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IS REDUCTION IN YIELD POTENTIAL OF SOME BRASSICACEOUS SPECIES DUE TO APHID INFESTATION ASSOCIATED WITH THE CHANGES IN STOMATAL FACTORS OF PHOTOSYNTHESIS?

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Abstract

Aphids cause heavy yield losses to Brassicaceous species by affecting various physiological and biochemical processes including photosynthesis. In the present study, seasonal activity of aphid population and its impact on some brassicaceous species was assessed. Three brassicaceous species (*Brassica campestris*, *Brassica carinata*, *Eruca sativa*) were grown in field following standard agricultural practices. Plants of control plots retained aphid free by insecticide spray, whereas treatment plots were freely allowed for aphid infestation. There was also intermediate treatment of partial aphid infestation where insecticidal spray was applied two times. Peak populations of both aphid species were observed in the 2nd week of March during which plant photosynthetic attributes were recorded. At the time of maturity, yield attributes were also recorded. From the results, it is obvious that application of insecticide significantly reduced the aphid populations on the three brassicaceous species and enhanced the crop yield. Yield losses due to aphid infestation were maximal in *Brassica campestris* followed by *B. carinata* whereas it was minimal in *Eruca sativa*. Yield losses in *Brassica campestris* and *B. carinata* were due to reduction in number of pods per plant, number of seeds per pod and size of seeds, whereas yield losses due to aphid infestation in *Eruca sativa* was mainly attributed to reduction in number of pods per plant. Although insecticidal spray reduced the aphid population and increased growth and productivity of all brassicaceous species, it did not change photosynthetic capacity of all plants except in *Eruca sativa*. Moreover, growth and yield reduction was not associated with stomatal factors of photosynthesis. Chlorophyll contents measured as SPAD values were reduced due to aphid infestation which is positively associated with yield reduction. Insecticidal spray increased chlorophyll contents in these three brassicaceous species by reducing aphid population. Application of insecticidal spray two times caused lower aphid infestation in brassicaceous species but it could not recover total yield losses. From these results, it is suggested that aphid infestation induced growth and yield reduction in the three brassicaceous species was due to some non-stomatal factors or due to alteration in metabolism of chloroplast. To affirm this, further research is needed.

Key words: Crop productivity, Canola, Tara mira, Chlorophyll, Losses, Pest population dynamics

Introduction

After cotton, rapeseed and mustard (*Brassica* spp.) are considered as second most important oil seed crops in Pakistan. According to estimates, 16-23% crop yield losses are due to by insects. Aphids cause significant loss in the yield which is up to 9-77%. For example, Razaq *et al*. (2011) reported that aphid infestation caused 70% yield losses in Brassica crops in Southern Punjab Pakistan. Similarly, Klem & Gadomski (1995) reported that aphids cause 11% reduction in oil contents of Brassica crops. Such high crop losses due to aphid infestation are mainly reasoned to their extremely rapid population growth. In contrast to other insect pests, embryonic development of aphids starts before the birth of their mother and thus nymphs of aphids become adult within five days (Tagu *et al*., 2005; Braendle *et al*., 2006). Depending on host plant physiology, highly fecund wingless aphid or less prolific winged progeny produced to new host plants. This dimorphism makes them well suited to colonize on host plants, particularly of monoculture crops (Goggin, 2007).

Aphid infestation caused substantial modification of host plant physiology and biochemistry either directly via secretion of chemicals or indirectly via host plant response (Burd, 2002). Aphids penetrate mouthparts in plant tissues via intercellular route and their impact on host plant is thought to be largely due to withdrawal of photoassimilates and injection of saliva that contains oxidases, pectinases and cellulases (Burd, 2002; Goggin, 2007). Aphid secretion can undergo long distance translocation in host plant and can cause toxic effects (Goggin, 2007). Using advance molecular biology and electron microscopy techniques Will *et al*. (2007) demonstrated that aphid saliva contains calcium binding proteins which prevents the sieve tube plugging and thus help aphids to remain on single feeding site for hours. Moreover, aphids increase the nutritional quality of their feeding sites by increasing the import of resources from other sites in the plant, mobilizing local resources, and blocking their export to other organs (Goggin, 2007). Such manipulation of resource allocation ability of aphids is deleterious to host plants or even it may be lethal.

Photosynthesis is central physiological processes that determine crop yield (Athar *et al*., 2009) and aphid infestation is known to reduce photosynthesis such as in cotton (Shannag *et al*., 1998; Lin *et al*., 1999a). Decrease in photosynthesis due to insect pest attack might have been due to damages to mesophyll cells, reduced chlorophyll content, impair the transport of photo-assimilates or any other regulatory switch that affect photosynthesis such as stomatal conductance (Lin *et al*., 1999a; 1999b; Athar *et al*., 2011; Zafar & Athar, 2013; Razaq *et al*., 2014a; 2014b; Hussain *et al*., 2015). Photosynthetic reduction in plants induced by phloem feeders has often been associated with stomatal conductance (Welter, 1993; Lin *et al*., 1999b; Razaq *et al*., 2014a; 2014b). These reports suggested that when plants are infested with insect pests, various physiological characteristics are impaired including photosynthesis. However, it is not clear that up to what extent level of aphid infestation is associated with yield reduction and photosynthetic capacity of plants. Gas exchange parameters have been considered as useful non-invasive technique to assess photosynthetic capacity and its relation with yield.

The major insect pests of mustard and rapeseed includes cabbage aphid (*Brevicoryne brassicae*), mustard aphid (*Lipaphis erysimi*), green peach aphid (*Myzus persicae*), cabbage butterfly (*Pieris brassicae*), mustard sawfly (*Athalia proxima*), painted bug (*Bagrada picta*), leafminer (*Chromatomyia horticola*), whitefly (*Bemesia tabaci*) and army worm (*Spodaptera litura*) (Ali & Munir, 1984; Verma *et al.,* 1993). Of these, crop damages due to mustard aphid (*L. erysimi*) and cabbage aphid (*B. brassicae*) in oilseed *Brassica* crops are large, particularly in the Asia, Australia, some states of USA and New Zealand (Buntin & Raymer, 1994; Hainan, 2007).

Various cultural practices, nutrient management, biological control, and chemical control are employed to manage insects (Athar *et al*., 2011; Razaq *et al*., 2014a; 2014b). Although chemical control is easy to apply with rapid action and mostly adoptable to all situations (Naik *et al*., 1993), it may alter plant physiology and crop yield. In view of all these reports and importance of *Brassica* oil seed crops, the present study was carried out to assess up to what extent pest infestation of two economically important aphids reduce plant yield of some Brassicaceous species. Moreover, it was also assessed that up to what extent level of pest infestation affecting photosynthetic activity that resulted in lowering in yield.

Material and Methods

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 in 2011. Seeds were sown manually with dibbling method on 25th November 2010 at recommended seed rate. The experiment was conducted in a randomized complete block design with four treatments *Brassica campestris* (local collected from farmers), *B. campestris* (var PARC), *Eruca sativa* (Tara Mira) and *B. carinata* (UCD) with three replications. 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. Rows were 5.0 m length. Row to row and plant to plant spacing was 45.0 cm and 10.0 cm respectively. As per requirement of the experiment two rows were sprayed twice (14th February and 2nd March, 2011) whereas two rows were kept free from aphids by applying insecticide on weekly basis (14th February to 13th March 2011). Remaining two lines were kept unsprayed to note aphid development without spray in each treatment plot. Insecticide Glitter (imidacloprid 20% SL, Warble Private Limited) was sprayed with a hand operated knapsack sprayer fitted with hollow cone nozzle by using a pressure of 3.0 bars at a given rate (80 mL/acre). At the time of insecticide application untreated rows of each plot were covered with plastic sheet to prevent the effect of insecticide drift on adjacent rows.

Data recording

Aphid population: Numbers of aphids were recorded per 10.0 cm of top plant inflorescence after an interval of one week from the beginning of flowering till crop maturation from six plants of each sprayed (twice) and non-sprayed rows from each treatment. The top 10 cm inflorescence was beaten gently 10 times with a stick of pencil thickness. The dislodged aphids were collected on sheet of white paper and counted (Razaq *et al*., 2012).

Gas exchange characteristics: Gas exchange characteristic were measured on single leaf basis, using third leaf from top or youngest and fully expanded leaves from each treatment. Gas exchange characteristics were measured using portable and open system infra red gas analyzer (LCA-4, ADC, Hoddesdon, UK). Photosynthetic rate, calculated internal CO_2 concentration, transpiration rate were obtained from IRGA (LCA-4, ADC, Hoddesdon, UK) from which water use efficiency (WUE = A/E) was calculated. Three plants were selected randomly from aphid protected and aphid infested rows in each treatment.

Chlorophyll contents: Two plants were selected randomly from each aphid free and infested rows in each plot. Chlorophyll content was measured with chlorophyll meter as SPAD relative values using SPAD-502 (Minolta, Japan).

Yield and its attributes: Yield attributes i.e. plant height, seed per pods, pod length, numbers of seeds per pod, were noted from three plants selected randomly from sprayed (twice), unsprayed and aphid free rows from each treatment. Seed yield was recorded from in plots of each treatment by harvesting one meter from a row of sprayed (twice), unsprayed and aphid free in each treatment and converted to kg per hectare.

Yield and yield component losses measurement: Yield of unsprayed plots was compared with yield of aphid free plots and percent loss in yield due to aphid infestation was calculated for each treatment by using the following formulae:

Loss in yield = sprayed plots yield −unsprayed plots yield (1)
Yield loss (%) =yield loss/sprayed yield × 100 (2)

Losses in other plant characters were also determined by using same formulae given above, by replacing yield with appropriate character of plant as given by Razaq *et al*. (2011) and to determine yield losses in sprayed and unsprayed conditions in late sown Brassicaceous species.

Statistical analysis: Aphid mean population per 10 cm inflorescence from unsprayed and sprayed (twice) plots was analyzed by analysis of variance (ANOVA) to compare aphid population among the *Brassica* species. Data on photosynthesis parameters was compared by t-test for aphid free and infested plants (Steel & Torrie, 1980). Data on yield and all the yield components were also analyzed by analysis of variance for sprayed (twice) aphid infested and aphid free treatments. Differences in means of yield and all the yield components were determined by LSD test among the each treatment in aphid free, sprayed (twice) and unsprayed plots, respectively.

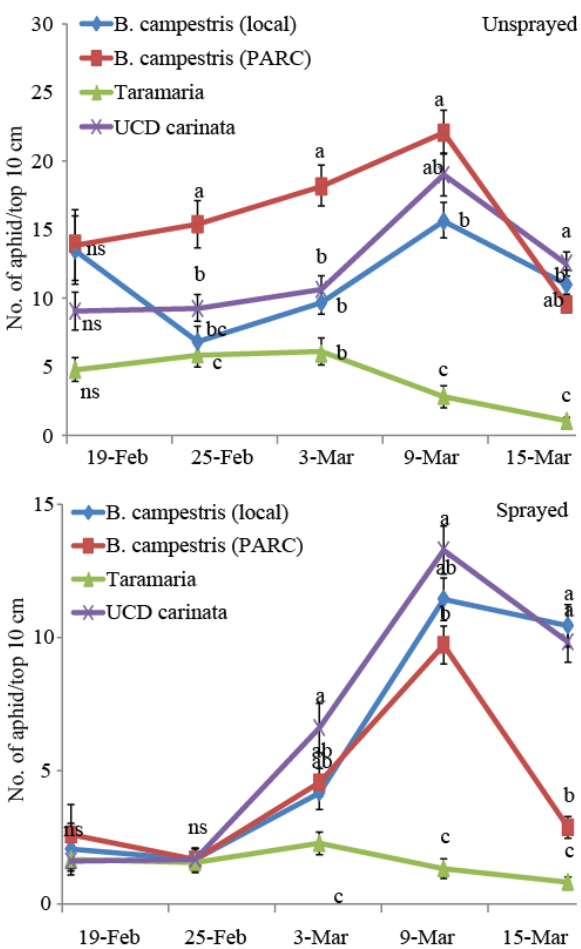

Fig. 1. Mean population of aphid per 10 cm inflorescence in unsprayed and sprayed twice (14th February and 2nd March) plots on *B. compestris* (local), *B. compestris* (PARC), *E. sativa* (Tara Mira) and *B. carinata* (UCD) at Multan in 2011.
Means followed by the same letters (date wise) on each treatment) are not statistically different at 5% level of significance, ns non significant at 5% level of significance.

Result and Discussion

Incidence and abundance of aphids: Of the two aphid populations, *L. erysimi* was the most abundant on all brassicaceous species whereas only few nymphs of *B. brassicae* were observed in some plots of examined species (Data not shown). It is therefore, data for nymphs of both aphid species were pooled. In unsprayed conditions, aphid population was 13.89 aphids on top 10 cm inflorescence per plant on 19th February. Due to rainfall (23th February and 3mm) aphid population decreased in *B. campestris* plots when noted on 26th February. Aphid population increased as observed on 5th and 12th March. Peak aphid population was recorded on 12th March on *B. campestris* (local). Later due to high temperature and maturity of the crop aphid population decreased when sampled on 19th March. Aphid population was significantly different on all sampling dates among all the species/cultivars except for 19th February in the unsprayed conditions at 5% significance level (Fig. 1). In sprayed plots, the highest aphid population on first sampling date (19th February) was 2.61 aphids per inflorescence. A slight decrease in aphid population was noted on second sampling date (26th February). Aphid population increased when noted on 5th and 12th March. Peak aphid population was recorded on 12th March on *B. carinata* (UCD). Aphid population was significantly different on three sampling dates 5th, 12th and 19th March on all the species in the sprayed conditions at 5% significance level (Fig. 1). In this present study, the peak population in both unsprayed and sprayed was found in the 2nd week of March on different *Brassica* species. Highest populations of aphid species (*L. erysimi* and *B. brassicae)* have been reported in 2nd week of March in previous studies on *Brassica napus* L. and *Brassica juncea* L. (Amer *et al*., 2009).

Effects of aphid feeding on plant photosynthesis and Chlorophyll content: Photosynthesis rate in all *Brassica* species did not change due to aphid infestation except in plants of *B. carinata* where it was slightly increased. Brassica species also differed significantly in their photosynthetic rate (Table 1). Of all *Brassicaceous* species, *Brassica carinata* (Var UCD) followed by *Eruca sativa* had greater photosynthetic rate. Both cultivars of *Brassica campestris* had lower photosynthetic rate under aphid infestation and non-infested conditions. Such difference in their photosynthetic rates might have been due to differences in their genetic potential as well as differences in duration of vegetative growth phase. These results are similar to earlier has been observed by Meyer & Whitlow (1992) who reported that no remarkable change in photosynthesis rate occur in goldenrod (*Solidago altissima* L.) due to leaf-feeding beetles (*Trirhabda* spp.) or aphids (*Uroleucon caligatum*) even at high population densities. However, these results are in contrast with those of Lin *et al*. (1999a) who reported that photosynthesis rate of the cotton plants was reduced when feed by Silver leaf whitefly *Bemisia argentifolii*. Similarly, in another study with rice, Watanabe & Kitagawa (2000) reported that photosynthesis rate of rice plants was reduced when feed by plant hopper (*Nilaparvata lugens*). Non-significant changes in photosynthetic rate in all *Brassica* species due to aphid infestation were positively associated with non-significant changes in internal CO_2 and transpiration rate. These results are similar with those of Lin *et al*. (1999a) who reported that no significant difference was found in the intercellular CO_2 concentration of the cotton plant leaves when attacked by whitefly *B. argentifolii*. Similarly, Macedo *et al*. (2009) observed no significant difference in transpiration rate of non-infested and infested plants of wheat with Russian wheat aphid (*Diuraphis noxia*). Water use efficiency measured as *A*/*E* did not changed due to aphid infestation. Moreover, *Brassica* species were also similar in their WUE under both aphid infested and non-infested conditions.

Chlorophyll contents were reduced due to pest infestation in all brassicaceous species except in *Eruca sativa* (Table 1). These results are similar to some of earlier findings in which it was found that insect or pest attack altered the chloroplast biochemistry and physiology by damaging thylakoid membranes and degrading chlorophylls. For example, Watanabe & Kitagawa (2000) reported that reduction in the chlorophyll contents in rice plants occurred due to attack of *N. lugens*. Similarly, infestation of Jassid, *Amrasca devastans* (Dist.) in okra (*Abelmoschus esculentus*) has been reported to greatly reduce chlorophyll contents (Razaq *et al*., 2014a). However, it is not clear whether such degradation of photosynthetic pigments is localized near or at the site of feeding or it is an overall response of plants. Moreover, non-significant difference in chlorophyll content in *Eruca sativa* due to aphid infestation can be explained in view of its greater tolerance to aphid infestation. Another explanation can be given as *E. sativa* become mature earlier (pod formation stage) than other species, which is more tolerant growth stage than the other growth stages of *E. sativa*.

Effects of aphid feeding on yield and yield attributes: Plant height was significantly different in sprayed (twice) and aphid free conditions in all the species. Insecticide application did not affect plant height as sprayed (twice), aphid infested and aphid free conditions in each species were non-significant except for *B. carinata* that might be due to the late maturity as we observed plants were still green and not mature at the time of harvesting (Fig. 2a). Numbers of pods were significantly different in aphid infested and aphid free plots among all species. Insecticide application increased significantly pods per plants in all the species except *B. campestris* (PARC) (Fig. 2b). Seeds per pod and thousand seed weight were statistically different in sprayed (twice), aphid infested and aphid free conditions among all the species. Insecticide application had significant effect on both parameters as sprayed (twice), aphid infested and aphid free conditions within the each species except for *E. sativa* was non-significant (Fig. 2c, 2e). Insecticide application had significant effect on yield and pod length. The loss in yield was significant in the treatments where two insecticides were applied as compared to four insecticides application (aphid free) in all species (Fig. 2d, 2f).

Table 1. Photosynthesis activity and its related parameters of *B. campestris* (local), *B. campestris* (PARC), *E. sativa* (Tara Mira) and *B. carinata* (UCD) under aphid free and aphid infested plots.

		B. camp (local)	B. camp (PARC)	UCD	Tara Mira	F value	P value	LSD value
Photosynthetic rate (µmol CO_2 m^{-2} s^{-2})	Aphid free	4.70±0.55b	5.23±0.76b	9.92±0.4a	8.18±0.6a	14.99	0.00	1.17
	Aphid infested	6.23±0.58c	4.03±0.6d	12.26±0.38a	7.52±0.74b	103.82	0.00	1.18
	t value	1.57ns	1.03ns	4.25	1.24ns			
	P value	0.19	0.36	0.01	0.28			
Water use efficiency (µmol CO_2/mmol H_2O)	Aphid free	2.47±0.14b	1.79±0.16c	3.33±0.27a	2.47±0.22b	12.34	0.00	0.625
	Aphid infested	2.40±0.23ab	1.66±0.2b	3.07±0.22a	2.04±0.15b	7.44	0.01	0.763
	t value	0.20ns	0.48ns	0.79ns	1.17ns			
	P value	0.85	0.65	0.47	0.30			
Transpiration rate (mmol H_2O m^{-2} s^{-1})	Aphid free	1.94±0.28b	3.07±0.43a	3.06±0.22a	3.41±0.33a	8.15	0.01	0.776
	Aphid infested	2.64±0.17	2.72±0.66	4.03±0.16	3.74±0.29	2.66	0.14	ns
	t value	1.69ns	0.47ns	4.83ns	0.66ns			
Internal CO_2 (µmol CO_2)	Aphid free	362.85±8.91	359.57±8.09	347.37±6.42	349.4±4.4	0.41	0.75	ns
	Aphid infested	360.68±8.45	358.11±6.87	342.88±6.11	351.87±5.72	1.22	0.38	ns
	t value	0.93ns	0.10ns	0.44ns	0.36ns			
Chlorophyll (SPAD values)	Aphid free	28.92±1.12b	28.37±0.91b	32.57±0.8a	30.78±0.66a	5.4	0.03	2.9
	Aphid infested	18.75±1.03c	19.77±1.77c	24.58±1.84b	28.8±1.04a	102.73	0.00	2.04
	t value	8.45	7.47	8.39	0.97			
	P value	0.00	0.00	0.02	0.30ns			

Means followed by the same letters in rows are not statistically different at 5 % level of significance, ns non significant at 5% level of significance. T-value for each parameter indicates significant difference between aphid infested and aphid free plants

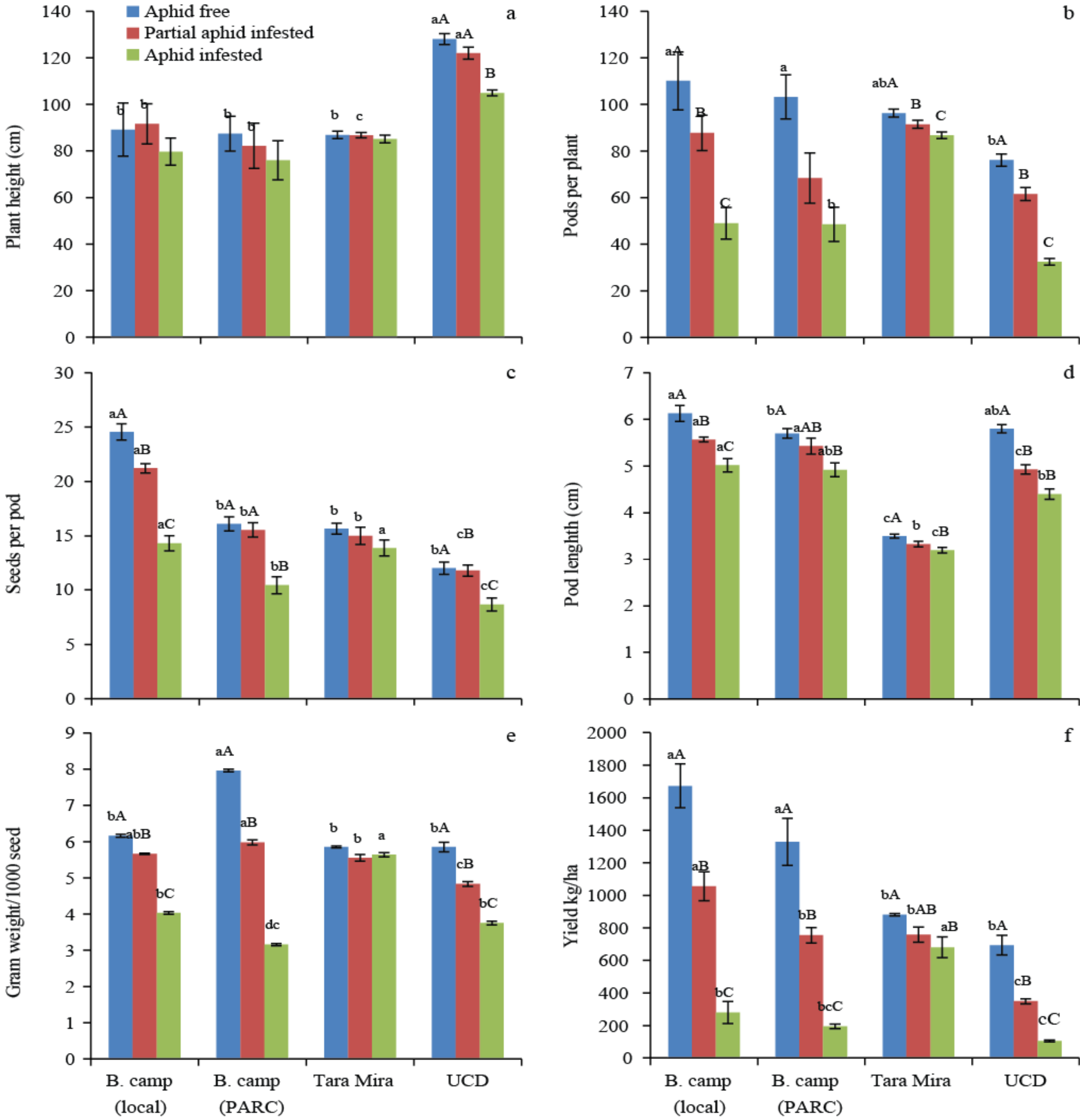

Fig. 2. Growth, yield and yield attributes of *B. campestris* (local), *B. campestris* (PARC), *E. sativa* (Tara Mira) and *B. carinata* (UCD) plants infested with or without aphids.

Means followed by the small letters for each parameter are not statistically different for same level of aphid incident conditions at 5% level of significance; means followed by the capital letters are not statistically different for each species at 5% level of significance.

Table 2. Percent loss in plant height, pods per plant, number of seeds per pod, pod length, pod weight, thousand seed weight and yield per hectare of *B. campestris* (local), *B. campestris* (PARC), *E. sativa* (Tara Mira) and *B. carinata* (UCD) in aphid infested plots at Multan in 2011.

Treatments	B. camp (local)	B. camp (PARC)	Tara Mira	UCD
Plant height	13.08	7.57	1.92	14.03
Pods per plant	44.18	28.93	5.22	47.37
Seed per pod	32.47	32.86	7.4	26.4
Pod length	9.87	9.39	3.9	10.75
Thousand seed weight	28.83	47.07	-1.57	22.33
Yield	73.52	74.18	10.37	69.88

Percent yield reduced in *B. campestris* (PARC) was 74.18% followed by 73.52% in *B. campestris* (local), 69.88% in *B. carinata* (UCD) and 10.37% in *E. sativa*. In other yield determining attributes like pods per plant, seed per pod and thousand seed weight, highest losses were observed in pods per plant ranging from 5.22 to 47.37% (Table 2). These parameters have positive correlation with the yield (Ali *et al*., 2003). The results are also in agreement with Sarwar *et al*. (2004) who reported that aphid population per plant was negatively correlated with plant height and number of pods per plant. Decrease in plant height, pods per plant, seeds per pod and test weight of grains has been reported due to increase in *L. eyrsimi* in India on mustard (Malik & Deen, 1998; Roy & Baral, 2002). Also significant increase in pods per plant and seed yield was observed in insecticides treated plots as compared to untreated plots on late sown *Sinapis alba, B. juncea*, *B. napus* and *B. rapa* in USA (Brown *et al*., 1999). In late sown *Brassica* crops, prolonged feeding of aphids caused heavy yield losses. Similar results have already been reported in India in which it was described that young tender and juicy shoots of brassicaceous plants provide favorable condition for aphids to colonization at the early stage of the crop till its maturity that resulted in yield reduction **(**Chattopadhyay *et al*., 2005). Aphids, *Schizaphis graminum* Rond. and *Rhopalosiphum padi* L. (Homoptera: Aphididae) cause more losses to late sown wheat as compared earlier sown (Shahzad *et al*., 2013). Application of insecticides is only option to reduce the yield losses by aphids because in available cultivars of *Brassica* lack plant resistance and natural enemies of aphids appear too late to reduce aphid population (Aslam & Razaq, 2007; Aslam *et al*., 2009; Amer *et al*., 2009). Although natural enemies of aphids on alternative host plants play an important role in reducing pests on crops (Razaq *et al*., 2015), role of alternative hosts in lowering in populations of aphids (*L. erysimi* and *B. brassicae*) still needs to be determined.

In this present study, impact of degree of aphid infestation in growth and yield reduction was assessed in late sown *Brassica* species. It was found that lower rate of aphid infestation by two applications of insecticides on the three brassicaceous species also caused significant yield reduction. In other words, two insecticidal sprays were not sufficient to reduce the aphid infestation below to threshold level where yield reduction is minimal. Similar results have already been reported in late sown *B. napus* varieties that two sprays were insufficient to keep plants aphid free (Razaq *et al*., 2014a). Recently, alternative to insecticidal spray, it is advocated that insecticide application as seed treatment could be more effective in lowering in pest population and also safer for natural enemies (Saeed *et al.*, 2016). Efficiency of insecticide treatment through seed treatment needs to be elucidated on brassicaceous crops.

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