Pour the solution (leaf +ethanol) in 2 ml Eppendorf tube and vortex for 10 minutes Then transferred the supernatant in a fresh 2 ml Eppendorf tube and add more 1ml (80% ethanol) into the old tube which has leaf extract and again centrifuge at 10,000rpm for 10 minutes then transfer the supernatant in Eppendorf, supernatant in Eppendorf tube in the oven at 60 ºC until gets dried overnight. After drying samples add 1ml distilled water and leave it until it gets dissolved. Now take 100 microliters from a flask with the help of a pipette in an Eppendorf tube then add 400 microliters Anthrone Reagent. The Eppendorf tube keeps boiling at water bath 100 ºC for 8 minutes and cools until it comes at room temperature and then takes the absorption at 630nm in the spectrophotometer.

Estimation of reducing sugar

Take 25 mg leaf sample at 80 days after sowing and crush it in liquid nitrogen. In 200ml beaker dissolve 0.5 gm 3, 5 dinitro salicylic acid, slowly add 10ml 2M NaOH and 15gm NaK tartrate make up 50ml dilute solution to final volume with water. Then 100 microliter sample in Eppendorf tube adds 400ul distilled water and 100 microliters DNS reagent. Then mix and shake by vortex for 10 minutes after keep boiling at Water bath 100 ºC for 10 minutes and cool until it comes at room temperature and take absorbance at 540nm in the spectrophotometer.

Estimation of non-reducing sugar

Non-reducing sugar = Total sugar – reducing sugar

Statistical analysis

The correlation coefficient analyzed by the statistical software "SPSS V 19" between phenol (%), wax (%), lipid (%), total sugar (mg/g), reducing sugar (mg/g), and non-reducing sugar (mg/g) and average mean no. of aphids/10cm apical twig/plant. Statistical analysis was done for biochemical profiling. Dendrogram analysis was done by using the function package in NTSYS software.

Results and Discussion

The phenol content varied from the minimum of 1.1% in JM-2 to maximum of 1.9% in Varuna (Table-2). The correlation coefficient between phenol content and aphid infestation was found negative and significant (Table-3). Similar findings were reported by Yadav and Rana (2018) [19] observed that the lower phenol content varieties were infested with a higher aphid population and present findings showed that correlation coefficient between phenol content and aphid infestation were negative and significant. Mishra *et al.* (2019) [20], Kumar *et al.* (2017) [11] also found the same. The amount of wax content in leaves varied from the minimum of 3.1% in JMWR 908-1 to maximum of 4.4 in Shradha. The correlation coefficient between wax content and aphid infestation was found negative and non-significant. These results are in close agreement with finding reported by Yadav and Rana (2018) [19] correlation coefficient between waxes content in leaves of plant and mustard aphid infestation was negative and non-significant. The amount of lipid content in leaves varied from the minimum of 2.3% in NRCHB-506 to maximum of 3.8% in Varuna, Vasundhra. The correlation coefficient between lipid content and aphid infestation was found negative and non-significant. Similar findings reported by Singh and Sinhal (2011) [15] observed that the correlation coefficient lipid content and aphid infestation were negative and non-significant. The amount of total sugar content in leaves varied from the minimum of 39.7mg/g in Varuna to maximum of 43.8mg/g in JM-2. The correlation coefficient between total sugar content and aphid infestation was found positive and significant. Similar results were obtained by Kumar *et al.* (2020) [10] that the correlation coefficient between total sugar content and aphid infestation positive and significant. The amount of reducing sugar content in leaves varied from the minimum of 22.5 mg/g in Pusa Jai kishan to maximum of 29.9 mg/g in JM-3. The correlation coefficient between reducing sugar content and aphid infestation was found positive and significant. The amount of non-reducing sugar content in leaves varied from the minimum of 13.1 mg/g in NRCHB-506 to maximum of 20.1 mg/g in Pusa Jaikishan. The correlation coefficient between phenol content and aphid infestation was found negative and significant. Biochemical quality parameters are important aspect for understanding the nutritional and antinutritional values and has been reported in several crops (Sahu *et al.*, 2020; Sharma *et al.*, 2021; Rathore *et al.*, 2021) [14, 16, 13] Phylogenetic analysis between mustard varieties for nutritional, and antinutritional profile revealed three major clusters based on Dendrogram (Fig.1) we can classify 20 varieties in two major groups A and B having 15 and 5 varieties respectively. Group A is further divided into A_1 and A_2 having 4 and 11varieties respectively, whereas group B is divided into B_1 and B_2 which consisted of 3 and 2 varieties and further division goes on. It is clear the cluster diagram that Variety JM-3 is most diverse to variety Varuna.

Table 1: Screening of mustard varieties against mustard aphid during 2019-20 & 2020-21

S. No.	Varieties	Number of aphids/10 cm apical twig/plant		
		2019-20	2020-21	Pooled Mean
V_1	SEJ- 2	62.31 (7.93)*	60.17 (7.79)	61.24 (7.86)
V_2	NRCHB- 506	65.51 (8.12)	63.62 (8.01)	64.57 (8.07)
V_3	Shradha	49.23 (7.05)	47.48 (6.93)	48.35 (6.99)
V_4	Vardhan	55.39 (7.48)	52.92 (7.31)	54.15 (7.39)
V_5	Pusa Jaikishan	53.62 (7.36)	51.40 (7.20)	52.51 (7.28)
V_6	Vasundhra	52.05 (7.25)	50.16 (7.12)	51.10 (7.18)
V_7	Krishna	48.67 (7.01)	46.87 (6.88)	47.77 (6.95)
V_8	DMH-1	46.47 (6.85)	44.49 (6.71)	45.48 (6.78)
V_9	JM- 1	66.57 (8.19)	64.31 (8.05)	65.44 (8.12)
V_{10}	Albeli	49.65 (7.08)	47.30 (6.91)	48.48 (7.00)
V_{11}	JMWR 908-1	53.01 (7.32)	49.63 (7.08)	51.32 (7.20)
V_{12}	Swaran jyoti	44.91 (6.74)	42.89 (6.59)	43.90 (6.66)
V_{13}	Pusa mahak	52.58 (7.29)	50.54 (7.14)	51.56 (7.22)
V_{14}	RH- 406	48.05 (6.97)	45.72 (6.80)	46.88 (6.88)
V_{15}	Maya	50.65 (7.15)	48.68 (7.01)	49.66 (7.08)
V_{16}	JM-3	69.95 (8.39)	67.85 (8.27)	68.90 (8.33)
V_{17}	JM-4	67.70 (8.26)	66.00 (8.15)	66.85 (8.21)
V_{18}	JM-2	75.06 (8.69)	72.91 (8.57)	73.99 (8.63)
V_{19}	NRCHB-101	64.00 (8.03)	61.98 (7.90)	62.99 (7.97)
V_{20}	Varuna	38.80 (6.27)	37.12 (6.13)	37.96 (6.20)
SE(m)±:		0.05	0.05	0.03
C.D		0.13	0.15	0.10

*Figures in parentheses indicated $\sqrt{x + 0.5}$ transformed value

Table 2: Biochemical constituents of varieties in relation to mustard aphid infestation

S. No.	Varieties	Phenol (%)	Wax (%)	Lipid (%)	Total sugar (mg/g)	Reducing sugar (mg/g)	Non-reducing sugar (mg/g)	Avg. Mean number of aphids/10cm apical twig/plant
V_1	SEJ- 2	1.4	3.6	3.1	42.9	29.3	13.6	61.24
V_2	NRCHB- 506	1.4	3.8	2.3	41.5	28.4	13.1	64.57
V_3	Shradha	1.8	4.4	3.4	41.1	26.1	15.0	48.35
V_4	Vardhan	1.2	3.6	3.6	42.8	24.6	18.2	54.15
V_5	Pusa Jaikishan	1.3	3.8	2.7	42.6	22.5	20.1	52.51
V_6	vasundhra	1.7	4.1	3.8	41.2	26.0	15.2	51.10

V_7	Krishna	1.6	3.9	2.9	42.9	28.1	14.8	47.77
V_8	DMH-1	1.6	3.6	2.4	42.7	27.6	15.1	45.48
V_9	JM- 1	1.2	3.4	3.7	41.8	26.1	15.7	65.44
V_{10}	Albeli	1.8	3.8	3.0	40.7	24.6	16.1	48.48
V_{11}	JMWR 908-1	1.3	3.1	3.1	42.5	23.1	19.4	51.32
V_{12}	Swaran jyoti	1.8	3.2	3.6	41.7	25.1	16.6	43.90
V_{13}	Pusa mahak	1.7	3.6	3.2	42.6	23.9	18.7	51.56
V_{14}	RH- 406	1.3	3.7	3.7	41.7	24.2	17.5	46.88
V_{15}	Maya	1.2	4.1	3.2	43.7	24.8	18.9	49.66
V_{16}	JM-3	1.5	3.4	3.1	43.4	29.9	13.5	68.90
V_{17}	JM-4	1.6	3.6	3.4	42.5	27.5	15.0	66.85
V_{18}	JM-2	1.1	4.1	3.4	43.8	29.7	14.1	73.99
V_{19}	NRCHB-101	1.6	3.7	2.7	41.6	26.7	14.9	62.99
V_{20}	Varuna	1.9	3.6	3.8	39.7	24.2	15.5	37.96

Table 3: Correlation coefficient between biochemical constituents of aphids/10cm apical twig/plant

Biochemical constituents	Phenol	Wax	Lipid	Total sugar	Reducing sugar	Non-reducing sugar	Avg. mean no. of aphids
Phenol	1	.034	.054	-.657**	-.085	-.238	-.512*
wax		1	-.019	-.022	.159	-.180	-0.07
Lipid			1	-.244	-.247	.141	-.162
Total sugar				1	.359	.119	.473*
Reducing sugar					1	-.884**	.642**
Non-reducing sugar						1	-.446*
Avg. mean no. of aphids							1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level 2-tailed).

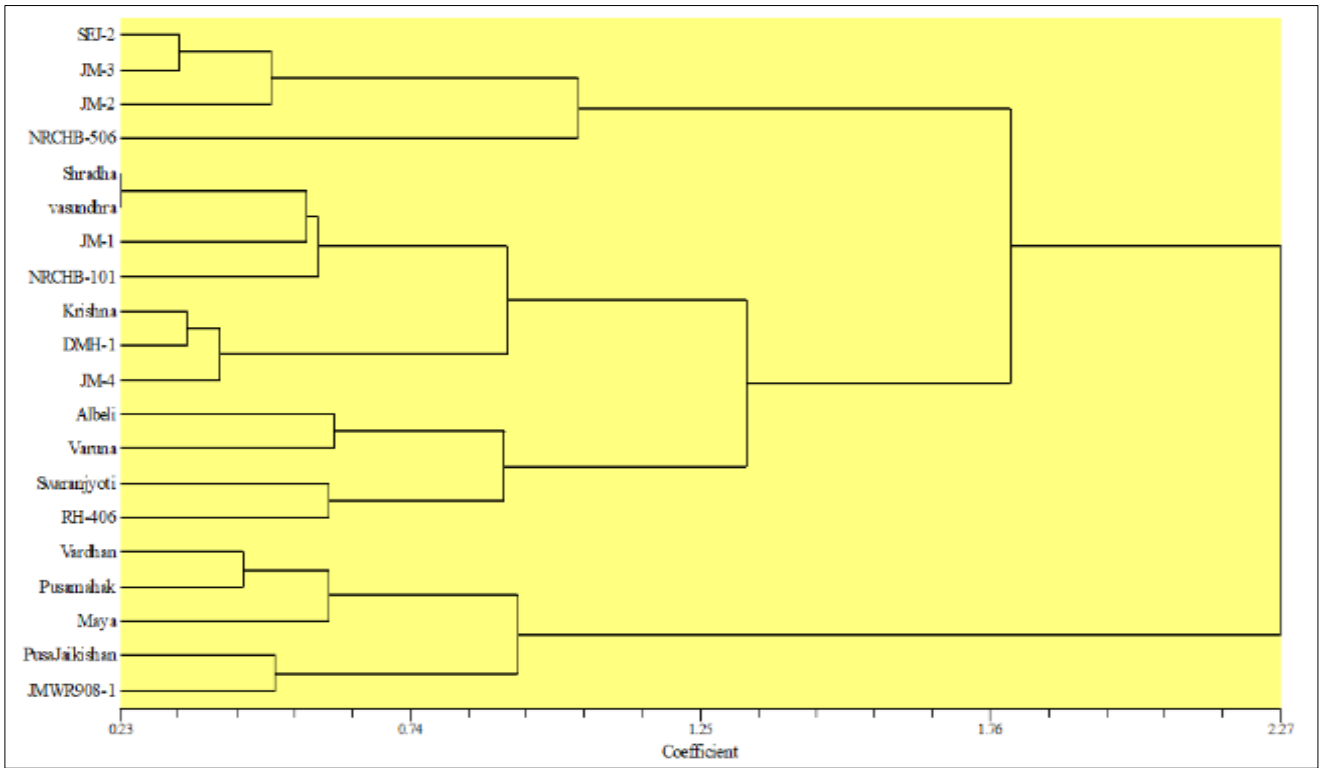

Fig.1: Dendrogram of mustard varieties based on biochemical characterization

Conclusion

The phenol content varied from the minimum of 1.1% in JM-2 to maximum of 1.9% in Varuna, wax content minimum of 3.1% in JMWR 908-1 to maximum of 4.4% in Shradha, lipid content minimum of 2.3% in NRCHB-506 to maximum of 3.8% in Vasundhra, total sugar content minimum of 39.7mg/g in Varuna to maximum of 43.8 mg/g in JM-2, reducing sugar content minimum of 22.5 mg/g in Pusa Jaikishan to maximum of 29.9 mg/g in JM-3 and non-reducing sugar content minimum of 13.1 mg/g in NRCHB-506 to maximum of 20.1 mg/g in Pusa Jaikishan.

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Conflict of Interest

The Author do not have any conflict of interest.

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