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Stress driven divergence: Biochemical and enzymatic insights into aphid morph diversity

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Abstract

Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) is a vital oilseed crop in India, yet its productivity is severely impacted by the mustard aphid (*Lipaphis erysimi* Kaltenbach), a major pest causing substantial yield losses up to 10-90 %. Understanding the population dynamics and physiological adaptations of apterous and alate morphs of mustard aphid under field conditions is vital for developing effective pest management strategies. This study aimed to investigate the seasonal incidence, biochemical composition, and enzymatic responses of winged (alate) and wingless (apterous) morphs of *L. erysimi* to abiotic stress. Field monitoring from December 2023 revealed a negative correlation between aphid incidence and maximum temperature (r = -0.682**) and a positive correlation with predator populations such as ladybird beetles (r = 0.923*) and spiders (r = 0.586*). Biochemical analyses indicated differential resource allocation, with apterous aphids accumulating more glucose and protein, while alates exhibited higher triglyceride levels. Spectrophotometric assays showed elevated antioxidant enzyme activity (Superoxide Dismutase and Catalase) in alates, reflecting enhanced oxidative stress defences during dispersal. Lipid peroxidation was significantly higher in alates, underscoring the physiological cost of flight. These findings highlight the role of abiotic factors and natural enemies in shaping aphid populations and provide insights into stress adaptations, crucial for designing ecologically sustainable pest control strategies.

Keywords: Indian mustard, *Lipaphis erysimi*, Seasonal incidence, Biochemical composition, Enzymatic responses

Indian mustard (*Brassica juncea*), a vital oilseed crop in India, plays a significant role in national oilseed production. In 2023-24 season, rapeseed-mustard became the leading oilseed crop, yielding 13.2 million tons with an average productivity of 1,443 kg/ha, surpassing the overall oilseed crop average of 1,314 kg/ha (Directorate of Rapeseed-Mustard Research, 2024)[1]. Despite this, mustard productivity remains constrained by insect pests like mustard sawfly (*Athalia lugens proxima* Klug.), Painted bug (*Bagrada cruciferarum* Kirk.) and Flea beetle (*Phyllotreta cruciferae* Goeze)[2] etc. Among them, the mustard aphid (*Lipaphis erysimi* Kalt.) is particularly devastating in India causing yield losses up to 10-90 % in *B. juncea* and a reduction in oil content of mustard seed in the range of 4.92-8.14 %[3].

Both nymphs and adults of *L. erysimi* feed on plant sap, causing stunted growth and reduced vigour[4]. Aphid population dynamics are closely linked to host plant quality[5], as nutrient availability influences their growth and reproduction[6]. Insect herbivores adapt physiologically and behaviorally to nutritional variation[7], and diets deficient in essential nutrients like biotin, Vitamin B6 (pyridoxine) etc. can lead to premature aging and higher mortality[8]. Environmental factors such as temperature, humidity, and rainfall significantly influence aphid activity in the field. Populations peak from late January to early February at optimal temperatures of 28.85°C (max) and 12.1°C (min), with morning and evening humidity of 86.5 and 37.5% respectively in Kolana village, Rewari , Haryana[9]. Temperature is particularly crucial for dispersal and reproduction[10]. Winged morphs specialise in dispersal, while wingless morphs maximise reproduction[11], complicating management efforts.

Temperature stress leads to the buildup of harmful molecules called reactive oxygen species (ROS), which can damage cells if not balanced by the insect's natural defences[12]. Excessive ROS can damage nucleic acids, proteins, and lipids, leading to mutations and cell death[13]. To mitigate oxidative stress, insects rely on non-enzymatic molecules and antioxidant enzymes[14], with studies confirming increased antioxidant enzyme activity under heat stress[15]. While chemical control is effective, its negative impact on natural enemies and the environment necessitates eco-friendly strategies. This study investigates the seasonal incidence of

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L. erysimi, its interactions with biotic and abiotic factors, and the nutrient assimilation and enzyme activity changes in alate and apterous morphs under heat stress. By linking ecological patterns with physiological adaptation, the findings offer novel insights that can inform the development of climate-resilient, sustainable pest management strategies aimed at improving mustard crop productivity while minimising ecological harm[16].

Materials and Methods

Aphid colony in field conditions

To investigate the population dynamics of mustard aphid (*Lipaphis erysimi*), the study was conducted at the Agricultural Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University which is situated in Southeastern part of Varanasi city at 25° 15′ North latitude and 82° 59′ East longitude at an elevation of 75.5 m above the mean sea level. The experimental site featured sandy loam soil with adequate irrigation provided through a tube well. A review of the field's cropping history indicated that mustard had been cultivated during the rabi season for the past 11–12 years.

The variety selected for the study was *Brassica juncea* (L.) Varuna (T-59), a high-yielding cultivar moderately susceptible to mustard aphids. This variety matures within 120–125 days, is tall (1.50–1.75 m), and exhibits a semi-spreading growth habit. It is bold-seeded with an average oil content of 42%, produces petiolate smooth leaves, and has moderate branching with numerous pods. Under optimal conditions, its yield ranges from 15–20 quintals per hectare. T-59 is widely cultivated in Uttar Pradesh, Gujarat, Bihar, and West Bengal.

The experimental fields were prepared using a soil-turning plough, followed by two passes with a cultivator, and subsequently levelled before sowing. The experimental plot size was 300 m², with a row-to-row spacing of 40 cm and a plant-to-plant spacing of 15 cm. A 1-meter border area was maintained between replication blocks to accommodate irrigation channels. Mustard seeds (T-59) were sown on November 24, 2023 in randomized block design with 3 replications, during the year of experimentation. Certified seeds were treated with the fungicide Captan at a rate of 2.5 g per kg of seed. The treated seeds, at a sowing rate of 5 kg per hectare, were placed into prepared furrows to ensure uniform germination and optimal crop establishment. No pesticides were sprayed.

Monitoring of insect pests and data collection

Aphid Population Monitoring

Mustard plants were systematically monitored at regular intervals from the flowering stage to pod maturity. The aphid population began to build in the first week of January and peaked around the first week of February. Weekly observations were conducted in the central rows of each plot, covering one square meter (Fig. 1). Aphid morphs were identified immediately in the field and collected using a needle brush and stored in Petri dishes for further analysis. The monitoring period was categorised into early, mid, and late flowering and fruiting stages, and aphid incidence was measured accordingly.

Data Collection

Observation and collection of aphid and predator population

To record aphid and generalist predator populations like ladybird beetles and spiders on the *L. erysimi*-infested plants, five plants were randomly selected and tagged within each plot. Weekly observations began with the initial appearance of aphids and continued until their final disappearance. The method followed was adapted from the All India Coordinated Research Project on Oilseeds[17]. During the flowering stage, aphids were removed from 10 cm of the terminal shoot of five tagged plants using a camel hairbrush onto a white paper sheet, and the total number of aphids was counted[18]. Number of ladybird beetles and spiders were counted similarly. Both wingless and winged morphs were segregated and collected for subsequent laboratory analysis.

Observation and collection of meteorological data

Meteorological data were gathered to analyse the correlation and regression between *L. erysimi* population dynamics and abiotic factors. Data on maximum and minimum temperatures, average relative humidity (RH), bright sunshine hours and rainfall were obtained from the meteorological observatory and the Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Daily and weekly meteorological data were recorded, covering a time frame from 7 days before to 7 days after the aphid infestation period.

These systematic observations provided a detailed dataset for analysing the impact of abiotic factors on aphid population trends and their infestation dynamics.

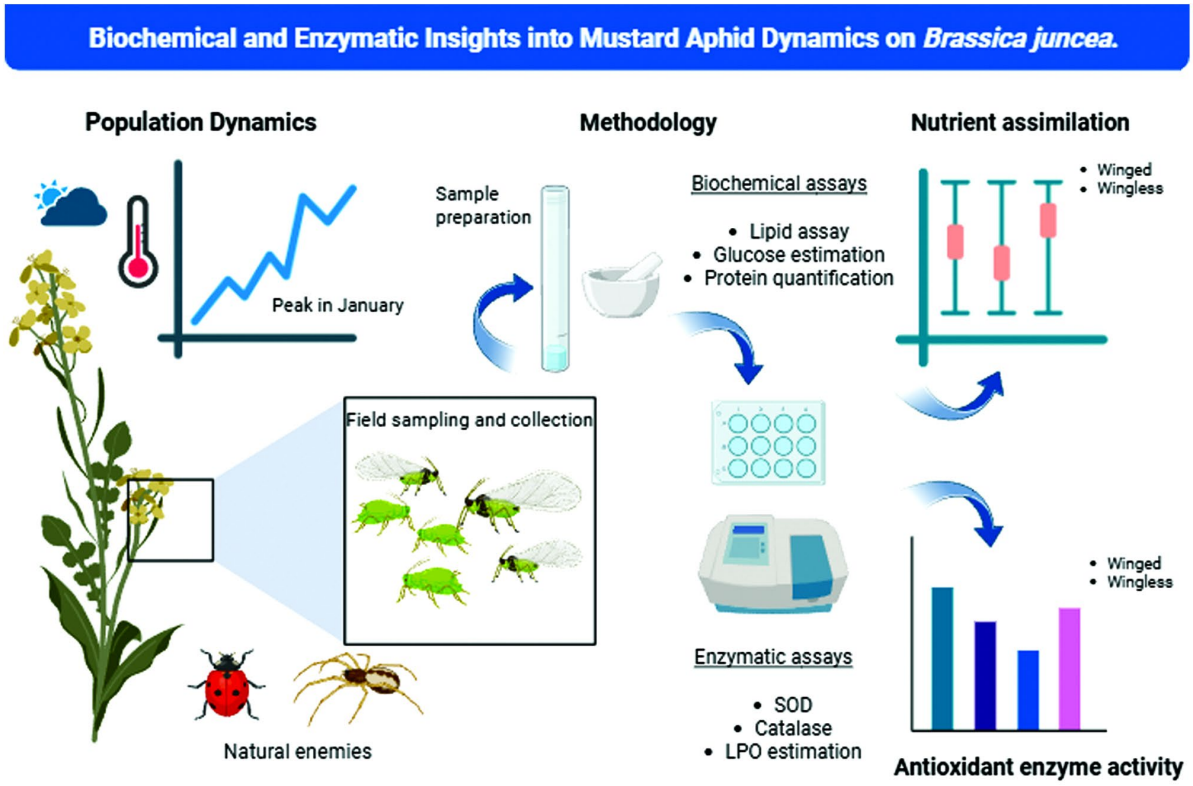

Fig. 1 — Graphical summary of the study.

Preparation of the Sample Solutions

A 10% (w/v) whole-body homogenate was prepared in ice-cold 0.1 M phosphate buffer saline (PBS) in a 1.5 mL centrifuge tube. The homogenates were centrifuged at 12,000 rpm for two minutes in a cooling centrifuge. The supernatant was collected, incubated at 70°C for 10 minutes, and centrifuged again under the same conditions. The final supernatant was transferred to fresh tubes and stored at −20°C until further use. Nymphs and adults were homogenised separately in ice-cold PBS (pH 7.0; SRL, India) in 1.5 mL centrifuge tubes at 10% (w/v) ratio using a tissue homogeniser (Precision Scientific, Varanasi, India) for 30 seconds at 4ºC. Homogenates were centrifuged at 10,000 g for 10 minutes at 4ºC in a centrifuge (REMI; NEYA 16R Refrigerated centrifuge HIGH SPEED, 4× 175mL, 16000 rpm). The supernatant from each sample was transferred to a fresh centrifuge tube, and the samples were stored at −20°C in a mini cooler until used as the sample solution.

Biochemical assays (Nutrient assimilation by) of *L. erysimi*

Glucose and lipid quantification in whole-body homogenates was conducted using a coupled colourimetric assay using Liquizyme Glucose and Triglycerides Kits respectively (*Beacon Diagnostics* Pvt Ltd, India)[19].

Protein concentration was determined according to the Bradford method with bovine serum albumin as the standard[20]. The Bradford reagent, made with Coomassie Brilliant Blue G-250 (SRL, India) and orthophosphoric acid (Merck, USA), was used. The protein standard was prepared by serially diluting 1 mg/mL BSA (SRL, India). 1 µL of diluted standard was mixed with 200 µL of the Bradford reagent to a flat bottom 96-well microplate (Tarsons, India) and incubated for 10 minutes at 25°C. The sample was then covered with aluminium foil and absorbance at 595 nm was measured using a microplate reader (Biotek Instruments Inc., ELX 800, USA). Using absorbance and concentration, the standard curve was plotted ($R^2 = 0.997$).

1 µL of the respective supernatant was mixed with 9 µL of distilled water and transferred to a flat bottom 96-well microplate (Tarsons, India). 10 µL of triple distilled water was used as a blank. Thereafter, 200 µL of Bradford reagent was added to each well containing sample or blank, and incubated for 10 minutes at 25°C. Each sample reading was taken in

triplicates and absorbance at 595 nm was measured using a microplate reader (Biotek Instruments Inc., ELX 800, USA). Standard curve prepared earlier was used for the determination of protein concentration in the unknown samples.

Enzyme Activity Assays of *L. erysimi*

In a fresh centrifuge tube, 700 µL of the reaction buffer was prepared using PBS = 550 µL, L-methionine = 40 µL (20 mM; SRL, India), Triton X-100 = 20 µL (1%; SRL, India), hydroxylamine-hydrochloride = 40 µL (10 mM; SRL, India) and ethylenediaminetetraacetic acid (EDTA) = 50 µL (50 mM; SRL, India). Within the reaction buffer, 50 µL of the sample was added, whereas in reference and blank solutions, 50 µL PBS was added. The above tubes were transferred to an incubator for preincubation at 37°C for 5 min. Thereafter, 40 µL (50 µM) of riboflavin (SRL, India) was added to both the sample and the reference solutions. Then the reaction mixture tubes containing the samples were exposed to 15 W CFL light (Philips, India) for 10 minutes and subsequently 500 µL of freshly prepared Greiss reagent was added to all the tubes. The UV-visible spectrophotometer was set upto measure SOD activity at 543nm and calibrated with the blank solution, and the absorbance of the sample and reference was recorded. The SOD activity calculation was done using the following formula:

$$\text{SOD (U/mg)} = \{(\text{Vo/V}) - 1/\text{mg of protein}\}$$

where Vo is the absorbance of the reference solution and V is the absorbance of the sample solution. The enzyme activity was expressed as U/mg protein.

The CAT activity was estimated using the protocol given by Aebi (1984). CAT activity was measured by assessing the reduction in H_2O_2 during its decomposition. Approximately 10 µL of the enzyme extract diluted with PBS (690 µL) was added to a centrifuge tube containing 300 µL of 30 mM H_2O_2 (Avantor Performance Materials, India) and the tube was quickly placed in a UV–visible spectrophotometer (UV–VIS Spectrophotometer 119, Systronics India limited) kept at 240 nm to record the absorbance with a recorder at 30 seconds intervals for 3 minutes. The readings were recorded in triplicate (for each larval/adult sample), and the average of the three readings was considered as a single value for further statistical analysis. Calculations of CAT activity were done with the help of the following formula:

$$\text{CAT activity (U/mg)} = \frac{(A_0 - A_{180}) \times V_t}{\varepsilon_{240} \times d \times V_s \times C_t \times 0.001}$$

where $(A_0 - A_{180})$ is the difference between the initial and final absorbance; V_t is the total volume of the reaction (1 mL); ε_{240} is the molar extinction coefficient for H_2O_2 at OD 240 nm (34.9 mol^{-1} cm^{-1}); d is the optical path length of the cuvette (1 cm); V_s is the volume of the sample (10 µL); C_t is the total protein concentration in the sample; and 0.001 is the absorbance change caused by 1 U of enzyme per minute at 240 nm OD. One unit of CAT activity corresponds to the breakdown of 1 nmol H_2O_2 per second per mg protein (U/mg protein).

The TBARS assay is a common method used to measure lipid peroxidation[21]. The assay quantifies the amount of malondialdehyde (MDA), a byproduct of lipid peroxidation, present in the sample. The thiobarbituric acid (TBA) reacts with MDA, forming a pink-coloured complex. Approximately 50 µL of the supernatant from each sample was added to 825 µL of thiobarbituric acid (TBA) reagent (Sigma Aldrich, Germany) prepared using 50 µL (8%) of sodium dodecyl sulfate (SDS) (SRL, India), 375 µL (20%) of acetic acid (Merck, USA), 375 µL (0.8%) of an aqueous solution of thiobarbituric acid and 25 µL (0.8%) of butylated hydroxy toluene (SRL, India). The reaction mixture containing the sample was incubated at 95°C in a water bath, which resulted in the appearance of a pinkish colour. After 1 hour, the tubes were taken out, cooled and centrifuged at 3000 *g* for 10 minutes to remove any debris. The supernatant (800 µL) was collected and the absorbance was measured at 532 nm in a UV–visible spectrophotometer. The amount of TBARS present was calculated using the following formula:

$$\text{nmoL TBARS/mg protein} = \frac{V \times OD}{156 \times C}$$

where V is the reaction mixture volume (µL); OD is the absorbance at 532 nm; 156 is the extinction coefficient of the MDA-TBA complex at 532 nm (mmol−1 cm−1); C is the protein concentration (mg).The LPO activity was expressed as nmol of TBARS produced per mg protein.

All readings were recorded in triplicate. The average of three readings was considered as a single

value for statistical analysis. Protein content was used to quantify SOD and CAT activities, with absorbance values measured using a full-wavelength spectrophotometer (ELX800UV, Bio-Tek Instruments Inc., Winooski, VT, USA).

Statistical analysis

The data on biochemical (glucose, triglycerides and proteins) parameters of the mustard aphid (winged and wingless forms) were subjected to one-way ANOVA and followed by Tukey′s post-hoc comparison of means at a level of significance (α) = 0.05, considering MINITAB-18 (Minitab Inc., State College, Pennsylvania, United States of America) as the statistical software. This corresponds to a 95% confidence level for interpreting the results. All graphs were plotted in Excel sheets (MS-EXCEL). All data were represented by a line graph and bar charts with values considering Mean±Standard Errors (SE).

Results and Discussion

The results indicated that the incidence of *L. erysimi* began during the 52nd Standard Meteorological Week (SMW) of 2023, reaching its peak in the 5th SMW of 2024, with 72.67 aphids per 10 cm terminal shoot (Fig. 2). The initial low aphid population observed in the third week of December gradually increased, peaking by the end of January, before declining in early March. These population fluctuations were strongly influenced by variations in temperature and humidity, which are known to significantly impact aphid biology and population dynamics.

In the present study, aphid populations exhibited a significant negative correlation with maximum temperature (r=-0.682**) and a non-significant negative correlation with minimum temperature (r=-0.432^{NS}). Moreover, aphid populations demonstrated a positive but non-significant correlation with morning (r=0.523 ^{NS}) and evening relative humidity (r=0.510 ^{NS}) percentages (Table 1).

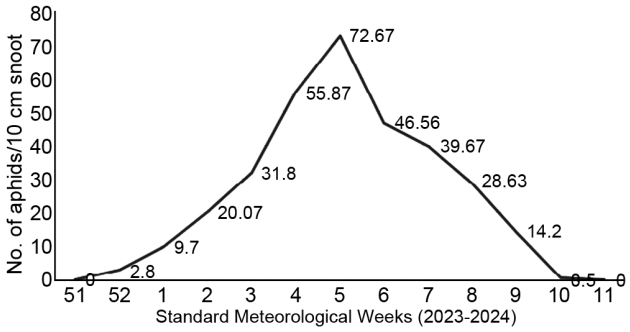

Fig.2 — Seasonal fluctuation of *Lipaphis erysimi* population on *Brassica juncea* based on aphid count per 10 cm terminal shoot: The figure depicts the weekly variation in aphid population density from the 52nd Standard Meteorological Week (SMW) of 2023 to the 10th SMW of 2024. Population buildup commenced in the third week of December, peaked during the 5th SMW of 2024 (late January), and declined thereafter.

Table 1 — Pearson′s correlation coefficient between number of predators per plant and weather parameters with population of *Lipaphis erysimi* during *rabi* 2023-24

Parameters	Number of ladybird beetles per plant	Number of spiders per plant	Mustard aphid per 10 cm of terminal twigs	Maximum temperature (°C)	Minimum temperature (°C)	Evening relative humidity (mm)	Morning relative humidity (mm)	Wind speed (km/ hour)	Rainfall (mm)	Bright sunshine hour (Hour)
Aphid population per 10 cm of terminal twigs	0.923*	0.586*	1.00							
Maximum temperature (°C)	-0.245^{NS}	-0.225^{NS}	-0.682**	1.00						
Minimum temperature (°C)	0.142^{NS}	0.140^{NS}	-0.432^{NS}	0.660**	1.00					
Morning relative humidity (%)	0.355^{NS}	0.426^{NS}	0.523 ^{NS}	-0.678**	-0.381^{NS}	1.00				
Evening relative humidity (%)	0.636*	0.485^{NS}	0.510 ^{NS}	0.062^{NS}	0.719**	0.120^{NS}	1.00			
Wind speed (km/hour)	-0.436^{NS}	-0.444^{NS}	-0.468^{NS}	0.594*	0.567*	-0.896**	0.153^{NS}	1.00		
Rainfall (mm)	-0.194^{NS}	0.128^{NS}	-0.274^{NS}	0.231^{NS}	0.416^{NS}	-0.126^{NS}	0.280^{NS}	0.185^{NS}	1.00	
Bright sunshine hour (Hour)	-0.438^{NS}	-0.614*	-0.519*	0.192^{NS}	-0.453^{NS}	-0.455^{NS}	-0.746**	0.221^{NS}	-0.540*	1.00

[NS- Non-significant, *Significant at 5% level, **Significant at 1% level]

In case of predators, number of ladybird beetles per plant showed positive and significant correlation with evening relative humidity (r = 0.636*) while all the other weather factors had non-significant relationship with ladybird beetle population. Number of spiders per plant showed a negative and significant relationship with bright sunshine hour (r = -0.614*) while all the other weather parameters did not have a significant effect on the population of spiders (Table 1).

From results we can find that ladybird beetles (r = 0.923*) and spiders (r = 0.586*) had a positive and significant correlation with the number of aphids per 10 cm of terminal twigs. The correlation analysis between prey and predators revealed a significant positive interaction, indicating that an increase in aphid population leads to a corresponding rise in predator numbers. This pattern aligns with the Lotka-Volterra model, which predicts that higher prey growth rates contribute to an increase in predator equilibrium density.

Glucose concentration

Although one-way ANOVA values revealed non-significant effect (F(1, 14) =2.17; P=0.164), of glucose concentration within the two morphs (winged vs wingless) of mustard aphid, however, comparison of means further exposed that the concentration of glucose in the apterous forms (0.877±0.130) was higher than in the alate forms (0.601±0.117). This result is consistent with the understanding that wingless forms often exhibit reduced locomotor activity and metabolic demands compared to their winged counterparts. Studies on various insects have demonstrated that winged forms generally have lower glucose levels due to their higher metabolic rates associated with flight. Many species show significant depletion of carbohydrates, including glucose, during long flights, indicating high energy expenditure in winged forms.

Triglyceride concentration

While, statistical analysis revealed non-significant effect (F(1, 14) =3.57; P=0.081), of triglyceride concentration within the two morphs (winged vs wingless) of mustard aphid. However, the triglyceride concentration in alate forms (0.536±0.163) was higher than in apterous forms (0.284±0.020). Triglycerides are crucial for sustained energy release, particularly in insects that engage in flight. The winged morphs store more lipids compared to the wingless morphs possibly owing to the need for high energy reserves for flight endurance. The high triglyceride concentration in alates may therefore be an adaptive trait enabling prolonged flight, crucial for dispersal and colonisation of new habitats.

Soluble protein concentration

The concentration of soluble proteins in apterous forms (9.174±0.814 mg/mL) was substantially higher than in alate forms (4.547± 1.461 mg/mL), and the differences were statistically significant (F(1, 12) =8.88; P=0.013). Soluble proteins are vital for various physiological functions, including stress responses and development. Higher protein concentrations in wingless forms could indicate a greater investment in growth and development processes, possibly at the expense of locomotor functions. Moreover, apterous aphids invest more in somatic growth and reproduction compared to their alate counterparts, which are more focused on dispersal. The relation between the OD values (y) and the BSA concentrations (x) was y= 0.0409x + 0.148 with an R of 0.99786.

The changes in the glucose, triglyceride and protein content of *L. erysimi* in the two morphs are presented in Fig. 3. Glucose concentration did not differ significantly between the alate and apterous morphs of mustard aphid (F(1,14) = 2.17, P = 0.164), although the mean value was higher in apterous forms (0.877 ± 0.130) than in alate forms (0.601 ± 0.117). Similarly, triglyceride concentration showed no significant difference between morphs (F(1,14) =

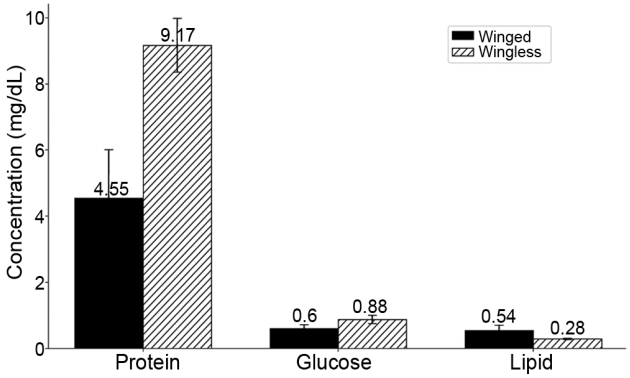

Fig.3 — Comparison of protein, glucose, and lipid between Wingless and Winged morphs: The figure illustrates comparative concentrations of (A) soluble proteins, (B) glucose, and (C) triglycerides between winged (alate) and wingless (apterous) forms Apterous aphids exhibited higher levels of glucose and soluble protein, reflecting investment in growth and reproduction, whereas alate aphids showed elevated triglyceride reserves, indicative of energy storage for flight.

3.57, P = 0.081); however, alate forms exhibited a higher mean triglyceride concentration (0.536 ± 0.163) compared with apterous forms (0.284 ± 0.020). In contrast, soluble protein concentration differed significantly between morphs (F(1,12) = 8.88, P = 0.013), with apterous forms showing markedly higher levels (9.174 ± 0.814) than alate forms (4.547 ± 1.461).

These findings (Fig. 3) show differential allocation of biochemical resources between the alate (winged) and apterous (wingless) forms of *L. erysimi* suggesting distinct metabolic adaptations associated with their morphological forms.

Superoxide dismutase (SOD) activity

SOD activity was relatively higher in alate forms (0.405±0.042) compared to apterous forms (0.314±0.067). The higher SOD activity in alate forms further suggested that winged insects have a greater need to neutralize ROS generated during flight.

Catalase (CAT) activity

Catalase activity was relatively higher in alate forms (0.046±0.011) compared to apterous forms (0.023±0.006). Catalase is an enzyme responsible for breaking down hydrogen peroxide into water and oxygen, serving a vital function in safeguarding cells against oxidative stress. The higher catalase activity in alate forms suggests that winged insects experience greater oxidative stress, likely due to the high metabolic demands of flight. Increased metabolic rates during flight may lead to elevated ROS production, necessitating higher catalase activity to mitigate oxidative damage.

Lipid peroxidation (LPO) activity

LPO activity was also relatively higher in alate forms (0.210±0.035) compared to apterous forms (0.068±0.004). LPO quantifies lipid peroxidation levels, serving as an indicator of oxidative stress and cellular membrane impairment. The increased LPO activity in alates suggests greater oxidative damage, aligning with their heightened metabolic demands. Organisms with higher metabolic rates tend to exhibit greater oxidative damage, supporting the observed higher LPO levels in winged forms.

The comparison of enzymatic activity between alate (winged) and apterous (wingless) forms (Fig 4) revealed non-significant differences at the 0.05 level in the activity of superoxide dismutase (F(1, 9) = 1.41, P = 0.269), catalase (F(1, 12) = 3.26, P = 0.098), and lipid peroxidation (F(1, 7) = 4.63, P = 0.075).

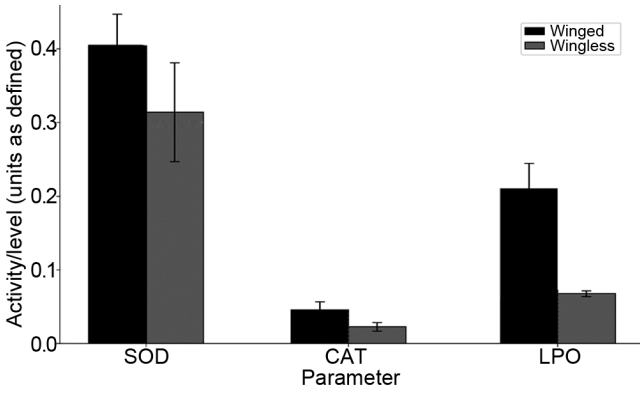

Fig.4 — Comparison of SOD, CAT and LPO in Winged and Wingless morphs: The figure illustrates comparative enzymatic activities of (A) superoxide dismutase (SOD), (B) catalase (CAT), and (C) lipid peroxidation (LPO) between winged (alate) and wingless (apterous) forms. Enzymatic assays revealed greater antioxidant activity (SOD, CAT) and LPO in alates, suggesting increased oxidative stress due to dispersal-related metabolic demands.

The present findings highlight the intricate relationship between climatic factors and aphid population dynamics, particularly in relation to temperature and humidity. Consistent with earlier reports by Sarkar *et al.*[22], Dotasara *et al.* [23], and Samantaray & Singh[24], a significant negative correlation was observed between mustard aphid (*Lipaphis erysimi*) populations and temperature. Kumar *et al*.[25] further corroborated these findings, identifying negative correlations with both maximum and minimum temperatures, while reporting a positive correlation with morning relative humidity. Together, these studies validate the thermosensitivity of aphid populations and highlight the predictive value of climatic parameters for forecasting pest outbreaks. Temperature extremes can disrupt aphid physiology, influence reproductive capacity, and affect their developmental rates, thereby regulating population growth. Relative humidity, particularly in the morning, may provide favorable microclimatic conditions that enhance aphid survival and feeding behavior, facilitating population persistence during early diurnal periods.

The study also identifies key natural enemies of *L. erysimi*, including the predatory ladybird beetle (*Coccinella septempunctata*) and a guild of spiders such as the jumping spider (*Plexippus paykulli*), Adanson's house jumper (*Hasarius adansoni*), wolf spider (*Wadicosa fidelis*), and crab spider

(*Thomisus projectus*)[26]. These predators represent a crucial component of natural biological control in mustard ecosystems. Their presence suggests a functioning trophic interaction network where natural enemies can exert regulatory pressure on aphid populations. The behavioral adaptations and hunting efficiency of these predators, especially under varying environmental conditions, warrant further investigation to integrate them into ecologically-based pest management frameworks.

The biochemical profiling of apterous and alate morphs of mustard aphid revealed distinct differences in nutrient assimilation patterns. Significant variation in glucose, triglyceride, and soluble protein concentrations between morphs reflects physiological adaptations linked to their dispersal and reproductive roles. These metabolic distinctions, when considered alongside predator efficiency and climatic influences, highlight the multifactorial regulation of aphid population dynamics. Integrating biochemical, ecological, and environmental variables offers a holistic perspective essential for sustainable pest management. Future research should focus on how seasonal and climate-induced changes in aphid nutrient composition influence predator-prey interactions, with the goal of optimizing ecologically resilient biocontrol strategies in mustard agroecosystems.

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Conflict of interest

The authors declare no competing interest.

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