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# Effects of intraguild predation on the life history traits and progeny of the ladybird beetle *Hippodamia variegata*

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## Abstract

The simultaneous use of multiple biological control agents requires sufficient information of intraguild predation (IGP). In this work, the prey preferences of immature stages of the ladybird beetle *Hippodamia variegata* (Goeze) (Coleoptera Coccinellidae) for non-parasitized and parasitized adults of *Aphis gossypii* Glover (Hemiptera Aphididae) by the parasitoid wasp *Lysiphlebus fabarum* (Marshall) (Hymenoptera Braconidae) were investigated at the laboratory choice experiments. Moreover, the effects of these different feeding regimes of the immature stages were determined for the life history traits of emerging *H. variegata* and its progeny. Results revealed that the second and third instar larvae of *H. variegata* revealed no preference for the non-parasitized and parasitized aphids. The immature developmental time of *H. variegata* fed on the parasitized aphids was significantly shorter than those fed on the non-parasitized aphids; however, the weight of the emerged adults did not differ significantly. Contrary to this, in the next generation, the egg and hatched larval size of *H. variegata* fed on the parasitized aphids were significantly larger than those fed on the non-parasitized aphids, whereas the mean and total number of eggs laid by *H. variegata* fed on the parasitized aphids was significantly smaller than for those fed on the non-parasitized aphids. The feeding regimes at maternal rearing conditions revealed a significant effect on the progeny, as the weight of the emerging male and female adults increased. Combined with the simultaneous application of the two biological control agents, the negative effects resulting from feeding on the IG prey, *L. fabarum*, may be compensated by the fitness gained by the IG predator, *H. variegata*, and its progeny. Hence, the asymmetric IGP may not have a negative impact on the biological control of *A. gossypii*.

**Key words:** Aphidiinae, *Aphis gossypii*, *Lysiphlebus fabarum*, maternal rearing condition, prey preference.

## Introduction

Parasitoids and predators compete with each other for similar and limited food resources during a prey-predator interaction (Holt and Polis, 1997). This competition can occur in a food web where parasitoids or predators as members of the guild, prey upon each other within the framework of intraguild predation (IGP) (Polis *et al*., 1989; Devee *et al*., 2018). IGP between predators and parasitoids is generally asymmetrical (Meyhofer and Klug, 2002; Gkounti *et al*., 2014), particularly in the parasitoids that grow inside hosts and are more vulnerable to predation (Brodeur and Rosenheim, 2000). However, several laboratory studies indicate that the feeding preference from parasitized and non-parasitized hosts varies depending on the predator species. Certain predators have no preference for non-parasitized and parasitized hosts (Duran Prieto *et al*., 2018); some exclusively feed on the non-parasitized hosts (Rosenheim *et al*., 1995), or reveal a preference for these hosts (Sunderland *et al*., 1997); while few selectively feed only on parasitized hosts (Fritz, 1982).

Quality of prey is an intrinsic property determined by the quantitative (e.g., body size) and qualitative (e.g., physiological status) differences of a prey (Mackauer *et al*., 1996). The quality of a prey, particularly that of aphids can be affected by many factors like host species (Harvey and Vet, 1997; Sampaio *et al*., 2008), age and growth stage of the host (Sequeira and Mackauer, 1992), the microorganisms in the aphids (Schmid *et al*., 2012), to be parasitized or not (Fu *et al*., 2017), and environmental conditions (Li and Mills, 2004; Xu *et al*., 2008). Studies by Rotem *et al*. (2003) reported that changes in the host quality caused by parasitism could affect the intraguild (IG) predator and its progeny. Likewise, in aphidophagous ladybird beetles, changes in host quality by parasitism can affect the biological and reproductive characteristics of the predator (Blackman, 1967; Hodek and Honek, 1996; Sugiura and Takada, 1998; Cabral *et al*., 2006). Takizawa *et al*. (2000) reported that the survival rate of the fourth instar larvae of *Coccinella septempunctata* L. was reduced by feeding on mummies of *Aphis craccivora* Koch, parasitized by *Aphidius colemani* Viereck (Braconidae). It has also been reported that IG predators usually change their reproductive allocation based on the nutrients present in the environment or in response to the host traits, and accordingly, adjust the egg size and number of eggs laid (Fox *et al*., 1997; Rotem *et al*., 2003; González-Teuber *et al*., 2008). This can have a significant effect on the progeny fitness and life traits (González-Teuber *et al*., 2008; Livnat *et al*., 2005).

Ladybird beetles and aphidiinae wasps frequently prey on common aphids (Hagen, 1986; Majerus, 1994). However, ladybird beetles can also feed on the parasitized aphids (Colfer and Rosenheim, 2001; Meyling *et al*., 2004; Roger *et al*., 2001), and cause an asymmetric IGP (Meyhofer and Klug, 2002). Nevertheless, whether the parasitized aphids can be a proper source of food for the ladybird beetle as compared to the non-parasitized aphids remains unclear, as the answer to this question may seem difficult due to the differences in food intake according to the quality of the prey (Giles *et al*., 2002).

The cotton aphid, *Aphis gossypii* Glover (Hemiptera Aphididae), is considered as one of the most important pests of the cucumber plants in the tropical and subtropical regions (Leclant and Deguine, 1994). *A. gossypii* usually causes significant damage to the plants, which may be amplified as a result of improper application of the pesticides and emergence of the pesticide-resistant populations. One of the best strategies for protecting the cucumber plants against this aphid is the use of biological control agents, particularly the simultaneous use of two or more agents (Desneux *et al*., 2007). *Hippodamia variegata* (Goeze) (Coleoptera Coccinellidae) has been widely used for the biological control of sucking pests in the Palearctic and Nearctic regions (Obrycki and Orr, 1990). This ladybird beetle is crucial in reducing the populations of 12 aphid and Psylla species in Australia (Franzmann, 2002), and also the citrus mealy bug, namely, *Parlatoria blanchardi* (Targioni Tozzetti), *Pseudococcus citri* (Risso) and *Phenacoccus aceris* (Signoret), in Northern Iran (Vojdani, 1964; Sadeghi, 1991).

*Lysiphlebus fabarum* (Marshall) (Hymenoptera Braconidae Aphidiinae) is a koinobiont endoparasitoid (Nuessly *et al*., 2004) with a wide range of aphid hosts (Carver, 1984), including more than 100 different aphid species (Yu *et al*., 2013). It prefers the hosts that belong to genus *Aphis*. Based on its reproduction, *L. fabarum* has two strains i.e. arrhenotokous (sexual) and thelytokous (asexual) (Belshaw *et al*., 1999). This parasitoid wasp reproduces mainly through thelytokous in central Europe (Nemec and Stary, 1985; Kavallieratos *et al*., 2008). Both asexual (Rasekh *et al*., 2011) and sexual strains (Rakhshani *et al*., 2013) have been reported in Iran.

In the present study, the preference of the second and third instar larvae of the IG predator, *H. variegata*, was examined for non-parasitized and parasitized aphids by *L. fabarum*. Thereafter, the effect of feeding of the immature stages of *H. variegata* on parasitized *A. gossypii*, as compared to the non-parasitized aphids, was inspected for the fitness components (i.e., developmental time of immature stages, adult weight, fecundity, and egg area) of the emerged adult ladybird beetle and its progeny. We hypothesized that feeding the immature stages of *H. variegata* on non-parasitized and parasitized aphids may affect the biological characteristics of the emerged adults and their progenies (i.e., adult weight, body length, and developmental time of immature stages). The specific research questions in this study are: (i) Do immature larvae of *H. variegata* reveal a preference for non-parasitized and parasitized aphids? (ii) Do these different feeding regimes affect the fitness components of the emerged adults and fitness gain in the progenies?

## Materials and methods

### Insect colonies

The initial population of *A. gossypii* was obtained from the cucumber fields at Ahvaz County (31°18'14.82"N 48°39'40.16"E). A stock colony of *A. gossypii* was established on the cucumber plants (*Cucumis sativus* cv. Super $N_3F_1$), in a ventilated cage (60 × 60 × 120 cm), under laboratory condition at 22 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod. Cucumber seeds were planted in 2 L plastic pots filled with sawdust and soil at a ratio of 2:3.

In the preliminary experiments, we observed the high predation and parasitism rates by *H. variegata* and *L. fabarum*, respectively, on the cotton aphid. This study is part of a larger project to use *H. variegata* and *L. fabarum* (mass reared on broad bean, *Vicia faba* L., infested with *A. fabae*) as biological agents to control *A. gossypii* on cucumber. The initial population of *A. fabae* was obtained from broad bean fields located at Ahvaz County, Khuzestan province. Adults of sexual strains of *L. fabarum* were obtained from the mummies of black bean aphid, collected from bean fields. The stock colony of *L. fabarum* was established on potted broad bean plants, grown in pots of fertilized sawdust (N:P:K = 20:20:20) infested with *A. fabae*. Arrhenotokous reproduction was confirmed in this strain by sexing the progeny produced by virgin females. The initial population of *H. variegata* was obtained from the alfalfa fields at Ahvaz County. A stock colony of this ladybird beetle was established on *A. fabae* in ventilated plastic boxes (20 × 15 × 12 cm) under a laboratory condition at 25 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod.

### Production of synchronous cohorts

In order to produce synchronous cohorts of *L. fabarum*, 2-days-old mated females, without prior exposure to the aphids, were introduced to an aphid cohort of *Aphis fabae* Scopoli in a 1:5 ratio (one wasp for each five aphids) in a ventilated plastic cylinder (8.0 cm diameter × 15.0 cm height). After 24 hours, the wasps were removed and the parasitized aphids were reared on potted bean seedlings until mummies formed. These mummies were maintained until emergence of wasps. The emerged wasps were regularly provided with droplets of honey (diluted 30% in distilled water) on a strip of wax paper and water on a cotton ball. These wasps were used to parasitize the *A. gossypii*. The synchronous cohorts of *L. fabarum* were introduced into a ventilated cage (40 × 40 × 80 cm) containing four potted cucumbers, infested with different nymphal stages of cotton aphid. The parasitoid wasps were removed after 24 hours and nymphs left in situ to develop. After 3 days, the parasitized aphids (bearing the first or second instar wasp larvae) were used in the experiments. In these cages, the nymphs born during the second and third days were not parasitized because of removal of the parasitoid wasps after 24 hours. Accordingly, the aphids of these pots were visited daily to remove these newly born nymphs. In order to provide food for ladybird beetles of control treatment, non-parasitized cotton aphids were also produced. The above process to produce non-parasitized and parasitized aphids was performed daily.

In order to produce synchronous cohorts of ladybird *H. variegata*, the eggs laid by the 5-days-old mated females, were collected. These ladybird beetles were being fed by cohorts of non-parasitized adult *A. fabae*. After hatching, the larvae were reared on cohorts of black bean aphid. The progenies of the emerged ladybirds were used in the experiments as synchronous cohorts. Production of all above synchronous cohorts was performed under a laboratory condition at 25 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod.

### Prey preference of *H. variegata* larvae between non-parasitized and parasitized aphids

In order to determine the prey preference, the second instar larvae of *H. variegata* (n = 20) were each provided a choice of 10 parasitized and 10 non-parasitized *A. gossypii*, on a cucumber leaf in a Petri dish (9.0 cm diameter) for a period of 2 hours. Prior to testing, the larvae had been deprived of access to food for 8 hours in order to standardize the level of hunger. The host aphids were labeled by snipping one antenna at its base with fine scissors (control aphids were snipped in half the replicates, and treated aphids in the other half). The aim of using antennectomy as a tool was to mark parasitized vs. non-parasitized *A. gossypii*, in the choice test. An experiment, conducted separately with both the second and third instar larvae, showed that amputation of an aphid antenna did not affect its acceptability as a host for *H. variegata* (Toosi *et al*., 2016). After 2 hours of foraging, larvae of the ladybird beetle were removed, and the number of aphids eaten per treatment was recorded. The aforementioned experiment was also repeated for the third instar larvae of *H. variegate*.

### Development of *H. variegata* under different feeding regimes

This experiment was performed to determine whether feeding the immature stages of *H. variegata* on non-parasitized and parasitized aphids affect the biological characteristics of the emerged adults and their progenies. For this purpose, *H. variegata* larvae (n = 47) from the first instar were randomly chosen from the stocks and each reared on the parasitized *A. gossypii*, on a cucumber leaf in a Petri dish (9.0 cm diameter). The control larvae (n = 47) were fed on non-parasitized aphids. Following, the larval and pupal developmental time were determined. The fresh weights of the emerged male and female adults (one day after the emergence) were measured by a scale with 0.0001 g precision. Thereafter, a pair of ladybird beetles emerging from the same treatments (parasitized aphids fed: n = 14; non-parasitized aphids fed: n = 16) was each coupled and introduced into a Petri dish (9.0 cm diameter). The ladybird beetles of both treatments were fed on non-parasitized *A. gossypii*, until the end of the laying period. In both treatments, with onset of oviposition, the number and area of eggs laid on the 3rd, 6th, 9th, and 12th days were obtained for each adult female. In order to measure the egg areas in each replicate, four eggs selected randomly, were each photographed under a stereomicroscope equipped with a digital camera (Nikon Coolpix S10; Nikon Corporation, Tokyo, Japan) attached to a binocular microscope at 100 × magnification. All photographs were measured using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland) with a precision of 0.003 mm, and the average egg area of four eggs was determined for each adult female. Subsequently, head width of newly emerged first instar larvae (as above mentioned method), and developmental time of larval and pupal stages was obtained. Finally, the weights of male and female adults, one day after the emergence, were determined in both treatments. All experiments were performed in a growth chamber at 25 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod.

### Statistical analysis

Kolmogorov-Smirnov and Levene tests were used to determine the normal distribution of the all data and homogeneity of variance, respectively. The Manly's $\beta$ preference index was used to test for differences in numbers of non-parasitized or parasitized aphids eaten (Manly, 1974).

$$\beta = \frac{\log \bar{P}_i}{\sum_{j=1}^{m} \log \bar{P}_j}$$

where $\beta$ = Manly's $\beta$ for the two types of prey (non-parasitized or parasitized aphids); $P_i$ = proportion of prey remaining at the end of the experiment relative to the number at the beginning ($i$ = 1, 2, 3, 4,…, m); $P_j$ = proportion of all types of prey remaining at the end of the experiment relative to the number at the beginning ($j$ = 1, 2, 3, 4, …, $m$); and $m$ = number of different kinds of prey, Manly's $\beta$ can vary between zero and unity. For two prey, non-parasitized or parasitized aphids of *A. gossypii* ($m$ = 2), a value of 0.5 represents no preference, a value larger than 0.5 indicates a preference for a prey and smaller than 0.5 indicates a preference for another prey. This method takes into account the depletion in prey density due to predation during the experiment (Manly *et al*., 1972; Sherratt and Harvey, 1993). Data from prey preferences of ladybird larvae were analysed using a one sample t-test (Alaee and Allahyari, 2013).

Data about biological data (fecundity, egg area, head width of the hatched larvae) were analysed using two-way ANOVA for the effects of 'different feeding regimes' and 'the day of oviposition' as independent fixed factors. Data from parents including developmental time and adult weights were analysed independent student's t-test (SPSS, 1998). Sex ratio data was analysed using a generalized linear model (GLM) with a binomial error distribution (Crawley, 1993).

## Results

### Prey preference of *H. variegata* larvae between non-parasitized and parasitized aphids

The second instar larvae of *H. variegata* showed no significant preference ($t_{1,18}$ = 0.13, $P$ = 0.89) for non-parasitized aphids or (1.1 ± 0.15) parasitized aphids (1.0 ± 0.16) (Manly's preference index: 0.49 ± 0.07 vs. 0.51 ± 0.07, respectively) (figure 1). The same was also true for the third instar larvae ($t_{1,18}$ = 0.58, $P$ = 0.56) feeding on the parasitized (3.4 ± 0.23) and non-parasitized aphids (3.2 ± 0.27) (Manly's preference index: 0.52 ± 0.03 vs. 0.48 ± 0.03, respectively) (figure 1).

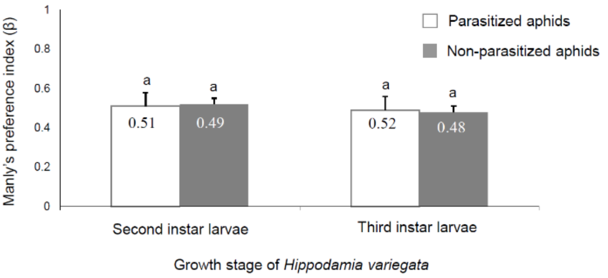

**Figure 1.** Mean (± SE) Manly's preference index ($\beta$) of second and third instar larvae of *H. variegata* when had access to 10 non-parasitized or 10 parasitized cotton aphids, *A. gossypii* for 2 hours. Values bearing the same letter in each growth stage were not significantly different (independent Sample *t*- test: $P > 0.05$).

## Development of *H. variegata* under different feeding regimes

The developmental times of male and female *H. variegata* larvae, pupae, and whole immature period fed on the parasitized aphids were significantly shorter than those fed on the non-parasitized aphids (table 1), whereas no significant difference was observed between the fresh weights of male (4.65 ± 0.17 vs. 4.64 ± 0.14, $\times 10^{-3}$ grams, respectively) and female (5.2 ± 0.22 vs. 4.64 ± 0.14) *H. variegata* reared on different feeding regimes (non-parasitized and parasitized aphids, respectively).

In the total of four days, the mean number of eggs laid by the female ladybirds feeding on the non-parasitized aphids was greater compared to those feeding on parasitized aphids ($t_{1,144}$ =9.49, $P$ = 0.003), and also on the 3rd ($t_{1,26}$ = 4.31, $P$ = 0.04), 9th ($t_{1,28}$ = 5.23, $P$ = 0.03), and 12th ($t_{1,27}$ = 4.55, $P$ = 0.04) oviposition days (figure 2).

Significant effects of age were observed on the fecundity in both treated ($F_{3,50}$ = 6.41, $P$ = 0.001) and control ($F_{3,58}$ = 3.317, $P$ = 0.026) *H. variegata* females (figure 2). A significant reduction was observed in number of eggs of females feeding on parasitized aphids, with increasing age (3rd vs. 12th oviposition day: $t_{1,25}$ = 4.32, $P$ = 0.04; 6th vs. 12th oviposition day: $t_{1,25}$ = 10.54, $P$ = 0.001).

In the total of four days, the area of eggs laid by females of the two treatments showed a significant difference ($t_{1,386}$ = 4.76, $P$ = 0.03), so that the area of the eggs laid by females feeding on parasitized aphids (0.31 ± 0.0002 mm$^2$) was greater than that of those parents fed on non-parasitized aphids (0.30 ± 0.0002) (figure 2). No effect of oviposition day was observed on the area of eggs laid by *H. variegata*, reared on the non-parasitized ($F_{3,224}$ = 1.92, $P$ = 0.127) and parasitized aphids ($F_{3,156}$ = 0.719, $P$ = 0.542) (figure 2).

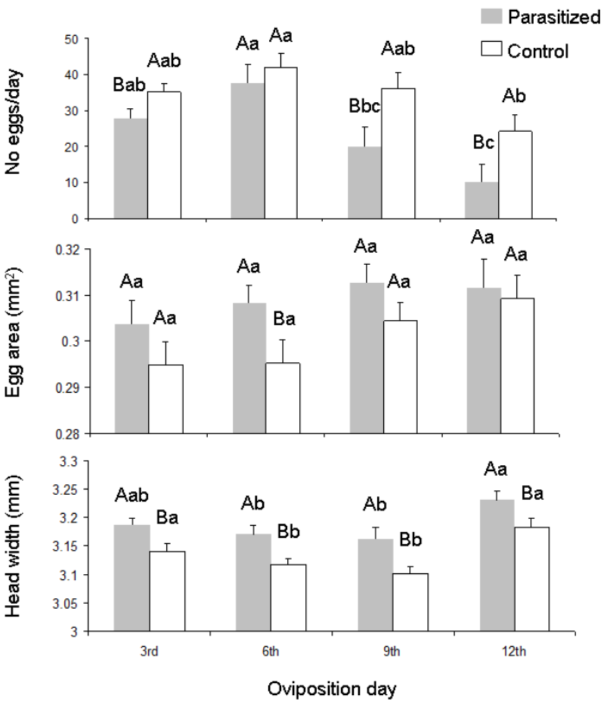

**Figure 2.** Mean (± SE) daily fecundity, egg area, and head width of the hatched larvae of *H. variegata*, feeding on the parasitized (72 hours before the experiment) or non-parasitized aphids (control), on their 3rd, 6th, 9th, and 12th days of oviposition. Means within the same day of oviposition between treatments with different feeding regimes bearing the same upper case letter (Independent Sample *t*- test), and means within a feeding regime between different days of oviposition bearing the same lower case letter (One-way ANOVA, followed by Tukey) were not significantly different (both $P > 0.05$).

The head width of the first instar larvae from the parents who fed on parasitized aphids was greater than that for the progeny of parents who fed on non-parasitized aphids on the 3rd ($t_{1,101}$ = 5.53, $P$ = 0.02), the 6th ($t_{1,94}$ = 8.05, $P$ = 0.006), the 9th ($t_{1,60}$ = 7.42, $P$ = 0.008), and the 12th ($t_{1,40}$ = 4.66, $P$ = 0.037) days (figure 2). The

**Table 1.** Mean (± SE) developmental time (hours) of *H. variegata* males and females, feeding on the parasitized (72 hours before the experiment) or non-parasitized aphids (control). Values bearing the different letter in each row were significantly different (independent Sample *t*- test: $P < 0.05$).

| | | Treated | Control | *t* | *df* | *P* |
|---|---|---|---|---|---|---|
| Larval development | female | 142.6 ± 0.16 b | 147.8 ± 0.32 a | 170.7 | 1, 33 | <0.001 |
| | male | 142.7 ± 0.13 b | 147.7 ± 0.15 a | 592.8 | 1, 57 | <0.001 |
| Pupal development | female | 72.2 ± 0.10 b | 77.1 ± 0.11 a | 905.2 | 1, 33 | <0.001 |
| | male | 72.1 ± 0.05 b | 76.9 ± 0.08 a | 2598.7 | 1, 57 | <0.001 |
| Immature development | female | 214.8 ± 0.17 b | 224.9 ± 0.32 a | 608.5 | 1, 33 | <0.001 |
| | male | 214.8 ± 0.16 b | 224.6 ± 0.20 a | 1447.2 | 1, 57 | <0.001 |

**Table 2.** Mean (± SE) developmental time (hours) of *H. variegata* progenies reared on non-parasitized aphids, whose parents were being fed on the parasitized (72 hours before the experiment) or non-parasitized aphids (control). Values bearing the same letter in each row were not significantly different (independent Sample *t*- test: $P > 0.05$).

| | | Treated | Control | *t* | *df* | *P* |
|---|---|---|---|---|---|---|
| Embryo development | female | 57.1 ± 0.15 a | 56.9 ± 0.14 a | 0.73 | 1, 108 | 0.39 |
| | male | 56.5 ± 0.17 a | 56.7 ± 0.09 a | 1.23 | 1, 106 | 0.26 |
| Larval development | female | 147.9 ± 0.12 a | 147.7 ± 0.11 a | 1.09 | 1, 108 | 0.29 |
| | male | 147.7 ± 0.14 a | 147.8 ± 0.10 a | 0.03 | 1, 106 | 0.86 |
| Pupal development | female | 77.7 ± 0.09 a | 77.7 ± 0.09 a | 0.009 | 1, 108 | 0.92 |
| | male | 77.8 ± 0.12 a | 77.7 ± 0.08 a | 0.85 | 1, 106 | 0.35 |
| Immature development | female | 225.6 ± 0.16 a | 225.4 ± 0.14 a | 0.57 | 1, 108 | 0.45 |
| | male | 225.6 ± 0.17 a | 225.5 ± 0.12 a | 0.21 | 1, 106 | 0.64 |

head width of the newly hatched larvae from the treated ($F_{3,124} = 3.4$, $P = 0.047$) and control ($F_{3,171} = 4.35$, $P = 0.006$) parents were positively influenced, with increasing female age (control: 6th vs. 12th day: $t_{1,30} = 4.05$, $P = 0.037$; 9th vs. 12th day: $t_{1,30} = 4.38$, $P = 0.045$; treated: 6th vs. 12th day: $t_{1,73} = 5.23$, $P = 0.027$; 9th vs. 12th day: $t_{1,64} = 6.38$, $P = 0.005$) (figure 2).

Developmental stages of the embryo, larvae, and pupae of male and female progeny were not affected by the different feeding regimes of the parents (table 2), whereas the male and female adults whose parents fed on the parasitized aphids revealed significantly heavier weight (4.9 ± 0.12 and 5.3 ± 0.13, $\times 10^{-3}$ grams, respectively) than the adults from the control parents (4.5 ± 0.08; $t_{1,94} = 5.73$, $P = 0.01$; 4.9 ± 0.12; $t_{1,86} = 4.98$, $P = 0.02$, respectively).

## Discussion

Several studies indicate that the predator preferences for feeding on parasitized or non-parasitized hosts vary and depend on various factors including species (Provost *et al.*, 2003) and growth stages of IG predator (Kutuk *et al.*, 2011), nutritional characteristics, rate of mobility, defensive behaviour, and growth stages of the IG prey (Hindayana *et al.*, 2001; Kutuk *et al.*, 2011), as well as the density of extra-guild prey (Lucas *et al.*, 1998). Brodeur and Rosenheim (2000) argued that the predator preference can also be affected by different intensities of defensive behaviour between parasitized and non-parasitized hosts. The results of this study revealed that the second and third instar larvae did not display any preference between the aforementioned two feeding regimes (figure 1). Other researchers revealed that ladybirds (*H. axyridis* and *C. septempunctata* larvae and adults) consumed non-parasitized aphids as readily as newly parasitized ones, but did not prefer consuming mummified aphids (Xue *et al.*, 2012; Fu *et al.*, 2017). However, Synder *et al.* (2004) found that *H. axyridis* adults, not larvae, on another IG prey species, did not show any preference for killing and consuming aphids when compared to mummies. So it seems that the predator preference is complicated and depends on various factors, as stated above.

The current study suggested that larval, pupal, and immature developmental time *H. variegata* larvae feeding on parasitized aphids in male and female were significantly shorter than those feeding on non-parasitized aphids. These indicated that indicate the higher quality and nutrient content of the parasitized aphids as compared to non-parasitized aphids. Faster development in aphidophagous ladybird beetles may be adaptive for resource tracking, with a lower mortality rate from food shortage (Osawa, 2002) and decreasing exposure to predators (Harvey and Strand, 2002). The developmental time of the fourth instar larvae fed on parasitized *A. craccivora* was not significantly different from that of larvae fed on the non-parasitized aphids in *Harmonia axyridis* Pallas (Takizawa *et al.*, 2000). Further, similar to the results of this study, they observed no significant effects on the weights of adults fed on parasitized aphids by *A. colemani* (Takizawa *et al.*, 2000). Additionally, Royer *et al.* (2008) reported that feeding of third and fourth instar larvae of *C. septempunctata* and *Hippodamia convergens* Guerin-Meneville on mummified aphids *Schizaphis graminum* (Rondani) showed a longer developmental time, higher mortality, and lower survival rate of larvae compared to the larvae fed on the non-parasitized aphids. Contrary to this, Fu *et al.* (2017) reported heavier weights of the adult ladybirds fed on the non-parasitized *M. persicae* than the mummified aphid (parasitized by *Aphelinus asychis* Walker), which could be related to the greater amount of carbohydrate in non-parasitized aphids compared to mummified ones.

The quality of available hosts and their nutritional suitability are crucial in the survival of larval stages of ladybird beetles (Takizawa *et al.*, 2000), as well as in the fecundity, egg size, and progeny size of the emerged adults (Riddick *et al.*, 2011; 2014; Riddick and Wu, 2012). It is reported that the oviposition capacity of *Adalia bipunctata* (L.) feeding on the eggs of the flour moth, *Ephestia kuehniella* Zeller was significantly higher than other prey species (Bonte *et al.*, 2010). Further, the extent of oviposition of *C. septempunctata* depended on the feeding regimes on different aphid species, suggesting diversity in the composition of the host's body (Michaud, 2000). In the present study, feeding on parasitized aphids as a higher quality food affected the reproductive strategy of *H. variegata*, such that adult females increased the volume of their eggs (to have the larger progenies), although the mean number of eggs laid by these females diminished. Riddick and Wu (2012) reported that the newly-born larvae of *Stethorus punctillum* (Weise) were fed with a higher quality of *Tetranychus urticae* Koch to lay larger eggs at maturity than the ladybird beetles feeding on low-quality hosts. Larger eggs usually hatch earlier than smaller eggs (Rossiter, 1991; Wallin *et al*., 1992) after which they produce a larger progeny, with higher survival rates in the early stages of life, and faster development rates than those developing from smaller eggs (Wallin *et al*., 1992; Fox, 1994; Bernardo, 1996). Furthermore, females hatching from these larger eggs attain sexual maturity earlier than females hatching from smaller eggs (Bernardo, 1996).

With ageing in *H. variegata* females, the results revealed a reduction in the number of daily laid eggs; instead the head width of the newly hatched larvae was positively enlarged (figure 2). Vargas *et al*. (2013) also showed that *H. convergens* mothers may invest more in later offspring than in earlier ones, although some opposite behaviour has also been reported (Mousseau and Dingle, 1991; Fox and Czesak, 2000).

The results of the current study revealed that maternal rearing conditions significantly affected the weight of the progeny adults in both genders emerging from the treated parents, relative to the offspring from which their parents were fed on the non-parasitized aphids. This response could protect the offspring against environmental stressors (Mousseau and Dingle, 1991; Agrawal *et al*., 1999), because along with the changes in local environmental conditions, mothers match the phenotype of their offspring to the changes through these transgenerational signals (Vargas *et al*., 2013; Najafpour *et al*., 2018). Considering the role of females in reproduction, body size is one of important direct fitness-related traits, with several life history traits, e.g., egg load and longevity, being positively linked to female size (Thornhill and Alcock, 1983; Ameri *et al*., 2014). Furthermore, other traits such as the ability to capture and subdue a host might vary with the body size. In regards to males, a direct relationship was observed between size and mating frequency (Osawa and Nishida, 1992; Osawa *et al*., 2015).

## Conclusions

Collectively, the feeding of IG predator (*H. variegata*) reduced the density of the parasitoid wasp *L. fabarum*, and in turn the efficiency of the simultaneous application of these two biological control agents. However, it seems that inappropriate effects resulting from feeding on the IG prey may be compensated by the fitness gained by the predator and its progeny. Hence, considering the species used in this study, the occurrence of IGP is not necessarily a negative phenomenon. It is suggested that in a supplementary work, the mortality of IG prey be investigated against the fitness gained by IG predator in a trade-off process.

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