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Effects of honey and flower on the longevity and parasitization of *Cotesia vestalis* Haliday (Hymenoptera: Braconidae) on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract Potential effects of honey and yellow cosmos (*Cosmos sulphureus*) on the longevity and parasitization of *Cotesia vestalis* were investigated. Results showed that when comparing diets from different honey-diluted solutions to water, 50% honey-diluted solution significantly increased longevity for both male and female parasitoid *Cotesia vestalis.* Mean longevities of male and female parasitoids were 10.16 $\pm$0.59 days and 10.8 $\pm$0.76 days, respectively. Similar results were obtained when using yellow cosmos flower (*C. sulphureus*), 50% honey-diluted solution, mixed honey and pollen, and honey-pollen to evaluate the longevity of *C. vestailis* parasitoids. Moreover, honey-pollen treatment significantly increased the parasitism efficiency of *C. vestalis* on the diamondback moth *Plutella xylostella* (L.) larvae, which gave the highest mean number of emergent parasitoids per day compared to the control. Results indicated that diets of honey and yellow cosmos (*C. sulphureus*) pollen extended longevity and benefitted the parasitization of *C. vestalis*.

Keywords: *Cotesia vestalis*, longevity, *Plutella xylostella*, pollen, yellow cosmos (*Cosmos sulphureus*)

Introduction

The diamondback moth, *Plutella xylostella* (L.) causes crop losses at over 90% (Verkerk and Wright, 1996) and has developed resistance to many insecticides. *Cotesia vestalis* (Kurdjumov) (Hymenoptera: Braconidae) has been reported to be a powerful agent and highly specific for controlling population density of *P. xylostella* (Cameron *et al*., 1997, Talekar and Yang, 1993; Kawaguchi and Tanaka, 1999; Noda *et al.*, 2000) in many countries. Nhi *et al*. (2014) indicated that *C. vestalis* showed dominant control of *P. xylostella* in cruciferous VietGAP (Vietnam Good Agriculture Practice) vegetable fields

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in Vietnam. To maintain survival and lifetime reproductive success, many adult parasitoid species feed on sugar sources which include floral nectar, extrafloral sources, and honeydew excreted by homopteran insects (Heimpel *et al.*, 1997). In agricultural systems, maintaining food sources enhances survival while also increasing host-searching activity and reproductive success of adult female parasitoids. Many flowers have been intensively studied to determine the effects of floral nectar on longevity of *Cotesia* such as *Centaurea cyannus* L., *C. jacea* L., *Origanum vulgare* L., and *Trifolium pratense* L. (Winkler *et al*., 2009), and on longevity of the *Anagrus nilaparvatae* parasitoid as *Turnera subulata* and *Cosmos sulphureus* (Sugiharti *et al*., 2018). Moreover, select plant species that provide nutritional benefits to the parasitoid play an important role when designing ecological agriculture. However, information on maintaining *C. vestalis* populations in the field by applying ecological engineering to provide plant-derived foods of pollen and nectar which greatly influence natural enemy longevity, fecundity, and behavior and positively impact on reducing *P. xylostella* (L.) numbers is limited (Mitsunaga *et al*., 2006). Here, we investigated the influence of honey-diluted solutions and flower supply on longevity and parasitization ability of *C. vestalis*, and discussed the role of food supply for effective utilization against insect pests.

Materials and methods

Insect rearing

Insects were reared and maintained at 28±1°C, 60±10% relative humidity (RH), and a photoperiod of light/dark (LD) 12:12 at the Laboratory of Plant Protection, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam. *Plutella xylostella* larvae were collected in Hoc Mon area, Ho Chi Minh City, Vietnam and reared on *Brassica integrifolia* for at least five generations before the moths were used for experiments. *Cotesia vestalis* parasitoids were established from larvae of *Plutella xylostella* collected in Hoc Mon area, maintained in a net cage 50x50x50 cm and cultured for at least three generations before the cocoons were collected and used for experiments. *Cotesia vestalis* parasitoids were identified by the morphological terminology follows Whitfield (1997), Goulet and Huber (1993) and PCR-COI based DNA barcoding sequencing analysis (Accession number LC179618, LC179620, LC179622).

Plants

Flowers of yellow cosmos (*Cosmos sulphureus*) were obtained by sowing the seed in polybags 50x50 cm in size and flowers in full bloom were used for the experiment. Pollen used for the experiment was collected from the yellow cosmos flowers by cutting the anthers.

Influence of honey-diluted solutions on longevity and parasitization ability of Cotesia vestalis

Longevity of *Cotesia vestalis*

To measure the longevity of unmated newly emerged wasps, males and females were placed in separate cages (50x25x25 cm) and allowed to feed on 1) water, 2) 10% honey solution (H10), 3) 20% honey solution (H20), 4) 30% honey solution (H30), 5) 50% honey solution (H50), and 6) 70% honey solution (H70). Parasitoids were allowed to feed on cotton saturated with honey-diluted solutions. A total of 25 males and 25 females were used for each treatment. Longevities of individual virgin males and females were recorded every 24 h until the wasps died.

Parasitization ability of *Cotesia vestalis*

One-month-old mustard leaves of *Brassica integrifolia* were placed in a net cage (50x25x25 cm). Fifty second instar larvae of *Plutella xylostella* were allowed to feed on *Brassica integrifolia* and exposed to mated female wasps for 24 h. After that, all larvae of *Plutella xylostella* were collected and rearing was continued for the emergence of wasps. Fifty new second instar larvae of *Plutella xylostella* were replaced every day and the experiment was continued until the female wasps died. A total of 25 mated females were used for each treatment. The number of emerged wasps was recorded to calculate parasitism percentage, number of emerged parasitoid wasps and sex ratio. Parasitism percentage was calculated as No. Parasitised host remains/(No. Non parasitised host remains + No. Parasitised host remains) (Russell, 1987).

Effect of honey-diluted solution and flower resources on longevity and parasitization ability of Cotesia vestalis

Longevity of *Cotesia vestalis* on honey-diluted solution and flower resources

Unmated male and female wasps were placed in separate cages (50x25x25 cm) and allowed to feed on 1) water (control), 2) yellow cosmos (*Cosmos sulphureus*), 3) 50% honey solution (H50), 4) mixed honey and pollen (weight/weight) honey-pollen (2 g for each, honey and pollen were put separately). A total of 25 males and 25 females were used for each treatment to measure their longevity. Longevities of individual virgin males and females were recorded every 24 h until the wasps died.

Parasitization ability of *Cotesia vestalis*

One-month-old mustard leaves of *Brassica integrifolia* were placed in a net cage (50x25x25 cm). Fifty second instar larvae of *Plutella xylostella* were allowed to feed on *Brassica integrifolia* and exposed to mated female wasps for 24 h. After that, all larvae of *Plutella xylostella* were collected and rearing was continued for the emergence of wasps. Fifty new second instar larvae of *Plutella xylostella* were replaced every day and the experiment was continued until the female wasps died. A total of 25 mated females were used for each treatment. The number of emerged wasps was recorded to calculate parasitism percentage, number of emerged parasitoid wasps and sex ratio. Parasitism percentage was calculated as No. Parasitised host remains/(No. Non parasitised host remains + No. Parasitised host remains) (Russell, 1987).

Carbohydrate, lipid, and protein analysis

Honey solution and pollen from the yellow cosmos flowers were sent to the Quality Assurance and Testing Center 3 (QUATEST 3), 49 Pasteur Street, Nguyen Thai Binh Ward, District 1, Ho Chi Minh City, Vietnam for analysis of the carbohydrates, lipids and proteins.

Statistical analysis

Data were analyzed using an ANOVA model (SPSS 22.0) followed by Dunnett's test. Parasitism percentage was statically scrutinized by completely randomized design one factor analysis after arcsine transformation.

Results

Effect of honey-diluted solutions on longevity of Cotesia vestalis

There were significant differences in the longevity of Cotesia vestalis at different honey-diluted solutions compared to water (male: $F=195.38$. $df=5$, $P<0.0001$; female: $F=168.45$ $df=5$, $P<0.0001$). Honey-diluted solution at 50% significantly increased longevity for both male and female parasitoid Cotesia vestalis. Mean longevities of male and female parasitoids at honey- diluted solution of 50% were 10.16 ± 0.59 days and 10.8 ± 0.76 days, respectively (Fig. 1). Longevity of fed wasps was about 5-fold that of water-fed conspecific species at honey-diluted solution of 50%.

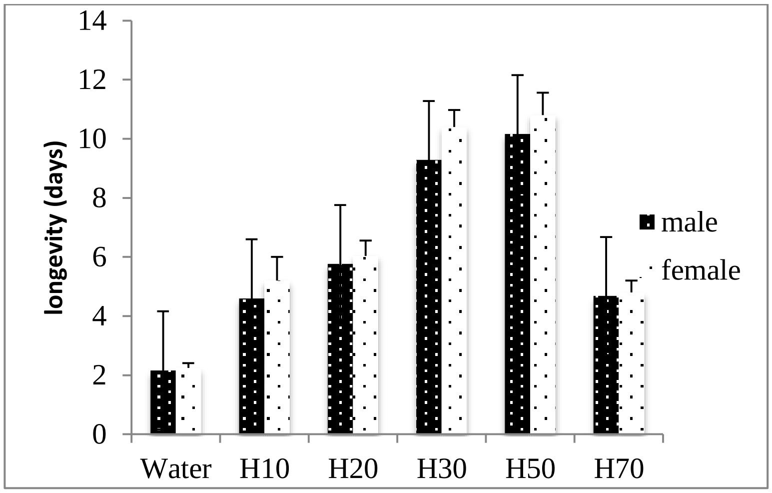

Figure 1. Longevity of *Cotesia vestalis* at different honey-diluted solutions

Table 1. Effect of honey-diluted solutions on parasitization ability of *Cotesia vestalis*

Diet	Number of successfully emerged parasitoids	Parasitization rate (%)	Female percentage (%)
Water (control)	16.8 ±1.03 f	20.99 ±1.2 d	48.36 ±6.39
H10	34.6 ±4.03 d	31.22 ±2.41c	49.04 ±6.78
H20	58.8 ±5.04 c	39.70 ±3.43 b	48.98 ±7.35
H30	73.6 ±8.39 b	50.25 ±6.85 a	49.22 ±4.44
H50	102.8 ±6.68 a	50.53 ±4.41 a	48.96 ±5.25
H70	28.2 ±3.05 e	26.53 ±3.55 c	48.56 ±5.43

The same letters within a column are not significantly different by Dunnett's test following ANOVA ($P<0.05$).

Mean numbers of emergent parasitoids were significantly affected by 50% honey-diluted solutions in comparison to water as diet, which also gave the highest mean number of emergent parasitoids per day (102.8 ± 6.68, n=50) compared to other treatments. Parasitism percentage was 50.53 ± 4.41 per female (Table 1). There was no significant difference in the offspring sex ratios of parasitoids compared to the control. We found no difference in the proportion of males/females (df=5, F=0.01, $P>0.05$) and the ratio was 1:1 for all treatments (Table 1).

Influence of honey-diluted solutions and flower resources on longevity and parasitization ability of Cotesia vestalis

Results in Fig. 2 indicated that all the treatments significantly increased longevity for both male (F=681.49, df=4, P<0.0001) and female (F=375.13 df=4, P<0.0001) Cotesia vestalis parasitoids compared to the control. Longevity of wasps increased when fed with honey-diluted solution at 50% and honey-pollen treatments.

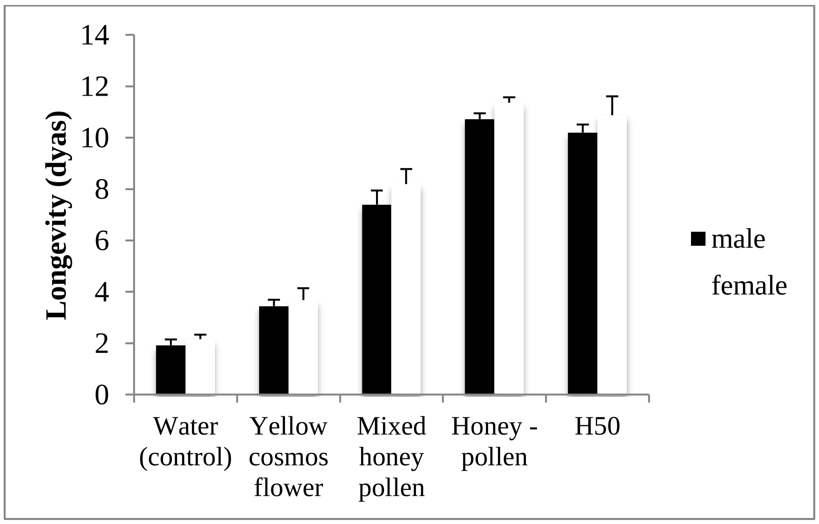

Figure 2. Effect of honey-diluted solutions and flower resources on longevity of *Cotesia vestalis*

Lifetime fecundity, as estimated by the total number of offspring of each parasitoid reaching cocoon stage and successful emergence, was significantly increased by the presence of honey or flower ($P<0.0001$). The mean fecundity with access to honey-pollen treatment was highest at 105 ± 2.62 offspring, compared with a mean of 18.2 ± 1.07 offspring produced in the control treatment. There was no significant difference in the offspring sex ratios of parasitoids compared to water as diet. We found no difference in the proportion of males/females and the ratio was 1:1 for all treatments (Table 2).

Table 2. Effect of honey-diluted solutions and flower resources on parasitization ability of Cotesia vestalis

Diet	Number of successfully emerged parasitoids	Parasitization rate (%)	Female percentage (%)
Water (control)	18.2 ±1.07 e	22.42 ±1.53 d	48.02 ±12.28
Yellow cosmos flower	35.6 ±2.79 d	30.38 ±1.94 c	48.78 ±8.10
Honey 50%	99.8 ±3.56 b	49.31 ±3.50 ab	49.12 ±5.90
Mixed honey pollen	55.7 ±5.14 c	39.49 ±3.77 b	49.25 ±8.07
Honey-pollen	105 ±2.62 a	53.72 ±3.48 a	49.60 ±4.97

The same letters within a column are not significantly different by Dunnett's test ($P<0.01$).

Discussion

Although honey is the most common diet used in the laboratory for rearing *Cotesia vestalis* (Kurdjumov) (Hymenoptera: Braconidae), the sugar content (95-99% per dry matter; FAO 1996) is assumed to be inadequate for maximum performance in a synovigenic parasitoid. Many synovigenic parasitoids gain nutrients such as proteins and vitamins by feeding on host body fluids, or by the provision of a protein supplement to an artificial diet to achieve maximum lifetime reproduction (Heimpel and Collier, 1996). Synovigenic females emerge with a fraction of their total egg complement and the eggs continue to mature (Jervis *et al*., 1986).

Table 3. Carbohydrate, lipid, and protein contents in diets for *Cotesia vestalis*

Diet	Carbohydrate (g/100g)	Lipid (g/100g)	Protein (g/100g)
Honey solutions	79.3	-	0.8
Pollen of yellow cosmos flower	23.6	7.0	11.9

In this study, carbohydrate content contained in honey and the pollen of yellow cosmos flowers was 79.3 g/100 g and 23.6 g/100g, respectively with protein at 0.8 g/100 g and 11.9 g/100 g, respectively (Table 3). This increased longevity and fecundity and produced an apparently normal sex ratio in *C. vestalis* compared with water. Many studies showed that longevity and fecundity of parasitoids increased when using honey or flowers (Mitsunaga *et al.*, 2006; Sugiharti *et al*., 2018; Winkler *et al.*, 2009) and our results concurred with these findings.

Longevity is crucial to the success of biological control (Roitberg *et al.*, 2001; Rivero and West 2002). The results of our study showed that flower-supplied wasps lived significantly longer than those fed on water. Onagbola *et al.* (2007) found a reduction in longevity of unfed wasps. Wasps lived for just two days when fed water, suggesting that resources acquired during immature development were rapidly depleted for somatic maintenance. Parasitoid sex ratio was not affected by using flowers as diets in our study although it was affected by many factors such as inbreeding, superparasitism and host size (Godfray, 1994). Therefore, provision of protein sources as supplemental food could be the key to improving mass rearing efficiency of *C. plutellae*. *Cotesia vestalis* eggs mature during their adult life and the wasp requires food by searching, indicating that availability of a good diet source is crucial to the biological success of this parasitoid.

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