



Phytochemicals in *Brassica juncea* distressing developmental and reproductive biology of mustard aphid (*Lipaphis erysimi*)

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ABSTRACT

The present study was carried out to decipher the variation in plant phytochemicals, and their effect on developmental, reproduction and survival of *Lipaphis erysimi* (Kalt.) on diverse *Brassica juncea* cultivars. These studies revealed significant differences in total nymphal, reproductive and developmental periods, fecundity and offspring survival of *L. erysimi* on the test *B. juncea* cultivars. Development period was significantly longer on DRMR 150-35, PM 26, RLC 3 and PM 25, while fecundity and survival were lower on PM 27, RLC 3, NRCHB 101, RH 749, Pusa Tarak, RH 0406 and PM 30, except in a few cases. Total proteins, antioxidants, tannins, phenols and FRAP were significantly higher, and sugars lower in DRMR 150-35, RLC 3, PM 26 and NRCHB 101 as compared to other *B. juncea* cultivars. Total proteins, total tannins and antioxidants exhibited a significant and positive, while total sugars showed negative correlation, and explained 86.1% variability in total developmental period of *L. erysimi*. Total sugars revealed a significantly positive and FRAP negative correlation, explaining 35.5% variability in fecundity of *L. erysimi*. Furthermore, total proteins and total antioxidants also showed significant and negative correlation with offspring survival, and total phenols and antioxidants explained 28.9% variability in offspring survival of *L. erysimi* on the test *B. juncea* genotypes. Present study suggests that DRMR 150-35, RLC 3, NRCHB 101 and PM 26 have greater amounts of antinutritional plant defense compounds which adversely affect the developmental and reproductive biology of *L. erysimi*, and thus could be used in *Brassica* improvement programme for sustainable crop production.

Keywords: Fecundity, Mustard aphid, Nutritional factors, Reproductive biology

Rapeseed-mustard (*Brassica* spp.) is the third most important oilseed crop after soybean and oil palm, and contributes 27.8% to the total Indian oilseed production (Kumrawat and Yadav 2018). In India, mustard is cultivated on over 8.06 million hectares (mha) area, producing 11.75 million tonnes (mt) with an average productivity of 1458 kg/ha (ASG 2022). The productivity of Indian mustard, *Brassica juncea* (L.) Czern and Coss is limited by various abiotic and biotic factors. Among the biotic factors, mustard aphid, *Lipaphis erysimi* (Kalt.) causes 11.4–71.0% yield losses, which can be prevented by up to 10.2–61.1% with appropriate management practices (Dhillon *et al.* 2022). Aphids are currently being managed by application of insecticides, which have some limitations such as detrimental effect on natural enemies, pollinators and the environment. Therefore, it becomes imperative that available pest management tactics should be such that they provide effective and economical control of the pest without any adverse effect on the environment. The insect resistant plants have the unique advantage of providing inherent

insect control in the crop, and could be the best alternative for the management of aphids (Kumar and Banga 2017). However, for the development of insect resistant cultivar, precise knowledge on available sources of resistance to insect pests is of primary importance.

The resistance to pests in most cultivars depends on their genetic makeup, biochemical profile and overall chemical defence package, and these factors are inter-dependent. Further, plant resistance to herbivores also depends on the interplay of biochemical factors like absence or insufficient amount of essential nutrients (nutritional factors) and presence of toxic substances and antimetabolites (antinutritional factors), which adversely affect the food digestion and utilization. Phenolic compounds are the specialized defence compounds produced by host plants against pest insect attack. Other biochemical constituents such as total antioxidants and tannins have also been found to result in adverse effects on the reproductive period, fecundity and survival of mustard aphid in *B. juncea* (Samal *et al.* 2021). Furthermore, the presence of differential amounts of nutritional elements such as proteins and sugars decide the fitness of insects on the host plant (Kumar *et al.* 2020). Hence, understanding the significant influence of different nutritional and antinutritional compounds on the

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establishment of *L. erysimi* is of great significance for their utilization in *B. juncea* breeding programme. The objective of the present study was to find sources of insect resistance/tolerance from the elite *B. juncea* cultivars, if any and use them in Brassica improvement program. Therefore, present studies were carried out on effect of commercially released *B. juncea* cultivars on developmental and reproductive fitness of *L. erysimi* vis-à-vis role and contribution of constitutive biochemical compounds in variable plant defence against mustard aphid.

MATERIALS AND METHODS

Crop raising: Twenty-three *B. juncea* cultivars were grown in 5 row plots of 5 m length, having 30 cm row to row and 15 cm plant to plant spacing in the experimental fields of the Indian Agricultural Research Institute, New Delhi during the 2021–22 cropping season. All recommended agronomic practices, except insecticidal application were followed to raise the crop. Ten randomly selected plants of each test cultivar were tagged for biological and biochemical studies.

Developmental and reproductive biology of *L. erysimi* on test *B. juncea* cultivars: The biological studies of *L. erysimi* on test *B. juncea* cultivars were carried out at 15±3°C temperature, 60–70% relative humidity and 12L: 12D photoperiod under controlled conditions in the laboratory. Mustard aphids, *L. erysimi* were collected from the field and reared on mustard siliquae in glass Petri dishes of 10 cm diameter and 2 cm height under laboratory conditions. Newly hatched nymphs obtained from the laboratory reared aphids were collected and transferred to siliquae of each test *B. juncea* cultivar with the help of a fine moist camel hair brush. The experiment was laid out with 15 replications for each test *B. juncea* cultivar in a completely randomized design. The observations were recorded on total nymphal period (birth of first instar to end of fourth instar), total reproductive period (birth of first nymph to last nymph), total developmental period (birth of the nymph to death of resulting female), and fecundity (number of nymphs produced by each female). Further, the total offspring produced were observed and survival of nymphs was also calculated after 48 h of emergence and expressed as survival (%) per female.

Estimation of constitutive phytochemicals in test *B. juncea* cultivars: The siliquae of earlier tagged three plants of each test *B. juncea* cultivars were collected in polythene zip bags separately and brought to the laboratory for the estimation of nutritional (total sugars and total proteins) and antinutritional [total tannins, total phenols, total antioxidants and Ferric Ion Reducing Antioxidant Power (FRAP)] biochemical constituents. Two-gram tissues from siliquae of aforesaid test *B. juncea* cultivars were crushed in liquid nitrogen separately and added with 10 ml of 50 mM phosphate buffer (pH 7.8). The slurry was transferred to centrifuge tubes and centrifuged at 12000 rpm for 20 min at 4°C. The supernatant was collected and stored in 2.5 ml micro-centrifuge tubes at -20°C in the deep freezer for estimation of aforesaid biochemical constituents. There were

three replications for each test genotype and biochemical constituent in a completely randomized design. Total sugars and proteins were estimated using the methods given by Dubois *et al.* (1956) and Bradford (1976), respectively, and expressed in mg/g of plant tissue. Total antioxidant content was estimated by the method given by Prieto *et al.* (1999), tannins by Amorim *et al.* (2008), total phenol by Singleton and Rossi (1965) and ferric ion reducing antioxidant power (FRAP) by Benzie and Strain (1999), and the values obtained were expressed in mg/g of plant tissue.

Statistical analysis: The data on biological and biochemical parameters were subjected to one-way analysis of variance (ANOVA). The significance of differences in the test cultivars was tested by F-test, and the treatment means were compared by least significant differences at P=0.05 using the statistical software SPSS version 16.0. The Pearson correlation, multiple linear and stepwise regression analyses between the plant biochemical constituents and *L. erysimi* biological parameters were done using RStudio analysis software.

RESULTS AND DISCUSSION

Developmental and reproductive biology of *L. erysimi* on test *B. juncea* cultivars: The studies revealed that the nymphal period varied between 77.95–86.15 h, and was significantly longer ($F_{22, 322} = 5.83$; $P < 0.001$) on DRMR 150-35, PM 26, PM 25, RLC 3, NRCHB 101, PM 32, PM 27, PDZM 31 and RH 749 as compared to other *B. juncea* cultivars (Table 1). The reproductive period, total developmental period, fecundity and offspring survival of *L. erysimi* on the test *B. juncea* cultivars varied between 187.53–397.27 h, 270.55–457.49 h, 34.67–77.87 nymphs/female and 33.1–79.4%, respectively (Table 1). The reproductive ($F_{22, 322} = 6.565$; $P < 0.001$) and total developmental ($F_{22, 322} = 6.51$; $P < 0.001$) period were significantly longer on DRMR 150-35, PM 26, RLC 3, NRCHB 101 and PM 25 as compared to other *B. juncea* cultivars (Table 1). Earlier studies also reported significant variability among the *B. juncea* genotypes for nymphal, total developmental and reproductive period of *L. erysimi* (Samal *et al.* 2021). The fecundity ($F_{22, 322} = 6.509$; $P < 0.001$) and the offspring survival ($F_{22, 322} = 7.66$; $P < 0.001$) were significantly lower on RLC 3, NRCHB 101, DRMR 150-35, Pusa Tarak, RH 749 and PM 27 as compared to other *B. juncea* cultivars, except in few cases (Table 1). Deleterious effect of *B. juncea* cultivars having speciality phenotypic traits like purple mutant was also reported on the lifespan and fecundity of *L. erysimi* (Rana 2005). Similarly, Samal *et al.* (2021) also reported lower fecundity and offspring survival of *L. erysimi* on a low erucic acid *B. juncea* genotypes, RLC 3. The varying developmental duration, reproductive period, fecundity and survival of the insects could be due to differences in the genetic makeup and/or expression of defence biochemical compounds in the host plants. Earlier studies have also reported the contribution of plant genetic makeup in imparting defence against *L. erysimi* in wild relative derived *B. juncea* introgression lines (Palial *et al.* 2022).

Table 1 Developmental and reproductive biology of *Lipaphis erysimi* on siliquae of diverse *Brassica juncea* cultivars

| Cultivar | Total nymphal period (h) | Reproductive period (h) | Total developmental period (h) | Fecundity (Nymphs/female) | Offspring survival (%) |
|---------------------|--------------------------|-------------------------|--------------------------------|---------------------------|------------------------|
| RH 0761 | 78.1 | 206.4 | 344.5 | 70.9 | 62.6 |
| RH 30 | 76.0 | 192.0 | 320.8 | 63.0 | 61.6 |
| RLC 3 | 84.5 | 249.6 | 389.4 | 43.7 | 33.1 |
| DRMIJ 31 | 77.7 | 177.6 | 312.9 | 58.3 | 66.3 |
| DRMR 1165-40 | 78.2 | 153.6 | 296.6 | 60.7 | 63.0 |
| NRCHB 101 | 84.1 | 249.6 | 383.9 | 46.1 | 46.8 |
| Radhika | 76.9 | 192.0 | 345.7 | 64.1 | 73.9 |
| DRMR 150-35 | 86.2 | 312.0 | 457.5 | 61.2 | 41.3 |
| PM 28 | 77.3 | 177.6 | 336.5 | 67.6 | 55.1 |
| Pusa Tarak | 78.2 | 172.8 | 327.8 | 49.9 | 42.3 |
| Chhattisgarh Sarson | 77.6 | 206.4 | 353.6 | 68.5 | 61.7 |
| RH 725 | 76.8 | 211.2 | 345.6 | 77.9 | 53.0 |
| RH 0406 | 77.9 | 172.8 | 320.3 | 50.8 | 79.4 |
| Pusa Vijay | 76.9 | 177.6 | 324.1 | 63.5 | 75.5 |
| RH 749 | 82.2 | 192.0 | 353.4 | 48.7 | 48.7 |
| DRMRIJ 16-38 | 76.7 | 144.0 | 304.9 | 54.3 | 52.5 |
| PM 32 | 83.7 | 187.2 | 333.4 | 56.1 | 50.6 |
| PDZM 31 | 83.3 | 129.6 | 270.6 | 57.7 | 70.3 |
| PM 25 | 85.8 | 227.2 | 387.4 | 58.2 | 62.4 |
| PM 26 | 86.0 | 272.0 | 430.0 | 54.9 | 65.7 |
| PM 30 | 79.8 | 192.0 | 343.7 | 53.3 | 59.7 |
| PM 27 | 83.6 | 129.6 | 285.2 | 34.7 | 57.6 |
| RVM 1 | 78.3 | 163.2 | 314.2 | 62.7 | 60.5 |
| F-probability | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| LSD (P=0.05) | 4.10 | 48.04 | 47.88 | 10.51 | 12.65 |

Constitutive phytochemicals in test B. juncea cultivars:

The total protein and sugars in the siliquae of test *B. juncea* cultivars varied from 1.7–5.3 and 0.5–2.9 mg/g, respectively (Table 2). Total protein content was significantly greater ($F_{22,46} = 41.632$; $P < 0.001$) in the siliquae of PM 26, NRCHB 101, Chhattisgarh Sarson, DRMR 150-35 and RLC 3 as compared to rest of the cultivars (Table 2). Rehman *et al.* (2014) also reported higher protein content in Alankar cultivar of mustard, which shows some resistance against aphid. Total sugar content was significantly lower ($F_{22,46} = 95.547$; $P < 0.001$) in the siliquae of RLC 3, PM 27, PM 25, DRMR 150-35 and PM 30. Earlier studies also reported greater sugar content in the aphid susceptible *B. juncea* genotypes (Kumar *et al.* 2020). Antinutritional factors like total phenols ($F_{22,46} = 112.383$; $P < 0.001$), tannins ($F_{22,46} = 33.517$; $P < 0.001$), antioxidants ($F_{22,46} = 59.009$; $P < 0.001$) and FRAP ($F_{22,46} = 29.751$; $P < 0.001$) significantly varied in the test *B. juncea* cultivars, and were significantly greater in the siliquae of NRCHB 101, RLC 3, DRMR 150-35, PM 26 and PM 27 as compared to other cultivars, except in a few cases (Table 2). Likewise, Samal *et al.* (2021) also reported greater quantity of total phenols, tannins, antioxidants and FRAP in the resistant genotypes of *B. juncea*. Kumar and

Sangha (2013) found that the biochemical constituents like total phenols and ortho-dihydroxy phenols in mustard provide defence against aphids. Further, the mustard lines NDRS-9-2 and NDRS2001-1 were recorded with minimum phenol content and maximum aphid infestation (Mishra *et al.* 2019).

Association between constitutive phytochemicals and L. erysimi biological parameters: Total sugars in the *B. juncea* siliquae showed significant and negative correlation with total nymphal period ($r = -0.48^*$), reproductive period ($r = -0.44^*$) and total developmental period ($r = -0.45^*$), while significant and positive correlation with fecundity ($r = 0.44^*$) of *L. erysimi*, indicating that the greater sugar content has a positive impact on the development and reproductive biology of *L. erysimi* (Table 3). Earlier studies also reported the positive relationship between total sugar and *L. erysimi* multiplication (Kumar *et al.* 2017, Sharma *et al.* 2022). Further, total protein in the siliquae showed significant and positive correlation with total nymphal period ($r = 0.41^*$), reproductive period ($r = 0.80^{**}$) and total developmental period ($r = 0.82^{**}$), while significant and negative correlation with per cent survival ($r = -0.40^*$), which could be due to certain defence proteins in the test

Table 2 Amounts of various constitutive phytochemicals in siliquae of diverse *Brassica juncea* cultivars

| Cultivar | Total proteins (mg/g) | Total sugars (mg/g) | Total phenols (mg/g) | Total tannins (mg/g) | Total antioxidants (mg/g) | FRAP (mg/g) |
|---------------------|--------------------------|------------------------|-------------------------|-------------------------|------------------------------|----------------|
| RH 0761 | 3.7 | 2.2 | 2.9 | 3.8 | 5.8 | 0.7 |
| RH 30 | 2.5 | 1.2 | 3.9 | 3.3 | 4.8 | 0.8 |
| RLC 3 | 4.0 | 0.5 | 5.0 | 4.2 | 6.8 | 0.9 |
| DRMIJ 31 | 2.8 | 1.7 | 5.2 | 2.3 | 4.3 | 1.1 |
| DRMR 1165-40 | 2.2 | 2.7 | 4.5 | 2.4 | 4.4 | 0.9 |
| NRCHB 101 | 4.7 | 1.3 | 5.3 | 4.8 | 6.1 | 1.3 |
| Radhika | 3.2 | 1.0 | 3.6 | 3.1 | 3.1 | 0.7 |
| DRMR 150-35 | 4.5 | 0.9 | 3.4 | 4.2 | 7.8 | 1.0 |
| PM 28 | 3.5 | 2.9 | 2.2 | 3.2 | 5.3 | 0.8 |
| Pusa Tarak | 3.6 | 1.7 | 1.0 | 2.6 | 4.3 | 0.8 |
| Chhattisgarh Sarson | 4.6 | 1.4 | 5.0 | 2.9 | 1.2 | 0.7 |
| RH 725 | 2.9 | 2.1 | 2.3 | 3.4 | 4.1 | 1.0 |
| RH 0406 | 2.8 | 1.7 | 4.6 | 2.7 | 4.3 | 0.9 |
| Pusa Vijay | 1.9 | 1.1 | 2.1 | 2.3 | 2.1 | 0.7 |
| RH 749 | 2.6 | 1.2 | 2.9 | 1.6 | 4.1 | 0.8 |
| DRMRIJ 16-38 | 3.0 | 2.6 | 2.7 | 2.7 | 4.3 | 1.1 |
| PM 32 | 3.0 | 1.8 | 2.0 | 3.7 | 2.2 | 0.6 |
| PDZM 31 | 1.7 | 2.1 | 4.6 | 2.4 | 2.7 | 0.7 |
| PM 25 | 3.5 | 0.8 | 1.1 | 3.3 | 4.0 | 1.2 |
| PM 26 | 5.3 | 1.2 | 4.8 | 5.5 | 6.6 | 1.0 |
| PM 30 | 2.8 | 0.9 | 1.5 | 3.3 | 2.9 | 0.6 |
| PM 27 | 2.4 | 0.7 | 0.9 | 4.7 | 1.4 | 1.4 |
| RVM 1 | 2.6 | 1.4 | 1.5 | 3.0 | 3.4 | 0.7 |
| F-probability | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| LSD (P=0.05) | 0.41 | 0.19 | 0.40 | 0.46 | 0.64 | 0.12 |

FRAP, Ferric ion reducing antioxidant power.

Table 3 Association of different constitutive phytochemicals with *Lipaphis erysimi* biological parameters

| Constitutive biochemical | Correlation coefficient (r) | | | | |
|--------------------------|-----------------------------|----------------------------|-----------------------------------|------------------------------|---------------------------|
| | Total nymphal period (h) | Reproductive period (h) | Total developmental period (h) | Fecundity (Nymphs/female) | Offspring survival (%) |
| Total proteins | 0.406* | 0.795** | 0.817** | -0.012 ^{NS} | -0.401* |
| Total sugars | -0.484* | -0.438* | -0.450* | 0.440* | 0.127 ^{NS} |
| Total phenols | 0.059 ^{NS} | 0.283 ^{NS} | 0.177 ^{NS} | 0.012 ^{NS} | 0.125 ^{NS} |
| Total tannins | 0.570** | 0.575** | 0.560** | -0.241 ^{NS} | -0.291 ^{NS} |
| Total antioxidants | 0.313 ^{NS} | 0.693** | 0.659** | -0.006 ^{NS} | -0.444* |
| FRAP | 0.371 ^{NS} | 0.154 ^{NS} | 0.163 ^{NS} | -0.445* | -0.203 ^{NS} |

*, ** = Correlation coefficients significant at P = 0.05, 0.01, respectively. NS = Nonsignificant at P = 0.05. FRAP, Ferric ion reducing antioxidant power.

B. juncea genotypes (Table 3). There was a significant and positive correlation between total phenols in the siliquae of test *B. juncea* genotypes and the total nymphal period of *L. erysimi* ($r = 0.57^{**}$). Total tannins and antioxidants showed significant and positive correlation with reproductive ($r = 0.58^{**}$ and 0.69^{**} , respectively) and total developmental ($r = 0.56^{**}$ and 0.66^{**} , respectively) period of *L. erysimi* (Table 3). However, there was FRAP content showed significant and negative correlation with fecundity ($r = -0.45^*$) and total antioxidant with per cent survival ($r = -0.44^*$) of *L. erysimi*. These findings indicate that the total

phenols, tannins, antioxidants and FRAP negatively impact the development, reproduction and survival of *L. erysimi* in *B. juncea* (Table 3). Earlier studies also reported negative correlation of these antinutritional elements in *B. juncea* with the developmental and reproductive biology of *L. erysimi* (Samal *et al.* 2021). Further, highly significant and negative correlation was observed between phenol content and aphid multiplication (Kumar *et al.* 2017, Sharma *et al.* 2022).

The multiple linear regression analysis of total protein (X_1) total sugars (X_2), total phenols (X_3), total tannins (X_4), total antioxidants (X_5) and FRAP (X_6) in *B. juncea* siliquae

indicated that these compounds contribute to 46.8, 84.1, 86.1, 38.1 and 34.7% variability for total nymphal period ($75.41 + 0.10X_1 - 1.91X_2 - 0.03X_3 + 1.09X_4 + 0.29X_5 + 3.08X_6$; $R^2 = 46.8$), reproductive period ($130.75 + 22.94X_1 - 24.53X_2 - 0.15X_3 - 0.37X_4 + 11.94X_5 - 22.54X_6$; $R^2 = 84.1$), total developmental period ($282.64 + 29.41X_1 - 24.82X_2 - 3.97X_3 - 5.23X_4 + 10.96X_5 - 13.89X_6$; $R^2 = 86.1$), fecundity ($59.78 + 1.99X_1 + 6.05X_2 - 0.32X_3 - 0.49X_4 + 0.06X_5 - 17.51X_6$; $R^2 = 38.1$) and per cent offspring survival ($70.44 - 3.81X_1 + 1.23X_2 + 2.75X_3 + 1.29X_4 - 2.87X_5 - 3.03X_6$; $R^2 = 34.7$) of *L. erysimi*. However, the stepwise regression analysis suggested that the total sugars and tannins contributed to 41.96% variability in total nymphal period ($77.35 - 1.79X_2 + 1.72X_4$; $R^2 = 42.0$). Further, total protein, sugars and antioxidants explained 82.9% variability in reproductive period ($110.99 + 22.62X_1 - 23.10X_2 + 11.26X_5$; $R^2 = 82.9$), and 83.4% variability in total developmental period ($257.27 + 25.38X_1 - 22.95X_2 + 9.39X_5$; $R^2 = 83.4$) of *L. erysimi*. The total sugars and FRAP explained 35.5% variability for fecundity ($64.02 + 5.69X_2 - 17.13X_6$; $R^2 = 35.5$), while total phenols and total antioxidants contributed to 28.9% variability in offspring survival ($66.24 - 2.43X_3 - 3.72X_4$; $R^2 = 28.9$), suggesting that these phytochemicals in test *B. juncea* cultivars contribute to varying effects on development, reproduction and survival of *L. erysimi*. Earlier studies also found detrimental effects of total antioxidants and total tannins on the developmental period, progeny production and survival of *L. erysimi* on *B. juncea* (Samal *et al.* 2021).

The success or failure of aphid multiplication is ascertained by the genetic make-up and/or expression of defence biochemical substances in the host plant. The current investigation revealed that the siliquae of DRMR 150-35, RLC 3, NRCHB 101, PM 26 and PM 25 have greater titres of total proteins, phenols, tannins, antioxidants and FRAP, impart deleterious effect on the growth and reproductive efficiency of *L. erysimi*, and can be used in the *Brassica* improvement program.

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