

LACK OF PLANT RESISTANCE AGAINST APHIDS, AS INDICATED BY THEIR SEASONAL ABUNDANCE IN CANOLA, (*BRASSICA NAPUS* L.) IN SOUTHERN PUNJAB, PAKISTAN

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Abstract

The objective of the study was to develop insect pest management strategy by exploring host plant resistance in canola, *Brassica napus* L., and seasonal abundance of aphids at Multan, Bahawalpur and Dera Ghazi Khan in Southern Punjab, Pakistan. Two aphid species, *Brevicoryne brassicae* L., and *Lipaphis eyrsimi* Kalt. were observed as the most devastating pests. Populations of *B. brassicae* were more than that of *L. eyrsimi*. All the varieties evaluated were found susceptible and weekly population of both the species of aphids did not differ significantly from their appearance till maturity of the crop. Appearance of aphids at all the locations was not uniform. However, highest population was recorded during last week of February to second week of March. The seasonal activity of the aphids is described and it is recommended that application of insecticides is inevitable to avoid economic damage. Development of insect pest management strategy against aphids by exploring sources of plant resistance and seasonal abundance of aphids on *B. napus* is reported from this region for the first time.

Introduction

Cultivation of rapeseed and mustard in the Indian Sub-continent dates back to 2000-1500 BC. Among these crops canola-quality rapeseed, *Brassica napus* L., is one of the world's leading edible oil crops. Its seed contains less than 2% erucic acid and $>30\mu\text{Mg}^{-1}$ of glucosinolates in the oil free meal. Furthermore, canola oil is lower in saturated fats (5-8%) than any other vegetable oil (Raymer, 2002). Currently local production of edible oil in Pakistan accounts for 31% of total requirement while remaining 69% is met through imports (Anon., 2006). Damage due to insect pests is a major constraint in production of *Brassica* crops. Damage by insects varies in different production regions of India (Bakhetia, 1990). Greatest loss reported in yield only due to mustard aphid, (*Lipaphis eyrsimi* Kalt.) is 83% to rapeseed and mustard in India (Mandal *et al.*, 1994). Canola is sown in Punjab, Sindh and North West Frontier Province (NWFP) of Pakistan. Five insect pests have been reported to damage this crop in Pakistan (Ali & Munir, 1984). Cabbage aphid, *Brevicoryne brassicae* L. damages oilseed brassicas at flowering and pod formation stages, whereas sawfly, *Athalia proximia* Klug., attacks before flowering in early stage of crop in NWFP and Sindh (Rustamani *et al.*, 1988; Anwar & Shafique, 1999). Losses due to insect pests are estimated to be 70-80% in Pakistan. But in case of severe infestation in years of sporadic attack there may be no grain formation at all (Rustamani *et al.*, 1988; Khattak *et al.*, 2002).

There is little published research on incidence of aphids and resistance of varieties on *B. napus* in Multan, Bahawalpur and Dera Ghazi Khan Districts of Southern Punjab. Although in previous studies, the incidence of *L. eyrsimi* and comparative resistance of *B. napus* varieties against *B. brassicae* in Multan region, based on observations in a single season, has been reported (Aslam *et al.*, 2002; Aslam *et al.*, 2005). Literature reports

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controversial results regarding resistance or susceptibility of *Brassica napus* against aphids (Bakhetia, 1990; Sehkun & Ahman 1992). There are many plant characters, which are responsible for host plant resistance. The plant structures may have positive as well as negative effect on herbivores and their natural enemies (Afzal & Bashir, 2007; Afzal *et al.*, 2009). Most of the work on mechanism of resistance against aphids has been reported in *Brassica* species other than *B. napus*. Morphological characters responsible for resistance against *L. erysimi* in crucifers include smaller and hardy inflorescence with loosely packed buds, (Rai & Seghal, 1975), darker leaves, non-waxy plant, more branches with wider angle of orientation and hairiness of plant (Sehkun & Ahman, 1992). Among the biochemical characters, presence of higher contents of flavonoids, total sugars and reducing sugars, low quantities of total ash, nitrogen and phosphorus contents in *B. napus* plants induce resistance (Bhowmik, 2003). Higher concentration of protein and free amino acids have been reported to be responsible for susceptibility to *L. erysimi* in 20 cruciferous species. Ascorbic acid and glucosinolates contents in *Brassica* species are negatively correlated with aphid population (Malik, 1981; Labana *et al.*, 1983). Therefore, main objective of this study was to generate comprehensive information on incidence and seasonal abundance of aphids and to evaluate available varieties of *B. napus* for comparative resistance against aphids.

Materials and Methods

Experimental sites: The experiments were planted at the Agriculture Farm, University College of Agriculture, Bahauddin Zakariya University, Multan during 2003 and 2004, Regional Agricultural Research Institute, Bahawalpur during 2003 and Experimental Farm, College of Agriculture, Dera Ghazi Khan during 2005 of the Punjab province of Pakistan. These areas are arid and receive mean annual rainfall of about 125mm. The winter season extends from November to February. There is short spring during March. Minimum temperature recorded was at Multan 8.2 and 5.5°C during December 2004 and 2005, respectively, whereas maximum temperature was 31.2 and 26.0°C during March 2004 and 2005, respectively. But during crop year 2005 exceptionally higher temperature (27.1°C) recorded was during the month of November that might not be too important for aphid population build up. Average of 10 years from these three districts depicts that there is little difference in temperature of Multan and two other districts (Anon., 1998; Cheema & Ahmad, 2003).

Experimental design: During 2003 the experiments were planted at the Agriculture Farm, University College of Agriculture, Bahauddin Zakariya University, Multan on 5th November and Regional Agricultural Research Institute, Bahawalpur on 28th October. Twelve varieties of *B. napus* (Abaseen, CON-I, CON-II, CON-III, Dunkald, KS-75, Oscar, Rainbow, Shiralee, Wester, 19-H and 20-E) were sown in a Randomized Complete Block Design with three replications. Each treatment consisted of four rows of 4.57 m length and 45.0 cm apart. Treatments were 1.0 m apart. Standard recommended cultural practices were followed.

All the varieties tested during 2003 at both the locations had non-significantly different aphid populations. Therefore, during next year only four canola varieties (Abaseen, CON-III, CON-I and KS-75) were planted on 5th November 2004 at Multan. All the varieties tested at Multan during crop season 2004-05 were planted at Dera Ghazi Khan on 14th November, 2005. At Dera Ghazi Khan, canola variety CON-III was replaced with CON-II. The number of replicates, size of plot and distance between

treatments was the same as during 2003. No insecticide was applied on the trials in any of the three years.

Insect sampling: Crops were monitored from germination to maturity for recording insect pests and natural enemies. For this purpose fields were observed twice a week in the beginning for recording occurrence of insects and once a week when populations of insects were sufficient to be recorded. Aphids (*B. brassicae* and *L. erysimi*) were observed as major insect pests. Populations of aphids were recorded from six plants in two middle rows of each treatment with three plants selected randomly from each row. For this purpose the top 10 cm of the central inflorescence of a plant was beaten gently ten times with a 15 cm stick of pencil thickness. Aphids were collected on a piece of white plastic sheet and counted. This method has also been employed in sampling *L. erysimi* in India on *Brassica juncea* (Singh *et al.*, 1989; Chattopadhyay *et al.*, 2005). This method is efficient and saves time, labour, effort and resources.

The following procedure developed by Bakhietia & Sandhu (1977) was adopted as criteria for resistance or susceptibility of varieties based upon aphid infestation for categorization of *Brassica* material at full bloom stage under field conditions.

“Score/grade	Description
0.	Free from aphid infestation. Even when a single wingless aphid was present the plant was considered as infested. Plants showed excellent growth.
1.	Normal growth no curling or yellowing of leaves, only a few aphids present but little or no symptoms of injury. Good flowering and pod setting on almost all the branches.
2.	Average growth, curling and yellowing of few leaves. Average flowering and pod setting on all the branches.
3.	Growth below average, curling and yellowing of the leaves on some branches. Plants showing some stunting, poor flowering and very little pod setting.
4.	Very poor growth, heavy curling and yellowing of the leaves, stunting of plants, a little or no flowering and pod formation of only few pods. Heavy aphid colonies.
5.	Severe stunting of plants, curling, crinkling and yellowing of almost all the leaves. No flowering and pod formation, plants full of aphids.

Varieties were graded according to their aphid infestation index as given below:

Aphid infestation index	Designation
Up to 1.0	Highly resistant
1.1-2.0	Resistant
2.1-3.0	Moderately resistant
Above 3	Susceptible”

Statistical analysis: The data of mean aphid population per 10 cm shoot were subjected to analysis of variance (ANOVA) by using MSTATC, computer software (MSU, 1982). Means of aphid population on different varieties were separated by Least Significant Difference Test at $p = 0.05\%$.

Table 1. Mean population of *B. brassicae* (*B.b*) and *L. erysimi* (*L.e*) on top 10 cm inflorescence per plant of *Brassica napus* at Multan on different varieties during 2004.

Varieties	Sampling dates													
	Feb. 11		Feb. 18		Feb. 25		Mar. 03		Mar. 10		Mar. 17		Mar. 24	
	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>
Dunkald	1.50	0.40	10.60	1.60	17.90	8.10	11.60	31.80	29.00	22.10	5.60	0.80	2.30	0.70
Rainbow	9.50	1.70	11.30	2.90	10.60	14.70	15.20	28.70	34.20	15.70	3.70	1.40	2.40	0.50
Oscar	2.50	0.30	9.50	5.60	19.50	7.10	14.40	24.60	37.00	11.80	5.40	1.60	2.80	0.50
Wester	3.20	0.70	7.50	2.80	11.50	13.30	21.20	15.40	31.50	15.20	3.50	1.50	2.40	0.40
Shiralee	5.00	0.80	12.00	1.00	13.20	14.20	21.50	26.00	36.20	13.40	8.60	1.80	3.20	1.00
CON-I	5.00	1.90	9.30	1.90	23.20	9.60	17.60	21.60	31.80	15.20	5.50	0.90	3.30	1.00
CON-II	3.10	1.80	8.20	3.00	15.80	13.00	14.80	21.70	34.20	13.90	4.30	1.40	13.30	0.60
CON-III	5.50	5.30	4.70	3.70	17.30	9.20	18.90	11.90	29.70	12.20	3.00	1.70	3.30	0.50
KS-75	6.70	0.90	8.70	0.90	15.20	15.70	13.70	19.90	30.20	17.40	6.20	1.90	3.90	0.80
Abaseen	15.50	3.80	11.30	2.20	14.20	14.90	22.00	17.80	27.30	19.50	5.50	1.00	3.50	0.40
19-H	2.90	1.60	7.00	2.20	18.70	11.90	18.90	21.80	29.22	22.80	3.70	1.90	3.30	1.70
20-E	5.50	2.60	10.40	10.20	19.90	16.30	21.90	33.20	45.20	17.40	4.10	1.70	2.00	1.00

Difference in population among varieties was not significant at $p=0.05$.

Results and Discussion

Incidence of aphids: Cabbage aphid, *B. brassicae*, and mustard aphid, *L. erysimi* were recorded as major insect pests at three locations. The population of *B. brassicae* was higher than that of *L. erysimi* during three years at each location. Aphids were observed for the first time on February 4, 2004 at Multan. First winged aphids appeared on 1 January, 2005 but nymphs of both the species were observed on 17 January along the border of the field at this location. Aphids were observed for the first time on 1st February, 2004 at Bahawalpur. After one week of appearance, only 25 nymphs of *L. erysimi* were noted on a plant. However, the population of aphids was sufficient to be recorded by 15th of February. Appearance of aphids at Dera Ghazi Khan was quite late as compared with Multan and Bahawalpur. Seven nymphs of *L. erysimi* and 12 of *B. brassicae* were observed in the whole trial on 21st February, 2006 at this location.

Resistance against aphids: Weekly populations of aphids were found non-significantly different ($p=0.05$) on 12 varieties throughout the crop season during 2004 at Multan and Bahawalpur (Table 1 and 2). However, 4 varieties (Abaseen, CON-I, CON-III and KS-75) tested at Multan had a significantly different population ($LSD=21.50$ at $p=0.05$) of *L. erysimi* only on 26 February out of six sampling dates (Table 3). *B. napus* varieties (Abaseen, CON-I, CON-II and KS-75) were also found to have a significantly different population ($LSD=36.96$ at $p=0.05$) of *L. erysimi* only on 21 March out of four sampling dates at Dera Ghazi Khan (Table 4). *B. napus* varieties evaluated in present research (except 19-H and 20-E) were found to have non-significantly different populations of *B. brassicae* and *L. erysimi* at Bahawalpur during 2003 (Faheem, 2004). Similarly the same varieties are also reported to have statistically similar populations of *B. brassicae* at Multan (Aslam *et al.*, 2005).

All the varieties tested in our experiments were found to have an aphid infestation index of approximately 3 as described by Bakhetia & Sandhu (1977) and explained in materials and methods section. We observed growth below average, curling and yellowing of the leaves on all branches. Plants showed some stunting. Flowering and pod setting was above average but grains were few and of reduced size. Therefore, no variety can be declared as resistant based on the present results. Although resistant / tolerant varieties of *B. napus* against aphids have been reported in the past (Bakhetia, 1990), but it has been advocated that resistance is probably not sufficient to prevent significant losses in yield (Sehkon & Ahman, 1992).

Table 2. Mean population of *B. brassicae* (*B.b*) and *L. erysimi* (*L.e*) top on 10 cm inflorescence per plant of *Brassica napus* on different varieties at Bahawalpur during 2004.

Varieties	Sampling dates											
	Feb.15		Feb.22		Feb.29		Mar.07		Mar.14		Mar.21	
	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	58.5	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>
Dunkald	0.7	0.8	24.3	8.8	31.6	22.2	63.8	27.0	27.0	18.8	0.7	1.7
Rainbow	0.3	0.7	17.1	9.9	31.7	14.0	47.9	29.5	35.8	18.5	0.7	2.0
Oscar	0.6	0.8	18.3	8.2	14.5	25.1	43.4	15.8	19.9	8.6	1.7	2.1
Wester	0.8	1.5	11.0	7.9	20.5	14.8	58.9	20.0	47.5	15.6	0.6	3.1
Shiralee	3.3	0.4	18.0	10.0	28.1	13.6	39.4	19.7	40.9	17.3	1.4	2.4
CON-I	0.5	0.8	12.9	11.1	25.1	11.4	61.1	22.5	36.2	16.7	0.9	3.0
CON-II	1.2	0.8	8.7	16.0	31.5	18.3	63.9	19.2	38.3	20.3	0.9	4.0
CON-III	0.7	1.4	19.2	8.3	28.6	15.3	66.2	17.2	14.8	15.7	1.7	0.8
KS-75	0.0	1.5	10.3	12.3	16.3	26.2	38.4	22.8	38.9	12.5	0.9	1.2
Abaseen	0.8	6.3	18.0	3.6	23.3	11.9	67.7	26.7	40.0	10.5	2.5	1.3
19-H	1.2	0.3	4.2	4.7	20.0	8.1	46.0	7.7	18.7	12.3	1.5	2.3
20-E	0.9	0.9	21.1	19.1	39.0	21.3	47.4	29.3	38.4	19.0	0.3	2.3

Difference in population among varieties was not significant at $p=0.05$.

Table 3. Mean population of *B. brassicae* (*B.b*) and *L. erysimi* (*L.e*) on top 10 cm inflorescence per plant of *Brassica napus* on different varieties at Multan during 2005.

Varieties	Sampling dates											
	Feb. 18		Feb. 26		Mar. 6		Mar. 13		Mar. 20		Mar. 27	
	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>
KS-75	22.0	26.3	49.0	21.0a	62.0	39.0	2.7	41.3	9.0	7.0	1.67	4.00
Abaseen	32.0	28.0	54.0	32.7ab	40.7	55.7	41.3	34.3	18.7	14.7	3.00	1.67
CON-III	19.7	35.0	41.0	49.3b	39.7	32.7	39.3	49.7	10.7	8.00	1.00	5.33
CON-I	26.0	23.3	50.0	41.3ab	39.0	41.0	42.3	58.0	9.00	6.7	2.33	1.33

Difference in population among varieties was not significant at $p=0.05$ except for *L. erysimi* population recorded on Feb. 26, where means in the columns following the same letter are non-significantly different from each other (LSD=21.50).

Table 4. Mean population of *B. brassicae* (*B.b*) and *L. erysimi* (*L.e*) on top 10cm inflorescence per plant of *Brassica napus* on different varieties at D.G. Khan during 2006.

Varieties	Sampling dates							
	Mar.01		Mar.07		Mar.13		Mar.21	
	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>	<i>B.b</i>	<i>L.e</i>
CON-1	87.77	92.40	128.8	132.6	88.9	109.4	77.80	87.27ab
CON-11	99.40	81.10	117.5	103.5	109.8	123.9	80.03	58.57b
KS-75	77.23	85.90	107.1	130.8	104.3	130.0	113.3	105.2ab
Abaseen	92.44	97.58	132.2	129.4	108.7	109.8	109.4	113.5a

Difference in population among varieties was not significant at $p=0.05$ except for *L. erysimi* population recorded on Mar. 21, where means in the columns following the same letter are non-significantly different from each other (LSD=36.96).

B. napus varieties have also been evaluated for resistance against *L. erysimi* at Islamabad, Pakistan. Aphids were counted from the whole plant. No criterion was used to designate varieties as resistant. Varieties were declared on the basis of population as resistant, intermediately susceptible and susceptible. It is interesting to note that 21.70 to 38.86 and 21.80 to 43.30 aphids per plant were present at flowering and seed formation stage on the varieties declared as resistant by Jatoi *et al.*, (2002). The economic threshold level (ETL) in Rajasthan state of India is 23-25 aphids per plant (Anon., 1999), whereas population of *L. erysimi* was very close to or well above this level in varieties declared as resistant in a study by Jatoi *et al.*, (2002).

Seasonal abundance: Peak period of activity of aphids was recorded during last week of February to second week of March. Generally populations of both the species were highest on 10th March and 26th February during 2004 and 2005, respectively at Multan (Tables 1 and 3). However, highest aphid number was observed on 7th March during 2004 and 2006 at Bahawalpur and Dera Ghazi Khan (Table 2 and 4). Highest population of aphids was observed on 13th and 15th March during 1999 and 2003 at Multan in previous studies (Aslam *et al.*, 2002; Aslam *et al.*, 2005). In Sindh province, peak period of activity had been reported to be the first week of March during 1995 and 1996, and third week of February during 1997 (Anwar & Shafique, 1999). Early appearance of a peak of aphids in Sindh province might be due to early onset of the summer season. The appearance of aphids on *B. juncea* has been recorded to be positively correlated with maximum temperature between 20-29°C in preceding week and also to morning relative humidity (RH > 92%) and daily mean RH >75% in different regions of India for appearance of *L. erysimi*. Minimum temperature (>5°C) also favoured *L. erysimi* infestation in India on *B. juncea* (Chattopadhyay *et al.*, 2005 and references cited herein from India). We could not find such correlation between appearance of aphids and above parameters in this study. Maximum and minimum temperature was 13.5-22.0°C and 4.0-10.5°C during 2004 in the week preceding the appearance of aphids, whereas maximum and minimum temperature was 16.0-21.5°C and 3.0-7.0°C during 2005 at Multan. Similarly, morning relative humidity was also above 92% for some days but daily mean relative humidity was close to that observed in research of Chattopadhyay *et al.*, (2005). The reason might be the difference in species of aphid and crop phenology. In India, only *L. erysimi* is reported but two species were recorded in our study. Average weekly maximum and minimum temperature observed for active development of *B. brassicae* in Sindh was 22.0-27.0°C and 7.0-12.0°C during 1995-1997 (Anwar & Shafique, 1999). However, it was not described either at the time of appearance or at the time of peak activity. Temperature at Multan was somewhat close to that in the research mentioned above during mid February to mid March i.e., at the time of peak period of aphid activity. Further studies will be directed towards forecasting of appearance of aphids on *B. napus* and other oilseed brassicas based upon weather parameters.

L. erysimi has been reported as an important insect pest of cruciferous crops in India (Bakhetia, 1990; Sehkon & Ahman, 1992; Patel *et al.*, 2004; Chattopadhyay *et al.*, 2005). *B. brassicae* and *L. erysimi* have been reported from Australia and Brazil (Mussurry & Feranandes, 2002; King *et al.*, 2006). *B. brassicae* has been reported (Khan *et al.*, 1985; Rustamani *et al.*, 1988; Anwar & Shafique, 1999; Khattak *et al.*, 2002) from Pakistan other than southern Punjab. *L. erysimi* has also been reported from Islamabad (Jatoi *et al.*, 2002), but both species of aphids were present during three years of research in Southern Punjab. The population of *B. brassicae* was higher than that of *L. erysimi* at Multan and Bahawalpur. In the present study, in three different experiments at Multan and Bahawalpur, the population of *L. erysimi* was higher only on one sampling date (10 March 2005). However, during 2006 at Dera Ghazi Khan population of *L. erysimi* was nearly equal to or higher than that of *B. brassicae*.

There was no difference in aphid population among the varieties tested. Therefore, no further research was undertaken towards morphological or biochemical characters considered responsible for resistance. Aphid infestation has been proved so prolific in India that none of the germplasm accession is free from it (Arunachalam & Katiyar, 1990). Extensive screening efforts of *Brassica* germplasm have failed to identify any source of resistance for aphids in India (Dhillon *et al.*, 1992). It has been argued that even

though some species and varieties among the *Brassica* oilseeds are less susceptible to major insect pests, their resistance is probably not sufficient to prevent significant yield losses due to insect attack. The goal of breeding for resistance has not yet been achieved in India, as aphids present on crops every year cross the ETL determined so far (Chattopadhyay *et al.*, 2005). Therefore, research on breeding for resistance against aphids in *Brassica* oilseeds is urgently required in Pakistan and results reported in present study about the susceptibility of varieties hold well. If resistant varieties are developed, this will provide insecticide residue free oil for human consumption.

ETL has not yet been determined for aphids on canola in Pakistan. However based upon the population of the aphids and crop situation, insecticides should be applied during second week of February. Furthermore, insecticide application needs caution as application of insecticide on rosette stage is reported to increase the yield on canola in Georgia. However, insecticides applied at pod filling stage did not increase yield and insecticide treatments at blooming stage also enhanced the yield in some trials but not in all (Buntin & Raymer, 1994). Therefore, proper time of application needs to be determined based upon treatments of insecticides at particular stage of canola and their effect on yield.

From the present research it is concluded that *B. brassicae* and *L. erysimi* are devastating insect pests in Southern Punjab. Varieties tested in the present research lack resistance against aphids. Therefore, insecticides should be applied to manage the aphids to avoid economic damage.

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