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Predatory Potential of *Coccinella septempunctata* L. against Four Aphid Species

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Authors' Contributions
MA and FH conceived and designed the study. RS and NI performed the experiments and collected raw data. MA, FH and HAAK analyzed the data, and wrote the articles.

Key words
Feeding potential, Biological control, Vegetable aphid, Ladybird beetle, Predator-prey relationship, IPM.

ABSTRACT

The relationship between a coccinellid beetle (*Coccinella septempunctata* L.) and four different aphid species (spinach aphid, *Aphis fabae* Scopoli; coriander aphid, *Hyadaphis coriandri* (Das); cabbage aphid, *Brevicoryne brassicae* L.; pea aphid, *Acyrthosiphon pisum* Harris) was evaluated in the laboratory in no choice and free choice feeding assays. In the no choice feeding assay, the stages of beetle (adults, 3rd and 4th instar) consumed more aphids than early stages (1st and 2nd instar). In the free choice feeding assay, the consumption of pea aphid (77.647) was statistically high followed by spinach (66.276), coriander (66.14) and cabbage (61.48) aphids. Manly's preference index suggest that pea aphid was the most preferred (by all stages of *C. septempunctata*) aphid species followed by coriander, and the cabbage aphid was the least preferred species. The results of the present study revealed predatory potential of *C. septempunctata* against different aphid species under the laboratory conditions. The results could be helpful in designing biological management strategies for aphids.

INTRODUCTION

Vegetables are herbaceous plants, comprised chiefly of mineral contents, carbohydrates and vitamins (Robinson, 1990). The vegetables like cabbage, pea and spinach are not only sources of energy for body building and maintenance but also curative and repair the body. In Pakistan, vegetables are always grown at huge risk because these mainly attacked/damaged by sucking and chewing insect pests. Sucking insect pests play a substantial part as yield declining agent. Out of all insect species, aphids are considered as notorious pest of different vegetables (Aheer *et al*., 2008; Minks and Harewijn, 1987; Ahmad *et al*., 2016). Aphids insert their proboscis into phloem vessel and suck the cell sap from different parts of the plants. Resultantly, the affected leaves turn pale yellow and start to wilt. Aphids also secrete honey dew due to which sooty mold appear on the leaves (Kindler *et al*., 1995).

Different chemicals are available for the control of aphids (Messelink *et al*., 2013) but these chemicals have negative effects on the environment (Khan *et al*., 2015, 2016). Therefore, there is a need to explore alternate measures for the management of aphids. Bio-control agents like spiders, syrphids and coccinellid species are commonly present in the environment. These agents have been reported as potential candidates for the management of aphids (Singh *et al*., 2001).

Coccinellid beetle, *Coccinella septempunctata* L., (Coccinellidae: Coleoptera) is a multicultural having extensive dispersal power with the ability to predate aphids and many soft bodied insects of valuable vegetables (Ali and Rizvi, 2009). In the same preview, *C. septempunctata* consumed more aphids (Soni *et al*., 2004) than *Brumoides suturalis*, *Cheilomenes sexmaculata, Menochilus sexmaculatus*. Both young ones (grubs) and adults of *C. septempunctata* consume about 40-173 aphids daily (Akram *et al*., 1996; Suhail *et al*., 1999). There are some Coccinellid beetles that have preference only to consume certain aphid species but others attack many species because of their capacity to survive (Habeck *et al*., 1990). Biological control agents (Coccinellids) consist of an important constituent of many incorporated pest management programs but many synthetic pesticides affect them negatively (Moddue and Blackwell, 1993). To avoid such harmful effects, there is a need to use the natural

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enemies. The present studies were therefore conducted to determine the predatory potential of *C. septempunctata* against spinach, coriander, cabbage and pea aphids under the laboratory conditions.

MATERIALS AND METHODS

Insects

The *C. septempunctata* adults were collected from vegetable fields (University of Agriculture, Faisalabad and Ayub Agricultural Research Area) and placed in plastic cups and vials under controlled conditions in the Insect Biodiversity and Biosystematic laboratory, Department of Entomology, University of Agriculture, Faisalabad. The mates were placed in separate cups with mouth of the cups covered with muslin cloth. Eggs were placed in petri dishes and maintained in the laboratory conditions set at 25±2°C and 62±5% relative humidity. Four aphid species, *Aphis fabae* Scopoli, *Hyadaphis coriandri* (Das), *Brevicoryne brassicae* L. and *Acyrthosiphon pisum* Harris were collected from spinach, coriander, cabbage and pea, respectively, and identified using an online database for aphids taxonomic identification (http://aphid.aphidnet.org/).

No choice assay

Newly hatched grubs of *C. septempunctata* were placed singly in separate cup with a Whatmann-No 1 filter paper. Counted numbers of aphids (200 nymphs of 3rd / 4th instar) of each species were provided in each cup daily. Predatory potential was calculated by counting left over aphids in the cups. The grubs were also checked daily for their moulting. Similarly duration of 2nd, 3rd and 4th instars were noted by observing the sheded skin of the beetle grubs**.** This practice continued till pupation. The same was continued for adults. Average consumption rate was calculated by formula (aphid consumed/aphid offered × 100).

Free choice assay

Four aphid species were offered to each life stage (such as 1st, 2nd, 3rd, 4th instar and adult) of *C. septempunctata*. The experiment was carried out using free choice feeding tests. During this test, a single instar/stage of *C. septempunctata* were released separately into plastic jars (10 × 05 inch) lined with muslin cloth (for aeration), and maintained under the laboratory conditions set at 25 ± 2˚C and 62±5 % relative humidity. A mixture of 300 nymphs of each aphid species (spinach, coriander, cabbage and pea) was introduced in each jar and fresh leaves of each vegetable were provided for aphids. Daily consumption was calculated by subtracting the number of aphids left from number of individuals brushed in the jar. The numbers of aphids consumed were counted after every 24 h till the end of each instar/stage of *C. septempunctata*.

Statistical analysis

The normality of data was checked by applying Kolmogorov-Smirnov test before statistical analysis. The difference in predatory potential of *C. septempunctata* (four instars of grubs and one adult stage) was compared by using two-way analysis of variance (ANOVA) with the help of computer based software "Statistix v8.1" (Analytical Software, 2005), and a least significant difference test ($\alpha = 0.05$) was employed to separate the means (Khan *et al*., 2012). A paired *t*-test was applied to compare the predatory potential of every stage of *C. septempunctata* against different aphid species. The preference of different aphid species was estimated by calculating the Manly's Preference Index (αP) in free choice assay (Manly *et al*., 1972).

$$\alpha p = \frac{ln\left[\frac{n_p - r_p}{n_p}\right]}{ln\left[\frac{n_p - r_p}{n_p}\right] + ln\left[\frac{n_u - r_u}{n_u}\right]}$$

Where; n_p and r_p were the initial numbers of different aphid species (*i.e.,* 200), r_p and r_u were the consumed after 24 h duration, respectively. The preference of *C. septempunctata* (four instars of grubs and one adult stage) were calculated separately. This index gives the value from 0 to 1, where 0.5 indicates no preference. However, we tested our null hypothesis of no preference for a specific aphid species by a *t*-test.

RESULTS

The results indicated that consumption rate (during no choice assay) of different instars and adults *C. septempunctata* on different aphid species (spinach, coriander, cabbage and pea) is almost similar with each other (Fig. 1). However, 1st, 2nd and 3rd instar grub took three days to complete its stage and shifted to the next one, whereas 4th instar and adults took two and five days respectively. Feeding/consumption was increased with the age of test insect. In addition to this, consumption of 1st instar grub was similar to in all aphid species. In case of 2nd instar, the consumption was almost equal for first two day but considerably different on the last day *i.e*. spinach, cabbage, coriander and pea (19.4, 19.8, 24.4 and 27.4, respectively). This feeding / consumption of pea aphid preference continued to the 3rd and 4th instar. The pupal stage arrived and lasts for four days. In the continuation of adult life span, consumption was increased rapidly with spinach and coriander aphid but gradually for cabbage

and pea. The feeding pace of adults on coriander aphid was maximum (100.8, 116.2, 148, 152.8 and 160.2) followed by spinach and pea aphids (on 12th to 16th day, respectively), whereas cabbage aphid was consumed less in number during all five days, respectively (Fig. 1).

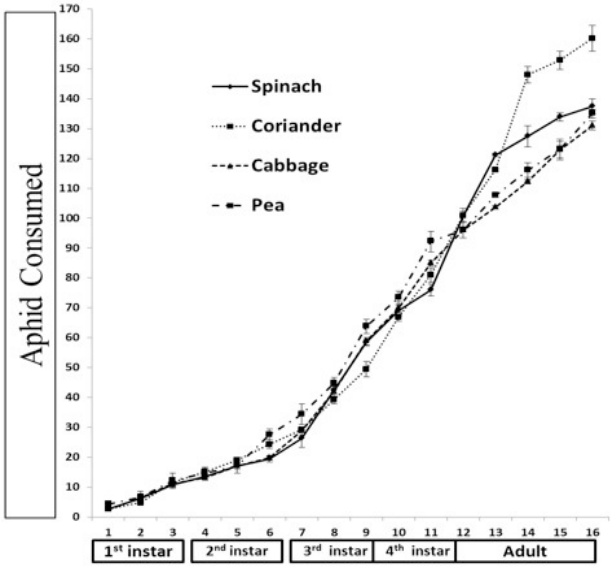

Fig. 1. Consumption of different aphid species *i.e.* spinach, coriander, cabbage and pea by different instars of *C. septempunctata*.

In the choice feeding tests, higher consumption of aphid was observed at each successive instar. However, the consumption of pea aphid was statistically higher in 1st (3.46), 2nd (6.46), 3rd (12.90), 4th (24.13) and adults (30.68) of *C. septempunctata* followed by spinach, coriander and pea (Table I).

Table I.- Consumption of aphid species by different life stages of *C. septempunctata* under free choice bioassay.

Stages of *C. septempunctata*	Aphid species			
	Spinach	Coriander	Cabbage	Pea
1st instar grub	1.63± 0.50^{bc}	2.1± 0.87^{b}	1.6± 0.61^{c}	3.46± 0.98^{a}
2nd instar grub	4.13± 0.40^{c}	5.86± 0.37^{b}	4.0± 0.64^{c}	6.46± 1.67^{a}
3rd instar grub	9.3± 1.32^{b}	7.0± 0.46^{c}	9.4± 1.03^{b}	12.9± 0.40^{a}
4th instar grub	18.93± 01.96^{b}	14.13± 1.75^{c}	18.6± 1.50^{b}	24.13± 2.70^{a}
Adult	32.28± 1.49^{b}	37.04± 3.80^{a}	27.88± 1.65^{d}	30.68± 1.74^{c}

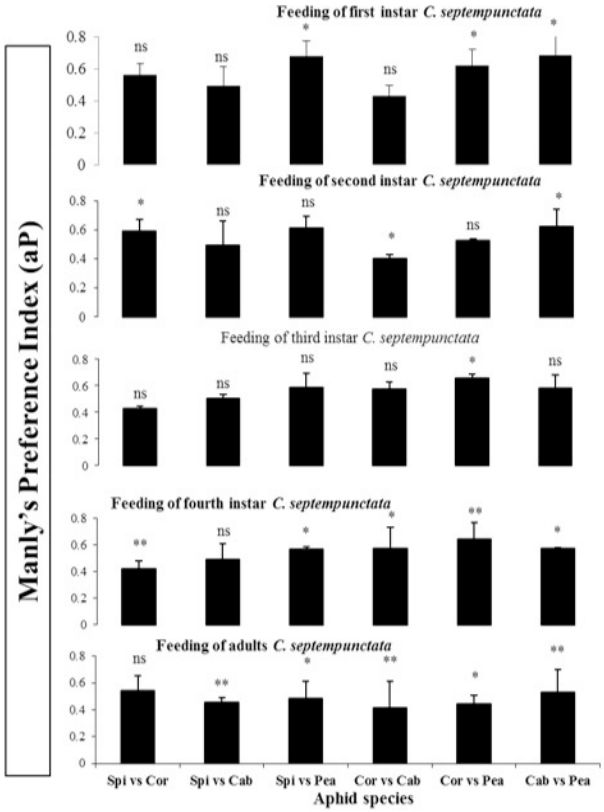

Fig. 2. Manly's preference index (aP) of different instars of *C. septempunctata* on different aphid species (abbreviated as: spi, spinach; cor, coriander; cab, cabbage). The bars with asterisks are significantly different from the predicted index at 0.5 by t-test whereas 'ns' values are non-significant or similar.

Manly's preference index

When 3rd instar aphid species (*i.e.* spinach vs. coriander, spinach vs. cabbage, spinach vs. pea, coriander vs. cabbage, coriander vs. pea, cabbage vs. pea) were offered to different life stages (first, second, third, and fourth instar, and adult) of *C. septempunctata* simultaneously, the beetle consumed all aphid species but preferred pea, coriander and spinach respectively (Fig. 2). Index values > 0.5 represents preference for aphid species (written after vs. in Figure 2; positive switching), while those < 0.5 represent non preference for aphid species (written before "vs." in Figure 2; negative switching). The first instar grub of *C. septempunctata* showed marked preference of pea aphid over spinach (t=-2.89, df=4, P= 0.05), coriander (t=-5.25, df=4, P=0.03) and cabbage (t=-4.25, df=4, P=0.05). Likewise second instar grub also showed significantly higher preference for coriander aphid over spinach and cabbage (t=3.61, df=4, P=0.05; t=3.52, df=4, P=0.05, respectively), and pea over cabbage (t=-3.25,

df=4, P=0.05). When the third instar grub was compared for predation potential for various aphids species, pea aphid was consumed significantly greater over (t=-5.9, df=4, P=0.01). In case of fourth instar grub, pea aphid was consumed greater over spinach, coriander and cabbage whereas spinach and cabbage aphid were preferred over coriander. However, adults of *C. septempunctata* under comparison of predation potential showed that spinach aphid was greatly preferred over cabbage and pea, whereas coriander preferred over cabbage and pea, and pea preferred over cabbage.

DISCUSSION

Ecosystem is made up of living and non-living environment. However, non-living environment *i.e.* ecology directly derive population fluctuations and energy flow by means of different food webs. In the same way, predators pose direct impact on prey population by consumption (Peckarsky *et al*., 2008; Arshad *et al*., 2015). Feeding of insects has some economical worth, so it is vital to identify the number of prey killed while determining the effects of predators in fluctuation of prey (Messina and Sorenson, 2001).

Our results suggest that *C. septempunctata* has great potential against aphids. Moreover, different stages also had significant effect on consumption rate. Our findings are partial agreement with scientists working sub-tropical regions (Sharma and Joshi, 2010; Singh and Singh, 2013) who found that *C. septempunctata* showed more preference to *Lipaphis erysimi* (under no choice assay). A steady increase in consumption/predation was observed with successive developmental stages. This difference in predation might be due to recognition of most suitable prey by its respective morphological (Dixon, 2000), physiological and chemical variations (Omkar *et al*., 2004). The phenomenon by which predators seek, locate and / recognize their suitable/ palatable prey is still unknown. This line of research is wide open and required to resolve complications in recognition and discrimination of prey. The results of Singh and Singh (2013) are in conformity with ours that voracity of *C. septempunctata* increased with the age and predate all stages (Jindal and Malik, 2006; Bilashini and Singh, 2009). In our experiments, adults consume more aphids than grubs, such investigations are in consonance with judgment of (Singh *et al*., 1994; Ali and Rizvi, 2007, 2009). In addition to this, the above findings yielded the following order preference: pea aphid > coriander aphid > spinach aphid > cabbage whereas Bilashini *et al*. (2007) while working in India, yielded this order *Lipaphis erysimi* > *M. persicae* > *B. brassicae.*

CONCLUSIONS

It could be concluded from the present findings that different aphid species had a significant effect on the lifelong consumption by *C. septempunctata*. However, morphological features of prey (aphids) entice a predator (*C. septempunctata*) that's why pea aphid was highly preferred under lab conditions in terms of development and predation as compared with the rest of the aphid species (spinach, coriander and cabbage). Hence, it offers comprehensive information to introduce *C. septempunctata* in biological-based management plans of aphids.

Statement of conflict of interest

Authors have declared no conflict of interest.

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