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**Imtiaz Ali Khan**  
Department of Entomology,  
The University of Agriculture,  
Peshawar-Pakistan.

**Fang-Hao Wan**  
<sup>2</sup>State Key Laboratory for  
Biology of Plant Diseases and  
Insect Pests, Institute of Plant  
Protection (South Campus),  
Chinese Academy of Agricultural  
Sciences, 12, South Street of  
Zhong-Guan-Cun, 100081  
Beijing, PR China.

**Correspondence:**  
**Imtiaz Ali Khan**  
Department of Entomology,  
The University of Agriculture,  
Peshawar-Pakistan.

## Prey consumption of *Encarsia Formosa* Gahan (Hymenoptera, Aphelinidae) un-parasitized and parasitized *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) biotype B prey by *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) predator

Imtiaz Ali Khan, Fang-Hao Wan

### Abstract

The cotton whitefly *Bemisia tabaci* is a major pest, causing serious losses to many agricultural crops. The ladybird beetle *Coccinella septempunctata* is an important polyphagous predator of *B. tabaci* worldwide. In the present study, prey consumption of *Encarsia Formosa* not parasitized and parasitized *B. tabaci* biotype B prey by the predator was determined on fresh tomato leaves as arena of observation under a no-choice condition. The experiments were conducted under laboratory conditions at a temperature of  $25 \pm 2$  °C, relative humidity of 60±5%, photoperiod of 16:8h (L: D) and an artificial light intensity of about 4000 lux. *E. Formosa* did not parasitize *B. tabaci* eggs and N<sub>1</sub> but the subsequent developmental stages of the prey were parasitized. There were no differences in the consumed number of *B. tabaci* eggs and N<sub>1</sub>, not exposed and exposed to *E. Formosa* for parasitization, by the predator larvae and adults. *C. septempunctata* larvae as well as adults consumed significantly higher mean number of not parasitized N<sub>2</sub>, N<sub>3</sub>, N<sub>4</sub> and pupal prey than parasitized by *E. Formosa*. Prey consumption, not parasitized as well as parasitized, increased and parasitized by *E. Formosa*, than the males. Intraguild predation (IGP) naturally occurs in all kinds of ecosystems in which the natural enemies attack each other and compete for same pest (s) species. Such interactions among natural enemies may have additive, disruptive or no effect on overall pest suppression. The present results provide high feasibility of using together *C. septempunctata* and *E. Formosa* in pest management programs against *B. tabaci*. But, IGP of *C. septempunctata* on *B. tabaci* nymphs parasitized by *E. Formosa* might weaken the overall pest management program.

**Keywords:** *B. tabaci*, *C. septempunctata*, *E. Formosa*, prey consumption, intra-guild predation

### 1. Introduction

The cotton whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), also known as tobacco or sweet potato whitefly, is a highly polyphagous pest of more than 500 plant species [1, 2] belonging to more than 60 plant families [3, 4, 5], which includes important field and greenhouse crops [3, 6, 7, 8], and horticultural crops [9] world-wide. It also vectors more than 50 Gemini viruses among which tomato yellow leaf curl virus (TYLCV), tomato mottle virus (TMOV), and bean golden mosaic virus (BGMV) [10, 11] are some of the important ones.

*B. tabaci* has been declared a difficult pest to control because of its high reproductive rate and many generations per year [12, 13], and preferred habitat on the undersurface of leaves [14]. Massive spraying of chemicals against the pest has not only resulted in development of resistance to many insecticides [15, 16, 17, 18] but also has severely damaged and killed its natural enemies too, which has further complicated the problem [19]. During the last few decades considerable efforts have been made towards the discovery and development of safe alternatives for management of this insect with reduced hazards to the environment and human health. Biological control is one of such safe alternatives for the pest suppression. According to [20] biological control is an ecological phenomenon, which can provide environmentally Harmonious and economical pest management.

Coccinellids have been widely used in Biological control for more than a century. Augmentative releases of several coccinellids species are well documented and have been

found effective [21]. *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is an important polyphagous predator of aphids, thrips, whiteflies, mites and Lepidopteran eggs [22, 23, 24]. Additionally, mass rearing of *C. septempunctata* is easily achieved [25, 26].

Investigation of biology and prey consumption of a predator is utmost important before using it in a biological control program. Its preference for different pest(s) stages as well as its interaction with other natural enemies should also be determined [27]. *Encarsia Formosa* Gahan (Hymenoptera: Aphelinidae) is used in many countries of the world for commercial control of whiteflies especially in glasshouse crops. *E. Formosa* parasitizes several whitefly species including *Trialeurodes vaporariorum* [28, 29] and *B. tabaci* [30].

Some authors [31, 32] have found intraguild predation (IGP) naturally occurring in many ecosystems. Many previous researchers [33, 34, 35, 36] have found IGP very important in insect biocontrol. According to them many natural enemies may attack and compete for same pest(s) species. Furthermore, interactions between these natural enemies may interfere, enhance or have no effect on overall pest suppression.

In this study, prey consumption of *E. Formosa* un-parasitized and parasitized *B. tabaci* different stages by *C. septempunctata* larvae and adults was determined under laboratory conditions.

## 2. Materials and Methods

### 2.1 Insect and Plant Stock Culture

For establishing stock culture of *B. tabaci* biotype B, few individuals were obtained from a previously maintained colony on cotton plants, variety Shi Yuan 321. The culture was maintained on tomato plants, variety Zhong Za No. 9, in a glasshouse of the Chinese Academy of Agricultural Sciences (CAAS) Beijing, China in 2007. The rearing took place in rectangular aluminum cages (80×50×60 cm) meshed with muslin cloth from four sides for ventilation. The cages were stored in a climatically controlled chamber at the Institute of Plant Protection (IPP), South Campus, CAAS Beijing, at a temperature of 25±2 °C, relative humidity of 60±5% and a photoperiod of 16:8h (L:D) with an artificial light intensity of about 4000 lux. Tomato plants were grown in small plastic pots (10 cm diameter and 8 cm height) in a glasshouse.

Tomato plants were exposed to adult's *B. tabaci* infestation for a couple of days in the stock culture. Thereafter, the whitefly adults were removed and the plants were incubated as per above climatic conditions and observed daily until the individuals reaching the desired stage for the experiments. The old plants were used to infest new ones and to feed the predators. The old plants were replaced with new ones whenever more *B. tabaci* were needed for the experiments.

Stock culture of *C. septempunctata* was initiated from few individuals obtained from a culture maintained for other laboratory experiments at IPP, CAAS. The rearing took place in cages in the climatic chambers as per *B. tabaci*. *Aphis craccivora* infested bean plants were served as substrate plants and prey for rearing the predators. Aphid infested bean leaves were frequently replaced inside the cages for maintaining regular and adequate prey supply. *C. septempunctata* eggs, larvae and pupae as well as adult females and males were obtained from the cages and used in the different experiments. The prey consumption experiments were conducted on uniformed sized fresh tomato leaves in the clip on cages (3.5 cm diameter at mouth, 2.0 cm diameter at base and 4.0 cm long).

### 2.2 Prey Consumption by the Larval Instars

For prey consumption by the larval instars of *C. septempunctata*, newly hatched larvae at their first instar (L<sub>1</sub>) were picked up using a camel-hair brush and kept individually in the clip on cages mentioned above and daily offered with 20 eggs, 10 first nymphal instar (N<sub>1</sub>), 10 second nymphal instar (N<sub>2</sub>), 5 third nymphal instar (N<sub>3</sub>), 5 fourth nymphal instar (N<sub>4</sub>) or 5 pupa. From the stock culture, uniform sized tomato leaves infested with batches of similar aged eggs, nymphs and pupae of *B. tabaci* were randomly selected, and extra individuals were removed with a fine camel-hair brush. The subsequent larval instars, i.e., second instar (L<sub>2</sub>) was offered with 50 eggs, 30 N<sub>1</sub>, 20 N<sub>2</sub>, 15 N<sub>3</sub>, 10 N<sub>4</sub> or 10 pupa; third instar (L<sub>3</sub>) with 90 eggs, 50 N<sub>1</sub>, 40 N<sub>2</sub>, 20 N<sub>3</sub>, 15 N<sub>4</sub> or 10 pupa; and fourth instar (L<sub>4</sub>) with 100 eggs, 80 N<sub>1</sub>, 70 N<sub>2</sub>, 60 N<sub>3</sub>, 50 N<sub>4</sub> or 40 pupa of *B. tabaci* prey on fresh uniform sized tomato leaf in the cages. During the experiments, the larvae were transferred daily into new cages with fresh prey and the consumed prey individuals in the old cages were recorded. All immature stages of *C. septempunctata* used in the different experiments were less than 12h old and starved for 12h before use. They were transferred to fresh uniformed sized tomato leaves in individual clip cages. The experiments were replicated 20 times per each prey stage.

### 2.3 Prey Consumption by the Adults

For these experiments *C. septempunctata* adult females and males (max. 24h old and starved for 12h) were used. Each predatory individual was singly caged and daily offered 100 eggs, 80 N<sub>1</sub>, 70 N<sub>2</sub>, 60 N<sub>3</sub>, 50 N<sub>4</sub> or 40 pupa on fresh tomato leaves as described above. The test individuals were daily transferred into new cages with fresh prey and the consumed number of nymphs or puparia was recorded from 1<sup>st</sup>-10<sup>th</sup> day of both the adults with feeding on all stages of *B. tabaci*. The experiment was replicated twenty times for each predator sex and prey stage.

### 2.4 Prey Consumption of Parasitized Prey

Before using *C. septempunctata* in a biological control program, it was noteworthy to investigate its prey consumption on a parasitized *B. tabaci* prey by another natural enemy. *E. Formosa* (obtained from an old stock of whitefly infested cotton plants at IPP, CAAS) was reared on *B. tabaci* infested tomato plants in the rearing cages and climatic conditions mentioned above. In a series of experiments, newly oviposited eggs and 1-4<sup>th</sup> instar emerged nymphs and pupa of *B. tabaci* were separately exposed to freshly emerged *E. Formosa* adults for oviposition on 2-3 weeks old tomato plants.

For investigation prey consumption of parasitized prey, the *B. tabaci* different stages were used after two day of exposure to *E. Formosa* adults. Two days exposure of *B. tabaci* was enough for parasitization by the parasitoid (Khan and Wan, 2008, un-published). The experimental procedure and number of parasitized *B. tabaci* prey offered to the immature as well as adult predators was the same as for not parasitized prey. Data of prey consumption was recorded during the entire development of L<sub>1</sub>-L<sub>4</sub> and 1<sup>st</sup>-10<sup>th</sup> days of adult's longevity with feeding on all stages of *B. tabaci*. The experiments were replicated twenty times for each immature stage as well as adult females and males per prey stage.

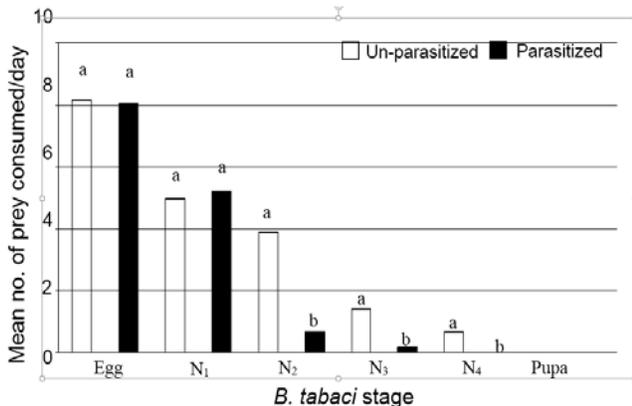
### 2.5 Statistical Analysis

The data obtained of prey consumption of *E. Formosa* un-parasitized and parasitized *B. tabaci* nymphs and pupae by *C. septempunctata* larvae and adults were subjected to T-test and significance levels determined at p ≤ 5%.

### 3. Results and Discussion

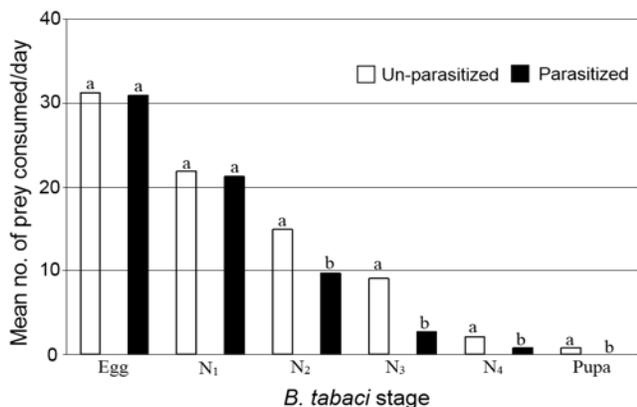
#### 3.1 Prey Consumption by the Larval Instars

Egg and N<sub>1</sub> of *B. tabaci* were un-parasitized by *E. Formosa*, but the subsequent developmental stages were parasitized by it. The number of *B. tabaci* eggs and N<sub>1</sub>, both exposed and not exposed to *E. Formosa* for parasitization, consumed by *C. septempunctata* L<sub>1</sub> were not significantly different (Fig 1). The L<sub>1</sub> consumed significantly higher mean number of un-parasitized *B. tabaci* N<sub>2</sub> (3.9), N<sub>3</sub> (1.4) and N<sub>4</sub> (0.7) than the parasitized these prey stages (0.7, 0.2, 0.0, respectively) by *E. Formosa*. The L<sub>1</sub> did not consume any not parasitized or parasitized *B. tabaci* pupa by *E. Formosa*.



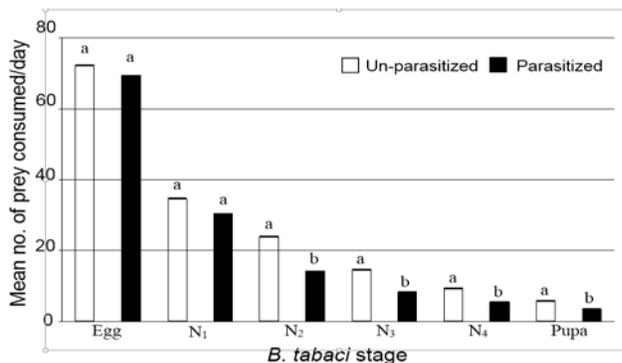
**Fig 1:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* L<sub>1</sub> at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

The *C. septempunctata* L<sub>2</sub> also consumed non-significantly different number of *B. tabaci* eggs and N<sub>1</sub>, both exposed and not exposed to *E. Formosa* for parasitization (Fig 2). The mean number of un-parasitized *B. tabaci* N<sub>2</sub> (15.1), N<sub>3</sub> (9.1) and N<sub>4</sub> (2.1) and pupae (0.9) were significantly higher than the parasitized one (9.8, 2.8, 0.8, 0.0, respectively).



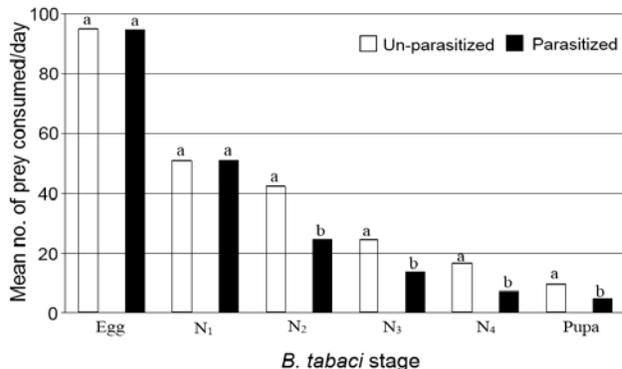
**Fig 2:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* L<sub>2</sub> at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

Similar to the L<sub>1</sub> and L<sub>2</sub>, the *C. septempunctata* L<sub>3</sub> also consumed non-significantly different number of *B. tabaci* eggs and N<sub>1</sub>, both exposed and not exposed to *E. Formosa* for parasitization (Fig 3). The predator consumed significantly higher mean number of un-parasitized *B. tabaci* N<sub>2</sub> (24.0), N<sub>3</sub> (14.5) and N<sub>4</sub> (9.4) and pupae (5.7) than parasitized (14.2, 8.1, 5.4, 3.5, respectively) by *E. Formosa*.



**Fig 3:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* L<sub>3</sub> at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

The number of *B. tabaci* eggs and N<sub>1</sub>, both exposed and not exposed to *E. Formosa* for parasitization, consumed by *C. septempunctata* L<sub>4</sub> were non-significantly different (Fig 4). But, the consumed mean number of un-parasitized *B. tabaci* N<sub>2</sub> (42.4), N<sub>3</sub> (24.7) and N<sub>4</sub> (16.7) and pupae (9.8) by the predator was significantly higher than the parasitized (24.6, 13.5, 7.2, 4.6, respectively) ones.



**Fig 4:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* L<sub>4</sub> at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

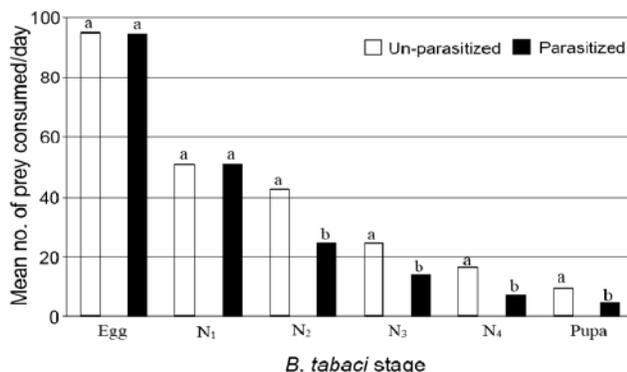
*Encarsia Formosa* parasitized all four instars of the *Trialeurodes vaporariorum* and was able to complete development [37]. But, in the present experiments *E. Formosa* didn't parasitize *B. tabaci* in the embryonic and first nymphal stage.

There were found no significant differences in the consumed number of *B. tabaci* eggs and N<sub>1</sub>, both not exposed or exposed to *E. Formosa* for parasitization, by the different immature stages of the predator. However, *C. septempunctata* different immature stages consumed significantly higher number of un-parasitized N<sub>2</sub>, N<sub>3</sub>, N<sub>4</sub> and pupal prey than the parasitized by *E. Formosa*. The number of prey consumed increased in the subsequent predator's immature instars.

*C. septempunctata* adults consumed all stages, both un-parasitized and parasitized by *E. Formosa*, of *B. tabaci*. The consumed number of *B. tabaci* eggs and N<sub>1</sub>, both not exposed and exposed to *E. Formosa* for parasitization, by both the adults were not significantly different. The adult predators consumed significantly higher number of un-parasitized N<sub>2</sub>, N<sub>3</sub>, N<sub>4</sub> and pupal prey than parasitized by *E. Formosa*, where the adult females consumed higher number of all prey stage than the males.

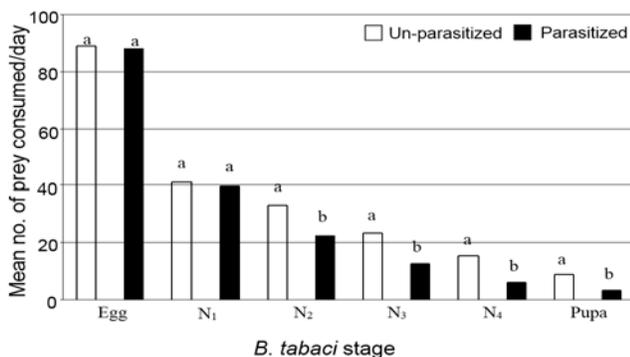
### 3.2 Prey Consumption by the Adults

Similar to the immature results, the *C. septempunctata* adult males also consumed non-significantly different number of *B. tabaci* eggs and N<sub>1</sub>, both exposed and not exposed to *E. Formosa* for parasitization (Fig 5). The predator consumed significantly higher mean number of un-parasitized *B. tabaci* N<sub>2</sub> (29.6), N<sub>3</sub> (20.8) and N<sub>4</sub> (11.6) and pupae (5.7) than parasitized (19.9, 10.1, 4.4, 2.0, respectively) by *E. Formosa*.



**Fig 5:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* adult male at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

The *C. septempunctata* adult females similar to the males consumed non-significantly different number of not exposed *B. tabaci* eggs and N<sub>1</sub> or exposed to *E. Formosa* for parasitization (Fig 6). The mean number of un-parasitized *B. tabaci* N<sub>2</sub> (33.2), N<sub>3</sub> (23.3) and N<sub>4</sub> (15.3) and pupae (8.7) were significantly higher than parasitized these prey stages (22.6, 12.7, 6.1, 3.3, respectively) by *E. Formosa*.



**Fig 6:** Mean number of *Encarsia Formosa* un-parasitized and parasitized *Bemisia tabaci* prey consumed/day by *Coccinella septempunctata* female at 25±2 °C, 60±5% RH, 16:8h photoperiod. Bar heads with different letters are significantly different at p ≤ 5% (t-test).

*C. septempunctata* female adults as potent predators of *T. tabaci* and *T. vaporariorum* on tomato leaves in controlled environments at proportions near 1:30 (predator/total prey) [38]. In greater pest densities, however, effective predation and successful biological control was difficult to achieve [38, 39], which required more predators to control the pest. They concluded that *C. septempunctata* is a capable predator, which can be used for the biological control of *B. tabaci* in glasshouse as well as under field conditions.

Several authors have found trophic interactions between natural enemies attacking *B. tabaci*, e.g., while *Delphastus pusillus* (LeConte) consume parasitized whitefly hosts

containing younger stages of aphelinid parasitoids indiscriminately, it avoids preying on hosts containing older parasitoid larvae and pupae [33, 40]. Two species of the coccinellid *Serangium* were found avoiding preying on older aphelinid parasitoid larvae and pupae within *B. tabaci* hosts [27, 41]. The mirid predator *Dicyphus hesperus* Knight is an indiscriminant IG predator of *E. formosa* pupae in greenhouse whiteflies [42]. This behavior of the predator was not affected by the parasitoid age [43].

Many authors have investigated IGP on immature parasitoids in a number of predator/parasitoid/prey systems [32, 44]. They found that the test predators readily preyed upon various stages of immature parasitoids within whitefly hosts even under choice condition of non-parasitized prey.

Experiments conducted on intraguild interactions between two natural enemies of *B. tabaci*, an oligophagous predator, *Delphastus catalinae* (Horn), and a parasitoid, *Encarsia sophia* (Girault and Dodd), under no-choice and choice experiments, revealed that predation was generally lower on *B. tabaci* nymphs containing *E. sophia* pupae than on larval stages or on un-parasitized host nymphs [45]. In choice tests, adult *D. catalinae* did not discriminate between prey types. In both choice and no-choice tests, the second instar *D. catalinae* larvae discriminated *B. tabaci* nymphs containing parasitoid larvae, and the third and fourth instar predator larvae attacked less the host nymphs containing parasitoid pupae than larvae.

Free-choice and no-choice laboratory experiments conducted on three generalist predators, *Geocoris punctipes* (Say), *Orius insidiosus* (Say), and *Hippodamia convergens* Gue'rin-Me'neville, by offering fourth instar *B. tabaci* nymphs and nymphs parasitized by *Eretmocerus sp. nr. Emiratus* showed significant preferences for larval and pupal stage parasitoids over early fourth instar nymphs, but *G. punctipes* and *O. insidiosus* did not discriminate when offered a choice of larval parasitoids and late fourth instar nymphs [46].

Both prior and current studies gave conflicted results that the coccinellid predators avoid or prefer to feed on the parasitized prey offered or preferred not parasitized over the parasitized prey. But, the present results enhance options of using *C. septempunctata* in pest management programs in conjunctions with parasitoids. Since the predator feed more on not parasitized prey, there is high potential for integration of both natural enemies in a biological control program against *B. tabaci* to provide greater level of pest suppression. But, predation by the *C. septempunctata* immature and adults on parasitized *B. tabaci* nymphs, so that intraguild predation of the parasitoid by the predator occur, might weaken the overall pest management program.

IGP is detrimental to biological control because it interfered with the predation/parasitization abilities of the natural enemies, e.g., exerting suppressive effects on pest populations [36]. However, there also were instances where addition of an IG predator didn't pose any effect on pest suppression [47] or even enhanced overall biological control [34, 48].

### 4. Acknowledgment

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