

EVALUATION OF ORTHO-DIHYDROXY PHENOL IN DIFFERENT LINES AGAINST MUSTARD APHID ON MUSTARD CROP

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Abstract

The Department of Plant Physiology at I.A.S., B.H.U., Varanasi conducted a detailed investigation to pinpoint the biochemical factors that contribute to resistance in mustard against the mustard aphid, *Lipaphis erysimi* (Kalt.). The analysis showed that the lines NDR08-14-1, NDR08-1, and NDYR-8 contained the highest amounts of ortho-dihydroxy phenols, a compound closely linked with natural defense. On the other hand, the lines NDRS-9-2 and NDRS2001-1, which suffered the heaviest aphid attack, recorded the lowest levels of this biochemical marker. This pattern confirmed a strong negative association between aphid incidence and ortho-dihydroxy phenol content. Such resistant lines highlight the value of host plant resistance in integrated pest management, offering farmers a reliable, low-cost and environmentally friendly method to suppress pest pressure.

Introduction

Mustard is cultivated in many parts of the world and serves multiple purposes, including human consumption, livestock feed, and various medicinal uses. Oilseed crops hold a major place in the agricultural system of India, covering close to thirteen percent of the total cropped area. They contribute roughly five percent to the national GDP and make up about eleven percent of the total value of agricultural produce. Among these, mustard stands alongside groundnut and soybean as one of the country's three most important oilseed crops, supplying nearly one-fourth of India's total oilseed production. The crop is grown on about 6.70 million hectares, producing nearly 7.96 million tonnes with an average yield of 1188 kg per hectare (DOAC 2014).

Despite its importance, mustard production in India faces heavy losses due to the mustard aphid (*Lipaphis erysimi* Kalt.), a persistent pest across major rapeseed-mustard regions. These aphids feed by extracting sap from leaves, stems, inflorescences, and pods, weakening the plant over time. Their attack begins as early as the vegetative stage and may continue until pod formation (Sachan 1990). Severe infestations have been reported to cause up to 96 percent yield reduction and a drop of 5-6 percent in oil content (Shylesha *et al.* 2006). Losses may range from 66-99 percent in *Brassica campestris* and around 27-28 percent in *Brassica juncea* (Bakhietia 1979), with oil content falling by as much as 15 percent (Verma and Singh 1987). Under field conditions, avoidable losses of 20-50 percent are common, while extreme situations can push the losses as high as 78 percent (Prasad and Phadke 1980).

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Host plant resistance plays a central role in integrated pest management because it offers protection without disturbing the environment or harming beneficial insects. It allows crops to defend themselves naturally, reducing the need for frequent chemical sprays. Painter (1951) first outlined three fundamental mechanisms through which plants resist insect attack: non-preference, later refined as antixenosis by Kogan and Ortman (1978), where insects avoid the plant; antibiosis, where the plant adversely affects insect biology; and tolerance, where the plant can withstand damage without major loss in yield.

Through long-term co-evolution with herbivores, plants have developed an impressive variety of secondary metabolites and biochemical compounds that contribute to these mechanisms. A single plant may contain hundreds of natural chemicals, many of which influence insect behaviour, growth, or reproduction. Some deter feeding, others interfere with digestion or development, and some strengthen plant tissues to reduce damage. Together, these biochemical defences form an important foundation for developing crop varieties that can thrive under insect pressure with minimal chemical dependence.

Phenolic compounds play a major role in several plant processes, including the regulation of plant hormones, and they serve as important elements in the plant's built-in defence against insect pests. These compounds can reinforce plant tissues, interfere with an insect's ability to feed, or activate internal signalling systems that prepare the plant for attack. With the advancement of modern biochemical and molecular tools, it has become far more practical to investigate how these substances contribute to natural resistance. This information is especially valuable for breeding programs that seek to develop varieties capable of handling pest pressure with reduced dependence on insecticides.

Based on this background, the present investigation was designed to explore the relationship between the mustard aphid (*Lipaphis erysimi*) and the biochemical makeup of various mustard lines. By measuring the concentrations of important defensive compounds and comparing them with the intensity of aphid infestation, the study aims to pinpoint traits that may serve as reliable indicators of resistance. Such insights can support the identification or development of mustard genotypes that show stronger tolerance or natural protection against aphid attack.

Materials and Methods

During the flowering period, when aphid activity is usually at its peak on mustard inflorescences, the study was carried out to assess aphid buildup and plant responses. Earlier studies indicate that flowering stems often show stronger defence against herbivores, likely because any injury to reproductive tissues can reduce the plant's overall fitness. With this in mind, experiments were conducted at the Vegetable Research Farm, Institute of Agricultural Research Sciences, Banaras Hindu University, to evaluate the feeding preference of *Lipaphis erysimi* on a range of mustard lines over two consecutive seasons, 2015-16 and 2016-17.

The mustard lines were sown late in the season in plots measuring 3×4.8 meters. A spacing of 45 cm between rows and 15 cm between plants was maintained to ensure uniform growth. Once seedlings emerged, all routine agronomic practices were followed through the season. No insecticidal treatments were used anywhere in the experimental field so that natural aphid colonization could occur without disturbance. The list of mustard lines included in the study is provided separately.

Aphid monitoring began as soon as the first colonies appeared. Observations were taken at three different crop stages to track changes in infestation. From each plot, five plants were selected at random, giving a total of fifteen plants per mustard line. These plants were tagged and observed

regularly until the end of the season. Aphid density was estimated by counting the insects on a standardized 10 cm segment of each plant, ensuring uniformity across all treatments.

Seventy days after sowing, biochemical sampling was carried out. From each mustard line, the top 10 cm of fully developed floral shoots was collected from vigorous, randomly chosen plants. Each sample was kept in a separate polythene bag and transported to the laboratory to prevent contamination. The samples were then dried in a hot air oven at 70°C for three days. After complete drying, they were finely ground and stored in labelled polythene bags for biochemical analysis. The dried material from the top 10 cm inflorescence portion was used to determine the concentration of ortho-dihydroxy phenols, expressed as milligrams per gram of dry tissue.

The estimation of ortho-dihydroxyphenols followed the method outlined by Nair and Vaidyanathan (1964). From each sample, 5 ml of the methanol extract was taken and evaporated to dryness. The residue was dissolved in 1 ml of distilled water, followed by the addition of 0.5 ml of 10 per cent TCA, 1 ml of sodium tungstate, 0.5 ml of 0.5 N HCl, and 1 ml of freshly prepared 0.5 percent sodium nitrite. This mixture developed a yellow colour. After five min, 2 ml of 0.5 N sodium hydroxide was added, producing a light cherry coloration. The absorbance of the solution was measured exactly 15 min later at 540 nm, using a blank containing only water and reagents.

The amount of ortho-dihydroxyphenols present in each sample was determined using a standard curve prepared with catechol concentrations ranging from 10 to 100 µg/ml. To understand how these biochemical traits relate to aphid pressure, correlation coefficients were calculated between total phenol content (mg/g) and the average number of aphids per plant for all twenty-two mustard lines. The statistical software OPSTAT was used for this analysis.

Results and Discussion

The data generated on the ortho-dihydroxy phenol content across the mustard lines are summarized in Table 1 and illustrated in Figs. 1, 2 and 3. The screening revealed considerable variation among the twenty-two lines tested. The highest concentration of ortho-dihydroxy phenols (1.11 mg/g) was recorded in the line NDR08-14-1, which emerged as the most promising genotype in terms of biochemical defense. This line was closely followed by NDR-08-1, NDYR-8, NDR1-11, MCN14-24, MCN14-32, MCN14-33, MCN14-31, MCN14-25, NDR07-2, MCN14-23, MCN14-26, MCN14-27, MCN14-29, NDR-8501, VARUNA, NDR-7, MCN14-30, DIVYA-33, NDRS2010, NDRS2001-1, and NDRS9-2, with corresponding phenol levels of 1.09, 1.08, 1.03, 1.03, 1.02, 1.01, 1.01, 1.00, 0.99, 0.98, 0.98, 0.98, 0.98, 0.96, 0.89, 0.86, 0.80, 0.77, 0.72, 0.63, 0.63, and 0.55 mg/g.

A closer statistical examination showed that NDR08-14-1, although the highest in absolute value, did not differ significantly from NDR-08-1, NDYR-8, NDR1-11, MCN14-24, and MCN14-32. These lines therefore form a top-performing group with respect to phenolic content. Similarly, NDR-08-1, NDYR-8, NDR1-11, MCN14-24, MCN14-32, MCN14-33, MCN14-31, and MCN14-25 exhibited statistically similar phenol levels, though NDR-08-1 still showed an edge over several other lines in the dataset.

Several mid-ranking lines, including MCN14-31, NDR1-11, MCN14-24, MCN14-27, MCN14-33, MCN14-23, NDR07-2, MCN14-26, and MCN14-25, also formed statistically comparable groups. These genotypes did not differ significantly from each other, but most of them recorded higher phenolic content than the lower-performing lines. The cluster involving MCN14-29, NDR8501, and VARUNA displayed no significant internal differences, although MCN14-29 showed slightly higher phenol levels compared with the remaining members of this set.

Further down the ranking, the varieties VARUNA, NDR-7, and MCN14-30 were statistically similar; however, VARUNA maintained marginally higher phenolic levels than several of the

other lower-performing lines. NDR-7 and MCN14-30 did not differ from each other but showed significantly higher phenolic concentrations than DIVYA-33, NDRS2010, and NDRS2001-1. The latter three lines were statistically alike, but DIVYA-33 performed better than NDRS9-2, which recorded the lowest phenol content in the screening.

Table 1. Biochemical constituents in inflorescence part of various mustard lines in relation to aphid population during 2015-16-and 2016-17(Pooled data).

Lines	Different biochemical constituents with average aphid population	
	Ortho-dihydroxy phenol (mg/g)	Aphid population/top10 cm inflorescence
MCN14-23	0.98	37.02 (1.56)
MCN14-24	1.03	29.39 (1.48)
MCN14-25	1.00	55.29 (1.81)
MCN14-26	0.98	48.64 (1.68)
MCN14-27	0.98	41.70 (1.62)
MCN14-29	0.96	57.72 (1.82)
MCN14-30	0.77	111.09 (2.05)
MCN14-31	1.01	26.57 (1.42)
MCN14-32	1.02	51.68 (1.79)
MCN14-33	1.01	25.48 (1.41)
DIVYA-33(S Check) *	0.72	111.01 (2.04)
NDRS2001-1	0.63	152.80 (2.46)
NDRS9-2	0.55	154.47 (2.52)
NDRS2010	0.63	107.87 (1.99)
NDR08-1	1.09	16.18 (1.13)
NDR08-14-1	1.11	15.81 (0.97)
NDR 07-2	0.99	48.84 (1.69)
NDR-7	0.80	65.51 (1.81)
NDYR-8	1.08	22.34 (1.30)
NDR-8501	0.89	53.33 (1.74)
NDR1-11	1.03	28.27 (1.46)
VARUNA (R Check) *	0.86	67.87 (1.83)
C.D. ($P \leq 0.05$)	0.09	0.14
S.E.M. $\pm$	0.06	0.04
Correlation Coefficient	-0.97**	-

*S Check- Susceptible Check, * R Check- Resistance Check, **Correlation is significant at 0.01 level.

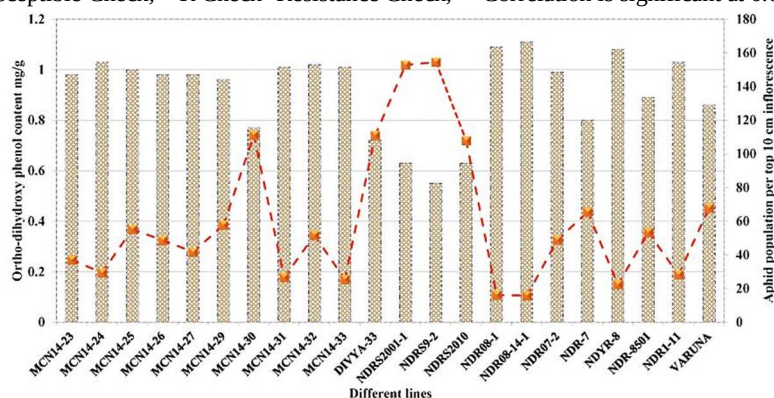


Fig. 1. Ortho-dihydroxy phenol content of different lines in relation to average aphid population during 2015-16 and 2016-17 (Pooled data).



Fig. 2. Resistance line observed during experiment.

Across all lines, the statistical analysis revealed a significant negative correlation between ortho-dihydroxy phenol content and aphid density, indicating that higher phenolic levels were generally associated with reduced aphid infestation. This finding aligns with several earlier studies. Kumar and Sangha (2013) observed lower aphid populations on lines T27 and DLSC2, attributing this to their elevated ortho-dihydroxy phenol concentrations. Kaur (2008) also reported that genotypes with higher phenol content tend to experience reduced aphid pressure.



Fig. 3. Susceptible line observed during experiment.

Similar trends have been documented for other Brassica species. Jensen *et al.* (2002) reported strong antibiosis resistance in *B. fruticulosa* against the cabbage root fly, *Delia radicum*, linked to elevated phenolic levels. Singh *et al.* (2000) also noted that mustard lines T-27 and RK-9501 had lower aphid infestation due to their higher phenolic content. Yadav and Rana (2018) found that genotypes with phenol levels exceeding 1.70% (such as RW-2-2, RW White Glossy, RC-199, Purple Mutant, and B-85 Glossy) had significantly lower aphid loads compared to genotypes like RH-30, Rohini, and Pusa Jaikisan, which had lower phenol concentrations.

The defensive role of ortho-dihydroxy phenols is well established. These compounds function as antifeedants, reducing the palatability of plant tissues, and act as toxins that interfere with insect metabolism. They inhibit key detoxification enzymes in insects, making it difficult for them to process plant defences. In addition, these phenols can induce oxidative stress by promoting the generation of reactive oxygen species, which damage insect cells. They also contribute to structural resistance by promoting lignin formation, strengthening plant cell walls and reducing insect feeding efficiency. Beyond these functions, ortho-dihydroxy phenols activate several defense-related genes, amplifying the overall resistance response of the plant.

Parallel observations were made by Kumar *et al.* (2017), who reported that varieties with higher phenol concentrations supported fewer aphids. Earlier work at Ludhiana (Anonymous 2007) showed a similar pattern in purple mutant, DLSC-2, and T-27. Sachan and Sachan (1991) also concluded that varieties with higher phenolic content tend to express greater resistance compared to those with lower levels.

Overall, the present findings reaffirm the importance of ortho-dihydroxy phenols as a key biochemical marker of resistance in mustard. Their consistent association with reduced aphid infestation highlights their role in breeding strategies aimed at developing robust, pest-tolerant mustard cultivars.

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