

Feeding Paradigm of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) on Parasitized Mustard Aphid, *Lipaphis erysimi* (Hemiptera: Aphididae) in Free Choice feeding trial

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Abstract

Understanding the interaction among natural enemies is a holistic approach to developing a more effective biological control program. The one crucial interaction is the consumption of parasitized prey by a predator. For instance, feeding on a parasitized aphid could affect predator and parasitoid species. The Ladybeetle, *Coccinella septempunctata* is a well-known natural predator of multiple agricultural pests including aphids. The current study evaluated the prey preference or aphidophagous behaviour of *C. septempunctata* on healthy and parasitized aphids in laboratory conditions at Temp. 25±2°C and R.H. 65±5%. The mustard aphid, *Lipaphis erysimi*, cabbage aphid; *Brevicoryne brassicae* and parasitized mustard aphid, *Lipaphis erysimi* were used in the study. Research work revealed the free choice feeding relationship between coccinellid beetle and different aphid species. The mean consumption of mustard aphids was statistically higher i.e., 193.19 followed by cabbage



(150.70) and parasitized mustard aphids (70.79). Female lady-beetle consumed more parasitized aphids (32.12) as compared to males and all larval stages. Specifically, male consumed 22.35 aphids while the consumption by larval stages was 6.56 aphids for the 4th instar followed by 3rd instar= 6.42, 2nd instar= 2.17 and 1st instar= 1.17. Adults of *C. septempunctata* were found to be more voracious, consuming more aphids than any of the larval instar stages. Manly's Preference Index indicated that healthy mustard aphid were highly preferred by all stages of *C. septempunctata*, followed by cabbage aphids, while parasitized mustard aphids were the least preferred. The least preference of *C. septumpunctata* towards parasitized aphids suggests that biological control programs can be optimized by targeting non-parasitized aphid populations. This could enhance the effectiveness of natural predators and reduce the risk of disrupting parasitoid populations.

Keywords: Coccinellids, parasitized aphid, preference, predation, biological control

Introduction

Mustard crop (*Brassica juncea* L.) is attacked by a multitude of insect pests including aphids, painted bug, *Bagrada cruciferae*; saw fly, *Athalia proxima* and pea leaf miner, *Chromatomyia horticola* but aphids are considered as a key pest (Agarwala and Datta, 1999; Patel *et al.*, 2019). Aphids are among the most serious sucking insect pests and play a significant role as yield-reducing agents (Sorensen, 2009; Khalique *et al.*, 2021) by sucking cell sap from leaves, stems, and inflorescence. Of the 4000 aphid species reported to colonize temperate flora, approximately 100 are recognized as pests of key economic importance (Blackman and Eastop, 2007). *Brassica* pods and seeds attacked by aphids remain stunted and resulting yield losses range between 30-35% that may rise to 70% in case of heavy infestation (Bhatti *et al.*, 1976; Phadke, 1985; Aheer *et al.*, 2008; Ahmad *et al.*, 2016). During feeding, aphids insert their stylets into plant cells to secrete phytotoxic saliva and extract the phloem sap from sieve elements (Tjallingii, 2006). Additionally, aphids excrete honeydew which promotes the growth of sooty molds, and also act as a vector for various plant viruses (Stevens and Lacomme, 2017; Alford, 2011).

Various chemical control measures are commonly used to suppress aphid populations. However, these insecticides are not environmentally friendly and pose significant risks to human health and the environment (Dedryver *et al.*, 2010; Khan *et al.*, 2016; Khalique *et al.*, 2018). Alternative measures include managing aphid population through natural enemies such as predators (spiders, syrphids and coccinellid), parasitoids and entomopathogenic fungi



(Kindlman *et al.*, 2007; Ahmad *et al.*, 2019). These agents have been identified as potential candidates for effective aphid management (Powell and Pell, 2007) especially the importance of coccinellids have long been recognized as aphid predators (Harrewijn and Minks, 1987). A predator's growth and reproduction largely depend on the availability of suitable prey (Dixon, 2000) and they are considered opportunistic generalists in terms of their life parameters (Michaud, 2000; Eubanks and Denno, 2001; Stamp, 2001). Ladybird beetle population appeared during the last week of January, reached a peak during the third week of February and then declined in the last week (Manzar *et al.*, 1998; Verma *et al.*, 1999; Kulkarni and Patel, 2001).

Coccinilid (Coccinilidae: Coleoptera) is a group of beetles comprising approximately 6000 species, distributed across 490 genera with a cosmopolitan distribution (Vanderberg, 2002; Slipinski, 2007). Most coccinellid species are predators of various insect pests (Kunznetsov, 1997) and play a crucial role in regulating pest populations in both agricultural crops and fruit plants (Kumar *et al.*, 2017).

The lady beetle, *Coccinella septempunctata* L., is a well-known effective predator with extensive dispersal ability, primarily preying on aphids and various other soft-bodied insect pests of valuable crops (Ali and Rizvi, 2010; Hodek *et al.*, 2012; Omkar and Pervez, 2016) whereas pollen, nectars and mildew are recorded as a secondary food. The choice of prey is mainly associated with the developmental and reproductive potential of coccinellid beetles (Pervez *et al.*, 2018). In this context, some coccinellid species prefer certain aphid species as prey, while others may exhibit different feeding preferences (Omkar and Mishra, 2005). Integrated pest management (IPM) includes various control strategies based on biocontrol agents and synthetic insecticides. Being a major component of IPM strategies, the objective of biological control is to suppress pest populations by using natural enemies, such as parasitoids, predators, and pathogens (Wratten and Van Emden, 1995: Ahmad *et al.*, 2019). Natural enemies such as coccinellids, have been successfully integrated into pest management programs to control aphid populations (Ferron and Deguine, 2009; Michaud and Sloderbeck, 2005).

In nature, host-specific species respond to the prey types, numbers and sizes but the generalist predators do not and this peculiarity is called prey preference. It has been reported that variety of prey species exposed to polyphagous predators revealed preferences for one or more prey types (Hassell, 1978). In previous studies, preference was measured by the

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difference in the proportion of prey species eaten out of the available prey species that indicated the preference level described (Maelzer, 1978; Elliott *et al.*, 2000). Moreover, it was found that among the coccinnelid beetles, *C. septempunctata* prey particularly on aphid species (Dixon, 2000; Hodek and Honêk, 2013). However, the effect of parasitized mustard aphids as a predator's diet has not been well studied and understood (Takizawa *et al.*, 2000). The present study was conducted to determine the feeding paradigm of *C. septempunctata* on different aphid species in a free-choice feeding trial.

Materials and Methods

The research was conducted at Entomological Research Institute (31.4049° N, 73.0505° E), Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Insect collection

Adults of ladybird beetles were collected from oilseed crop by sweeping aerial nets and hand-picking. Collected beetles were brought to the laboratory and shifted to acrylic cages (20 x 18 inches) with an opening (5-inch diameter) for aeration and covered with muslin cloth. Aphid population was provided as food for *C. septempunctata* and fresh mustard leaves for aphids rearing. Eggs laid by adult females were collected from the acrylic box and placed separately into the petri plates (6 x 0.5 inches) having Whatman No.10 filter paper and its mouth was covered with muslin cloth, tied with a rubber band and kept under in vitro conditions at $25\pm2^{\circ}$ C and $65\pm5\%$ relative humidity.

Brevicoryne brassicae, *Lipahis erysimi* and parasitized *Lipahis erysimi* were collected from the rapeseed or oilseed rape, *Brassica napus* and reared on freshly provided leaves in the laboratory. These aphids were identified using an online database of taxonomic identification (http://aphid.aphidnet. org/).

Bioassay

For the experiment, coccinellid larvae that newly emerged from eggs shifted individually into the petri plates containing filter paper using a soft camel hairbrush. Twenty-five replications, each provided with twenty different nymphal instars of the previously mentioned aphid species using a soft camel hairbrush. Feeding preferences were noted in all larval instar stages (1st, 2nd, 3rd and 4th) and adult males and females of *C. septempunctata*. The mouth of petri plates was covered with muslin cloth and tied with rubber bands for



aeration. The population number of aphids was increased by up to two hundred times (200x) for *C. septempunctata* stages. Daily consumption of aphids was calculated by subtracting the number of aphids left from the number of aphids brushed into the petri plates. Data was taken every 24 hrs till the end of each instar/ stage of *C. septempunctata*.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyse the differences in feeding potential of four stages of grubs, adult male and female stage with the help of computer-based software "Statistix v8.1" (Miller and Miller, 2005). The feeding potential of each stage of lady beetle against different aphid species was compared using Tuckey's test at α =0.05. The feeding preference of various stages of *C. septempunctata* against different aphid species was calculated by the following formula.

$$\alpha P = \frac{in\left[\frac{np-rp}{np}\right]}{in\left[\frac{np-rp}{np}\right] + in\left[\frac{nu-ru}{nu}\right]}$$

Where; 'np' and 'nu' were the initial numbers of different aphid species that provided (i.e., 200), 'rp' and 'ru' were the consumed aphids after 24 h duration. The preference of *C. septempunctata* (across all larval stages, adult males and adult females) for aphids was assessed separately on daily basis until the completion of each developmental stage.

Results

Results indicated that the consumption of various aphid species by all stages of *C*. *septempunctata* was relatively similar. However, the 1st, 2nd and 4th instars required three days to complete their stages and transition to the next, while the 3rd instar took only two days. Both adult males and females required twenty-five days to complete their respective stages. All larval instars showed greater preference for mustard aphids over cabbage and parasitized mustard aphids (Fig.1). After 4th larval instar it shifted into the pupal stage that lasted seven days. Consumption of aphids gradually increased by adult males and females (Fig. 2, 3).



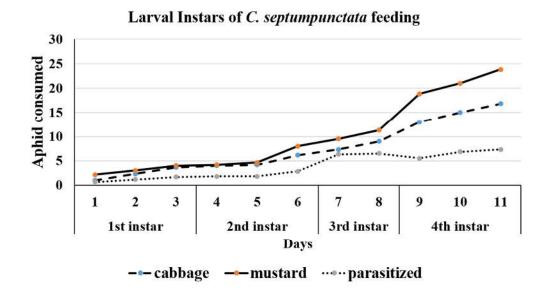


Fig. 1. Consumption of different aphids i.e. cabbage, mustard and parasitized aphid by larval instars of *C. septumpunctata*

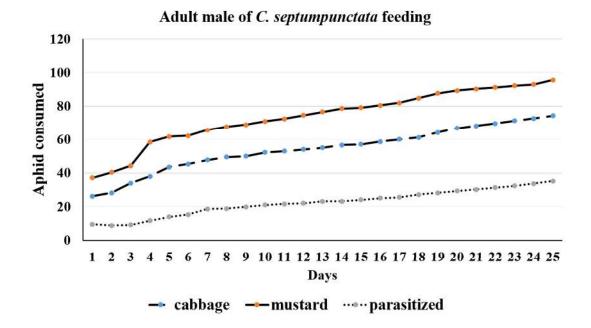


Fig. 2. Consumption of different aphids i.e. cabbage, mustard and parasitized aphid by adult male of *C. septumpunctata*



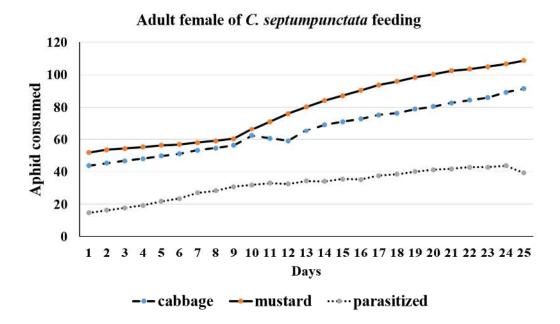


Fig. 3. Consumption of different aphids i.e. cabbage, mustard and parasitized aphid by adult female of *C. septumpunctata*

Aphid consumption increased progressively with each successive instar during feeding tests. However, the mean consumption of mustard aphids was significantly higher across all stages: 1st instar (3.06), 2nd instar (5.61), 3rd instar (10.42), 4th instar (21.22) adult males (73.83) and adult females (79.05) compared to both cabbage aphids and parasitized mustard aphids (Table-I).

Table-I: Mean consumption of aphids by different developmental stages of C. septempunctata

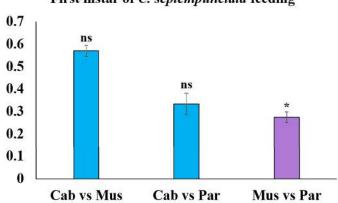
Stages of C. septempunctata	Aphid consumed		
	Cabbage	Mustard	Parasitized Mustard
1 st Instar grub	$2.33\pm0.77~dB$	$3.06 \pm 0.53 \text{ dA}$	$1.17 \pm 0.29 \text{ dC}$
2 nd Instar grub	$4.78\pm0.70~cdB$	$5.61 \pm 1.21 \text{ cdA}$	2.17 ± 0.33 cdC
3 rd Instar grub	$8.17 \pm 0.83 \text{ cdB}$	$10.42 \pm 0.92 \text{ cdA}$	$6.42 \pm 0.08 \text{ cdC}$
4 th Instar grub	$14.94 \pm 1.11 \text{ cB}$	$21.22 \pm 1.45 \text{ cA}$	$6.56 \pm 0.55 \text{ cC}$
Adult Male	$54.29 \pm 2.67 \text{ bB}$	73.83 ± 3.29 bA	22.35 ± 1.55 bC
Adult Female	$66.19 \pm 3.02 \text{ aB}$	$79.05 \pm 4.09 \text{ aA}$	32.12 ± 1.78 aC

Means with different lowercase letters within column and uppercase letters between columns are significantly different at $\alpha = 0.05$.



Manly's preference index

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to the first instar of C. septempunctata, the grub consumed all aphid species but preferred mustard aphid over cabbage and parasitized mustard aphids respectively (Fig. 4). The index generally indicated that 0.5 value represent the non-preference but here in this experiment, mustard aphid showed marked preference over parasitized (t=7.82, df=2, P=0.01).



First instar of C. septempunctata feeding

Fig. 4. Manly's preference index of 1^{st} stage of C. septempunctata on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to second instar of C. septempunctata, the grub consumed all aphid species but preferred cabbage aphid over parasitized mustard aphid and mustard aphid over parasitized mustard aphids respectively (Fig. 5). Index showed that cabbage aphid preferred over parasitized mustard aphid (t=7.17, df=2, P=0.02) and mustard was preferred over parasitized mustard aphid (t=3.96, df=2, P=0.05).

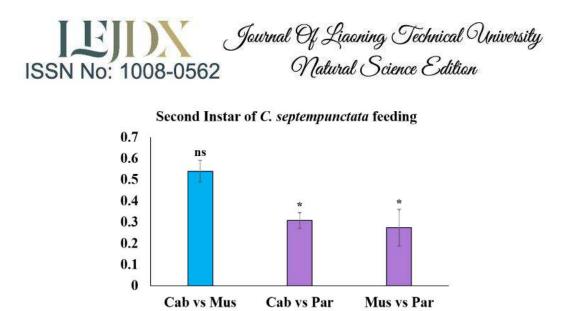
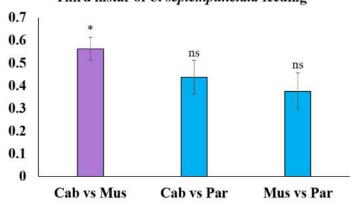


Fig. 5. Manly's preference index of 2nd stage of C. septempunctata on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to the third instar of C. septempunctata, the grub consumed all aphid species but preferred mustard aphid over cabbage aphid (Fig. 6). Index showed that mustard aphid preferred over cabbage (t=4.5, df=2, P=0.01).



Third instar of C. septempunctata feeding

Fig. 6. Manly's preference index of 3rd stage of C. septempunctata on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to fourth instar of C. septempunctata, the grub consumed all aphid species but preferred mustard aphid over



cabbage aphid (t=1.72, df=2, P=0.003) and parasitized mustard aphids (t=1.55, df=2, P=0.004) while cabbage aphid over parasitized mustard aphid (t=1.43, df=2, P=0.005) (Fig. 7).

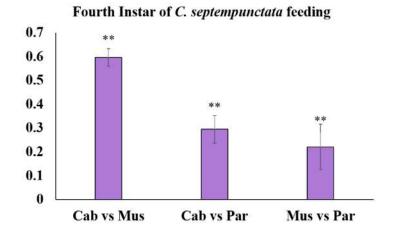


Fig. 7. Manly's preference index of 4th stage of *C. septempunctata* on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to adult males of *C. septempunctata*, the beetle consumed all aphid species but preferred mustard aphids (Fig. 8). Index showed significantly greater preference for mustard aphid over cabbage aphid (t=2.69, df=24, P=0.00) and parasitized mustard aphid (t=2.86, df=24, P=0.00) whereas, cabbage aphid preferred over parasitized mustard aphid (t=2.77, df=24, P=0.00).



Adult male of C. septempunctata feeding

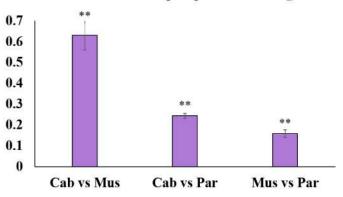


Fig. 8. Manly's preference index for adult male of *C. septempunctata* on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.

When different aphid species (i.e. cabbage vs. mustard, cabbage vs. parasitized mustard and mustard vs. parasitized mustard) were offered to adult female of *C. septempunctata*, the beetle consumed all aphid species but showed marked preference for mustard aphid over cabbage and parasitized mustard aphid (Fig. 9). Index showed significantly greater preference for mustard aphid over cabbage aphid (t=1.06, df=24, P=0.00) and parasitized mustard aphid (t=2.35, df=24, P=0.00).

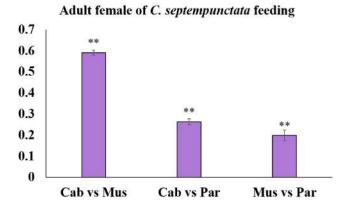


Fig. 9. Manly's preference index (aP) for adult female of *C. septempunctata* on different aphid species (abbreviated as: Cab, cabbage; Mus, mustard; Par, parasitized mustard). The bars marked with asterisks are significantly different from the predicted index at 0.5 based on t-test while those labelled 'ns' indicate non-significant or similar.



Discussion

Predation is an important biological association that plays a significant role in ecology and maintains the energy flow. Predators directly impact the prey population through consumption (Arshad *et al.*, 2015; Arshad *et al.*, 2017). Preying on insect pests by natural enemies holds an economic significance, so in determining the effects of the predatory potential of predators on host prey, it is very important to identify the quantitative prey consumption by predators (Messina and Sorenson, 2001).

For the successful incorporation of potential predators in integrated pest management programs, a complete understanding of the choice of host prey is crucial. The biology and ecology of *C. septempunctata* are widely studied among the coccinellid beetles (Omkar and Pervez, 2002). Our results suggest that *C. septempunctata* has a potential preference against parasitized aphids though they were least suitable as food prey in the presence of healthy aphid species. The quality of host prey greatly influences the overall development of *C. septempunctata* as reported by Majerus and Kearns (1989). Our findings are in partial agreement with Ali and Rizvi (2007); Sharma and Joshi (2010); Singh and Singh (2013) who reported that *C. septempunctata* showed more preference for *L. erysimi* as host prey than other aphid species. Dixon (2000) determined that the differences in predation rate might be attributed to the recognition of the most suitable prey morphologically. However, Omkar *et al.* (2004) reported differences in prey consumption rates solely due to physiological and chemical variations.

Results revealed that consumption rate increased with successive developmental stages of *C. septempunctata* while adults consumed more than larval stages (adult female= $177.36 > adult male= 150 > 4^{th} instar= 42.7 > 3^{rd} instar= 24.95 > 2^{nd} instar= 12.55 > 1^{st} instar= 6.5$). Moreover, the different larval stages of *C. septempunctata* also significantly affect the consumption rate irrespective of the aphid species which might be attributed to the size of each larval instar and is consistent with the results reported by Ali and Rizvi (2007). Manly's preference index also projected the preference of *C. septempunctata* on different aphids. Under controlled conditions, healthy *L. erysimi* was found most suitable as a prey host for *C. septempunctata* while parasitized aphids were the least suitable prey. This work was unusual for checking the preference of *C. septempunctata* but results suggested that parasitized aphids were also consumed by predators which might be due to a shortage of preferred aphids. In this experiment, adult males and females of *C. septempunctata* consumed more aphids than



grubs. Such results are agreed with the findings of Singh *et al.* (1994); Ali and Rizvi (2007); and Ali and Rizvi (2010). Furthermore, adult females were found more voracious than adult males. The difference in aphid consumption is mainly associated with the size of each developmental stage as it increases with age. Singh and Singh (2013) reported similar results that the voracity of *C. septempunctata* increased with age and all stages preyed on aphids (Jandial and Malik, 2006; Bilashini and Singh, 2009).

Farooq *et al.* (2020) conducted a comparative study on the biological parameters of *C. septempunctata* against four different aphid species: *Rhopalosiphum padi, Rhopalosiphum maidis, Sito-bion avenae,* and *Schizaphis graminum* and found *R. padi* as highly preferred in terms of optimal developmental period and adult longevity under in vitro conditions as compared to the rest of the aphid host species. Pervez and Chandra (2018) evaluated the effect of host-plant association on prey preference of the coccinellid beetle, *Menochilus sexmaculatus* and reported that the aphid consumption rate is greatly associated with the size of predatory stages.

Arshad (2017) investigated the preference and predatory potential of *C*. *septempunctata* against four different prey species: spinach aphid, coriander aphid, cabbage aphid, and pea aphid and among the four host species, pea aphid, and cabbage aphid were found as most suitable and least suitable preys in terms of overall development and predation under no choice and free choice feeding assays. The current study findings yielded the following prey preference for *C. septempunctata*: mustard aphid > cabbage aphid > parasitized aphid.

Conclusions

It is concluded from the above study that different aphid species pose a significant effect on lifelong consumption by *C. septempunctata*. Preference of one aphid species over the others might be due to morphological features of prey that entice a predator (*C. septempunctata*). That's why the mustard aphid was highly preferred in terms of predation as compared with the rest of the aphids (cabbage aphid and parasitized aphid) under lab conditions. However, consumption of parasitized aphids also increased with the passage of time and the next stage consumed more than the previous ones while consumption by adults was significantly higher than all stages of grubs (1st, 2nd, 3rd and 4th instars). Hence, findings provide information about *C. septempunctata* feeding on parasitized aphids in the presence of



non-parasitized aphids but further clarifications of tri-trophic interaction among aphids, parasitoids and coccinellids are still needed.

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