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EFFICIENCY OF THREE INDIGENOUS SPECIES OF COCCINELLID PREDATORS FOR CONTROLLING APHIDS AND WHITEFLIES ON CUCUMBERS IN GREENHOUSES

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Abstract
Cucumber plants can be infested with many insect pests such as aphids, whiteflies, and thrips, especially in greenhouses, resulting in significant damage to the crops. This study investigated the efficacy of three species of coccinellids (*Coccinella undecimpunctata*, *Coccinella septempunctata*, and *Hippodamia variegata*), indigenous to the Taif region of Saudi Arabia, to control *Bemisia tabaci* whiteflies and *Aphis gossypii* aphids on cucumbers in greenhouses. The study was conducted with a release rate of five second-instar larvae of each coccinellid species per plant. One week after the first release of coccinellids, there were no significant differences among the treatment groups in terms of infestation rate, but all three groups differed significantly from the control group. After the first week, the *B. tabaci* and *A. gossypii* infestation rates decreased significantly in the *H. variegata* group compared with the other two coccinellid species, a trend that continued until the end of the experiment. These results suggested that *H. variegata* was the most effective of the three species at decreasing the *B. tabaci* population. After the second release of each coccinellid species, the reduction rates were >90% in all three groups, being significantly higher in the *H. variegata* group. Thus, these results suggest that second-instar larvae of all three coccinellid species would be effective predators of *B. tabaci* and *A. gossypii* on cucumbers in greenhouses, with those of *H. variegata* showing the greatest efficacy. Future research should focus on the effects of these predators on outdoor cucumber fields and on other crops grown in the Taif region.

Keywords: Aphid. Biological pest control. Greenhouse. Ladybirds. Predators. Whitefly.

1. Introduction

Greenhouses are a mainstay of modern agriculture, providing protection and an appropriate cultivation environment for economically important plants. Cucumber, *Cucumis sativus* L. (Fam: Cucurbitaceae), is native to India and was imported to East Asia and from there to Southern Europe. It is now an important vegetable in many regions of the world. However, *C. sativus* has many insect pests, such as aphids, whiteflies, and thrips (Zahedi et al. 2019). Despite its name, *Aphis gossypii* (Hemiptera: Aphididae), the cotton or melon aphid is a pest of species from more than 92 families, including vegetable, fruit, ornamental, and field crops. The effects of *A. gossypii* feeding on plants include sap removal and the transmission of plant viruses. These can lead to significant levels of damage, including flower and fruit abortion, stunting, necrosis, wilting, chlorosis, leaf distortion, and defoliation (Ebert and Cartwright 1997). Such effects can cause substantial economic damage to *C. sativus* in fields and in greenhouses (Alizamani et al. 2017).

The whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) is also an important insect pest of various vegetable crops and causes direct damage following the removal of plant sap and secretion of honeydew. The latter acts as a substrate for sooty molds on leaves and fruits, causing indirect damage as a vector of viral diseases (Razze et al. 2016). This whitefly species infests over 600 of plant species, including vegetables, field crops, fruits, and ornamental plants (Kashima et al. 2015).

Chemical insecticides remain a priority method for insect pest control, because they are low-cost, good-quality compounds (De Bueno et al. 2017). However, most chemical insecticides have negative effects on parasitoids and predators. Thus, research has focused on developing alternative methods that could be safe for natural enemies and other beneficial organisms but effective against pest species (Rimoldi et al. 2012). There are various safe alternatives to chemical insecticides, such as natural enemies (predators and parasitoids), biodegradable compounds, and entomopathogenic bacteria, fungi, and viruses (Tauber et al. 2000; El-Shourbagy et al. 2023).

Coccinellid predators (Coccinellidae: Coleoptera) include important natural enemies of various phytophagous insect pests, including whiteflies, aphids, leafhoppers, thrips, mealybugs, and land lepidopteran caterpillars (Elheneidy et al. 2008; Khan et al. 2022). Coccinellids are the most common predators of aphids and have a worldwide distribution. The genera most predaceous on whiteflies and aphids are *Coccinella*, *Hippodamia, Adalia, Scymnus, Cheilomenes*, and *Oenopia*. These coccinellids have a long history of use as biological control agents with recognized success (Hodek and Honek 1996).

The predatory coccinellids *Coccinella undecimpunctata* L., *Coccinella septempunctata* L., and *Hippodamia variegata* Goeze are effective predators of different insect pests, including whiteflies and aphids, in both fields and greenhouses because of their high predation efficacy (Franzman 2002; Cabral et al. 2008). These three species are widely distributed in many parts of the world, including different regions of Saudi Arabia (El-Hawagry et al. 2013; Al-Deghairi et al. 2014; Sayed 2016). Generally, indigenous natural enemies are more suitable for controlling pests in their native regions when the level of control by resident populations of natural enemies is insufficient. Repeated mass introductions of natural enemies have been frequently used as a biological control approach in greenhouse systems (Pijnakker et al. 2020). However, new associations with the pest can develop based on multilevel interactions if effective native natural enemies are also present in the agroecological system (Yang et al. 2016). Moreover, reduction in the prey, which affect native and non-native coccinellid predators via greater competition, are likely to have an impact on the abundance of native predatory species over both the short and long term (Li et al. 2021). Thus, investigations of intraguild interactions are important, especially those involving higher trophic levels, to determine the efficacy of indigenous biocontrol agents and the potential threats, if any, to their efficacy (Gupta et al. 2021).

The current study examined three species of coccinellid predators (*C. undecimpunctata*, *C. septempunctata*, and *H. variegata*) indigenous to the Taif region of Saudi Arabia, for their efficacy against whiteflies and aphids, based on the percentage reduction in the levels of infestation on cucumber in greenhouses.

2. Material and Methods

Collection of predators

Female *C. undecimpunctata*, *C. septempunctata*, and *H. variegata* were collected from different fields cultivated with various species in the Taif region (21° 21' 44'' N and 40° 18' 12'' E), Saudi Arabia. Once females started to oviposit, they were placed in a cylindrical plastic vessel (8 cm height ×10 cm diameter) with a hole in the lid (4 cm diameter) and covered by gauze to maintain the ventilation inside the vessel. The females were fed eggs of *Ephestia kuehniella*. Oviposited eggs were collected daily and kept in fresh cylindrical plastic vessels, as described above.

Mass rearing of predators

Newly hatched larvae of all three coccinellid species were reared for three generations on *E. kuehniella* eggs. Rearing was carried out in the plastic vessels described above under laboratory conditions of 26°C ± 2°C, 60% ± 6% relative humidity (RH), and a 14:10 photoperiod (L:D). Second-larval instars of the third generation of each coccinellid species were used for the experiments.

Predator release in greenhouses

The study was performed in three greenhouses (3.75 x 12 m) in three different locations in Taif Governorate, Saudi Arabia (21° 19' 17'' N, 40° 21' 13'' E; 21° 20' 54'' N, 40° 19' 28'' E; and 21° 21' 37.5192'' N, 40° 20' 2'' E). The distance between each *C. sativus* plant was 75 cm. The study used a randomized complete block design (RCBD) in each greenhouse, with three treatment groups and a control group. Thus, each greenhouse was divided into four blocks (3.75 x 3 m) using nylon curtains, resulting in a total of three blocks per group, each of which had 12 plants. Five second-instar larvae of each coccinellid species were released onto each plant in each block on 28 April and 12 May. At the first release, the *C. sativus* plants had between four and six leaves, with a mean of five leaves and approximately 30 aphids and 13 whiteflies per plant. No chemicals were applied to the plants during the study period.

Aphid and whitefly counts

On 28 April, the plants were approximately 50 cm tall. The lower and upper surfaces of three randomized leaves from three randomized plants in each block of treated and control plants were examined to estimate the number of aphids and whiteflies per leaf before the first release of predators. A second predator release occurred 2 weeks later on 12 May. The numbers of the two insect pests on each plant were recorded weekly, using the same method as described above, until 3 weeks after the second release date (2 June).

Statistical analysis

Reductions in infestation rates were calculated by using the Henderson and Tilton formula. Infestation and reduction rates were analyzed using one-way ANOVA and Duncan's multiple range test in SPSS v. 23 (SPSS 2015) with a significance level of $P < 0.05$.

3. Results

Infestation rates of *A. gossypii* at the start of the study (28 April) on all plants (control and treatment groups) ranged from 31.82 to 34.52 individuals/leaf, with no significant difference among groups. The infestation rate gradually increased until the end of the study (63.42 individuals/leaf on 2 June). One week after the first release, there was no significant difference in the infestation rate among the three treatment groups, although all three treatments were significant lower compared with the control group (41.2 versus and 8.42, 10.51, and 11.39 aphids/leaf in the treatment groups) (Table 1). Two weeks after the first release, the infestation rate decreased significantly in the *H. variegata* treatment group (6.12 individuals/leaf) versus the *C. undecimpunctata* and *C. septempunctata* treatments groups (8.69 and 9.42 individuals/leaf, respectively). This difference was consistent until the end of the study.

The reduction in the number of aphids did not differ significantly among treatments after the first (range: 73.48%–80.62%, $F_{2,78} = 0.764$, $P = 0.423$) or second week (range: 81.12%–88.16%, $F_{2,78} = 0.839$, $P = 0.203$) following the first release (Figure 1). However, after the second release until the end of the study, the reduction rate in the *H. variegata* treatment group was >95% and significantly higher than those in either the *C. undecimpunctata* or *C. septempunctata* groups (range: 90.28%–92.22%; $P < 0.001$).

Table 1. Population density of aphids, *A. gossypii* on *C. sativus* inoculated with coccinellid predators under greenhouse conditions

Study group	Level of *A. gossypii* aphid infestation (mean no. of aphids/plant ± SE)					
	28 April	5 May	12 May	19 May	26 May	2 June
Control	31.82±3.38 A	41.20±3.23 A	49.62±4.37 A	55.22±4.82 A	57.13±3.04 A	63.42±5.04 A
H. variegata	34.52±2.75 A	8.42±1.83 B	6.12±1.05 C	3.04±0.43 C	2.18±0.81 C	2.21±0.27 C
C. undecimpunctata	33.21±3.36 A	10.51±1.35 B	8.69±1.54 B	5.23±1.12 B	5.14±1.10 B	5.94±0.59 B
C. septempunctata	33.45±2.29 A	11.39±2.01 B	9.42±1.91 B	4.91±0.47 B	4.92±0..56 B	5.43±0.92 B
F values (*df*=3,104)	0.34	21.33	82.66	104.51	142.13	155.26
P	0.648	<0.001	<0.001	<0.001	<0.001	<0.001

Means bearing different letters in the column are significantly different from each other (Duncan's multiple range test, $P < 0.05$).

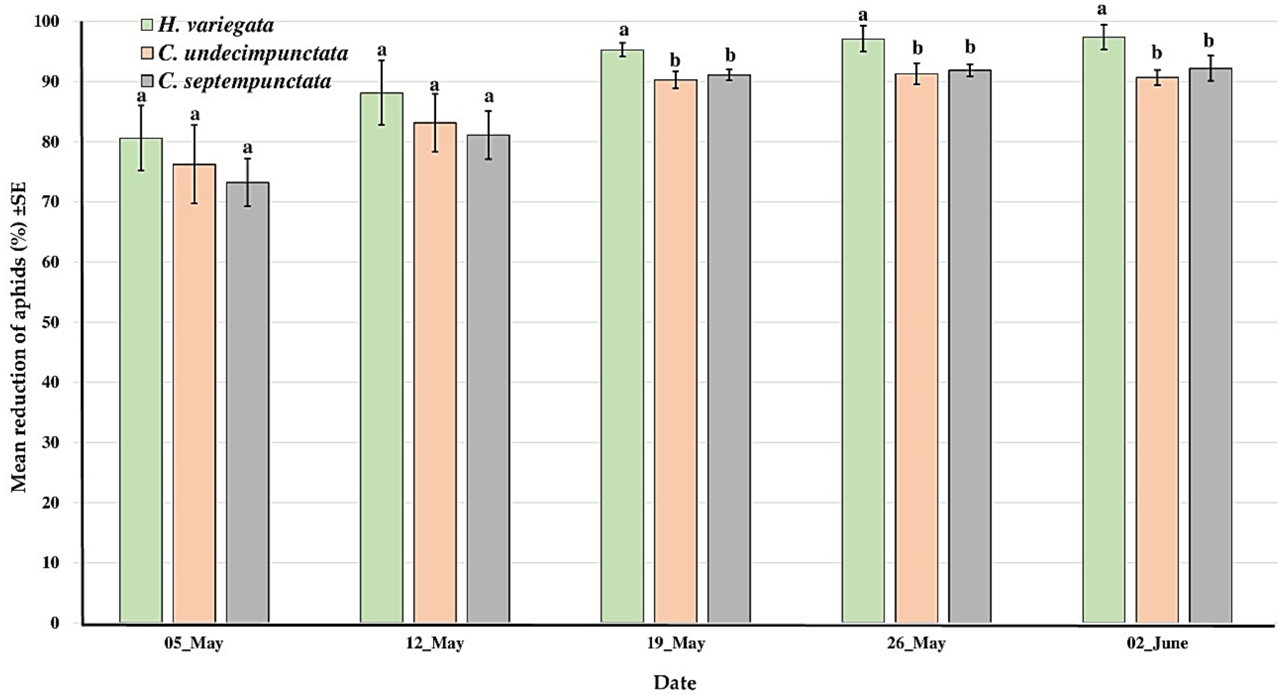

Figure 1. Percentage reduction (mean ± SE) in the number of *A. gossypii* on *C. sativus* inoculated with different coccinellid predators under greenhouse conditions. Different letters above columns indicate significant differences among the coccinellid groups on the same date.

Infestation rates of *B. tabaci* at the start of the study on all plants (control and treatment plants) ranged from 13.26 to 14.04 whiteflies/leaf, with no significant difference among groups. As seen with the aphid infestation, the *B. tabaci* infestation rate on control plants increased gradually until the end of the study (29.35 whiteflies/leaf on 2 June). One week after the first release, there was no significant difference in infestation rate among the three treatment groups, whereas all three groups were significantly different from the control (15.22 versus 5.11, 6.36, and 6.21 whiteflies/leaf in the treatment groups; Table 2). Two weeks after the first release, the *B. tabaci* infestation rate decreased significantly in the *H. variegata* group (2.19 individuals/leaf) compared with that of the *C. undecimpunctata* and *C. septempunctata* groups (3.91 and 3.55 individuals/leaf, respectively), mirroring that recorded for *A. gossypii.* This difference was consistent until the end of the study.

There was no significant difference in the reduction rates of *B. tabaci* among the treatment groups one week after release (range: 59.56%–67.42%, $F_{2,78} = 0.925$, $P = 0.534$). From after two weeks after the first release until the end of the study, the reduction rate in the *H. variegata* treatment group was 88.35%, which was significantly higher than in either the *C. undecimpunctata* or *C. septempunctata* groups (range: 80.36%–81.13%; $P = 0.035$). After second release until the end of the study, the reduction rate in the *H. variegata* group was >92.55%, which was again significantly higher than in either the *C. undecimpunctata* or *C. septempunctata* groups ($P < 0.001$).

Table 2. Population density of whiteflies (*B. tabaci*) on *C. sativus* inoculated with coccinellid predators under greenhouse conditions

Study group	Level of *B. tabaci* infestation (mean no. of whiteflies/plant ± SE)					
	28 April	5 May	12 May	19 May	26 May	2 June
Control	13.26±1.14 A	15.22±1.58 A	18.22±1.28 A	19.38±1.13 A	22.35±1.83 A	29.35±3.12 A
H. variegata	13.74±0.87 A	5.11±0.52 B	2.19±0.68 C	1.56±0.27 C	0.84±0.13 C	1.31±0.38 C
C. undecimpunctata	14.04±0.47 A	6.36±0.72 B	3.91±0.41 B	2.85±0.23 B	2.08±0.14B	3.04±0.81 B
C. septempunctata	13.43±0.91 A	6.21±0.59 B	3.55±0.77 B	3.01±0.31 B	2.21±0..24 B	2.78±1.05 B
F values (*df*=3,104)	0.26	12.44	41.35	65.81	98.54	124.14
P	0.832	<0.001	<0.001	<0.001	<0.001	<0.001

Means bearing different letters in the column are significantly different (Duncan's multiple range test, *P* < 0.05).

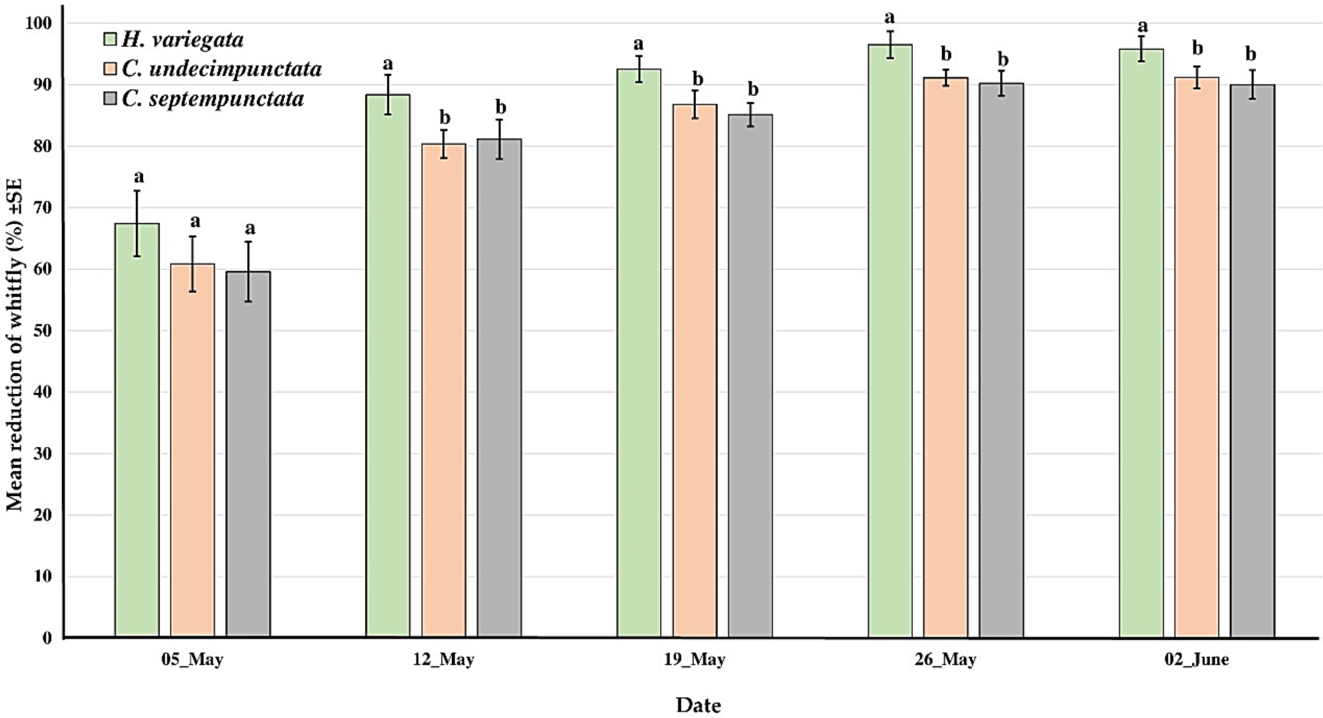

Figure 2. Percentage reduction (mean ± SE) in the number of *B. tabaci* on *C. sativus* inoculated with different coccinellid predators under greenhouse conditions. Different letters above columns indicate significant differences among the coccinellid groups on the same date.

4. Discussion

The current results showed that the release of second-instar larvae of *H. variegata*, *C. undecimpunctata*, and *C. septempunctata* on *C. sativus* in greenhouses was an effective control measure against both aphids and whiteflies, supporting previous research in fields or greenhouses that showed that the release of ladybirds suppressed aphid populations (Snyder et al. 2004; Flint and Dreistadt 2005). Various experimental setups of the interactions between coccinellids and aphids achieved reductions in aphid population, based on release and predation rates of the coccinellids (Flint and Dreistadt 2005). Consumption by ladybirds contributed to the reduction in aphid number in different agricultural systems (Flint and Dreistadt 2005). Coccinellid predators can both slow the growth, and reduce the size, of aphid populations at the start of the growing season and during crop growth (Pervez et al. 2020). In the current study, the aphid reduction rate by *H. variegata* was >95%, which was significantly higher than that achieved by either *C. undecimpunctata* or *C. septempunctata* after the second release. A previous study in the same geographical region as the current study achieved a >95% reduction in aphid numbers on rose leaves and flower buds after three releases of 30 larval *H. variegata* (Sayed and Alghamdi 2017). Moreover, the high values of finite and intrinsic rates of increase of *H. variegata* predating *A. gossypii* and *Aphis craccivora* highlighted their suitability for the mass rearing of this coccinellid (Powell and Pell 2007). Previous work showed that *H. variegata* fed A. *gossypii* had high fecundity and a long oviposition period, indicating the palatability, energetic value, and high nutritive content of this aphid (Keshavarz et al. 2015). In a previous investigation (Hämäläinen 1977), 9-m^2 plots were set up to test the efficacy of *C. septempunctata*, reporting that a single release of first-instar larvae at different release rates reduced *Myzus persicae* aphid populations on chrysanthemum and sweet pepper after 8–10 days. Another investigation of the control of *A. gossypii* and *Aphis craccivora* Koch on sweet pepper by *C. septempunctata* suggested that this species also contributed to aphid population reduction in the experimental greenhouses in contrast to the control greenhouse (Valério et al. 2007).

Similar findings to those for aphids were also recorded for the control of the whitefly *B. tabaci*, with reduction rates of >90%. More than 50 species of coccinellid predators attack the eggs and other immature stages of whiteflies (Gerling 1990). Thirteen species are predators of *Bemisia* spp. (Nordlund and Legaspi 1996). For example, *Hippodamia convergens* achieved nymphal mortality of 45.5% (Hagler and Blackmer 2013), *Delphastus pallidus* attained 68.0% and 55.1% egg and nymph mortality, respectively on leaf discs (Ahmed et al. 2017; Kumar et al. 2020). When added to plants at a rate of four individuals per plant, *Serangium parcesetosum* achieved a 60% fatality of *B. tabaci* (Razza et al. 2016) and was also reported to have potential for control of the whitefly *Bemisia argentifolii* in greenhouses, increasing whitefly mortality within 2 weeks of its release (Ellis et al. 2001). Another investigation evaluated the potential of *C. septempunctata, Chelomenes vicina, Diomers hottentota*, and *Diomers flavipes*) as predators of two whitefly species: *B. tabaci* and *Aleurodicus dispersus*. All four species achieved high efficacy against both whitefly species, although they significantly preferred *B. tabaci* nymphs (Atuncha et al. 2013). *Clitostethus brachylobus* showed efficient prey consumption against *B. tabaci*, suggesting the use of *C. brachylobus* in integrated management programs of this whitefly (Wang et al. 2020).

5. Conclusions

The current results showed that after the second release of *H. variegata*, *C. undecimpunctata*, and *C. septempunctata*, the reduction rates for both whiteflies and aphids were >90%, and significantly higher with *H. variegata* than with either *C. undecimpunctata* or *C. septempunctata*. Thus, the use of these three coccinellid predators but particularly *H. variegate* can suppress aphid and whitefly infestations on *C. sativus* in greenhouses. Further research should focus on these predators in *C. sativus* and other crops under field conditions.

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Ethics Approval: Not applicable.

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