Screening of Selected Canola Genotypes against Mustard Aphid (*Lipaphis erysimi* (K.) Hemiptera: Aphididae) through Antixenosis and Antibiosis Assays

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**ABSTRACT**

The study on categories of resistance in selected canola genotypes against mustard aphid (*Lipaphis erysimi*) was conducted under glasshouse conditions, The University of Agriculture, Peshawar during growing seasons of 2018-2019. During this study, a total of two different experiments were carried out where, antixenosis experiment, comprised three levels; seedling, flowering and pod stage. In all the three stages, only genotype ‘KS-75’ proved as antixenosis resistant against the aphid compared to susceptible genotype Abaseen. The second experiment was performed to test antibiosis resistance against *L. erysimi* among the selected genotypes. During the antibiosis experiment different life table parameters of aphids; developmental period, reproductive period, longevity and fecundity were studied. Based on the calculations, only ‘KS-75’ proved antibiosis resistant against *L. erysimi* among the selected genotypes. Thus, during our current studies, genotype KS-75 proved antixenosis and antibiosis characteristics based on less number of aphids attracted/sustained and low number of progenies produced as compared to the tested genotypes Abaseen, Omega and Zahoor showed strong vigor against mustard aphid and symptoms of attack/damages were observed.

**INTRODUCTION**

Mustard plants are attacked by various insect pests including cabbage caterpillar, leaf miner and mustard aphid in Pakistan (Aslam and Razaq, 2007). They suck cell sap from young leaves, twigs, buds, pods and flowers and pods of the plants. Consequently, the affected plants lose their vitality, vigor growth and become stunted. Both adults and nymphs may congregate on leaves, flowers, tender, stalks and pods that suck the cell sap and provide damage indirectly by secreting the honey-dew. Insect pests and diseases are important factors responsible for yield reduction in canola crops. The leaves become curly and turn pale yellowish resulting in premature fall of the flowers. Yield losses that occur only due to the mustard aphid (*L. erysimi*) in crop account for approximately 50-75% (Tolba, 2020).

Plants that are resistant to insect herbivores have unique characters that enable them to resist insect attack. Many factors are involved in plant resistance to insect pests including antixenosis, antibiosis and tolerance (Rana, 2005; Shylesha *et al*., 2006). Due to consumer acceptance and market demand, the varietal resistance has received priority in Integrated Pest Management programmes. Cultivation of resistant or tolerant varieties is the very effective and cheapest method of cultural control to save mustard crop from being attacked by insect pests. Utilization of resistant varieties/germplasms against aphids results in increased production and reduces harmful pesticides residue in the environment (Dey *et al*., 2005).

Several control strategies have been evolved so far to manage mustard aphids like physical, mechanical, cultural, biological, chemical and host plants resistant control. Injudicious use of chemical pesticides led to the development of resistance in several species of insect pests and also negatively affects survival and adaptation of biocontrol agents (Essani *et al*., 2020; Dwivedi and Singh, 2020). Application of mustard crops field with heavy chemicals can cause mortality of natural enemies and also may cause environmental pollution (Mpumi *et al*., 2020). The development of insecticide resistance in various species of insect pests has forced the plant protectnists to...
MATERIALS AND METHODS

The study was conducted in the glasshouse condition at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan during the crop growing seasons, 2018-19. The glasshouse trials were laid out in a complete randomized design with ten replications. Four selected genotypes viz. Abaseen, KS-75, Omega and Zahoor were used during the study.

Preparation of plant and insect culture

Plant culture was prepared to establish seedlings for aphid rearing. The seeds of four selected canola genotypes (B. napus) were individually sown in round mud trays. Already established of aphid dense colonies in the field, were carefully shifted on fresh plant (susceptible genotype) for multiplication to further use for the experimentation in the glasshouse. Trays were filled with soil as a substrate. All trays were kept in the sliding metal trays to enable exchange of water and aeration. With great care plants in glasshouse were maintained at specific conditions [20 ± 2°C, 60-65 % RH, and of 14:10 h (D:L) photo-period]. Plants cultures were observed for water levels and clean water was provided to the sliding metal trays if required, on a daily basis. In case when plants get damaged, these were exchanged with new plants and aphids were also transferred to new plants to maintain their colony for smooth running of the trial, while old plants were carefully destroyed to avoid cross contamination. Pots were filled with potting mix materials. Pots were kept in the iron-sliding tray to permit water interchange. After emergence, plants were maintained in a growth chamber. Single seedlings of each variety were planted in separate pots (16 cm x 32 cm height and circumference). Antibiosis resistance against the aphids was determined by caging an individual adult aphid on each genotype at four to six leaves stage (seedling stage). Susceptible canola plants (for raising aphid colony), when required, were substituted with a new plant and old plants were carefully destroyed to avoid cross contamination. When enough colonies of aphids started to establish, the L. erysimi were carefully introduced to fresh clean plants. Before transferring aphids to new plants, careful observations were made to check and identify the presence of mummified forms.

Antixenosis assay

During the antixenosis experiment, three similar tests were planned and conducted on selected genotypes of canola at different stages of plants against L. erysimi (six leaves, flowering and pods stage). During the antixenosis test, the procedure of Kumar et al. (2011) was followed. For this experiment, four trays were maintained in a CR...
design. After ten days of plant transplantation, wingless (apterous) *L. erysimi* from culture were taken using a fine camel hair brush into a petri dish containing filter paper and were counted, which were afterwards carefully transferred to the middle of each tray. Seeds of each variety were sown in circular mud trays filled up to 12cm height with mixed soil materials. A total of 100 virgin (last instars') aphids were released in the center of the tray at six leaves stage of the seedling. Data for preference and non-preference behavior of (*L. erysimi*) was started after 12 h, 24 h and 48 h post infestation of *L. erysimi* on selected canola genotypes (Abaseen, KS-75, Omega, and Zahoor). Similar procedure for the second and three tests was adopted on the analogy of the first test.

**Antibiosis assay**

Newly born nymphs raised up to the last instar stage collected from the insect culture stock were used in the experiment. When plants reached (seedling) four to six leaves stage, the aphid was caged on the midsection of the leaf. Cages were ventilated by nylon mesh cloth to prevent escape. Clip-cages were made with double sided 2.54 x 2.54cm foams mounted square, with circular inner side areas of 1.2cm square. Each plant leaf was infested with last instar aphids and was caged. The caged aphid was observed twice a day to observe the first nymph produced (F1). When the caged aphid (P) produced its first offspring (F1), the time and date was recorded was recorded. The aphids were then transferred to another leaf (same plant) and caged again and were maintained until F1 offspring were produced. The time duration (days) was recorded and the experiment was terminated. During experiment, the number of progenies produced by P were observed and counted as (M1). For each variety the natural intrinsic rate of increase (rm) was calculated by using the equation 

\[ r_m = 0.738 \ln(M/d) \]

where \( M \) is total number of progeny produced by (P1) mother aphid of F1, \( d \) is the time taken by F1 aphid from its birth till produce its 1st nympha, and 0.738 is the mean regression slope of Md/d for mustard aphid (Wyatt and White, 1977).

**Statistical analysis**

The data were arranged and statistically analyzed by using analysis of variance (STATISTIX 8.1 package). The F-value was calculated at the probability level (p< 0.05). The significant data were identified by calculating LSD (Steel and Torrie, 2004).

**RESULTS**

The results presented in Table I, indicated free choice test where preferences and non-preferences tendency of the *L. erysimi* against 4 canola genotypes were recorded at 6 leaves stage that exhibited statistically significant difference among the selected canola genotypes. The data recorded after 12 h revealed that numbers of aphids counted on Abaseen, Omega, Zahoor and KS-75 were 31.02, 31.00, 30.97 and 5.33 aphids/plant respectively while similar pattern was maintained after 24 h, which showed significant difference among the tested genotypes i-e Abaseen, Omega, Zahoor and KS-75 with respective 31.01, 30.99, 30.93 and 5.00 aphids/plant. At 48 h interval, the experiment was terminated and number of aphids per plant were finally counted and recorded on Abaseen (31.02 aphids/plant), Omega (30.99 aphids/plant), Zahoor (30.98 aphids/plant) and KS-75 (5.33 aphids/plant). Based on average number of aphids per leaf during post infestation period of all the time intervals (12, 24 and 48 h), the susceptible genotype Abaseen (31.01 aphid/plant) attracted significantly more aphids as compared to the genotype KS-75 (5.22 aphid/plant) sustained minimum number of aphids. Statistically there was no significant difference among the three tested genotypes Omega, Zahoor and susceptible Abaseen against *L. erysimi*.

Data were recorded for preferences and non-preferences tendency of *L. erysimi* against 4 selected canola genotypes at flowering stage (Table I). The data recorded after 12 h showed that number of aphids counted on Abaseen, Omega, Zahoor and KS-75 were 31.01, 30.97, 31.00 and 7.33 aphids/flower, respectively while the data recorded after 24 h post infestation of *L. erysimi* on different tested genotypes was counted 31.00, 30.93, 30.99 and 7.00 aphids/flower, respectively. After 48 h, the experiment was terminated and the number of aphids were finally counted and recorded on the tested genotypes Abaseen, Omega, Zahoor and KS-75 with 31.00, 30.96, 30.00 and 7.32 aphids/flower, respectively. Based on average number of aphids at flowering stage during post infestation period of all the time intervals (12, 24 and 48 h), maximum number of aphids were attracted by susceptible genotype Abaseen (31.01 aphids/flower) and significantly least number of aphids were sustained by genotype KS-75 (7.22 aphids/flower). The genotype KS-75 sustained a minimum number of aphids as compared to the susceptible Abaseen. Although the genotype KS-75 attracted significantly minimum numbers of *L. erysimi* compared to genotypes (Abaseen, Omega and Zahoor) while Omega and Zahoor were no-significant to each other, genotype Omega was also significantly different from Abaseen.
Table I. *Lipaphis erysimi* (Mean±SEM) on selected canola genotypes during 2018-19.

| Genotypes | Time interval (h) | Mean |
|-----------|------------------|------|

| Genotypes | 12  | 24 | 48 |
|-----------|-----|----|----|
| Abaseen   | 31.02 ± 0.58 a | 31.01 ± 0.57 a | 31.02 ± 0.58 a | 31.01 ± 0.57 a |
| KS-75     | 05.33 ± 0.48 c | 05.00 ± 0.45 c | 05.33 ± 0.48 c | 05.22 ± 0.48 c |
| Omega     | 30.97 ± 0.53 b | 30.93 ± 0.51 b | 30.98 ± 0.54 b | 30.96 ± 0.52 b |
| Zahoor    | 31.00 ± 0.56 ab| 30.99 ± 0.55 a | 30.99 ± 0.55 ab| 30.00 ± 0.50 ab|
| LSD       | 0.0384         | 0.0549        | 0.0352         | 0.0346         |

**Six leaves stage**

**Flowering stage**

| Genotypes | 12  | 24 | 48 |
|-----------|-----|----|----|
| Abaseen   | 31.01± 0.57 a | 31.00± 0.56 a | 31.00± 0.56 a | 31.01± 0.57a |
| KS-75     | 07.330 ± 0.32 c| 07.000 ± 0.30 c | 07.320 ± 0.31 c | 07.220± 0.31 c |
| Omega     | 30.97± 0.53 b | 30.93± 0.51 b | 30.96± 0.54 b | 30.96± 0.54 b |
| Zahoor    | 31.00± 0.56 ab| 30.99± 0.55 a | 30.00 ± 0.50 ab | 31.00± 0.56 ab |
| LSD       | 0.0384         | 0.0549        | 0.0352         | 0.0345         |

**Pod stage**

| Genotypes | 12  | 24 | 48 |
|-----------|-----|----|----|
| Abaseen   | 30.60 ± 0.52 a | 30.40 ± 0.51 a | 30.65 ± 0.52 a | 30.63 ± 0.52 a |
| KS-75     | 10.30 ± 0.32 c | 10.10 ± 0.30 c | 10.20 ± 0.31 c | 10.16 ± 0.31 c |
| Omega     | 29.90 ± 0.49 b | 29.90 ± 0.49 b | 29.98 ± 0.48 b | 29.96 ± 0.46 b |
| Zahoor    | 30.02 ± 0.52 b | 30.00 ± 0.50 b | 30.03 ± 0.53 b | 30.05 ± 0.50 b |
| LSD       | 0.0949         | 0.3028        | 0.1963         | 0.3101         |

Means followed by the different letters are significant difference (0.05).

Table I revealed antixenosis experiment at pod stage of the tested genotypes where tendency of the *L. erysimi* against selected canola genotypes were recorded. The data recorded after 12 h indicated that number of aphids counted on Abaseen were maximum followed by Omega, Zahoor and KS-75 with 30.60, 29.90, 30.02 and 10.30 aphids per pod, respectively. After 24 h post infestation, data counting on pods of the tested genotypes Abaseen, Omega, Zahoor and KS-75 was 30.40, 29.90, 30.00 and 10.10 aphids per pod, respectively. Similarly, after 48 h, the experiment was terminated and the number of aphid per pod were finally counted and recorded with maximum number on Abaseen (30.65 aphids/pod) followed by Zahoor (30.03 aphids/pod), Omega (29.98 aphids/pod) and KS-75 (10.16 aphids/pod).

**Table II** indicated the results of an antibiosis experiment when *L. erysimi* was caged on a single seedling on tested canola genotype to observe the feeding and breeding behavior of the tested insect against canola genotypes. The average progeny production (Md) during the caged period was observed and recorded. Significantly more number of nymphs was produced by *L. erysimi* on susceptible genotype Abaseen (28.01) as compared to the tested genotypes and minimum progenies production was recorded on KS-75 (7.459). The *L. erysimi* caged aphid produced (27.63) offspring on genotype Zahoor was non-significantly different from Abaseen but different from Omega and KS-75. The *L. erysimi* produced offspring (10.73) on genotype Omega lower than Abaseen but more than KS-75. The second life table parameter revealed that *L. erysimi* took statistically significant and more pre-reproduction time on KS-75 (9.40 days) as compared to other tested genotypes. This pattern was followed by Omega (8.20 days) while significantly minimum pre-reproduction time was observed on Abaseen (5.90 days) as compared to Omega, KS-75 and Zahoor (6.00 days), respectively. The third life table parameter (*r_m* natural intrinsic rate of increase) was also calculated based on (10.16 aphids/pod).
the previous two parameters. The minimum $r_m$ value was calculated for KS-75 (0.075) and maximum for Abaseen (0.497). The tested genotype Omega (0.285) calculated value statistically falls lower than Abaseen and upper than KS-75, but also different compared to Zahoor (0.486). Antibiosis resistance is represented in Table II which revealed that during post infestation period, L. erysimi produced significantly more number of progeny (28.01) on susceptible genotype Abaseen in a short time (5.90 days) and minimum number of progeny (7.45) was produced by the aphid on genotype KS-75 in 9.40 days. Furthermore, genotypes Abaseen (28.01) and Zahoor (27.63) were non-significant to each other (based on progeny production of the aphid on genotype KS-75 in 9.40 days). Furthermore, genotypes Abaseen (28.01) and Zahoor (27.63) were non-significant to each other (based on progeny production of caged aphid during post infestation period F1), but they were statistically different from the genotypes KS-75 and Omega. Although genotype Omega produced a minimum number of L. erysimi post the infestation period.

Table II. Number of progeny (Mean±SEM) produced by P1 adults, pre-reproductive period and $r_m$ of L. erysimi on tested canola genotypes during 2018-19.

| Genotypes | Life parameters | No of progeny produced by (P1) (Md) | No of nymph produced by (F1) (d) | Natural intrinsic rate increase (rm) |
|-----------|----------------|-----------------------------------|---------------------------------|-----------------------------------|
| Abaseen   |                | 28.01 ± 0.098 a                   | 5.90 ± 0.048 c                  | 0.497 ± 0.017 a                   |
| KS-75     |                | 7.459 ± 0.323 c                   | 9.40 ± 0.362 a                  | 0.075 ± 0.007 d                   |
| Omega     |                | 10.737 ± 0.301 b                  | 8.20 ± 0.323 b                  | 0.285 ± 0.012 c                   |
| Zahoor    |                | 27.63 ± 0.970 a                   | 6.00 ± 0.032 c                  | 0.486 ± 0.014 b                   |
| LSD       |                | 0.1580                            | 0.6497                          | 0.0231                            |

Means followed by the same letters are not significantly different (P≥0.05; LSD).

Table III represents different life parameters of the tested genotypes against aphids. Among the tested genotypes, highest mean developmental period (DP) was observed on KS-75 (9.40 days) followed by Omega (8.20 days) and Zahoor (6.00 days) and lowest on Abaseen (5.94 days). The average reproductive period (RP) observed on the tested genotypes was 12.11 days. The maximum reproductive period was observed on the variety Abaseen (15.06 days) followed by Zahoor (14.94 days) and Omega (10.24 days), while the lowest mean reproductive period was observed on KS-75 (8.200 days). As far as the fecundity of the tested insect is concerned, the average fecundity (F) produced on the tested genotypes was observed (28.01) on Abaseen followed by Zahoor (27.63), while statistically minimum fecundity was recorded on genotype KS-75 (7.459) and Omega (10.74). Similarly, the insect longevity parameter also showed the longevity (L) highest survival was observed on genotype Abaseen (21.07 days) followed by Zahoor (20.89 days) and Omega (18.84 days), while minimum survival/longevity was recorded on the genotype KS-75 (17.38 days). The last parameter of L. erysimi tested against the selected genotypes revealed that significantly maximum offspring’s per day were produced and observed on genotype Abaseen (1.85/day) followed by Zahoor (1.76/days) and Omega (1.03/days) respectively, while significantly minimum offspring’s were observed on KS-75 (0.91/day).

DISCUSSION

The response of the mechanism of host plant resistance towards insect behaviour has been explained by previous authors including Muhammad and Khan (2019) and Kishor et al. (2019) who investigated that plants revealing antixenotic may produce visionary repellent ability, which may be due to plants providing the smell (odor) insect pest keeping distance from the host. Furthermore, susceptible plants may also emit aversive odors and cause insect movement to cease in close proximity to the odor source (host). The interplay between the odors emitted by plant sources, the effects of the environment on these odors, the perception of the odors by insects and the resultant insect behaviors. The plant secondary metabolites either act as an insect repellent or serve as a host recognition using olfactory signals (Baldwin, 2010). Canola germplasms have been tested against aphid (L. erysimi) by various researchers (Matis et al., 2008; Sarwar, 2008; Rashid et al., 2009) regarding antixenosis test at different stages (vegetative, flowering and pod stage).

During the current antixenosis experiments, a total of 3 tests were conducted on selected canola genotypes against L. erysimi under laboratory conditions at different
During the first experiment (seedling stage), the susceptible genotype Abaseen sustained the maximum number of aphids per leaf on average time interval during post the infestations period and was followed by Zahoor, Omega and KS-75. The antixenosis experiment results proved that genotype Abaseen attracted significantly more aphids compared to the tested genotypes, while the genotype KS-75 attracted a minimum number of aphids comparatively. The second test was conducted at the flowering stage of plants. During flowering stage assay, a significantly minimum number of aphids was sustained by genotype KS-75 compared to susceptible genotypes Abaseen, Omega and Zahoor against L. erysimi. During the pod stage assay of the selected genotypes against L. erysimi, based on average number of aphids at pod stage post infestation period of all the time intervals (12, 24 and 48 h), the susceptible genotype Abaseen attracted significantly more aphids compared to KS-75, Zahoor and Omega. Our results are in good agreement with Shah et al. (2015) who assessed antixenosis test in terms of mean number of aphids/flower and mean number of aphids/pod and proved that germplasm G-9 shows more susceptibility as compared to the G-28, which was highly resistant out of total eight tested germplasms. Color factor was also argued by Shah et al. (2015) that aphids are vulnerable to yellow color and that L. erysimi is more attracted towards yellow than other tested colors (red, white and green). Our findings are in conformity with Kumar et al. (2011) by stating that L. erysimi preferred the excised leaves of Brassica species which are also in line with our second and third tests of antixenosis experiments where L. erysimi preferred excise leaves of canola and maximum aphids were attracted by the susceptible genotype Abaseen compared to KS-75. The genotype KS-75 was observed with dark green color and spar trichomes. Thus, our current findings are also supported by many researchers (Muhammad and Khan, 2022; Kishor et al., 2019; Kumari et al., 2009).

During our antibiosis experiment, phenotypic response was investigated in four selected canola genotypes and their response was observed during the antibiosis assay through different life table parameters including total offspring productions (Md), pre-reproductive period in days (d) and natural intrinsic rate of increase ($r_m$) value were observed and recorded. Among the selected canola genotypes, aphids produced statistically more average number of offspring and significantly minimum numbers of offspring were sustained by KS-75 in terms of progeny production on susceptible genotype Abaseen. During the second life table parameter (pre-reproductive time in days), maximum time of L. erysimi was taken by KS-75 and significantly minimum time was taken by aphid on susceptible genotypes Abaseen to produce offspring after caging the aphid on canola seedling. These results are in agreement with the previous researchers where the aphid (D. noxia) took a short time to produce its offspring on susceptible genotypes Vista compared to resistant genotype H871 (Khan et al., 2009). In our study, the life table parameter revealed that L. erysimi took significantly more pre-reproduction time on KS-75 in days compared to other tested genotypes and statistically less time on susceptible genotype Abaseen. Similarly, the rate of natural intrinsic increases ($r_m$) was also observed with lower $r_m$ value for KS-75 (0.07) and maximum for Abaseen (0.49). The calculated values of tested genotype Omega (0.28) statistically falls lower than Abaseen and upper than KS-75. Thus, our current findings are in conformity with that of Zauva et al. (2020) and Ram et al. (2020) by linking the behavior of canola aphids to physiological characters in its host on different genotypes and revealed that rates of naturals intrinsic increase were lowest on Zagiros genotype. Thus, it is concluded that genotype KS-75 proved strong antibiosis characteristics against L. erysimi both in terms of lower progeny production and a prolonged nymphal pre-reproductive period, which have been reflected in life table parameters. The natural rate of intrinsic increase value ($r_m$) of KS-75 was recorded statistically smaller than genotype Abaseen. Our results are in agreement with Voothuluru et al. (2006). However, further genetic analysis (antibiosis) of genotype KS-75 may provide detailed information regarding number(s) of resistant genes in canola seedling against L. erysimi.

CONCLUSION

During all three stages of the plant, the known susceptible genotype Abaseen attracted a maximum number of aphids while minimum were sustained by KS-75 genotypes. Thus, genotype KS-75 proved antixenosis and antibiosis resistance in term of low progeny production and pre-reproductive period with small value of ($r_m$) natural rate of intrinsic increase. The genotype KS-75 is highly recommended as standard for antixenosis in future screening programs on canola crop against L. erysimi and is recommended for canola growers in Pakistan.

ACKNOWLEDGMENT

The authors are highly grateful to the Higher Education Commission for providing all necessary facilities and funding to achieve milestone research activities during investigation.

Statement of conflict of interest

The authors have declared no conflict of interest.
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