

ASSESSMENT OF LEAF PROPERTIES ON MUSTARD VARIETIES EXHIBITING RESISTANCE TO APHID AND SAWFLY INFESTATION

Moshfiqur Rahman¹, Md Ruhul Amin¹,
Md. Mamunur Rahman¹, Mansura Afroz¹,
Md. Raihan Talukder², Antara Samiha^{1,3},
Ariful Islam^{1,4} & Md. Shamim Hossain^{1*}

¹Department of Entomology,
Bangabandhu Sheikh Mujibur Rahman Agricultural University,
Gazipur-1706, Bangladesh

²Department of Environmental Science,
Bangabandhu Sheikh Mujibur Rahman Agricultural University,
Gazipur-1706, Bangladesh

³Bangladesh Agricultural Research Institute,
Gazipur-1701, Bangladesh

⁴Bangladesh Jute Research Institute,
Manik Mia Avenue, Dhaka-1207, Bangladesh

*Corresponding author: shamim.ent@bsmrau.edu.bd

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ABSTRACT

The current study was conducted in the experimental field of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from November 2019 to March 2020 to explore host plant resistance of seven mustard varieties, namely BARI Sarisha-11, BARI Sarisha-12, BARI Sarisha-13, BARI Sarisha-14, BARI Sarisha-15, BARI Sarisha-16, and BARI Sarisha-17 against sawfly and aphid based on leaf biochemical parameters. Chlorophyll a, chlorophyll b, reducing sugar, protein, and proline of leaf showed significant variations but Soil Plant Analysis Development (SPAD) value showed no statistical variation among the varieties. The mean infestation rate of sawfly was the highest in BARI Sarisha-12 ($4.2 \pm 2.2\%$) and the lowest was in BARI Sarisha-13 ($0.0 \pm 0.0\%$). The infestation of aphid was also the lowest in BARI Sarisha-13 ($15.2 \pm 0.7\%$), but the highest was in BARI Sarisha-14 ($24.9 \pm 1.5\%$). The correlation matrix showed that the estimated biochemical contents of leaf except proline showed positive influence on the infestation of both sawfly and aphid, but only reducing sugar, protein and proline showed significant results. Among the studied varieties, BARI Sarisha-13 showed comparatively lower level of abundance and infestation of both sawfly and aphid.

Keywords: *Athalia lugens*, biochemical contents, *Brassica* spp., *Lipaphis erysimi*

ABSTRAK

Kajian semasa ini dijalankan di kawasan eksperimen di Jabatan Entomologi, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh dari November 2019 ke Mac 2020 bagi mengkaji tanaman perumah yang rintang ke atas tujuh varieti sawi iaitu BARI Sarisha-11, BARI Sarisha-12, BARI Sarisha-13, BARI Sarisha-14, BARI Sarisha-15, BARI Sarisha-16 dan BARI Sarisha-17 bagi mengawal Lalat Gergaji dan afid berdasarkan parameter biokimia daun. Klorofil a, klorofil b, pengurangan gula, protein dan pada daun menunjukkan variasi yang signifikan tetapi menunjukkan nilai *Soil Plant Analysis Development* (SPAD) menunjukkan tidak terdapatnya variasi statistik antara variasi. Purata kadar infestasi pada Lalat Gergaji adalah tertinggi pada BARI Sarisha-12 ($4.2 \pm 2.2\%$) dan yang terendah adalah pada BARI Sarisha-13 ($0.0 \pm 0.0\%$). Infestasi afid adalah terendah pada BARI Sarisha-13 ($15.2 \pm 0.7\%$), tetapi tertinggi pada BARI Sarisha-14 ($24.9 \pm 1.5\%$). Matrik korelasi menunjukkan jangkakan kandungan biokimia pada daun kecuali prolin menunjukkan kesan positif dalam infestasi pada Lalat Gergaji dan afid, tetapi menurunkan gula, protein dan prolin dengan nilai hasil yang signifikan. Antara varieti yang dikaji, BARI Sarisha-13 menunjukkan kelimpahan dan infestasi yang ketara rendah pada Lalat Gergaji dan afid.

Katakunci: *Athalia lugens*, kandungan biokimia, *Brassica* spp., *Lipaphis erysimi*

INTRODUCTION

Mustard (*Brassica* spp.) is an important oilseed crop in Bangladesh grown in winter season. It contains both polyunsaturated (linolenic and linoleic acid) and monounsaturated (oleic acid) fatty acids which are very important for human health (Kaur et al. 2019). The crop occupied an area of 309.1 thousand hectares of land with total production of 358.3 thousand metric tons during 2019-20 in Bangladesh (BBS 2021). The three species of mustard *B. campestris*, *B. juncea* and *B. napus* are mainly grown in the country (Sarker et al. 2021).

The mustard crop is very vulnerable to a wide variety of insect pests and the incidence of these pests is one of the most important constraints in stabilizing its yield (Aslan and Gok 2006). A number of insect pests such as aphid, cabbage aphid, sawfly, leaf hopper, painted bug and flea beetle were reported to infest mustard in Bangladesh (Mandal et al. 2018) while in India 38 insect pests were recorded to infest the crop (Dwivedi et al. 2018). Mustard aphid *Lipaphis erysimi* (Homoptera: Aphididae) was found as the most destructive one among them, which causes serious damage to mustard plant during vegetative to siliqua maturity stage (Das 2002). The nymphs and adults of aphids with their piercing-sucking type mouthparts suck saps from leaves, stems, inflorescence and pods, and the plant shows stunted growth, withered flower and deformed pod (Atwal & Dhaliwal 1997). Aphid infestation causes enormous qualitative and quantitative losses of seed, which in turn reduces the viability of the seed and the oil content. According to the research findings, the yield loss due to aphid infestation in mustard ranged from 53.7% to 71.5% in Bangladesh (Hossain et al. 2015). The sawfly *Athalia lugens* (Hymenoptera: Tenthredinidae) is other economical pest of mustard as well as other cruciferous plants, which causes qualitative and quantitative losses to the crop. It is a polyphagous insect and is considered as devastating pest of rapeseed-mustard in India, a neighboring country of Bangladesh (Pal et al. 2020). In Bangladesh, mustard sawfly is a major insect pest and causes a significant yield loss in the crop at seedling stage (Mandal et al. 2019).

Different crop protection strategies are frequently needed against insect pests to reduce yield loss. However, plant itself has different mechanisms of resistance to avoid, minimize, or tolerate the effects of pest attacks (Sarfraz et al. 2006). Screening of mustard varieties showed that different varieties of the plant had different levels of susceptibility to aphid infestation (Hossain et al. 2015). Therefore, the level of aphid and sawfly infestation and plant varietal characteristics can be the determining factors of crop susceptibility or resistance. Exploring the population dynamics, infestations and deleterious effects of mustard aphid and sawfly on different mustard varieties would be a mechanistic way to know how these crops are injured by these insects. So, the objectives of this study were to find out the rate of sawfly and aphid infestation in selected mustard varieties and to determine their chemical properties which influence the resistance in mustard against sawfly and aphid infestation.

MATERIALS AND METHODS

Study Location and Duration

The experiment was set in the research field of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. The study was carried out during the month of November 2019 to March 2020. Annual mean maximum and minimum temperatures, relative humidity and rainfall of the location are 36.0 and 12.7° C, 65.8% and 149.6 mm, respectively.

Mustard Varieties

BARI Sarisha-11 (*Brassica juncea*), BARI Sarisha-12 (*B. campestris*), BARI Sarisha-13 (*B. napus*), BARI Sarisha-14 (*B. campestris*), BARI Sarisha-15 (*B. campestris*), BARI Sarisha-16 (*B. juncea*) and BARI Sarisha-17 (*B. campestris*) were used in this experiment. The seeds were collected from the Oilseed Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

Experimental Design and Crop Cultivation

All the mustard varieties were cultivated in natural condition for the exposure to sawfly and aphid infestation following randomized complete block design with plot size 3.0×2.0 m² and three replications. There were 21 plots in total divided into three blocks. The spacing between block to block and plot to plot was 1.0 m in both cases. The seeds were sown on 18 November 2019 in 6 lines per plot following line sowing method where row to row distance was 30 cm and in rows seeds were sown continuously. Seeds were placed at 2 cm depth, and then rows were covered with loose soil properly. Plant to plant spacing was maintained 20 cm by thinning of weak plants 15 days after germination. Fertilizers were applied according to Fertilizer Recommendation Guide of BARC (2018) at the following rate: cow dung- 10 t ha⁻¹, N- 40 kg ha⁻¹, P- 12 kg ha⁻¹, K- 30 kg ha⁻¹ and S- 9 kg ha⁻¹. Farm yard manure was applied at the time of field preparation.

Observation of Insect Pest Incidence

Weekly observation was started 10 days after sowing. The population of sawfly was observed visually, and the number of aphid was counted with the help of a magnifying glass (FD75, Ballon Brand, China). Aphid population was counted from the top 10 cm shoot containing apical leaves, inflorescence and siliqua of 10 sample plants at 30 DAS, 45 DAS and 60 DAS, respectively, then the mean number of aphids per 10 plants was calculated. The counting of aphid was done on the top 10 cm shoot regardless of whether it was containing leaf, inflorescence or siliqua. The percent of aphid infested plant was calculated from the number of total plants and aphid infested plants per plot. For each 10 sample plants, the number of sawflies

per plot was counted at 30 DAS, 45 DAS and 60 DAS and then the mean number of sawflies was calculated. The percent of plant infestation was calculated based on the number of total plants and sawfly infested plants per plot.

Biochemical Analysis of Mustard Leaf

Data on Leaf SPAD. Fresh leaves were used for biochemical analysis and the processes were replicated five times for each of the varieties. Soil Plant Analysis Development (SPAD) value was recorded by using a SPAD machine (Minolta SPAD-502 Plus Chlorophyll Meter). A section from mid position of the middle-aged leaf was considered while taking the SPAD data.

Estimation of Leaf Chlorophyll. Chlorophyll a and chlorophyll b were also estimated from leaf samples. Fresh leaf (0.05 g) from each sample plant was taken in a test tube and 25 ml of 80% Acetone was added in it. The test tube was kept in dark for 72 hours. Then the absorption was taken in spectrophotometer (T80+ uv/vis spectrometer, PG Instruments Limited, UK) at 645 nm and 663 nm (Gagoi & Basumatary 2018). Following formulas were followed for the calculation of chlorophyll content.

$$\text{Chl a (mg/g FW)} = (12.7 \times D_{663} - 2.69 \times D_{645}) \times \text{DF}$$

$$\text{Chl b (mg/g FW)} = (22.9 \times D_{645} - 4.68 \times D_{663}) \times \text{DF}$$

Where, FW= Fresh weight, D_{645} = Absorbance at 645 nm wave length; D_{663} = Absorbance at 663 nm wave length; 12.7, 2.69, 22.9 and 4.68= Absorbance co-efficient, DF = Dilution factor = $25/1000 \times 0.05$

Estimation of Reducing Sugar Content in Leaf. Modified Bertrand's method (Kumar et al. 2011) was followed to estimate the reducing sugar content of leaf. Leaf (100 mg) from the sample plants was taken and sugar was extracted with hot 80% ethanol twice (5 mL each time). Supernatant was collected and evaporated by keeping it on a water bath at 80°C. Water and dissolve the sugars (10 ml) was added and pipetted out 0.2 mL aliquot to a separate test tube. Working standard solution (0.2, 0.4, 0.6, 0.8 and 1 mL) were pipetted out into a series of test tubes. The volume in both sample and standard tubes was made to 2 mL with distilled water and 2 mL distilled water was taken in a separate tube to set a blank. Then, 1 mL of alkaline copper tartrate reagent (anhydrous carbonate, sodium bicarbonate, Na-K tartrate, anhydrous sodium sulphate, copper sulphate and sulphuric acid) was added to each tube and placed in boiling water for 10 minutes. After cooling the tubes, 1 mL of arsenomolybolic acid reagent was added to all the tubes, volumed to 10 mL with water and absorbance of blue colour was taken at 620 nm.

Absorbance corresponds to 0.1 mL of test = x mg of glucose

Protein Content of Leaf. The estimation of nitrogen content of the leaves was done in Micro Kjeldahl method (Maehre et al. 2018). Chopped, oven dried leaves (1.0 g) from selected plants were weighed using a digital balance and transferred to Kjeldahl flasks. For each sample, concentrated H_2SO_4 (20 ml) was added in presence of 3-4 g catalyst mixture (K_2SO_4 - CuSO_4 ,

5:1). The flask was continuously heated until the solution became clear. The flask was then allowed to cool and 150 ml distilled water was added. A few zinc granules were added to the mixture. One hundred milliliters of 40% NaOH was then poured into the flask, which was immediately attached to a distillation set. An Erlenmeyer flask containing methyl red and methyl blue indicator was placed underneath prior to collecting the distillate. Approximately 150 ml of distillate was collected and titrated with standardized 0.1 N HCl. The nitrogen content was calculated using the equation:

$$N\% = (T-B) \times N \times 1.4 \times 100/S$$

Where, T is the sample titer (ml), B is the blank titer (ml), N is the concentration of HCl (0.1 N) and S is sample weight. The percentage of N in each sample was multiplied by 6.25 to obtain the percentage protein content.

Determination of Leaf Proline: Fully expanded uppermost leaves of each variety were collected and proline was estimated using the method of Bates et al. (1973). Plant materials (0.5 g leaf sample) were homogenized in 5 ml of 6% aqueous sulfosalicylic acid and the homogenate was centrifuged for 20 min at 4000 rpm. Two ml of supernatant was taken in Pyrex test tube with 2 ml acid ninhydrin and 2 ml of glacial acetic acid and covered tightly with aluminum foil. Then the test tubes were heated at 100°C for 60 min and the reaction terminated in an ice bath for 15 min. The reaction mixture was added with 4 ml toluene, mixed vigorously for 15-20 seconds. Keeping at room temperature for 10 min the toluene layer was separated and the absorbance was measured at 520 nm using toluene blank. A series of standard with pure proline (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ml distilled water) was run in a similar way and a standard curve was prepared. The proline concentration was determined from the standard curve and calculated on a fresh weight basis as follows:

$$\text{Proline content (g}^{-1} \text{ fresh wt.)} = \{ \mu\text{g proline ml}^{-1} \times \text{vol. of extr. sol. (ml)} \times \text{toluene used (ml)} \} / (115.13 \mu\text{g mole}^{-1} \times \text{g sample})$$

Data Analysis

Data on the biochemical characters and the rate of sawfly and aphid infestation were analyzed using one-way analysis of variance at 5% level of significance. The results were expressed as mean±SE and the Tukey HSD posthoc statistic was used to test for differences among the means. The Pearson's correlation was used to examine the relationship between aphid and sawfly infestation with leaf biochemical properties of the varieties. All the analyses were performed using IBM SPSS 21.0 (IBM SPSS statistics 21.0, Georgia, USA).

RESULTS

SPAD value and the amount of chlorophyll were estimated from the leaf of tested mustard varieties. No statistical variation but a decrease over time was observed in the leaf SPAD values of the selected mustard cultivars at 30 DAS, 45 DAS and 60 DAS (Table 1). On the other hand, significant variation was observed in 'chlorophyll a' and 'chlorophyll b' contents of leaf among the selected mustard varieties. BARI Sarisha-14 was found to contain the highest amount of both 'chlorophyll a' and 'chlorophyll b' (16.5±0.7 and 30.1±0.4 mg g⁻¹ fresh weight,

respectively) while BARI Sarisha-16 (12.6 ± 0.8 and 22.2 ± 0.6 mg g⁻¹ fresh weight, respectively) had the least.

Table 1. Performance of mustard varieties on the basis of leaf chlorophyll contents in Gazipur region during Rabi season 2019-20

Variety	SPAD value			Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)
	30 DAS	45 DAS	60 DAS		
BARI Sarisha-11	45.8±2.0a	43.2±1.1a	42.8±0.5a	13.6±0.4ab	23.5±0.2bc
BARI Sarisha-12	50.1±0.5a	46.4±0.8a	45.0±2.3a	15.4±0.2ab	26.5±2.2abc
BARI Sarisha-13	49.0±1.1a	44.2±1.1a	42.9±0.9a	14.3±0.3ab	24.1±0.4bc
BARI Sarisha-14	52.2±1.1a	47.0±0.5a	47.1±0.6a	16.5±0.7a	30.1±0.4a
BARI Sarisha-15	47.0±1.1a	41.6±0.8a	45.0±1.1a	13.8±0.1ab	27.2±0.6ab
BARI Sarisha-16	44.2±5.2a	39.8±4.1a	41.4±5.1a	12.6±0.8b	22.2±0.6c
BARI Sarisha-17	50.1±0.4a	45.3±0.6a	45.1±1.1a	15.3±0.8ab	23.1±0.6bc

* DAS= Days after sowing, *FW= Fresh Weight

Means within a column followed by same letter(s) are not significantly different by Tukey HSD posthoc statistic at <0.05.

There were significant variations in the reducing sugar content of mustard leaf among the selected varieties (Table 2). BARI Sarisha-14 (29.5 ± 0.8 mg g⁻¹ FW) showed the highest amount of reducing sugar and BARI Sarisha-17 (20.2 ± 0.6 mg g⁻¹ FW) had the lowest amount of it. The leaf protein content also showed significant variations among the selected mustard varieties. The leaf protein content was the highest in BARI Sarisha-12 (8.3 ± 1.4 mg g⁻¹ FW) while the lowest in BARI Sarisha-13 (4.6 ± 0.1 mg g⁻¹ FW). Significant variation was observed in leaf proline content among the selected mustard varieties. It was the highest in BARI Sarisha-16 (23.5 ± 0.8 mg g⁻¹ FW) and lowest in BARI Sarisha-14 (13.2 ± 0.6 mg g⁻¹ FW).

Table 2. Chemical composition of leaf of mustard varieties grown in Gazipur region during Rabi season 2019-20

Variety	Reducing Sugar (mg g ⁻¹ FW)	Protein (mg g ⁻¹ FW)	Proline (mg g ⁻¹ FW)
BARI Sarisha-11	21.4±0.7cd	4.7±0.1bc	17.4±0.1cd
BARI Sarisha-12	25.9±1.9abc	8.3±1.4a	14.6±0.4de
BARI Sarisha-13	20.3±0.3d	4.6±0.1c	19.4±0.8bc
BARI Sarisha-14	29.5±0.8a	7.3±0.1ab	13.2±0.6e
BARI Sarisha-15	26.1±0.6ab	6.5±0.1abc	16.8±0.4cd
BARI Sarisha-16	22.2±0.6bcd	6.1±0.0abc	23.5±0.8a
BARI Sarisha-17	20.2±0.6d	5.3±0.0bc	21.1±0.6ab

*DAS= Days after sowing, *FW= Fresh Weight

Means within a row followed by same letter(s) are not significantly different by Tukey HSD posthoc statistic at <0.05.

The abundance of sawfly (per plot) on the studied mustard varieties showed variations and markedly decreased over time from 30 DAS to 60 DAS (Table 3). At 30 DAS the highest abundance was observed on BARI Sarisha-14 (33.0 ± 1.5) and At 45 DAS again the highest abundance was observed on BARI Sarisha-14 (14.6 ± 1.4) along with BARI Sarisha-12 (11.0 ± 2.3). BARI Sarisha-13 (0.3 ± 0.3) had the lowest abundance of sawfly at 30 DAS and after that no sawfly infestation was observed on it. Moreover, no sawfly infestation was observed on

the varieties at 60 DAS except BARI Sarisha-12 (2.5 ± 2.5). Considering the rate of plant infestation, the variety BARI Sarisha-12 ($4.2 \pm 2.2\%$) had the highest infestation while BARI Sarisha-13 ($0.0 \pm 0.0\%$ (mathematically negligible; hence is set as zero)) experienced almost no infestation.

Table 3. Infestation of sawfly on different mustard varieties grown in Gazipur region during Rabi season 2019-20

Variety	No. of sawfly/plot			Rate of plant infestation (%)
	30 DAS	45 DAS	60 DAS	
BARI Sarisha-11	$9.0 \pm 0.5d$	$0.3 \pm 0.3b$	$0.0 \pm 0.0a$	$0.2 \pm 0.0ab$
BARI Sarisha-12	$23.8 \pm 1.0b$	$11.0 \pm 2.3a$	$2.5 \pm 2.5a$	$4.2 \pm 2.2a$
BARI Sarisha-13	$0.3 \pm 0.3e$	$0.0 \pm 0.0b$	$0.0 \pm 0.0a$	$0.0 \pm 0.0b$
BARI Sarisha-14	$33.0 \pm 1.5a$	$14.6 \pm 1.4a$	$0.0 \pm 0.0a$	$2.3 \pm 0.0ab$
BARI Sarisha-15	$14.6 \pm 1.4c$	$3.3 \pm 0.8b$	$0.0 \pm 0.0a$	$1.5 \pm 0.1ab$
BARI Sarisha-16	$15.6 \pm 0.6c$	$0.6 \pm 0.3b$	$0.0 \pm 0.0a$	$0.3 \pm 0.0ab$
BARI Sarisha-17	$6.3 \pm 0.8d$	$0.3 \pm 0.3b$	$0.0 \pm 0.0a$	$0.5 \pm 0.1ab$

* DAS= Days after sowing

Means within a row followed by same letter(s) are not significantly different by Tukey HSD posthoc statistic at < 0.05.

The studied varieties showed significant variations in aphid abundance per 10 shoots at 30 DAS, 45 DAS and 60 DAS (Table 4). The abundance of aphid was constantly higher on BARI Sarisha-14 throughout the period (19.3 ± 0.8 , 201.3 ± 5.8 and 166.6 ± 4.4 at 30, 45 and 60 DAS, respectively), and BARI Sarisha-13 showed the lowest number of aphids (9.0 ± 0.0 , 133.3 ± 3.5 and 96.0 ± 2.6 at 30, 45 and 60 DAS, respectively). Furthermore, the highest rate of plant infestation by aphid was also recorded in BARI Sarisha-14 ($24.9 \pm 1.5\%$), and the lowest was in BARI Sarisha-13 ($15.2 \pm 0.7\%$).

Table 4. Infestation of aphid on different mustard varieties grown in Gazipur region during Rabi season 2019-20

Variety	No. of Aphid/10 shoots			Rate of plant infestation (%)
	30 DAS	45 DAS	60 DAS	
BARI Sarisha-11	$10.3 \pm 0.8c$	$155.3 \pm 3.5ab$	$102.0 \pm 3.2bc$	$19.0 \pm 1.1bc$
BARI Sarisha-12	$17.1 \pm 0.1ab$	$160.8 \pm 27.1ab$	$132.0 \pm 22.8abc$	$22.6 \pm 1.1ab$
BARI Sarisha-13	$9.0 \pm 0.0c$	$133.3 \pm 3.5b$	$96.0 \pm 2.6c$	$15.2 \pm 0.7c$
BARI Sarisha-14	$19.3 \pm 0.8a$	$201.3 \pm 5.8a$	$166.6 \pm 4.4a$	$24.9 \pm 1.5a$
BARI Sarisha-15	$15.0 \pm 0.5b$	$172.6 \pm 4.6ab$	$141.0 \pm 2.3ab$	$23.1 \pm 1.1ab$
BARI Sarisha-16	$16.3 \pm 0.8ab$	$165.3 \pm 3.1ab$	$141.3 \pm 3.5ab$	$22.2 \pm 1.1ab$
BARI Sarisha-17	$10.0 \pm 0.5c$	$141.0 \pm 3.4b$	$119.3 \pm 3.7bc$	$18.8 \pm 1.1bc$

* DAS= Days after sowing

Means within a row followed by same letter(s) are not significantly different by Tukey HSD posthoc statistic at < 0.05.

The correlation matrix of the percent plant infestation by sawfly and aphid with the leaf biochemical contents of mustard varieties is presented in Table 5. Among the biochemical properties of leaves, only the protein and reducing sugar showed significant positive influence

on both sawfly and aphid infestations. On the contrary, proline had significant negative relationship with the sawfly infestation.

Table 5. Correlation matrix of the rate of infestation by sawfly and aphid with the leaf biochemical contents of mustard varieties

Parameters	SPAD value	Chlorophyll a	Chlorophyll b	Reducing sugar	Protein	Proline
Chlorophyll a	0.993**					
Chlorophyll b	0.671*	0.692*				
Reducing sugar	0.472 ^{NS}	0.524 ^{NS}	0.931**			
Protein	0.394 ^{NS}	0.463 ^{NS}	0.660 ^{NS}	0.818*		
Proline	-0.666 ^{NS}	-0.685*	-0.887*	-0.789*	-0.588 ^{NS}	
% sawfly infested plant	0.527 ^{NS}	0.579 ^{NS}	0.664 ^{NS}	0.735*	0.938**	-0.727*
% aphid infested plant	0.187 ^{NS}	0.256 ^{NS}	0.650 ^{NS}	0.869*	0.818*	-0.471 ^{NS}

NS = Non-significant; * = Significant, $P < 0.05$; ** = Highly significant, $P < 0.01$.

DISCUSSION

The mustard varieties used in the present investigation showed variations in the amount of their leaf biochemical contents, namely chlorophyll, reducing sugar, protein and proline. The infestation of aphid and sawfly on the varieties also showed variations where aphid infestation was constantly higher than sawfly infestation regardless of the varieties. A maximum of 201.3 ± 5.8 aphids per 10 shoots were recorded at 45 DAS (the flowering stage of the plants) in the present study, which was lower compared to the result of Kalita et al. (2016) who reported a maximum of 63.48 aphids per 10 cm central shoot during the 2nd standard week of mustard growth i.e. at the vegetative stage. The variation happened possibly due to the variation of crop growing stage. However, the current results showed close conformity with the findings of Mandal et al. (2018) who reported that mustard inflorescence showed significantly higher level of infestation compared to leaf and siliqua.

The mean infestation level of mustard leaf, inflorescence and siliqua as reported by Mondal et al. (2003) ranged from 34.1 to 59.3% under rainfed condition. The results differed from the current findings where we observed relatively lower level of aphid infestation on the tested varieties, which ranged from 15.2 ± 0.7 to $24.9 \pm 1.5\%$. It is known that variation in weather conditions, varieties used in the experiment and crop agronomic practices could influence the infestation of insect pest (Ghosh et al. 2020; Mesbah et al. 2002). Rahman (2014) found that BARI Shorisa-9 had the highest siliqua infestation by aphid (90.87%) and the lowest siliqua infestation (44.20%) by the insect was recorded in BARI Shorisa 13. The findings of the current study supported these results to some extent where BARI Shorisa 13 ($15.2 \pm 0.7\%$) was found to be the least infested variety irrespective of the infestation of leaf, inflorescence and siliqua. On the other hand, BARI Shorisa 14 ($24.9 \pm 1.5\%$) showed the highest aphid infestation and BARI shorisa-9 was not taken in the present study.

A significant difference was observed in the abundance and infestation of aphids and sawfly among varieties in the current study. The infestation of both aphid and sawfly infestation was higher in the varieties having higher leaf chlorophyll, reducing sugar and protein. On the other hand, a negative correlation with leaf proline depicted that a high level of leaf proline resulted in lower infestation by the insect. Amin et al. (2011) observed that the development,

foraging and reproduction of pest insect varied depending upon plant morphological features and biochemical contents. Yasmin et al. (2022) found a positive correlation between aphid infestation and leaf protein content (%) in brinjal i.e. higher amount of leaf protein of brinjal variety faced the higher infestation by aphid. Another research findings showed that aphids to fulfill their nutrient requirements choose the phloem sap that contains high amount of amino acid and sugar (Cao et al. 2018). The present findings also suggested that a high amount of leaf protein and reducing sugar positively influenced the infestation of aphid and sawfly in mustard.

CONCLUSION

It can be concluded that BARI Sarisha-13 faced the lowest infestation of both aphid and sawfly among the tested varieties. On the other hand, BARI Sarisha-12 and BARI Sarisha-14 were found to be more susceptible to the infestation of sawfly and aphid, respectively. Therefore, cultivation of BARI Sarisha-13 might be suggested to ensure the utilization of varietal resistance against aphid and sawfly infestation.

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AUTHORS DECLARATIONS

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Conflict of Interest

All authors declare that there is no conflict of interest among them.

Ethics Declarations

Ethics declarations are not applicable for this research.

Data Availability Statement

This manuscript has no associated data.

Authors' Contributions

M. Rahman: Conducting research and writing the draft manuscript; M.R. Amin: Reviewing and editing the manuscript, M.M. Rahman: Reviewing and editing the manuscript, M. Afroz: Data analysis, reviewing and editing the manuscript, M.R. Talukder: Reviewing and editing the manuscript; A. Samiha: Reviewing and editing the manuscript; A. Islam: Reviewing and editing the manuscript; M.S. Hossain: Designing the experiment, research supervision, reviewing and editing the manuscript.

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