Research Article

Food preference and comparative feeding efficacy of *Chrysoperla carnea* (Stephens) in population management of invasive Cotton mealybug, *Phenacoccus solenopsis* (Tinsley)

Bhugro Mal¹, Hakim Ali Sahito^{2*}, Abdul Hafeez Mastoi³, Shoib Rahim⁴, Attaullah Khan Pathan¹, Wali Muhammad Mangrio² and Tasneem Kousar²

1. PARC-Southern Zone Agricultural Research Centre, Karachi, (75270), Sindh, Pakistan

2. Department of Zoology, Shah Abdul Latif University, Khairpur (66111), Sindh, Pakistan

3. Department of Entomology, Lasbella University of Agriculture, Water and Marine Sciences, Uthal (80700), Balochistan, Pakistan

4. Department of Agriculture and Agribusiness, University of Karachi (75270), Sindh, Pakistan

*Corresponding author's email: hakim.sahito@salu.edu.pk

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Abstract

The knowledge of preference and feeding efficacy of a predator plays an important role in visualizing its role in pest management programs. The present research study was conducted to have a deep insight into the predatory potential and preference of *Chrysoperla carnea* on different instars of *P. solenopsis*, which was performed in the Bio-control Laboratory at PARC-SARC, Karachi. The cotton mealybugs were collected from the okra crop and further multiplied on potatoes sprouts at (26±2°C and 65±5% RH) for feeding the larvae of *C. carnea* and all larval stages expressed response when they fed over the different instars of *P. solenopsis*. Predatory performance increased with the advancement in the growth of the larval stage as the maximum performance was expressed by the third-stage larva of *C. carnea*. The successive instars (1st to 3rd) consumed per day on an average (50.89±0.51), (92±0.33), and (144.67±0.66) on first instars of *P. solenopsis*. Each larval stage took three days to enter into the next phase. A single larva right hatching from an egg to prepupal stage consumed an average of 866.67 first instars of cotton mealybug. All larval instars preferred first instars of *P. solenopsis* and exhibited less preference towards the second and third stages. The first stage larva did not at all consume the third instar of *P. solenopsis*. The findings of the present study suggested that; *C. carnea* is a very

successful predator of *P. solenopsis*, easy to rear in the laboratory, and can be exploited for the biological control of cotton mealybug under field conditions.

Keywords: Biological control; *Chrysoperla carnea*; Cotton mealybug; Feeding efficacy; IPM

Introduction

The Cotton mealybug, *Phenococcus solenopsis* (Tinsely) is a polyphagous invasive insect pest in Sindh, Pakistan has been recorded from more than 154 different species of plants including; cotton, okra, tomato, eggplant, sesame, sunflower, and ornamentals and it has also been found infesting different types of weeds as well [1] has been recorded from 35 different ecological zones of the world [2]. As a result of the sucking of sap, the growth of cotton plants is stunted, leaves turn yellow, and affected plants produce a lesser number of bolls [3]. The cotton mealybugs, in addition to sucking the sap from plants also excrete honeydew which serves as a medium for the development of sooty mold, which interferes with the process of photosynthesis and also reduces the market value of lint [4]. Ants also feed on honeydew secreted by mealybugs and play an important role in their spread and also deter natural enemies from playing their role [5]. Under favorable climatic conditions population of *P. solenopsis* is greatly increased and natural enemies available in the crop do not bring to below ETL, levels in the crop. Hence, chemical interventions become necessary to protect the crop from the ravages of pests. In this scenario, the development of the IPM package is necessary to reduce the pressure of pesticides [6]. The farmers mainly depend upon synthetic chemical pesticides for controlling insect pests in their crops; however, *P. solenopsis* due to the development of waxy secretions on its body is difficult to control with chemical pesticides [7]. Environment and biodiversity are also affected due to the intensive use of synthetic pesticides used for controlling various insect pests in crops [8]. The concept of pest management suggested

by [9] suggests the use of both biological and chemical control methods for pest control in agricultural systems. The growers seek alternatives to synthetic chemical pesticides for controlling different insect pests in their crops. Given this their interest in using natural enemies as an alternative to chemical pesticides has recently increased. The main component of the IMP is biological control and pest control can be effectively achieved utilizing biological control [10]. The mealybug infestations on different host plants can also be effectively brought under control using natural enemies [11].

Among the generalist predators that are available for controlling different insect pests, the *Chrysoperla carnea* is a very important one and can be effectively exploited for controlling different insect pests in IPM programs [12, 13, 14]. This predator is frequently found in cotton crops [15], Okra [16], and Oilseeds [17]. It has a wide host range like whiteflies, thrips, mealybugs, aphids, neonate lepidopterous larvae, and eggs of different arthropods. Larvae of this predator express cannibalism when there is a shortage of host/food. Female of this predator laid several hundred small eggs with stalks on the underside of leaves and small shoots during dark hours. Eggs of this predator hatch in three to six days and the larvae that emerge from eggs eat voraciously on their prey and molt three times [18, 19, 20]. This predator has high searching efficiency [21] and also has developed high resistance capability against many pesticides that are massively used for the control of a variety of pest insects [22]. The money spent on the import of pesticides can be reduced by utilizing this predator in pest management programs [23]. Nowadays, this predator is commonly reared in

biological control laboratories and is extensively used for biological control of different insect pests of agricultural importance throughout the world [24]. This predator has huge potential to be commercialized and for integration in IPM programs for the management of a variety of insect pests in different crops [25, 26, 27]. In Pakistan, this predator has been used for the management of cotton mealybugs and proved to be an efficient predator of cotton mealybugs [28]. Because of the importance of this predator in the management of cotton mealybug, this study was undertaken to know its preference, and consumption potential was confirmed on the different instars of cotton mealybug under laboratory conditions.

Materials and Methods

Study area

The studies on food preference and comparative feeding efficacy of different laboratory-reared and field captured larva of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on different stages of cotton mealybug, *Phenococcus solenopsis* (Tinsely) were undertaken in the biological control laboratory of Pest Management Research Institute, PARC, SARC, at $26\pm 2^{\circ}\text{C}$ temperature and $65\pm 5\%$ RH% during, 2020.

Rearing of *Phenococcus solenopsis* (Hemiptera: Pseudococcidae)

Cotton mealybugs were collected from the okra crop and further multiplied on sprouted potatoes. The potatoes with small buds were purchased from the market, brought in the laboratory, washed and dried, and put under soaked gunny bags for sprouting. The gunny bags were slightly sprinkled with water daily to maintain moisture for the quick sprouting of potatoes. The potatoes took 10-15 days to sprout. The cotton mealybugs were released on these sprouted potatoes for further multiplication. The cotton mealybugs multiplied freely on these potatoes and

colony established for feeding to *C. carnea* larvae.

Rearing of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

Chrysoperla carnea larvae were collected from okra crop grown at the SARC-PARC, Karachi from Experimental field brought under the laboratory and released on sprouted potatoes on which cotton mealybugs had been established. The potatoes were put in rectangular cages, made of a 6cm thick, transparent plastic sheet. When larvae of *C. carnea* turned into adults, the adults were provided with an artificial diet in the ratio of 70ml distilled water, one tablespoon honey, one tablespoon sugar, and a 5.5g yeast solution. The artificial diet was discarded after two days and a new one was prepared. The food was provided on the sides of cages in drops and also in a plastic strip with closed round holes. The soaked cotton swab was also placed in cages for fulfilling the water requirement of adults of green lacewing. The food was provided on daily basis. A black granulated paper underside the removable top of the cage was provided for oviposition purposes of *Chrysoperla carnea*. Eggs were harvested with a razor every morning and placed in 9cm diameter Petri dishes for hatching. The eggs took three to four days to hatch. The larvae hatched from eggs were put separately in Petri dishes to avoid the possibility of cannibalism due to voracious feeding in nature. The moistened filter papers were put in the base of Petri dishes to provide optimum conditions for the suitable growth of larva.

Consumption potential of predator to pest

In a bid to check the consumption potential of 1st to 3rd laboratory-reared, as well as field-collected stages of *Chrysoperla carnea* on first to third cotton mealybug instars and the different larvae stages of *C. carnea* were separately released into 9cm diameter Petri dishes and given a definite number of

specific instars of *P. solenopsis* in three replications. The specific instars of cotton mealybugs were separated with the help of camel hairbrushes from the general population and released into Petri dishes. To avoid the escape of mealybugs, the Petri dishes were covered with lids. The fresh okra leaves were placed in Petri dishes for feeding cotton mealybugs. Daily consumption of each stage of *Chrysoperla carnea* on each instar of *P. solenopsis* was examined by subtracting the daily number of consumed from the total number of mealybugs that were released into the Petri

dishes. The counting of numbers of *P. solenopsis* consumed by each larval stage was carried out every 24 hours until the larva entered the next phase (Fig. 1).

Statistical analysis

The differences in total consumption between 1st, 2nd, and 3rd instar of *C. carnea* on different stage instars of *P. solenopsis* were calculated by analysis of variance using the statistical software (SXW 8.1 USA). Further confirmation means were separated through the least significance test at a 5% significance level.

1st instar of *P. solenopsis* being consumed by the 1st stage of *C. carnea*

1st instar of *P. solenopsis* being consumed by the 2nd stage of *C. carnea*

1st instar of *P. solenopsis* being consumed by the 3rd stage of *C. carnea*

Figure 1. Food preference and comparative feeding efficacy of *C. carnea* in population management of *P. solenopsis* under laboratory conditions

Results

The consumption rate of laboratory-reared larva

The results of the present findings revealed that all larval instars of *C. carnea* expressed performance against the different stages of *P. solenopsis*. However, the 1st instars of *P. solenopsis* frequently preferred mostly all larval instars of *C. carnea*. The consumption strength of *C. carnea* larva significantly increased with its stage advancement. The 1st larval stages of *C. carnea* consumed on an average (152.67±1.56) first instar, (3.66±0.143) second instar, and (0) third instar *P. solenopsis* respectively in three days period. The second stage of *C. carnea* extremely used the double number of different *P. solenopsis* stages compared with the 1st star and consumed on an average (276±2.67) first instar, (7.32±1.78) second instar, and (0.99±0.89) third instar *P. solenopsis* in three days period respectively. The third instars were found to be a voracious feeder and consumed almost triple the more number of different stages of *P. solenopsis* than the first stage of *C. carnea* and consumed (437.01±1.66) first instars, (12.66±1.36) second instars, and (5.34±0.71) third instar of *P. solenopsis*, respectively in three days period before entering into prepupal stage (Table 1). Each instar took three days to enter into the next phase.

A single larva right from emerging from the egg up to entering into prepupal stage consumed on an average 866.67 first instar *P. solenopsis*. The mean per day consumption of successive larval instars of *C. carnea* on the 1st instars of *P. solenopsis* was (50.89±0.51), (92±0.33), and (145.67±0.66), respectively and significantly different from each other (DF= 2, 8; F=1852.394; P= 0.000). All larval instars of *C. carnea* exhibited less preference towards the second instars *P. solenopsis* as compared to first instar cotton mealybugs. The mean

per day consumption of successive larval stages of *C. carnea* on the second instar *P. solenopsis* was (1.22±0.192), (2.44±0.192), and (4.22±0.19) respectively and significantly different from each (DF= 2, 8; F=93.3; P= 0.000). All larval instars expressed extreme non-preference towards the third instar *P. solenopsis* and non-preference might be due to its bigger size and presence of waxy secretions on its body. The first instar did not at all fed on the third instar *P. solenopsis* due to its smaller size in comparison with it. The second instar consumed worth nothing and the third instar hardly consumed 1.78±0.51 third instar *P. solenopsis* per day. The mean per day consumption of successive larval stages of *Chrysoperla carnea* on third instars of *P. solenopsis* was (00), (0.33±0.192), and (1.78±0.51), respectively and significantly different from each other (DF= 2, 8; F=41.8; P= 0.0003). However, significant differences in feeding rate between 1st instars and 3rd instars and non-significant differences between 2nd and 3rd larval instars of *C. carnea* on 3rd instar of *P. solenopsis* were observed when means were compared through the least significance test (LSD) as shown in (Table 2).

The consumption rate of field captured *Chrysoperla carnea* larvae

The results of mean consumption rate of successive field captured larval stages of *Chrysoperla carnea* on successive instars of *P. solenopsis* were almost the same as those of laboratory-reared *Chrysoperla carnea* larva. The mean consumption rate of successive field-collected larval instars of *C. carnea* on first instars of *P. solenopsis* per day was (43.78±0.69), ((92.56±0.1.64) and (144.33±0.33), respectively and significantly different from each other (DF= 2, 8; F=15.500; P= 0.004). As such, the mean consumption rate of successive field captured larval instars of *C. carnea* on second instars of *P. solenopsis* per day was

(1.44±0.19), (2.33± 0) (4.11±0.69), respectively and significantly different from each other (DF= 2, 8; F=15.500; P= 0.004). The field captured first instar larvae of *C. carnea* also did not feed on third instars of *P. solenopsis*. The mean consumption rate of successive field captured larva of *C. carnea* on third instars of *P. solenopsis* per day recorded was (00), (0.33±0.192) and (1.44±0.19), respectively and significantly different from each other (DF= 2, 8; F=41.8; P= 0.0003). However, the (LSD) test at a 5% significance level found non-significance differences in consumption rate between the first and second stages of *C. carnea* on the third instars of *P. solenopsis* further justification is given in (Table 3).

Relative Comparison of consumption rate b/w laboratory-reared and field captured *Chrysoperla carnea* larva on *P. solenopsis*

The first stage of predator provided the 100 first stage, 200 to the second stage and 300 to the third stage of pest to both kinds of laboratory-reared and field captured

organisms from which, the lab-reared consumed (145.67±0.66) and field captured (144.33±0.33) with the highest pest consumption by the predator were observed under laboratory conditions. The significant differences (DF= 5, 17; F=2083; P= 0.000) in the consumption of mean rate between different stages of *C. carnea* larva on first instars of *P. solenopsis* were observed. However, non-significant differences between the same categories of *C. Carnea* except the first instar on the first instars of *P. solenopsis* were observed when means were subjected through the least significance test. In the same way, significant differences between different categories (DF= 5, 17; F=57.6; P= 0.000) and non-significant differences between the same categories on second instar mealybugs were observed when means were compared through the least significance test. Same results were obtained (DF= 5, 17; F=34; P= 0.000) on third instar *P. solenopsis* (Table 4).

Table 1. Three days mean consumption of different laboratory-reared larval instars of *C. carnea* on different instars of *P. solenopsis*

<i>Chrysoperla carnea</i> (stages)	<i>P. solenopsis</i> (instars)		
	First	Second	Third
First	152.67 ±1.56 ^c	3.66±1.43 ^c	0.00±0.00 ^b
Second	276.00±0.26 ^b	7.32±1.78 ^b	0.99±0.89 ^b
Third	437.01±1.66 ^a	12.66± 1.36 ^a	5.34±0.71 ^a

Means followed by the similar letters in columns are significantly different (P<0.05) using the LSD test

Table 2. Per day mean consumption of different laboratory-reared larval instars of *C. carnea* on different instars of *P. solenopsis*

<i>Chrysoperla carnea</i> (stages)	<i>P. solenopsis</i> (instars)		
	First	Second	Third
First	50.89±0.51 ^c	1.22±0.192 ^c	0.00±0.00 ^b
Second	92.00±0.33 ^b	2.44±0.192 ^b	0.33±0.192 ^b
Third	145.67±0.66 ^a	4.22±0.19 ^a	1.78±0.51 ^a

Means followed by similar letters in columns are significantly different (P<0.05) using the LSD test

Table 3. Per-day mean consumption different field captured larval instar of *C. carnea* on different instars of *P. solenopsis*

<i>Chrysoperla carnea</i> (stages)	<i>P. solenopsis</i> (instars)		
	First	Second	Third
First	43.78±0.69 ^c	1.44±0.19 ^c	0.00±0.00 ^b
Second	92.56±1.64 ^b	2.33±0.0 ^b	0.33±0.92 ^b
Third	144.33±0.33 ^a	4.11±0.69 ^a	1.44±0.19 ^a

Means followed by similar letters in columns are significantly different (P<0.05) using the LSD test

Table 4. Relative per day comparison between consumption potential of laboratory-reared and field captured larva of *C. carnea* on *P. solenopsis*

<i>P. solenopsis</i> (instars)	<i>Chrysoperla carnea</i> (stages)					
	Lab-reared 1 st stage	Field-captured 1 st stage	Lab-reared 2 nd stage	Field captured 2 nd stage	Lab-reared 3 rd stage	Field captured 3 rd stage
First	50.89±0.51 ^c	43.78±0.69 ^d	92.33±0.33 ^b	92.56±1.64 ^b	145.67±0.66 ^a	144.33±0.33 ^a
Second	1.22±0.192 ^c	1.44±0.19 ^c	2.44±0.19 ^b	2.33±0.00 ^b	4.22±0.19 ^a	4.11±0.69 ^a
Third	0.00	0.00	0.33±0.192 ^b	0.33±0.192 ^b	1.78±0.51 ^a	1.44±0.19 ^a

Means followed by similar letters in columns are significantly different (P<0.05) using the LSD test

Discussion

The results of this research study show that all *C. carnea* larval stages prey upon the different stages of instars of *P. solenopsis* and predatory performance of lava significantly increased with the advancement in the stage of larva and reached its maximum in the third stage. The various researchers like [29-33], have also documented the predatory performance of *C. carnea* increased with the advancement in the stages of larva, and the third larval stage consumed the maximum number of sucking pest species when used as prey. [34] their research findings also reported the 3rd larval stages of *C. carnea* consumed different nymph stages and the maximum number of *P. solenopsis* nymphs in comparison with the 1st and 2nd instar due to its increased nutritional requirements and bigger. In our research findings, all larval stages of *Chrysoperla carnea* expressed maximum preference towards 1st instar of cotton mealybug and consumed its maximum numbers in comparison with the 2nd and 3rd instar of *P. solenopsis* due to mobile in nature. On the first instar of *P. solenopsis*

the significant differences were observed in the consumption rate between the successive instars. The results of our research study are similar to the findings of [35], who documented significant differences in the rate of consumption between different larval stages of *C. carnea* on first instars of *P. solenopsis*. [34, 36] reported that different stages of *Chrysoperla carnea* preferred first instars of *P. solenopsis* to second and third instar.

The reason for the choice of 1st instar *P. solenopsis* by all larval stages of *Chrysoperla carnea* might be due to its smaller size and absence of waxy coating on its body and also its high mobility compared to the 2nd and 3rd instar whereas, *C. carnea* prefer to prey on fast-moving insects compared to slow ones [12]. Our findings are also in conformity with the hypothesis that predators mostly choose the stages of a host which could maximize their fitness for successful prey. Because of this the best control of *P. solenopsis* via this predator it would be better to release *C. carnea* in fields when cotton mealybug is in its first or second stage. The large-sized prey can be

overcome and weaken the predator species and may result in a reducing predator population, i.e., in coccinellid beetles that fed on soft-bodied insect species, their consumption rate ultimately decreased as their prey size increased. In anthocorid beetles, the consumption rate on prey also declined with the increase in the size of prey [37]. Thus; [38] observed the different insecticides against cotton mealybug as their effect on the natural enemies and [39] found residual impact under laboratory conditions [40] observed the varietal resistance of this invasive cotton mealybug pest. The results of the present research study showed non-significant differences in the rate of consumption between the same categories of laboratory-reared and field captured larval stages of *C. carnea* on the same stages of *P. solenopsis* (except first instar). The significant difference in consumption rate between laboratory-reared larvae and field captured first instar larva of *C. carnea* of the first instar *P. solenopsis* might be distance problematic since field captured first-stage larvae of *C. carnea* that have disturbed due to its smaller size while handling and bringing it from field to laboratory conditions.

Conclusion and Recommendations

The main motto of the present research study was to explore the food preference and predatory potential of *C. carnea* on *P. solenopsis* as a natural balancing ecosystem. The results of the research study revealed that *Chrysoperla carnea* under laboratory conditions can be easily mass-reared on cotton mealybug and best utilized for the efficient biological control of *P. solenopsis* in the IPM program. However, our research findings revealed that the third stage of *P. solenopsis* is least preferred by all the instars of *C. carnea*. Therefore, it is recommended that *Chrysoperla carnea* should be released in the field when *P. solenopsis* is in its first or second stage.

Authors contributions

The experiment designated and conceived: Bhugro M, Experiments performed: Shoib R, Kousar T, Data analyzed: HA Sahito, Contributed tools, materials, analyses: Pathan AK & Mastoi AH, Wrote the paper: Bhugro M & WM Mangrio.

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