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Effect of Insecticidal Application on Aphid Population, Photosynthetic Parameters and Yield Components of Late Sown Varieties of Canola, *Brassica napus* L.

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Abstract The study was carried out to determine the population growth of aphids and their effect on photosynthesis and yield components of late sown varieties of canola (*Brassica napus* L.) during the growing season of 2010-2011 at Multan, Pakistan. Canola varieties Can Raya, Con-I, Con-II and Westar were sown in a Randomized Complete Block Design with three replications. Six rows were planted in each plot of respective varieties. Two rows were sprayed with imidachloprid 20 SL @ 80ml/acre twice during the season, two were sprayed four times (aphid protected) and two were not sprayed (aphid infested) in each plot. The mustard aphid, *Lipaphis erysimi* (Kalt.) was the most abundant species on the crop. The cabbage aphid, *Brevicoryne brassicae* L. was also found in very small numbers. Peak aphid population was on 19th February on the varieties Can Raya and Con-I, whereas on Con-II and Westar the peak population was noted on 5th March. Aphid numbers per plant did not vary among different varieties in both sprayed and unsprayed plots. Photosynthetic rate and chlorophyll was lower in aphid infested plants as compared to that in aphid protected plants in all the varieties. However, water use efficiency, internal CO_2 concentration and transpiration rate did not differ in aphid infested and aphid protected plants in any of the varieties. There was a significant yield reduction in aphid infested plots when compared with plots where two sprays were applied in all varieties. Application of two insecticides to late sown *B. napus* varieties could not recover the total losses caused by aphids. Highest yield was recorded in variety Con I, where plants were sprayed twice and not sprayed. Westar suffered the highest losses in yield from aphids.

Keywords: *Brevicoryne brassicae*, *Lipaphis erysimi*, *Brassica napus*, photosynthesis, yield losses.

INTRODUCTION

Pakistan's economy is largely dependent upon the agricultural sector both directly and indirectly. Pakistan is acutely deficient in the edible oil production and spends millions of dollars on its import. Only 20% of edible oil need is met through domestic production and the balance is imported from other countries (Pakistan Oil Seed Products, 2010). Among oilseed crops, rapeseed and mustard and contribute 21% of national oil production. However, their oil is inferior in quality due to the presence of glucosinolates and ercuic acid (Vermorel *et al*., 1986). Canola (*Brassica napus* L.) refers to cultivars that produce seed with lower levels of glucosionolates and erucic acid which makes it suitable for edible oil extraction for human consumption (Syed *et al*., 1999). However, its crop productivity is greatly hampered by pest infestation. Of 21 insect pests of canola, aphids are most common and predominat pest (Lamb, 1989). Three aphid species that infest canola are the cabbage aphid, *Brevicoryne brassicae* L., the turnip aphid, *Lipaphis erysimi* (Kalt.) and the green peach aphid, *Myzus persicae* Sulzer. High populations of these aphid species can cause distortion and discoloration of leaves thereby resulting in reduction in growth and yield of canola (Blodgett and Johnson, 2001). In Southern Punjab, Pakistan cabbage and turnip aphids are main threatening pests of canola (Aslam and Razaq, 2007). Recently, it has been observed that crop losses to late sown *B. napus* due to cabbage and turnip aphi ds were more than 75% (Razaq *et al*., 2011). To reduce the aphid population and consequently lessening losses, non-chemical

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strategies are environmental friendly. But non-chemical control methods proved to be ineffective in reducing losses due to aphids in southern Punjab, as cultural control methods such as plant density (plant spacing) (Razaq *et al*., 2012). Moreover, available varieties lack sufficient resistance to aphid infestation (Amer *et al*., 2009; Razaq *et al*., 2011a). Therefore, aphids require application of insecticides on *B. napus*. But there are no guidelines for applying insecticides. Sucking insect pests may or may not affect photosynthetic activity and internal CO_2 and other related parameters of the infested plants (Lin *et al*. (1999). Keeping in view this information, present studies were conducted to assess the interactive effect of insecticide and aphid population on growth, yield and photosynthetic capacity of different canola cultivars.

MATERIALS AND METHODS

Experimental site and design

Research was conducted at the Warble Agricultural Farm of Bahauddin Zakariya University, Multan ($30^0$16'33.81 N and $71^0$30'51.57 E) Punjab, Pakistan during 2011. The crop was sown on 26th November 2010 using recommended seed rate. The experiment was conducted in a randomized complete block design with split plot design having three replicates. Four varieties of Canola were in the main plots whereas three insecticide application regimes were in the subplots. The total area of the experiment was 0.0214 ha. Each replication had an area of about 63.0 m^2. Each treatment plot had six rows with total plot size of 11.25 m^2. Treatments and replicates were 0.90 m and 1.40 m apart, respectively. Rows were 5.0 m long. Row to row and plant to plant spacing was 45.0 cm and 10.0 cm, respectively.

The varieties were *B. napus viz.*, Can Raya, Con-II, Con-I and Westar. *B. napus* is among the minor crops in the Multan region. There are no specific varieties recommended to be grown particularly for this region. Therefore, we selected four varieties from recommended varieties which are available for cultivation to the farmers. Moreover, from pest management point of view available varieties including those selected for present research have been proved to posses no resistance for aphids in several studies (*e.g.*, Amer *et al*., 2009; Razaq *et al*., 2011a). Among the six rows in each plot two rows were sprayed twice (14th February and 2nd March, 2011), two rows were kept free from aphids by applying insecticide on weekly basis starting from 14th February and the remaining two rows were kept unsprayed to allow aphid infestation for each variety in a main plot. The insecticide used for spraying was Glitter (imidacloprid 20% SL, Warble Private Limited, Multan, Pakistan) at 200 ml/hectare. Application of the imidacloprid was done with a hand operated knapsack sprayer fitted with hollow cone nozzle by using a pressure of 3.0 bars. At the time of insecticide application untreated rows of each plot were covered with plastic sheet to prevent drift on adjacent rows.

Aphid population

Numbers of *L. erysimi* and *B. brassicae* were recorded in the top10.0 cm of inflorescences of -+six plants at weekly intervals from flowering to crop maturity for each treatment. The top 10cm of inflorescence was beaten gently 10 times with a stick of pencil thickness. The dislodged aphids were collected on sheet of white paper and counted as by Razaq *et al*. (2012).

Photosynthesis parameters and chlorophyll contents

Measurements of net CO_2 assimilation rate (A), transpiration rate (E), water use efficiency (A/E), and sub-stomatal CO_2 concentration (C_i) were made on 3rd leaf from top of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). These measurements were made from 10:00 to 14.00 hours with the following specifications/adjustments: leaf surface area 11.25 cm^2, ambient CO_2 concentration (C_{ref}) 356 µmol mol^{-1}, temperature of leaf chamber (Tch) varied from 27.2 to 34.9^{o}C, leaf chamber volume gas flow rate (v) 296 mL min^{-1}, leaf chamber molar gas flow rate (U) 257 µmol s^{-1}, ambient pressure (P) 97.95 kPa, molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}, PAR (Q leaf) at leaf surface maximum up to 970 µmol m^{-2} s^{-1}.

For chlorophyll content measurement, plants of each variety were selected randomly from each treatment in each plot. Chlorophyll content was measured using chlorophyll content meter (SPAD-502, Konica-Minolta, Japan). Six independent SPAD measurements were made per treatment, using several different plants. The SPAD-502 meter is a hand-held device that is widely used for the rapid, accurate and non-destructive measurement of leaf chlorophyll concentrations. Photosynthesis parameters and chlorophyll contents were measured only from aphid infested plants (treatment where no insecticide was applied) and from plots where insecticide was applied weekly (aphid protected treatment).

Yield and its attributes

Yield attributes, *i.e.* plant height, pod length, numbers of seeds per pod, were noted from three plants selected randomly from each treatment and variety. Seed yield was recorded by harvesting one meter from a row of each treatment and variety and converted to kg per hectare.

Statistical analysis

Aphid mean population per 10 cm inflorescence from unsprayed and sprayed (twice) plots were analyzed by analysis of variance (ANOVA) to compare aphid population among the *B. napus* cultivars with randomized complete block design. Data on photosynthetic parameters were also subjected to two-way ANOVA and differences among the varieties were assessed by LSD test. Data on yield and all the yield components were analyzed for split plot design having two factors for sprayed (twice) aphid infested and aphid protected treatments separately for all the varieties. Differences in means of yield and all the yield components were compared by Tukey HSD test among the varieties themselves and also for each insecticide regime, i.e. in aphid free, sprayed (twice) and unsprayed plots. We did not apply LSD test as there were total 12 means for comparison LSD test is not recommended for more than six treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Incidence and abundance of aphids

Aphid population was non-significantly different at 5% significance level on all sampling dates in all the varieties in the unsprayed conditions. *L. erysimi* was the most abundant whereas only few nymphs of *B. brassicae* were observed in some plots. However, we present here pooled numbers of both the species of aphids. Peak number of aphids were noted 19th February in most of the varieties (Table I). Due to rainfall (3 mm on 23th February aphid population decreased in *B. napus* varieties when noted on 26th February. Aphid population increased on 5th March on all cultivars as compared to that recorded on 26th February. Later on aphid population decreased on 12th and 19th March.

Insecticide was applied on 14th February and then on 2nd March in sprayed plots. Aphids were non-significantly different among the varieties on all sampling dates, except that of 12th and 19th March, respectively. Application of insecticide reduced aphids but their numbers increased 10 days after second spray (Table I). In the present study, peak population in unsprayed plots was found in the 3rd week of February. Highest population of aphid species (*L. erysimi* and *B. brassicae)* have been reported from mid February to mid March in previous studies by Amer *et al*. (2009) on *B. napus* varieties. The mean aphid population was non-significantly different on many sampling dates in both sprayed and unsprayed conditions. Non-significant difference in mean aphid population was found on different sampling dates on canola varieties by earlier workers also (Amer *et al*., 2009).

Impact of aphid feeding on plant photosynthesis and chlorophyll contents

The mean photosynthetic rate and chlorophyll contents was significantly different between aphid protected and aphid infested treatments at 5 % level of significance on all cultivars, while water use efficiency, transpiration rate, internal CO_2 concentration among cultivars was non-significantly different between aphid free and aphid infested treatments. Application of insecticides did not significantly affect the water use efficiency, transpiration rate and total internal CO_2 of plants in all the plots as interaction between varieties and insecticides was non-significant. Variety Can Raya suffered significant losses in photosynthetic rate, whereas application of insecticides also increased rate when compared to other varieties (Table II).

Table I.- Comparative population of aphids (*B. brassicae + L. erysymi*) per 10 cm inflorescence in plots on varieties of *B. napus* with and without insecticides application at Multan during 2011.

Varieties	19-February	26-February	5-March	12-March	19-March
No insecticide treatment					
Can Raya	18.78±2.01	13.33±1.56	14.78±1.52	7.39±1.27	6.22±0.89
Con-II	10.56±1.48	12.33±1.40	15.78±1.74	11.78±2.67	9.89±0.76
Con I	17.56±3.53	12.50±1.28	13.22±1.15	11.29±2.02	7.56±0.69
Westar	8.67±1.30	9.56±1.00	10.94±0.73	10.5±2.49	8.83±0.75
F(df=3,6)	3.26	1.16	2.46	0.82	2.74
LSD	ns	Ns	Ns	ns	Ns
Insecticide treatment					
Can Raya	3.89±1.17	2.83±0.73	5.44±0.75	12.22±1.48 C	6.56±0.76A
Con-II	2.67±1.17	2.22±0.52	6.11±0.66	19.56±1.90 A	13.39±1.27B
Con-I	5.00±1.69	1.5±0.35	5.06±0.49	21.06±1.71 A	12.67±1.92B
Westar	4.94±1.43	2.11±0.44	5.28±0.68	16.00±1.82 B	10.00±1.14B
F(df=3,6)	0.25	1.41	0.57	19.28	4.46
LSD	ns	Ns	Ns	3.11	5.08

Means followed by the same letters in columns are not statistically different at 5 % level of significance ns indicates non-significant difference at 5 % level of significance.

The reasons for non significant difference between aphid infested and aphid free plants of *B. napus* cultivars for different photosynthetic parameters might be due to the maturity of the crop, as we recorded on 17th March 2011, and on this date the plants were at pod formation stage.

Photosynthesis rate *of B. napus* cultivars are in accordance to Lin *et al.* (1999), who reported a reduction in the photosynthesis rate of the cotton plants when infested by Silver leaf whitefly (*Bemsia argentifolii*). Also Watanabe and Kilagawa (2000) found that there was a reduction in the photosynthesis rate of rice plants when fed by plant hopper (*Nilapavarta lugens*) as compared to control conditions. Macedo *et al*. (2009) observed transpiration rate in the infested lines of wheat crop against feeding of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) and no significant difference was observed between the infested lines and uninfested counter parts of wheat plants. The internal CO_2 concentration of aphid infested and aphid free conditions are also in accordance to the Lin *et al*. (1999), as they observed no significant difference in the intercellular CO_2 concentration of the cotton plant leaves fed by Silver leaf whitefly *B. argentifolii*. These findings suggested that reduction in photosynthetic rate was not related with stomatal factors, and this might had been due to some perturbation in metabolism due to aphid infestation as has been observed recently in okra (Razaq et al., 2014). Further investigation is needed to assess how aphids reduce photosynthetic capacity at different stages of crop growth.

Effects of aphid feeding on yield and yield attribute

Insecticidal treatments affected significantly all the growth and yield parameters viz., plant height, pods per plant, pod length, number of seeds per pod, thousand seed weight and yield per hectare. Interaction between insecticide application and varieties was also significant. Moreover, two applications of immidacloprid could not recover the total losses due to aphid feeding as each of the parameter was significantly different from that of four sprays (aphid protected) (Table III).

A positive correlation between pods per plant, seed per pod and thousand seed weight with yield of *B. napus* has been reported by Ali *et al*. (2003). Mustard plants infested with *L. eyrsimi* had less plant height, produced less pods per plant, seeds per pod and test weight of grains in India (Malik and Deen, 1998; Roy and Baral, 2002). In addition, significant increase in pods per plant and seed yield was observed in plots treated with insecticides as

Table II.- Photosynthesis rate and related parameters of *B. napus* varieties in aphid protected plots (four sprays) and aphid infested plots (without application of insecticide) at Multan during 2011.

		CAN Raya	Con-II	Con-I	Westar
Photosynthetic rate	Aphid protected	15.53±1.23	10.24±0.92	12.15±0.67	13.1±0.70
	Aphid infested	4.91±0.74	11.95±1.06	9.42±0.88	11.20±0.90
Water use efficiency	Aphid protected	2.52±0.24	2.06±0.36	2.37±0.36	3.18±0.30
	Aphid infested	2.55±0.31	1.87±0.22	2.15±0.20	2.89±0.20
Transpiration Rate	Aphid protected	4.99±0.44	8.33±1.02	6.18±1.67	4.35±0.56
	Aphid infested	1.89±0.14	6.98±1.11	4.45±0.38	3.99±0.41
Total internal CO_2	Aphid protected	344.07±8.96	349.67±8.07	349.68±10.34	350.47±10.93
	Aphid infested	355.65±8.77	340.2±13.00	346.45±5.09	347.51±14.67
Chlorophyll contents	Aphid protected	29.7±1.90	31.40±0.81	33.58±0.78	29.48±1.02
	Aphid infested	27.2±0.58	30.26±0.62	26.03±1.48	29.33±1.49

ANOVA for photosynthesis rate and related parameters of *B. napus.*

	SOV	F value	P value	LSD	
				Between treatments	Within same treatment
Photosynthetic rate	Varieties	5.34	0.04		
	Insecticide	22.08	0.00		
	Variety*insecticide	9.66	0.00	3.86	2.63
Water use efficiency	Varieties	0.81	0.50		
	Insecticide	0.04	0.80		
	Variety*insecticide	0.92	0.48	Ns	Ns
Transpiration rate	Varieties	5.48	0.03		
	Insecticide	10.72	0.01		
	Variety*insecticide	1.76	0.23	Ns	Ns
Internal CO_2	Varieties	0.88	0.50		
	Insecticide	0.27	0.61		
	Variety*insecticide	0.54	0.66	Ns	Ns
Chlorophyll contents	Varieties	2.09	0.20		
	Insecticide	35.65	0.03		
	Variety*insecticide	4.95	0.03	3.65	2.86

Note: Degree of freedom for variety, insecticide level and variety*insecticide level are3, 1 and 3 respectively. ns=nonsignificant.

compared to those plots which were untreated in late sown *Sinapis alba*, *B. juncea*, *B. napus* and *Brassica rapa*in USA (Brown *et al*., 1999). In late sown *Brassica* crops, prolonged feeding of aphids causes heavy losses. Late sown Brassica crop remain tender for longer period, thus with the availability of favorable conditions, aphid feeding becomes prolonged and causes reduction in yield as reported by Chattopadhyay *et al*. (2005) and Razaq *et al.* (2011). Late sown wheat crop in the same region suffered from more losses in yield and growth by aphids, *Schizaphis graminum* Rond. and *Rhopalosiphum padi* L. (Homoptera: Aphididae)) as compared with early sown crop in recent study (Shahzad *et al*., 2013).

Table III.- Plant height, pods per plant, pod length, number of seeds per pod, thousand seed weight and yield per hectare in *B. napus* cultivars in plots where two sprays, no spray (aphid infested) and four sprays (aphid protected plots) at Multan during 2011.

Yield attributes	Varieties	Insecticide level		
		Two sprays	Aphid infested (no spray)	Aphid protected (Four sprays)
Plant height	Can Raya	107±1.20A	82.56±1.31BCD	112.44±2.28A
	Con-II	99.78±1.60AB	65.67±4.94DE	114.67±2.59A
	Con-I	92.44±2.24ABC	70±1.41DE	109.44±3.31A
	Westar	77.78±1.47CDE	59.69± 2.08E	76.78±1.19CDE
Pods per plant	Can Raya	81.89±2.11BC	40.33±2.56D	113.11±4.49A
	Con-II	56.11±3.30 CD	38.89±2.55 D	128.22±5.42A
	Con-I	58.78±3.08CD	34.22±3.20 D	75.11±2.46C
	Westar	74.22±6.39 C	35.33±2.93 D	104.44±3.32AB
Seeds per pod	Can Raya	9.44±0.60CDE	5.78±0.40E	11.78±0.60BCD
	Con-II	14.56±0.60B	8.22±0.46DE	19.44±0.90A
	Con-I	10.22±0.46BCDE	6.11±0.38 E	13.89±0.75BCD
	Westar	10.11±0.42BCDE	8±0.40 DE	14.22±0.85BC
Pod length	Can Raya	4.28±0.05EF	4.20±0.06F	4.63±0.05DEF
	Con-II	5.63±0.12BC	5.67±0.10ABC	5.97±0.21AB
	Con-I	5.49±0.45BCD	5.13±0.48BCDE	6.58±0.06A
	Westar	5.80± 0.16ABC	4.90±0.12CDEF	5.04±0.10BCDE
1000 seed weight	Can Raya	4.10±0.24C	3.54±0.14DE	5.76±0.04B
	Con-II	3.97±0.14B	3.52.03±0.21CD	6.17±0.37A
	Con-I	4.33±0.24D	2.78±0.22E	5.33±0.08C
	Westar	4.62 ± 0.03DE	3.48 ±0.06DE	4.8 ±0.03DE
Yield	Can Raya	568.20±38.61CD	92.86±10.14F	1261.18±114.35B
	Con-II	533.99±58.67CD	160.35±6.32EF	1960.71±95.92A
	Con-I	733.33±41.80C	427.06±36.26 D	1380.33±67.82B
	Westar	345.35 ±34.54DE	96.76± 8.75F	713.02 ±38.69C

ANOVA for plant height, pods per plant, pod length, number of seeds per pod, thousand seed weight and yield per hectare in *B. napus* cultivars.

	SOV	F value	P value	Tukey HSD Value	
				Same level of variety	Different levels of variety
Plant height	Varieties	47.86	0.00		
	Insecticide	74.07	0.00		
	Variety*insecticide	3.01	0.03	22.200	21.831
Pods per plant	Varieties	17.98	0.00		
	Insecticide	200.72	0.00		
	Variety*insecticide	8.06	0.00	25.729	26.068
Seed per pod	Varieties	87.03	0.00		
	Insecticide	259.3	0.00		
	Variety*insecticide	7.09	0.00	4.8984	4.8261
Pod length	Varieties	63.63	0.00		
	Insecticide	14.36	0.00		
	Variety*insecticide	6.46	0.00	0.8796	0.9146
1000 seed weight	Varieties	3.33	0.09		
	Insecticide	93.56	0.00		
	Variety*insecticide	4.53	0.00	1.3855	1.8331
Yield	Varieties	49.7	0.00		
	Insecticide	495.3	0.00		
	Variety*insecticide	228.29	0.00	197.02	233.92

Note: Degree of freedom for variety, insecticide level and variety*insecticide level are3, 2 and 6 respectively. ns=non-significant.

Highest yield was recorded in variety Con I where plants were sprayed twice and not sprayed, whereas Con II gave the highest yield in aphid protected plots. Westar suffered the highest losses in yield from aphids. Other yield contributing characters varied in all the varieties in three treatments. Therefore, variety Con-I can be recommended for farmers' cultivation (Table III).

In the present study, late sown *B. napus* cultivars were subjected to three different aphid management conditions *i.e.* aphid infested, where two sprays were applied to control aphid and third one in which four sprays were applied to control aphid completely. We observed that there were significant differences in yield between aphid protected plots (four insecticide applications) and in treatments where two applications of insecticides were made depicting that even two applications of insecticides cannot avoid total losses to late sown crops. *B. napus* is a minor crops in this region and farmers are not aware of losses due aphids. Therefore, the best approach will be to avoid late sowing of *B. napus*. If it is inevitable to avoid late cultivation of *B. napus* then application of insecticides is necessary however, fine tuning is required regarding application of insecticides.

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