

RESEARCH ARTICLE

Preferences and functional response of Coccinellidae to *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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ABSTRACT

An exploration study found that 4 out of 11 predominant predator species of *Bemisia tabaci* in Indonesia belong to Order Coleoptera, family Coccinellidae, i.e., *Menochilus sexmaculatus*, *Coccinella transversalis*, *Verania lineata*, and *Curinus coeruleus*. The current study was conducted to further determine the most effective predator species among those coccinellids. The evaluation included three consecutive assays, i.e., predation, preference, and functional response assay. The experiments were conducted in 2019 under controlled conditions in West Java. Predation assay using *B. tabaci* as the prey showed that *M. sexmaculatus*, *C. transversalis*, and *V. lineata* had comparable predation capacity to consume 46-48 nymphs d⁻¹ or 9 adults d⁻¹, and the *V. lineata* preference to *B. tabaci* was higher compared to other predators. Further logistic regression analysis (linear coefficient $P_1 = -0.1006$, $X^2 = 34.99$) showed that *V. lineata* had type II functional response characteristics. The analysis using the Hollings disc equation for type II functional response ($R^2 = 0.9239$) suggested that the searching rate (a) and the handling time (T_h) of *V. lineata* to *B. tabaci* nymphs were 0.3522 h⁻¹ and 0.151 h, respectively. These results indicate that *V. lineata* could be considered in pest management strategies and effectively regulate populations of *B. tabaci* in Indonesia.

Key words: *Bemisia tabaci*, chili pepper, Coccinellidae, functional response, predation.

INTRODUCTION

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important pests causing significant losses to a wide variety of agricultural commodities all over the tropical and subtropical regions of the world. In addition to feeding on more than 500 species of horticultural and agronomic crops in fields and greenhouses (Liu et al., 2020). The vegetable crops most affected by *B. tabaci* include chili, tomato, cabbage, cucumber, eggplant, gherkin, melon, squash, sweet potato, poinsettia, gerbera, and cotton (Shah and Liu, 2013; Cuthbertson, 2015). *Bemisia tabaci* has a high reproductive capacity and a destructive living habitat that allows it to cause severe damage through the secretion of honeydew that stimulates the rapid growth of molds (Gangwar and Gangwar, 2018), and the transmission of over 300 viral diseases in major economic eminent agricultural and vegetables crops (Gilbertson et al., 2015).

Generally, chemical controls have been widely used for *B. tabaci* management. Chemical control is an important part of crop protection in modern agriculture, although over-reliance on pesticides has resulted in environmental contamination, creating toxic conditions for beneficial insect species and higher costs for growers. In addition, *B. tabaci* has shown resistance to organophosphates, pyrethroids, and neonicotinoids (Longhurst et al., 2013; Naveen et al., 2016; Wang et al., 2021), in China (Wang et al., 2017), Malaysia (Shadmany et al., 2015), and USA (Prabhaker et al., 2014). Nowadays, *B. tabaci* has displayed resistance to more than 50 active ingredients of insecticides and several multi-resistant *B. tabaci* populations (IRAC,

2022). In addition, the processes of resistance in *B. tabaci* are analogous to those that have been defined from many other pest species, and they are frequently categorized as metabolic, involving esterase-, glutathione S transferase (GST)- or P450 monooxygenase-based detoxification, or point mutations in the target position (Horowitz et al., 2020).

Biological control is an integral part of the whitefly management program and has been considered an effective substitution for chemical applications without causing contamination of the environment. Smith and Krey (2019) reported that in the last five decades, biological control procedures have been efficaciously utilized to control whiteflies in a secure environment. Predators perform a crucial role in controlling pest populations and show great potential compared to parasitoids and pathogens in controlling *B. tabaci*. There are at least 150 arthropod species currently described as predators of *B. tabaci* (Abubakar et al., 2022), and dominated by ladybird beetles (Coleoptera: Coccinellidae), true bugs (Hemiptera: Anthocoridae and Miridae), lacewings (Neuroptera: Chrysopidae), and mites (Acarina: Phytoseiidae) (Al-Zyoud, 2014). Several hemipterans are frequent predators of the whitefly and can contribute to its control. *Dicyphus hesperus* could control a high population of the whitefly and at an early stage in the cropping cycle of tomatoes (Dumont et al., 2021). The genus *Orius* (Hemiptera: Anthocoridae) has many polyphagous species regarded as predators, with a partiality for attacking spider mites, thrips nymphs and adults, and whiteflies (Yamada et al., 2016). *Orius laevigatus* was proficient to prey on *B. tabaci* eggs, nymphal instars, and adults during fulfilling its immature stages, while in its mature growing phases with nymphs and eggs of the pest (Salama et al., 2022).

The plant-feeding habit of whitefly predators has to be known to maintain an available population for preying. Roda et al. (2020) reported that three mirid species, i.e., *Nesidiocoris tenuis*, *Macrolophus praeclarus*, and *Engytatus modestus* notably decreased the number of *B. tabaci* on tomato plants based on field cage studies in South USA. Multiple cropping of the tomato plants with *Sesamum indicum* indicated that mirid numbers increased despite a low number of preys, thus proving an advantage of the plant-feeding habit of these predators.

Udiarto et al. (2012) revealed that four main species of coccinellids, namely *Menochilus sexmaculatus*, *Coccinella transversalis*, *Verania lineata*, and *Curinus coeruleus* are generally found in most vegetable commodities in Indonesia. Apart from the high feeding potential and multiplication rates, coccinellids beetles are an important candidate for the whitefly biological control program.

Efficacious biological control of pest species depends on the fact that the predator destroys, kills, or consumes enough of the pest to keep its population below the economic threshold level. The study suggested four predominant coccinellids that were found in various vegetable commodities and exploration sites, i.e., *M. sexmaculatus*, *C. transversalis*, *V. lineata*, and *C. coeruleus*. Unfortunately, their usefulness as biological control agents of *B. tabaci*, including their predation capacity, preferences, and functional responsiveness are yet to be outlined. The current predation study aimed to determine effective biological effects by pinpointing predation capacity, prey preferences, and functional response of those four species of coccinellids to *B. tabaci*.

MATERIALS AND METHODS

The study was carried out in the Entomology Laboratory of the Indonesian Vegetable Research Institute (IVegRI), Lembang, West Java, from May to December 2019. All climate-controlled rooms were set at 24 ± 1.5 °C, 60%-85% RH and 12:12 h photoperiod.

Predation test of predator against nymphs and adults of *B. tabaci*

Coccinellids that were predominantly found during natural enemies' exploration in 2018 were tested in the study. The colonies of four coccinellids species i.e., *Coccinella transversalis*, *Menochilus sexmaculatus*, *Verania lineata*, and *Curinus coeruleus* were brought together from a field population and maintained in the laboratory at ambient temperature, humidity, and lighting on whiteflies before using in the bioassays. A completely randomized design (CRD) with four treatments and 10 replicates was set up under controlled

conditions in the Entomology Laboratory of IVegRI. A female adult of each predator was placed into a plastic jar with 15 cm diameter and 4 cm height containing chili (*Capsicum annuum* L.) leaves bearing 50 nymphs or adults of *B. tabaci*. The predator individuals fasted before the predation test. The number of *B. tabaci* individuals that survived in the jar was counted after a 24-h test. The data were analyzed using the examination of variance tracked by Duncan's multiple range test at the 5% confidence level once it had differences between treatments.

Preference test of coccinellids on various prey species

The preference of four coccinellids predators, i.e., *M. sexmaculatus*, *V. lineata*, *C. transversalis*, and *C. coeruleus* on four chili pests was evaluated. The first three predators showed a high predation capacity against *B. tabaci* in the previous predation test. The free-choice test method was performed using the 2-3 instar of nymphs of four chili pest prey species. The tested prey included, *Myzus persicae*, *Thrips parvispinus*, *Aphis gossypii*, and *B. tabaci*.

The 9 cm diameter Petri dish was divided into four equal-sized rooms, and each was separated using cardboard. Each part of the Petri dish was then filled with chili leaves bearing 50 nymphs of each prey to allow the tested predators to choose their preferred prey. Nymphs of *B. tabaci*, *M. persicae*, and *A. gossypii* used in this test were obtained from laboratory mass cultures, whereas *T. parvispinus* nymphs were collected from pesticide-free fields. Wet cotton was placed at the tip of the leaf stalk to prevent quick withering. Once it was set, one starved female *V. lineata* adult was released right in the middle of the Petri dish. The predator was fasted for 6 h before the test. These steps were performed for all three tested coccinellids and replicated ten times.

The groups of *B. tabaci* nymphs used in the preference study were obtained from a mass-rearing screen house. Ten pairs of coeval *B. tabaci* collected from the screen house were infested into chiffon cages covered for 6-8 wk after planting chili plants grown in 25 × 35 cm polybags. The insects and plants were thoroughly maintained. The chili leaves containing 50 nymphs of *B. tabaci*, 50 *M. persicae*, and 50 *A. gossypii*, and 50 *T. parvispinus* were selected for use in the preference test.

The number of remaining nymphs of *B. tabaci*, *M. persicae*, *A. gossypii*, and *T. parvispinus* were recorded at 3, 6, 12, and 24 h after predator release. The degree of preference (preference index) for prey was calculated using the formula $L_i = r_i - p_i$, where L_i is the index for prey selection, r_i is the proportion of prey consumed by predators, and p_i is the proportion of available prey. The L_i value ranges from (-1) to (+1). The maximum preference occurs when $r_i = 1$ and $p_i = 0$, and the maximum avoidance (rejection) when $r_i = 0$ and $p_i = 1$.

The data were statistically analyzed using ANOVA followed by Duncan's multiple range test at the 5% confidence level to determine the differences between treatments. The analysis was performed using the SAS System 9.0 (SAS Institute, Cary, North Carolina, USA).

Functional response of *V. lineata* to *B. tabaci*

Experiments were conducted under controlled conditions to determine the predation of whitefly nymphs by *V. lineata*. A completely randomized design (CRD) consisting of 10 treatments of prey density levels and 10 replications for each treatment was used in this experiment. The treatments comprised 10 populations of *B. tabaci*: 1, 2, 3, 4, 6, 8, 12, 16, 22, and 28 nymphs.

Chili leaves containing a certain number of 2-3 instar of *B. tabaci* nymphs depending on the treatment were placed into a 10 cm diameter and 1.5 cm height of Petri dishes. Twenty-four-hour-fasted female predators were then introduced into a Petri dish. The number of surviving nymphs was recorded every 3 h for 24 h. At each observation time, the number of lost or killed prey was added back, so the number of preys remained unchanged by the treatment.

The effect of the number of prey available on the average number of preys consumed was analyzed using ANOVA, followed by Duncan's multiple range test at the 5% confidence level. Logistic regression between the available prey density (N_0) and the proportion of prey consumed (N_c/N_0) was used to determine the type of predator functional response to *B. tabaci* nymphs. The following equation was applied (Juliano, in Rehman et al., 2020):

$$N_e/N_0 = \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)/1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)$$

These four parameters were estimated using the maximum likelihood method with the PROC CATMOD SAS procedure (SAS Institute). If the linear coefficient (P_1) is not significantly different from 0, the proportion of prey consumed is constant with increasing prey availability, and the suitable functional response model is type I. If P_1 is negative ($P_1 < 0$), the proportion of prey consumed decreases from the beginning as prey increases, which characterizes the type II functional response. When P_1 is positive ($P_1 > 0$), the proportion of prey consumed initially increases and then decreases, which characterizes type III functional dates.

Furthermore, functional response parameters of instantaneous prey searching rate (a) and prey handling time (Th) were determined using Holling's (in Rehman et al., 2020) disc equation model. The functional response type I followed Disc equation: $N_e = aN_0 + b$; Random equation $N_e = aTN_0$.

The functional response type II used Disc equation $N_e = aTN_0/(1 + aThN_0)$; Random equation $N_e = N_0 \{1 - \exp[a(ThN_e - T)]\}$.

The functional response type III used Disc equation $N_e = aTN_0^2/(1 + cN_0 + bThN_0^2)$; Random equation $N_e = N_0 \{1 - \exp[(d + bN_0)(ThN_e - T)/(1 + cN_0)]\}$, where N_e is the number of preys eaten per individual predator, N_0 is the number of available preys, T is the time the prey is exposed to the predator during the prey searching time, Th is the prey handling time, a is the instant search (attack) rate and b , c and d are inherited constant of parameter a (Hassell, in Bruzzone et al., 2022).

Since during the experiment, the prey consumed by predators was replaced according to its original density, thus the disc equation model from Holling was used to obtain parameters of functional response instant prey search rate (a) and prey handling time (Th) as follows:

$$N_e = aTN_0/(1 + aThN_0)$$

The parameter estimation values (a and Th) of the equation were obtained through non-linear regression using the GLIMMIX procedure in SAS/STAT software version 9.4 (SAS Institute).

RESULTS

Predation of various predators against nymphs and adults of *B. tabaci*

The predation of predators against *B. tabaci* nymphs and adults is shown in Figure 1. The result revealed that *C. transversalis*, *M. sexmaculatus*, and *V. lineata* had similar predation capacities which were significantly different from *C. coeruleus* ($P = 0.0108$) against both the nymphs and adults. The three coccinellids were able to consume nymphs and adults of *B. tabaci* up to 96.67% and 18.40% d^{-1} , respectively. On the other hand, *C. coeruleus* indicated the lowest predation ability compared to the three other predators against both nymphs and adults of *B. tabaci*.

Preference of coccinellids for various pest species

The average nymph numbers of insect pests preyed upon by coccinellids are indicated in Figure 2. The predator species seem to have different preference indexes for preying on insect pests. The preference indexes for different predator species are presented in Figure 3. *Verania lineata* had the highest preference for *B. tabaci* compared to *M. persicae*, *T. parvispinus*, and *A. gossypii*. However, *M. sexmaculatus* preferred *A. gossypii* and *M. persicae* and while *C. transversalis* preferred *T. parvispinus* over other chili pests. These results show that, although they are general predators, these three species have different prey preferences. The study showed that *V. lineata* had the highest preference for whiteflies compared to the other predators. This indicated that *V. lineata* had the potential as an effective biological control agent for whiteflies. Schartel and Schaubert (2016) mentioned some factors that play a role in determining the rate of predator consumption, including the preference for different prey species.

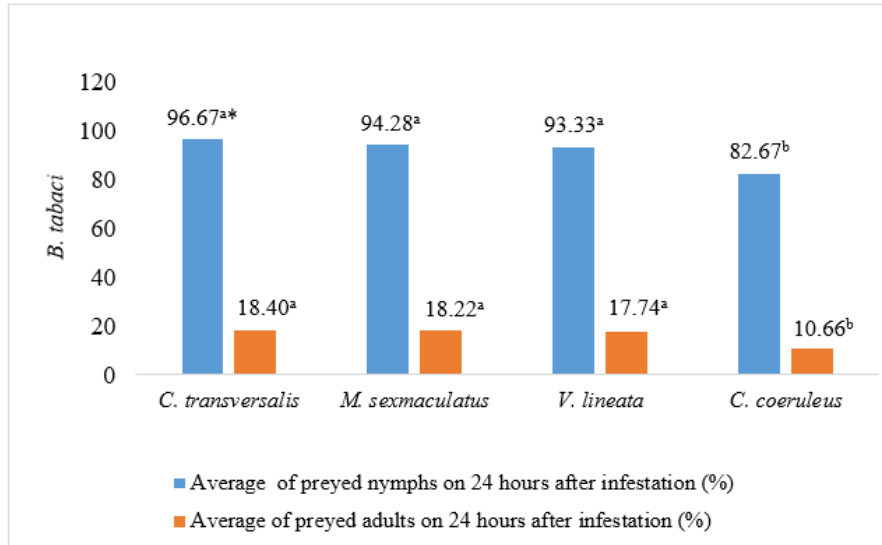


Figure 1. Predation of predators against *Bemisia tabaci* nymphs and adults in the laboratory. *Values followed with the same letter on blue or brown bars are not significantly different according to Duncan's multiple range test at the 5% confidence level. *Bemisia tabaci* (ordinate), *Coccinella transversalis*, *Menochilus sexmaculatus*, *Verania lineata*, and *Curinus coeruleus* (axis).

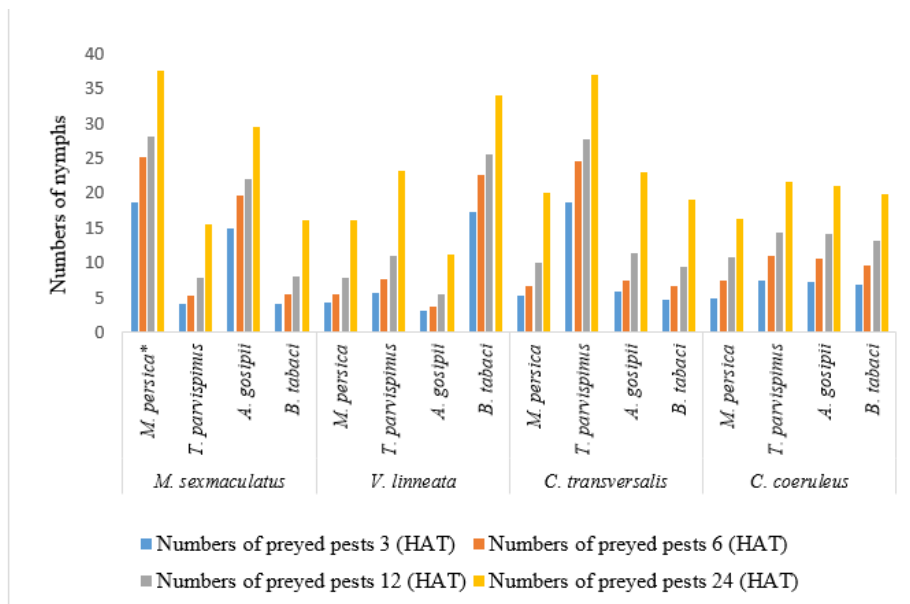


Figure 2. Average nymph number of insect pests preyed upon by coccinellids. *Insect pests (*Myzus persicae*, *Thrips parvispinus*, *Aphis gossypii*, and *Bemisia tabaci*); coccinellids (*Menochilus sexmaculatus*, *Verania lineata*, *Coccinella transversalis*, and *Curinus coeruleus*); HAT: hours after treatment.

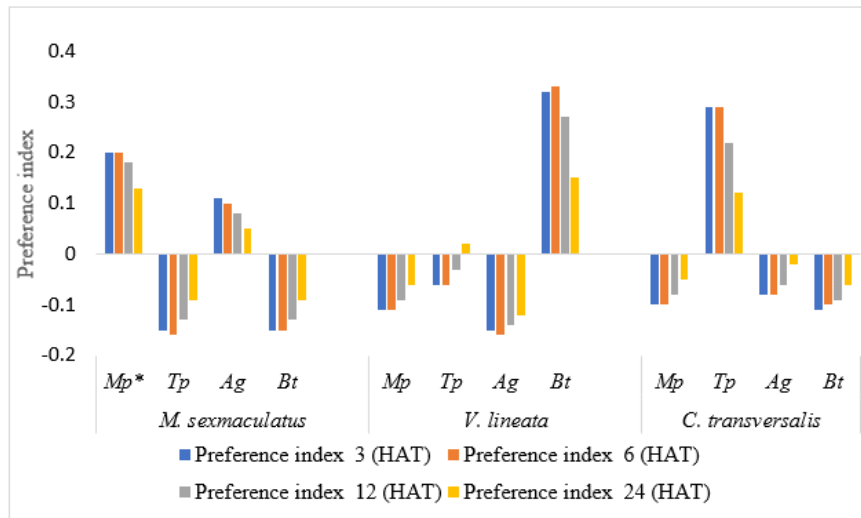


Figure 3. Preference indexes for different predator species. **Mp*: *Myzus persicae*; *Tp*: *Thrips parvispinus*; *Ap*: *Aphis gossypii*; *Bt*: *Bemisia tabaci*; coccinellids (*Menochilus sexmaculatus*, *Verania lineata*, and *Coccinella transversalis*); HAT: hours after treatment.

Functional responses of *V. lineata* at different whitefly densities

The mean number of consumed preys increased significantly with increasing prey density (Table 1). At low prey densities (1-3 nymphs), all available preys were consumed (100%). In addition, the number of consumed preys continued to increase, and at a density of 28 nymphs, the number of consumed preys was 19.

Table 1. Average of *Bemisia tabaci* consumed ($x \pm$ standard deviation) by *Verania lineata* at different nymph densities. Means followed by different letters in the same column show a significant difference according to Duncan's multiple distance test at the 5% confidence level.

Nymph density	Average of consumed prey
1	1.00 ± 0.00 ^a
2	2.00 ± 0.00 ^b
3	3.00 ± 0.00 ^c
4	3.80 ± 0.42 ^d
6	5.50 ± 0.53 ^e
8	7.10 ± 0.74 ^f
12	10.20 ± 0.79 ^g
16	13.20 ± 0.79 ^h
22	17.30 ± 0.82 ⁱ
28	19.20 ± 0.79 ^j

Logistic regression analysis using linear equations resulted from the linear coefficients ($P_1 = -0.1006$, $X^2 = 34.99$), which were negative and significantly < 0 , indicating that the proportion of consumed prey decreased with increasing prey density, as shown in Figure 4. This indicated that the functional response of *V. lineata* to *B. tabaci* nymph was classified as type II (Figure 5). The Type II model was derived from Linear equations $Y = 0,7014X + 1,0763$, with $R^2 = 0,9859$. This result is by what Kumar et al. (2020) stated that the feeding response of a predator increases with increasing prey density and saturates at a certain level.

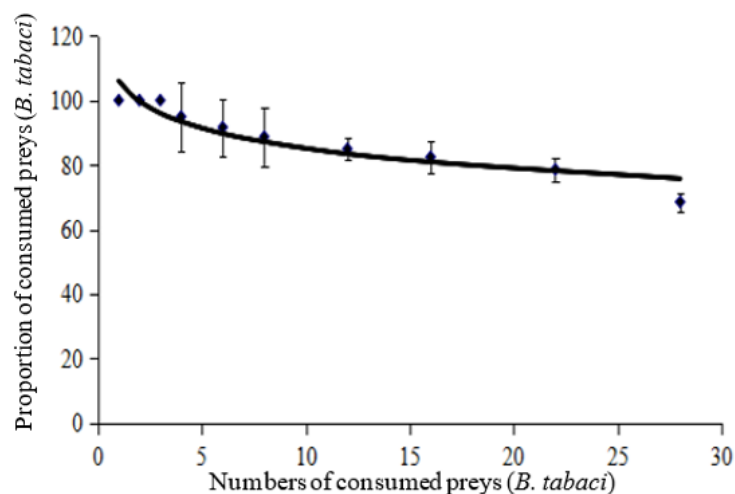


Figure 4. Average observed values for the proportion of *Bemisia tabaci* consumed prey (points) and predictors (lines) are based on the logistic regression analysis. Logistic regression analysis using linear equations resulted in the linear coefficients ($P_1 = -0.1006$, $X^2 = 34.99$).

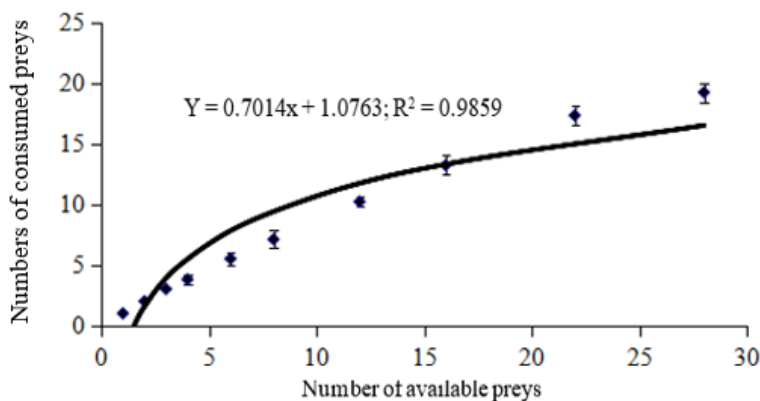


Figure 5. Curve of type II functional response of *Verania lineata* to the different densities of *Bemisia tabaci*.

DISCUSSION

The results of the prey-stage preference study indicated that the four coccinellid predators preferred *B. tabaci* nymphs to adults. *Bemisia tabaci* nymphs were easily caught by predators because they were commonly passively attached to the underside of the leaf, except for the first instar. On the other hand, active adults of *B. tabaci* were difficult for predators to catch. Kumar et al. (2020) reported that *Delphastus catalinae* Horn and *D. pallidus* LeConte (Coleoptera: Coccinellidae) prefer whitefly eggs over the early nymphal instars. Furthermore, Kheirodin et al. (2020) reported that predators of coleopterans favor *B. tabaci* eggs, whereas hemipterans choose *B. tabaci* nymphs and adults. This resulted from the differences in preferences for the two distinct prey species. Predators' preferences toward prey depend on various characteristics such as the capacity to detect prey, how easily the predator can access prey, the defenses exhibited by prey against predators, and the capacity to effectively feed on prey (Jaworski et al., 2013). This suggests that general whitefly predators may consume all life phases of *B. tabaci*, and synergism is probable

when chewing and sucking predators are found together, which would augment the repression of *B. tabaci* (Kheirodin et al., 2020).

Understanding predator-prey interactions in agroecosystems is very important for developing a successful biological control program. Kheirodin et al. (2022) reported the use of molecular gut content analysis (MGCA) to study predator-prey interactions. The utilization of the longest half-life primer resulted in abundant predators vigorously feeding on *B. tabaci* in cotton fields such as *Geocoris punctipes*, *Orius* spp., *Hippodamia convergens*, fire ants (*Solenopsis invicta*), and Thomisidae spider. Fire ants and *Orius* spp. were the most plentiful predator species and provided the highest potential for *B. tabaci* control.

Udiarto et al. (2012) reported that based on a laboratory study, several predator species including *M. sexmaculatus*, *C. transversalis*, *Harmonia* sp., and *Curinus* sp., which are bio-control agents of *B. tabaci*, belong to the family Coccinellidae. According to Jayanti et al. (2018), *C. transversalis* was able to consume 22 *T. parvispinus* nymphs or adults per day in a screen-house experiment. Previous studies have reported that Coccinellidae are important natural predators of several pest species, including whiteflies (Perring et al., 2018), mealybugs (Ferreira et al., 2020), psyllids (Canovai et al., 2019), and mites (Iskra et al., 2019). Hence, Coccinellidae are considered to be generalist predators because they can prey on several pest species. However, although they are generalists, they still prefer certain prey, even though reports of this are limited. Therefore, further research is needed regarding the preferences of coccinellid species for various pest species, such as *B. tabaci*, *A. gossypii*, and *T. parvispinus*, in chili and other crops.

The current study found that even though all predators evaluated in the study were general predators, these three species of predators had different prey preferences. Predators prefer prey because of its physical and chemical properties (Culshaw-Maurer et al., 2020). Other factors, including predation rates, population densities, and prey stage, can also help predators select the target prey (Martínez et al., 2021). In addition, physical factors, such as temperature, water availability, oxygen, salinity, light, food and nutrients, light, color, shape, and size may be important (Schartel and Schaubert, 2016), and chemical factors, such as secondary volatile compounds, also influence predator preference (Kumar et al., 2020).

The type II functional response was a common predator response when providing one prey species during the laboratory predation assay. In this assay, both predators and prey were close to each other in a limited space; therefore, it was easy for predators to find their prey. The effectiveness of predation can be partially determined by measuring the ability of a predator to find prey at lower prey densities and to consume more prey at higher prey densities. Holling's disc equation model II was used to evaluate the effect of prey density on the parameters of the functional response of *V. lineata*. Two important parameters of this model are prey search rate (a) and prey handling period (T_h). A natural enemy's handling period and attack rate are important indicators of their predatory behaviors (Kumar et al., 2020). The prey handling period is the time needed by a predator to recognize, chase and consume prey, and other activities associated with predation such as mouth cleaning and resting before the next prey hunting. The searching rate of *V. lineata* that indicated the proportion of the total area explored by the predator per foraging time unit was $a = 0.3522 \text{ h}^{-1}$ and $R^2 = 0.92$. The *B. tabaci* nymphs T_h of *V. lineata* was 0.151 h with $R^2 = 0.92$. In the abundance of prey, *V. lineata* could prey on a maximum of six nymphs of *B. tabaci* per hour.

CONCLUSIONS

The predators, i.e., *Menochilus sexmaculatus*, *Verania lineata*, and *Coccinella transversalis* had similar predation rates when *Bemisia tabaci* was their prey, except *Curinus coeruleus*. However, based on the estimated parameters, *V. lineata* can be considered the most efficient predator among the four coccinellids and preferred *B. tabaci* over the other prey. The other predators had a negative preference. *Verania lineata* could be mass-reared and developed as the biological control agent for *B. tabaci* in chili crops.

Author contribution

Conceptualization: B.K.U. Methodology: B.K.U., W.S. Validation: T.K.M., A.H., A.M. Investigation: R.M., A.M., T.K.M., W.S., A.H., I.S., N.G., E.K., T.U., T.Y.T., A.N., A.A., I.M. Resources: R.M., A.M., T.K.M., A.H., I.S., N.G., E.K., T.U., T.Y.T., A.N., A.A., I.M. Data analysis: B.K.U., A.M. Writing-original draft: B.K.U., R.M., W.S. Writing and editing: N.G., A.M., W.S. Supervision: B.K.U. All co-authors reviewed the final version and approved the manuscript before submission.

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