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Effect of parasitized prey on prey consumption, survival, growth, and development of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) under laboratory conditions

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Abstract

A study of interactions among natural enemies is important to develop a better biological control program. Among these interactions, one that is crucial is the consumption of parasitized prey by a predator. Feeding on aphid mummies could affect the predator as well as the parasitoid species. In this study, the effect of parasitized prey on prey consumption, survival, growth, and development of 4th instar larvae of the predatory species, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), as an active predator of aphids, was studied. Moreover, the effect of parasitized and mummified aphids on female fecundity was also evaluated. The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) was used. Obtained results showed that the parasitized aphids, as a predatory food, significantly affected the predator. The prey consumption was highest on parasitized (24.10 ± 0.53) and lowest on mummified (9.75 ± 0.40) aphids. The survival rate was in the order of non-parasitized > parasitized > mummified. The mass gained by the 4th instar larvae of *C. septempunctata* was in the order of parasitized ($26.25 \text{ mg} \pm 0.89$) > non-parasitized ($19.25 \text{ mg} \pm 0.86$) > mummified aphids ($10.20 \text{ mg} \pm 0.52$), when fed on different prey. Developmental time of the predator was recorded highest, when it consumed mummified aphids (13.6 ± 0.39). As well, female fecundity was affected positively. The female was observed to lay the most eggs (323.55 ± 1.57) to have the highest number of hatched eggs (38.70 ± 0.92), and to have the highest egg mass ($196.10 \mu\text{g} \pm 1.22$), when it consumed the parasitized aphids in contrast to non-parasitized and mummified aphids. It was also found that the type of prey did not affect the incubation period of the eggs.

Keywords: Foraging behavior, Aphid mummies, Parasitized prey, *Coccinella septempunctata*, Intraguild predation

Background

Extensive use of pesticides adversely affects the population of biological control agents in the field and green house crops (Tooker et al. 2020). As a result, the potential control that they can provide is also restricted, which may result in the outbreaks of different pest species decreasing

the crop yield (Douglas et al. 2015). Furthermore, resistance to different pesticides has been documented in many pest species like the aphid species, *Myzus persicae* Sulz. (Hemiptera: Aphididae) (Voudouris et al. 2017). In order to reduce the pest population and enhance the environmental safety effectively, Integrated Pest Management (IPM) including biological control, is the most appropriate strategy (Jalali et al. 2009; Skouras et al. 2017).

One of the advantages of predatory insects is their ability to feed on a range of non-preferred prey species, when their preferred prey is scarce. However, the ability

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of predators to feed on multiple prey species can sometimes create an intraguild competition among different biological control agents. One of these competitions is feeding on parasitized prey, which may reduce the biological control of the target pest species. There is a very little understanding of the effects of consumption of parasitized prey on predator's fitness as well as on the overall biological control program. Thus, exploring the predatory behavior of an insect predator on parasitized prey is useful for understanding its effects on predator's growth and development.

The green peach aphid, *Myzus persicae* Sulz. is a polyphagous aphid species that can be found worldwide attacking many crops and a widespread pest of brassica crops in the world (Blackman and Eastop 2000). This aphid was found to be an important prey for many predatory species such as lacewings and the coccinellids (Hodek and Honěk 2013). One of the major problems of *M. persicae* is damaging the photo-assimilates and transmits viruses, causing heavy losses of crop yield (Cao et al. 2016).

Insect predators have relatively longer life duration than parasitoids and consume a range of insects. Hence, they are indulged in the intraguild competition (Polis and Holt 1992; Rosenheim et al. 1995). Predators have shown a reduced biological control in the presence of entomoparasitoids (Fincham et al. 2019). Predators may reduce pest control by feeding on other bioagents present in the crops (Rosenheim et al. 1993; Snyder and Wise 2001). In a predator-parasitoid interaction, the predators mostly consume specialists, with a rare chance of reverse happening (Brodeur and Rosenheim 2000; Lucas et al. 1998). Thus, generalists could potentially decrease the biological control program (Snyder and Ives 2001).

To develop a better understanding of predators vs. parasitoid interactions in a biological control program, different experimental studies have been studied (Chang and Kareiva 1999). The predatory species, *Coccinella septempunctata* L., has a significant importance worldwide as a voracious aphidophagous predator (Soni et al. 2004). Both its larvae and adults are active aphidophagous predator. Prey scarcity and poor diet could lead towards lower developmental rate and survival (Sarwar and Saqib 2010). *C. septempunctata* has shown better performance than other predators as biological control agent in an intraguild predation competition (Afza et al. 2019). However, the effect of feeding a predator on parasitized prey has rarely been investigated. Several studies concluded that the quality of the aphids significantly affects the performance of ladybird beetles, especially at larval stages. Moreover, it was found that *C. septempunctata* is highly specific to the aphids' diet (Hodek and Honěk 2013). However, the effect of aphid mummies as a predator's diet has not well studied and understood (Takizawa et al. 2000).

The present study aimed to evaluate the effects of parasitized prey on development, survival, prey preference, and fecundity of *C. septempunctata*.

Materials and methods

Plant and insect cultures

Cabbage, *Brassica oleracea* L. (Brassicaceae) plants, were used to rear the predator (*C. septempunctata*) and the prey species (*M. persicae*). The plants were grown in beds and later transplanted in pots (100 mm radius) to provide them to insect culture. The cabbage plants were raised in an insecticidal-free environment and under natural light in a wired house, watering the plants daily or every other day depending upon the weather conditions.

The aphid, *Myzus persicae*

M. persicae culture was established by collecting the aphids from a cabbage field close to the Multan Road, Bahawalpur, Punjab, Pakistan. Collected aphids were screened for any chance of being parasitized for 3 days. After the 3 days, healthy aphids were shifted into cages (2 × 2 × 2.5 ft) and reared on cabbage at the entomology laboratory, UCA&ES, the Islamia University of Bahawalpur Punjab, Pakistan. The cages were placed under the laboratory conditions at 25 ± 2°C, 55–65% R.H. and artificial lighting. Cages were kept close to the window to provide natural light when possible. Plants were replaced with new ones when necessary.

The predator, *Coccinella septempunctata*

Adults of *C. septempunctata* were obtained from a cabbage field near Multan Road Bahawalpur, Punjab, Pakistan, and were reared at the entomology laboratory, UCA&ES, the Islamia University of Bahawalpur Punjab, Pakistan. The predator was reared on *M. persicae* in a cage (2 × 2 × 2.5 ft) up to the 4th instar larvae. Water was also provided in cages with a wet cotton wick. The 4th instar larvae were used in experiments of prey consumption, survival, growth, and development, while adult females were used for the test of fecundity. A couple of adult *C. septempunctata* were selected for oviposition and kept into the separate petri dishes to get the uniform aged batches of eggs for the experiments.

Experimental design

Effects of parasitized prey on prey consumption, survival, growth, and development as well as female fecundity of *C. septempunctata* under laboratory conditions were studied. Three types of prey (healthy (non-parasitized), parasitized (1 day-old parasitized), and mummified (aphid mummies) were tested. Small plastic container (120 mm diameter, 80 mm height) with holes in the lid for proper aeration was used to conduct the experiments. Each plastic container was provided by 30 early

4th instar nymphs of either non-parasitized, parasitized, or mummies of *M. persicae* placed on a cabbage leaf. A control treatment was not used in this experiment since preliminary tests showed more than 92% survival of the predator. Twenty-four hours starved and 1-day-old 4th instar larvae of *C. septempunctata* were used for the experiments. Same day egg batch was used to obtain a uniform population of 4th instar larvae of *C. septempunctata*. Aphid mummies collected from the cabbage field were allowed to complete their development until emerging as parasitoid adults and then they were allowed to sting healthy aphid's nymphs in a cage (2 × 2 × 2.5 ft). These newly parasitized aphids and aphid mummies were used in the experiments along with healthy aphids. The predators were placed singly in a petri dish to starve for about 24 h before the experiment. Each predator was tested only once. Each experiment was tested for exactly every 24 h at artificial lighting with 14L: 10D photoperiod and 25 ± 2 °C.

Twenty replicates were performed for each experiment. Prey consumption was recorded by counting the remaining aphid and aphid mummies after each experiment. Survival of *C. septempunctata* was considered successful when larvae developed to adults. The percentage of survived predators was calculated. The development of the predator was tested by subtracting the mass of each predator at the newly developed pupal stage from the mass at the start of the 4th instar larvae. Developmental time was recorded from newly molted 4th instar larvae to the development of adults in days. For the experiment of fecundity, adult couples of males and females were kept in a petri dish separately. The healthy, parasitized, or mummified prey were provided by a cabbage leaf as a substrate for egg-laying. The numbers of eggs laid were counted daily and a new leaf was provided as well. The trial was ended when no new eggs were laid at the end of the 24-h period. Total eggs laid were counted and 50 of these eggs were kept for further recording of the incubation period. Ten eggs were selected randomly from each treatment and weighed together and then the average egg mass per female was estimated.

Data analysis

PASW Statistics 18 (release 18.0.0) was used for data analyses of different biological and reproductive parameters. The significance level was tested by one-way ANOVA at $p < 0.05$ and Duncan's multiple range test was used for the comparison of the data. The homogeneity of variances test was also performed to confirm that the assumptions for ANOVA are met in the analysis. Graphs were constructed in Microsoft Excel. The survival rate (%) of the predator was tested, using the chi-square test with $p < 0.05$.

Results and discussion

Effect of feeding *C. septempunctata* on parasitized aphids Biological parameters

Average values of the effect of feeding *C. septempunctata* on parasitized prey on different biological parameters of the *C. septempunctata* are given in Table 1. The prey consumption by *C. septempunctata* on different prey types was in the order of parasitized > non-parasitized > mummified aphids. The consumption of parasitized prey was the highest (24.10 ± 0.53) followed by non-parasitized (22.65 ± 0.50), while the mummified aphids (9.75 ± 0.40) were the least for the predator. The consumption of each prey type was significantly different ($p \leq 0.05$) from the others.

The growth (mass gained) of 4th instar larvae was analyzed by considering the mass gain from the start of the 4th larval instar to the start of the pupal stage of the predator. Growth of 4th instar larvae of *C. septempunctata* was significantly different ($p \leq 0.00$) among different prey types and was in the order of parasitized > non-parasitized > mummified aphids. The highest mass gain (mg ± S.E) was observed in the parasitized prey (26.25 ± 0.89), followed by non-parasitized (19.25 ± 0.86) and then mummified (10.20 ± 0.52) aphid prey, respectively.

Aphidophagous generalists such as *C. septempunctata*, as well as different aphid parasitoids commonly played a significant role of suppressing aphids' population in different agro-ecosystems (Bilu and Coll 2007). However, these predators and parasitoids do not necessarily interact in a synergistic manner (Gontijo et al. 2015). Many of these aphidophagous generalists can also consume aphid parasitoids (Chacon and Heimpel 2010).

Obtained results showed that the diet containing aphid mummies was not preferred for the 4th instar larvae of *C. septempunctata*. The prey consumption was greatly reduced by using aphid mummies. These findings are coherent with Takizawa et al. (2000) who concluded that the prey consumption of *C. septempunctata* was significantly lower when fed on aphid mummies. Fu et al. (2017b) reported the same results in case of the coccinellid, *Harmonia axyridis*, when fed on un-parasitized and mummified *M. persicae*.

This study showed that parasitized aphids were preferred over non-parasitized ones. This could be due to the reduced defense and increased vulnerability of the affected aphids. Similar results were demonstrated in case of *Myrmedonota xipe* Mathis & Eldredge, beetles which consumed more parasitized ants than non-parasitized ones (Mathis and Tsutsui 2016). Another study on the predatory behavior of *Callinectes sapidus*, showed an around 20% increase in prey consumption when fed with parasitized prey as compared to the healthy prey (Gehman and Byers 2017). The results are coherent with Pirzadfard et al. (2020), who concluded

Table 1 Effect of feeding on parasitized, non-parasitized, and mummified aphids on the *C. septempunctata* biological parameters (mean \pm S.E)

| Prey type | Biological parameters | | |
|------------------------|--------------------------------|-------------------------------|----------------------------------|
| | No. of prey consumed | Mass gained (mg) | Developmental time (no. of days) |
| Non-parasitized aphids | 22.65 \pm 0.50 ^{bt} | 19.25 \pm 0.86 ^b | 12.15 \pm 0.34 ^b |
| Parasitized aphids | 24.10 \pm 0.53 ^a | 26.25 \pm 0.89 ^a | 12.55 \pm 0.35 ^b |
| Aphid mummies | 9.75 \pm 0.40 ^c | 10.20 \pm 0.52 ^c | 13.6 \pm 0.39 ^a |

†Means (\pm standard error of means) followed by different letters (a, b, and c) in the same column are significantly different according to Duncan's multiply range test ($p < 0.05$)

that while *Orius albidipennis* (Reuter) actively consumed both the parasitized and non-parasitized prey, a preference was observed for the parasitized nymphs of *Bemisia tabaci* (Genn.) over healthy ones. The obtained data is also well aligned with Fu et al. (2017a), who observed a 36% rejection rates for mummified prey in contrast to 2% rejection of healthy prey by *H. axyridis*.

In terms of developmental time (days \pm S.E), non-significant difference was recorded among parasitized (12.55 \pm 0.35) and non-parasitized (12.15 \pm 0.34) aphids. However, predator's developmental time on aphid mummies (13.6 \pm 0.39) was significantly longer than other prey types. Overall a significant effect ($p \leq 0.00$) of prey type was observed on developmental time of 4th instar larvae of *C. septempunctata*.

Many studies concluded that the nutritional deficiency of low-quality aphids (like aphid mummies) could not be replenished by mixing a good quality diet with poor diet (Nielsen et al. 2002). This study also demonstrated that overall aphid mummies were a low-quality diet and longer developmental time was observed on mummified aphid diet. Yu et al. (2020) reported that the larval

developmental time of *H. axyridis* was significantly prolonged when fed with mummified prey.

Obtained results showed that the survival of *C. septempunctata* was significantly higher when fed on non-parasitized aphids, followed by parasitized aphids, while feeding on aphid mummies greatly reduced its survival (Fig. 1). Chi-square (χ^2) test showed that the survival rate (%) of 4th instar larvae of *C. septempunctata* was significantly affected ($p \leq 0.037$) by the prey type. The present study also showed that the survival rate of *C. septempunctata* decreased when it fed on parasitized and mummified aphid. In a similar study conducted on *C. septempunctata* but different aphid species, the survival rate of the predator was significantly affected by the diet containing aphid mummies (Takizawa et al. 2000). Agarwala and Dixon (1992) also concluded that the low-quality prey (like aphid mummies) could lead to a poor larval performance and even death before reaching the adult stage. Sugiura and Takada (1998) reported similar results and showed that poor prey quality could lead to a lower survival rate, growth, and development of other coccinellids. Obtained results are in coherent

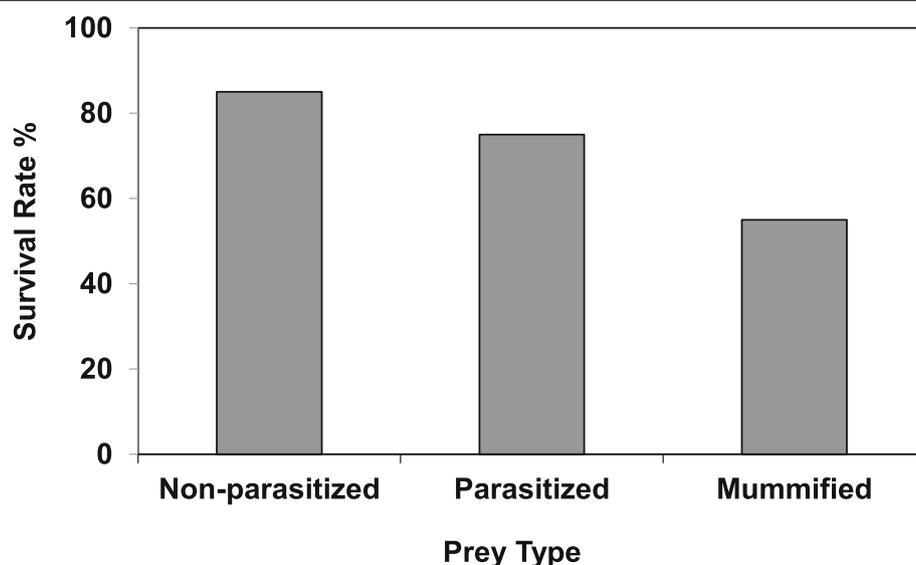


Fig. 1 Survival rate (%) of 4th instar larvae of *C. septempunctata* on non-parasitized ($n = 20$), parasitized ($n = 20$), and mummified ($n = 20$) aphids. X-axis represents type of the prey offered, while Y-axis shows the survival rate (%) of 4th instar larvae of *C. septempunctata* (χ^2 test, $p \leq 0.037$)

Table 2 Effect of feeding on parasitized, non-parasitized, and mummified aphids on the *C. septempunctata* reproductive parameters (mean \pm S.E)

| Prey type | Reproductive parameters | | | |
|------------------------|---------------------------------|-------------------------------|-----------------------------------|--------------------------------|
| | No. of eggs laid | No. of eggs hatched | Incubation duration (no. of days) | Egg mass (μ g) |
| Non-parasitized aphids | 295.90 \pm 1.61 ^{ct} | 24.85 \pm 0.61 ^c | 3.00 \pm 0.10 ^a | 187.05 \pm 1.26 ^b |
| Parasitized aphids | 323.55 \pm 1.57 ^a | 38.70 \pm 0.92 ^a | 3.20 \pm 0.09 ^a | 196.10 \pm 1.22 ^a |
| Aphid mummies | 314.85 \pm 1.04 ^b | 31.10 \pm 0.54 ^b | 3.35 \pm 0.10 ^a | 195.60 \pm 1.03 ^a |

†Means (\pm standard error of means) followed by different letters (a, b, and c) in the same column are significantly different according to Duncan's multiply range test ($p < 0.05$)

with Fu et al. (2017b) who concluded that by feeding on aphid mummies, the survival rate and development of coccinellids were significantly affected. Similarly, in another study, 13% decrease in the predator survival rate was observed, when fed on infected prey (Flick et al. 2016). In contrast, 1st and 3rd instars of the lacewing, *Chrysoperla rufilabris* (Burr.) showed non-significant difference in its survival, when fed on healthy vs. parasitized prey (Jessie et al. 2019).

Reproductive parameters

Experiments on the fecundity of *C. septempunctata* females were carried to find out the total number of eggs laid by the female, the number of hatched eggs, the incubation period of eggs, and egg mass when the predator was fed either on non-parasitized, parasitized, or mummified aphids (Table 2). The total number of eggs laid by *C. septempunctata* on different prey types was in the order of parasitized > mummified > non-parasitized aphids. The number of eggs laid by feeding on parasitized aphids was the highest (323.55 \pm 1.57), followed by mummified (314.85 \pm 1.04) and then non-parasitized (295.90 \pm 1.61) aphids, respectively. Overall, a significant difference ($p \leq 0.05$) was observed for the total number of eggs laid by feeding on different prey types.

Similar results were obtained for egg hatching by feeding different prey. Most of the eggs were hatched, when female *C. septempunctata* was fed on parasitized aphids (38.70 \pm 0.92), followed by mummified (31.10 \pm 0.54) and non-parasitized (24.85 \pm 0.61) aphids, respectively. However, a non-significant effect ($p > 0.05$) of prey type was recorded for hatching period of the selected eggs. Regarding the egg mass, a significant effect ($p \leq 0.05$) was observed among different prey types. The highest egg mass (μ g \pm S.E) was found, when female *C. septempunctata* was fed on parasitized prey (196.10 \pm 1.22), followed by aphid mummies (195.60 \pm 1.03) and non-parasitized (187.05 \pm 1.26) aphids.

Obtained results demonstrated that aphid mummies were more suitable for egg laying and development of eggs into adult aphids. Omkar and Srivastava (2003) concluded that female fecundity was directly affected by its diet. Similarly, Sugiura and Takada (1998) suggested that the good prey quality enhanced reproductive

potential and fecundity of coccinellids. The mummified aphids were not a good diet for the developing predator, but the fecundity of adult females was enhanced by feeding on aphid mummies. This could be due to the higher nutrition present in the aphid mummies as they contain developed pupae of the parasitoids inside. In another study, conducted on egg development of the coccinellid, *Hippodamia variegata*, it was concluded that the egg mass and egg size and no. of hatching larvae significantly increased, when fed on parasitized in contrast to non-parasitized prey (Toosi et al. 2019).

Conclusion

The present study evidenced that aphid mummies are not a preferred diet for *C. septempunctata*. Mummified aphid diet greatly reduced the survival and development rate of the 4th instar larvae of the predator. In the context of biological control, the less preference of aphid mummies by the predator favored the combined use of this biological control agent with parasitoids. However, further clarifications of interaction between the coccinellids and other natural enemies are still needed.

Abbreviations

R.H.: Relative humidity; UCA&ES: University College of Agriculture and Environmental Sciences; L: D: Light period (daylight): dark period (night); ANOVA: Analysis of variance; S.E: Standard error of mean

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Authors' contributions

AC carried out the experiments on the predatory behavior of *C. septempunctata* and analyzed the data. SN reared all plants and insects' culture, designed the study, and contributed in the write-up and proofreading of the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

Do not wish to share. The data is not very much organized to be presented here.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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