



Effects of floral resources on the longevity and parasitism of *Cotesia vestalis* Haliday (Hymenoptera: Braconidae) on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Vietnam

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ABSTRACT

Parasitoids are important biological control of crop pests. In Vietnam, *Cotesia vestalis* is a native wasp species that has demonstrated its applicability as a natural insect pest enemy. Many adult parasitoids require food resources such as nectar and pollen to optimize their life cycles. Potential effects of yellow cosmos (*Cosmos sulphureus*), shrub verbena flower (*Lantana camara*), common zinnia (*Zinnia elegans*), and coriander flower (*Coriandrum sativum* L.) on the longevity and parasitism of *C. vestalis* were investigated. Results showed that nutrition resources from yellow cosmos (*C. sulphureus*) and shrub verbena flower (*Lantana camara*) significantly increased the longevity of *C. vestalis* in comparison with water ($P < 0.001$). Kaplan-Meier estimates of survival functions of female *Cotesia vestalis* exposed to different food sources indicated a significant difference in the treatments ($P < 0.01$). Male and female wasps feeding on *Cosmos sulphureus*, *Lantana camara*, and *Coriandrum sativum* survived significantly longer than wasps fed on water or *Zinnia elegans*. Parasitism efficiency of *C. vestalis* was tested on diamondback moth *Plutella xylostella* (L.) larvae for different food resources. *Cosmos sulphureus*, *Lantana camara*, *Coriandrum sativum* treatments gave the different mean number of emergence parasitoids per day compared to water treatment. Results implied that food resources from flowers benefitted the longevity of *C. vestalis*.

1. Introduction

Many adult parasitoid species feed on sugar sources which include floral nectar, extrafloral sources, and honeydew excreted by homopteran insects to maintain survival and lifetime reproductive success (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.

The diamondback moth, *Plutella xylostella* (L.) causes crop losses at over 90% (Furlong et al., 2013) and has developed resistance to many insecticides. *Plutella xylostella* is also reported to be relatively tolerant to some classes of insecticides (Verkerk and Wright, 1997; Anjum and Wright, 2017). Natural enemies play an important role in achieving a successful integrated pest management (IPM) program. *Cotesia vestalis* (Kurdjumov) (Hymenoptera: Braconidae), a native solitary endoparasitoid, has been reported to be a powerful agent for controlling

population of *P. xylostella* (Talekar and Yang, 1993; Kawaguchi and Tanaka, 1999; Noda et al., 2000) in many countries. *Cotesia vestalis* (Haliday) [= *Cotesia plutella* (Kurdjumov)] was evaluated as highly specific to effective control of the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Cameron et al., 1997). In Vietnam, *Cotesia vestalis* showed dominant control of *P. xylostella* in Cruciferous VietGAP (Vietnam Good Agriculture Practice) vegetable fields (Nhi et al., 2014). 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 *Plutella xylostella* (L.) numbers is lacking (Mitsunaga et al., 2006). Many flowers have been intensively studied to determine the effects of floral nectar on longevity of *Microctonus hyperodae* Loan including phacelia (*Phacelia tanacetifolia*), buckwheat (*Fagopyrum esculentum*), alyssum (*Lobularia maritima*), coriander (*Coriandrum sativum*), white clover (*Trifolium*

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repens), red clover (*Trifolium pratense*) and white mustard (*Sinapis alba*) (Vattala et al., 2006), on longevity of *Cotesia* as *Centaurea cyanus* 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 field margins. Here, we investigated the influence of food supply on longevity and parasitization ability of *C. vestalis* and discussed the role of food supply for effective utilization against insect pests.

2. Materials and methods

2.1. Insect rearing

Insects were reared and maintained at 28 ± 1 °C, $60 \pm 10\%$ relative humidity (RH), and a photoperiod of light/dark (LD) 16:8 at the Laboratory of Plant protection, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam. *Plutella xylostella* was collected in Hoc Mon area, Ho Chi Minh City, Vietnam and reared on *Brassica integrifolia* (about 20 days old) for at least 5 generations before the moths were used for experiments. *Cotesia vestalis* were established from larvae of *Plutella xylostella* collected in Hoc Mon area, maintained in a net cage $50 \times 50 \times 50$ cm and cultured for at least 3 generations before the cocoons were collected and used for experiments. *Cotesia vestalis* parasitoids were identified by the morphological terminology follows Whitfield (1997), Goulet and Hubert (1993) and PCR-COI based DNA barcoding sequencing analysis (Accession number LC179618, LC179620, LC179622 were deposited in NCBI, DDBJ).

2.2. Plants

Flowers of yellow cosmos (*Cosmos sulphureus*), common zinnia (*Zinnia elegans*), and coriander flower (*Coriandrum sativum* L.) were obtained by sowing the seed in polybags 50×50 cm in size. Shrub verbena flowers (*Lantana camara*) were propagated by cuttings and flowers in full bloom were used for the experiment. All plants were flowering prolifically and had florets throughout the experiment.

2.3. Experiment 1: influence of floral resources on the longevity of *Cotesia vestalis*

To measure the longevity of unmated wasps (newly emerged wasp), males and females were placed in separate cages ($50 \times 25 \times 25$ cm) and allowed to feed on 1) water, 2) yellow cosmos (*Cosmos sulphureus*), 3) shrub verbena flower (*Lantana camara*), 4) Common zinnia (*Zinnia elegans*), and 5) Coriander flower (*Coriandrum sativum* L.). 5 females were released in one cage and 5 males in a separate cage for each treatment. Each treatment was replicated 5 times. Analyses of variance (ANOVA) followed by a Dunnett's test were used to determine differences. A total of 25 males and 25 females were used for each treatment. Longevity of individual virgin males and females were recorded every 24 h until the wasps died.

2.4. Experiment 2: influence of floral resources on the parasitization ability of *Cotesia vestalis*

Each flower treatment plant and 1-month-old mustard leaf *Brassica integrifolia* were placed together in a net cage ($50 \times 25 \times 25$ cm). Fifty second instar larvae of *Plutella xylostella* were allowed to feed on *Brassica integrifolia* and exposed to 5 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 all the female wasps died. A total of 50 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. Ten replicates were conducted for each host flower plant. The significant differences between treatments with control group were determined by Dunnett's test. Parasitism percentage was calculated as No. Parasitised host remains/(No. Non parasitised host remains + No. Parasitised host remains) (Russel Derek, 1987).

3. Analysis

Data were analyzed using an ANOVA model (SPSS 22.0). Survival curves were drawn using the Kaplan-Meier estimate of the survival functions and compared between treatments by Cox's proportional hazard regression model.

4. Results

4.1. Influence of floral resources on the longevity of *Cotesia vestalis*

Yellow cosmos (*Cosmos sulphureus*) and shrub verbena flower (*Lantana camara*) significantly increased longevity for both male ($F = 22.03$ df = 4, $P < 0.0001$) and female ($F = 24.18$ df = 4, $P < 0.0001$) parasitoid *Cotesia vestalis* in comparison with the water. Mean longevity of male and female parasitoids were 3.48 ± 0.23 days and 3.78 ± 0.26 days for yellow cosmos and 3.16 ± 0.43 days and 3.68 ± 0.61 days for shrub verbena flower, respectively (Fig. 1).

Fig. 2 shows survival distribution functions using Cox's Regression Model. Results indicated a significant difference in the treatments ($P < 0.01$). Male and female wasps feeding on *Cosmos sulphureus*, *Lantana camara*, and *Coriandrum sativum* survived significantly ($P < 0.05$) longer than wasps fed on water or *Zinnia elegans*.

4.2. Influence of floral resources on the parasitization ability of *Cotesia vestalis*

There was no significant ($P > 0.05$) difference in the offspring sex ratios of emerging adults parasitoids (Fig. 3). Mean numbers of emergence parasitoids were significantly affected by yellow cosmos, shrub verbena flower, and coriander flower in comparison to water as diet ($P < 0.001$). *Cosmos sulphureus* treatment gave the highest mean number of emergence parasitoids per day (39 ± 1.37 , $n = 50$) compared to other treatments and parasitism percentage was 32.33 ± 1.55 per female (Table 1). Female *C. vestalis* continued to parasitize until death.

5. Discussion

In our study, mean male and female longevity of *Cotesia vestalis* were enhanced relative to water only. Food supplements as flowers are very important for the effective utilization of parasitoids as biological control agents. Flowers's pollen and nectar which contains sugar, protein, amino acids, and lipids, could increase the fecundity of parasitoids (Vattala et al., 2006). Many studies reported increased longevity using honey or flowers as the food sources, compared with water or no food did not significantly extend the longevity of parasitoids (Mitsunaga et al., 2006; Winkler et al., 2009; Sugiharti et al., 2018), while carbohydrates are also a nutritional factor determining the longevity of parasitoid wasps. Leatemia et al. (1995) found significantly higher lifetime fecundities with carbohydrate than with non-carbohydrate foods in *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), and *Dolichogenidea tasmanica* (Berndt and Wratten, 2005) with similar results to our study, while Vattala et al. (2006) informed that coriander increased *Microctonus hyperodae* (Hymenoptera: Braconidae) longevity. Without food ingestion, *Cotesia plutella* cannot continue to search and attack hosts (Mitsunaga et al., 2006). Of the four flower species tested, flowers of common zinnia did not extend *C. vestalis* longevity relative to water. According to Wackers (1994), parasitoids are equipped with a number of mechanisms that enable them to forage effectively for floral nectar. Certain floral odours as

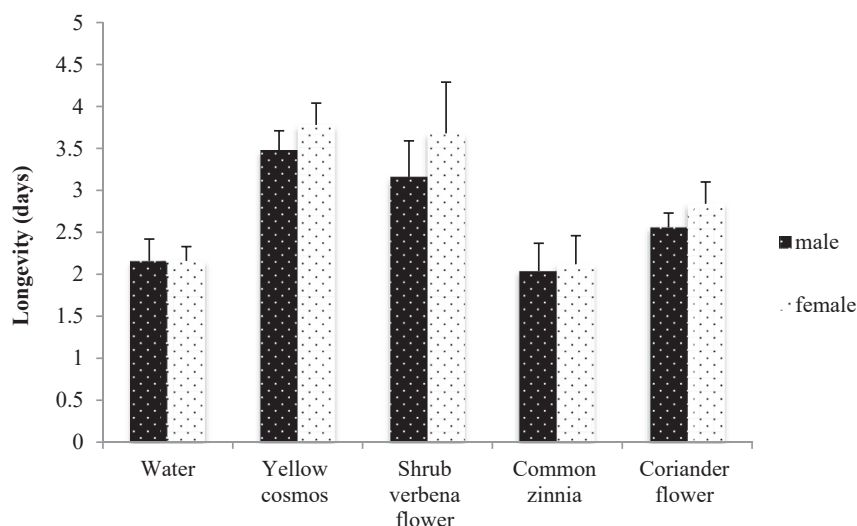


Fig. 1. Influence of flowers on the longevity of *Cotesia vestalis*. Column bar lines represent mean $\pm$ SD; solid columns are adult males, open columns are adult females.

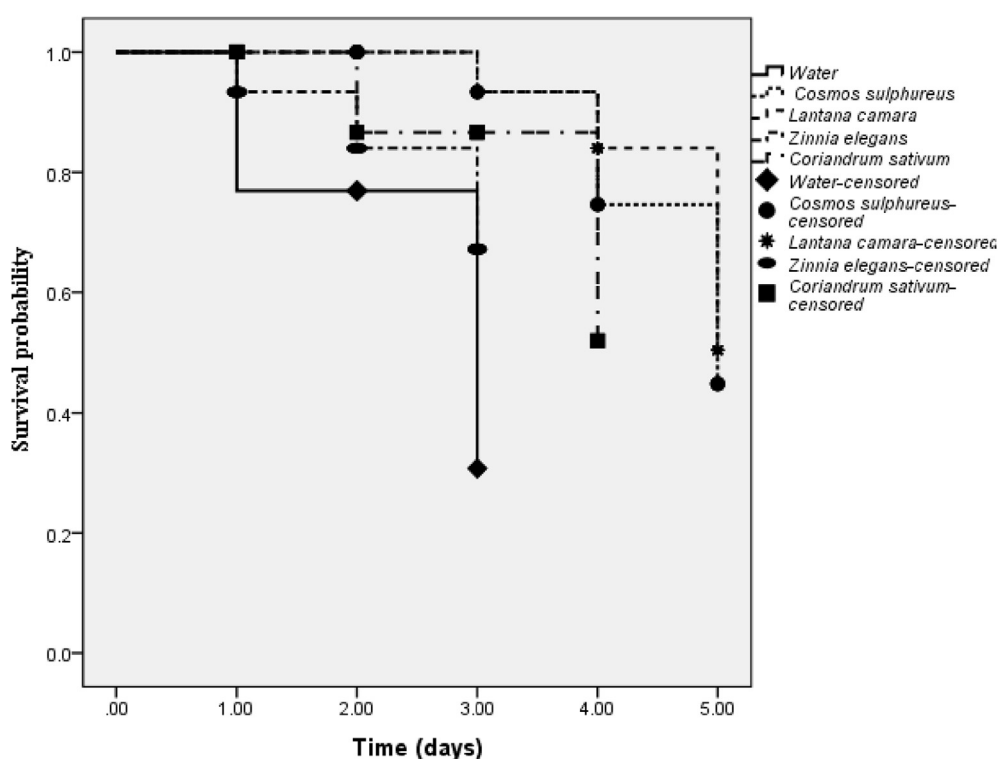


Fig. 2. Kaplan-Meier estimates of survival functions of female *Cotesia vestalis* exposed to different food sources.

well as flower colours and volatiles were attractive or deterrent to parasitoids. Some studies indicated that flower morphology affects parasitoid access to nectar, nectar quality, and utilization of floral nectar by parasitoids is highly dependent on flower and parasitoid mouthparts structure (Baker and Baker, 1983; Jervis and Kidd, 1986). Study by Charles and Paine (2016) indicated that the longevity and fecundity of Aphid parasitoid *Aphidius colemani* was markedly improved with the provision of floral resources of 6 different families, Apiaceae (*C. maculatum*), Rosaceae (*P. x freaseri*), Lamiaceae (*S. apiana*), Verbenaceae (*L. camara*), Oleaceae (*L. japonicum*), and Polygonaceae (*E. fasciculatum*); *Coriandrum sativum* L., enhance longevity of stinkbug parasitoid *Trissolcus basalis*. Yellow cosmos and shrub verbena flower greatly enhanced parasitization percentage of *C. vestalis* in our experiment. *Cosmos*

sulphureus flower significantly increased the number of offspring produced by parasitoid *Anagrus nilaparvatae* (Sugiharti et al., 2018). Longevity of *Cotesia glomerata* was slightly higher than in the control when exposed to *Centaurea cyanus*, *C. jacea* (Winkler et al., 2009). Most of braconid parasitoid has very short mouth parts so it could only access nectar from plants with shallow corollas (Russell, 2015). The Asteraceae with tubular florets between 1 and 2 mm deep (Van Rijn and Wackers, 2016) which can be explained an increase in longevity and parasitization ability of *Cotesia vestalis* in this study.

Cotesia vestalis mature eggs during their adult life and requires food for searching, indicating that availability of a good diets source is crucial to the biological success of this parasitoid. On the other hand, parasitoid sex ratio was affected by many factors such as inbreeding,

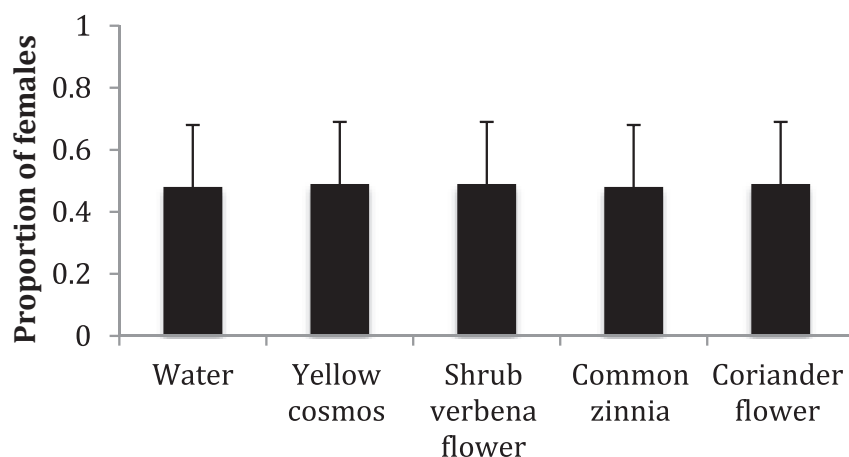


Fig. 3. Mean proportion of females in flowers and water treatments. Column bar lines represent mean $\pm$ SD (n = 50).

Table 1

Mean number of emergence parasitoids and parasitism rate in water and flower treatments.

Food	Number of emergence parasitoids	Parasitism rate (%)
Water	18.0 $\pm$ 3.71 d	22.10 $\pm$ 4.09 c
Yellow cosmos	39.0 $\pm$ 1.37 a	32.33 $\pm$ 1.55 a
Shrub verbena flower	33.6 $\pm$ 1.78 b	27.51 $\pm$ 1.30 b
Common zinnia	18.7 $\pm$ 1.65 d	23.41 $\pm$ 2.47 c
Coriander flower	26.6 $\pm$ 3.43 c	21.74 $\pm$ 2.68 c

Values are mean $\pm$ SD (n = 50). The same letters within a column are not significantly different by Dunnett's test (P < 0.05).

superparasitism and host size (Godfray, 1994) but it was not affected by using flowers as diets in our study.

This study provides evidence that yellow cosmos (*Cosmos sulphureus*), shrub verbena flower (*Lantana camara*), and Coriander flower (*Coriandrum sativum* L.) may be important nectar and pollen sources when choosing non-host food for *C. vestalis*. These three plants show potential as candidates for ecological engineering systems in Vietnam since parasitoids increase fecundity with greater longevity.

Declarations

Author contribution statement

Nguyen Ngoc Bao Chau: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nguyen Thi Phung Kieu: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Nguyen Van Tri Dung: Performed the experiments.

Nguyen Bao Quoc, Truong Kim Phuong: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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