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Functional responses of green lacewing *Chrysoperla nipponensis* (Neuroptera: Chrysopidae) reared on natural and herb based artificial diet

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Abstract
A functional response study of 3rd instar *Chrysoperla nipponensis* (Neuroptera: Chrysopidae) larvae reared on artificial diet and *Corcyra cephalonica* eggs was conducted. Different prey densities of aphid, *Aphis craccivora*, papaya mealybug, *Paracoccus marginatus* and whitefly, *Bemisia tabaci* were used. The larvae of *C. nipponensis* showed type II functional response to all prey species. The highest search rates (á) of larvae reared on artificial diet and *C. cephalonica* eggs were of 0.688 and 0.404 on mealybug and whitefly, respectively. Both, *C. cephalonica* eggs and artificial diet reared larvae showed maximum and minimum handling time on aphid (2.133 and 2.051) and mealybug (0.459 and 0.410), respectively. The maximum and minimum predation rate of artificial diet and *C. cephalonica* eggs reared larvae was recorded on aphids (0.487 and 0.468) and mealybug (2.433 and 2.177) respectively.

Keywords: *Chrysoperla nipponensis*, functional response, artificial diet, *Corcyra cephalonica*, prey densities

1. Introduction
The common green lacewing, *Chrysoperla carnea* (Stephens) is a generalist predator of a wide range of soft-bodied insect pests [1]. The predator voraciously feeds on insect pests such as aphids, mealybugs, whiteflies, thrips, leafhoppers, psyllids, caterpillars, insect eggs, mites and it is considered as an important component of Integrated Pest Management (IPM) program [2]. The predator has a high searching ability, easy to mass produce, wide geographical distribution and high tolerance to environmental factors [3]. In nature, the adult lacewing is non-predacious feeding on pollen, nectar and honeydew and has a high reproductive ability with long oviposition period [4].
Biological control is an effective means of controlling insect pests in the field of agriculture by the use of natural enemies because it is an environmentally sound and effective means of mitigating pest density [5, 6]. Recently, there has been increasing interest of growers to utilize this beneficial predator as an alternative to insecticides to manage insect pests of field and horticultural crops in IPM programs. The predation is assumed to be one of the significant biotic mortality factor reducing insect pest populations, and their use in insect pest management programs has received increased attention due to the current need to reduce the exclusive use of insecticides for pest control [7]. Due to growing economic and environmental concerns involved in the use of synthetic chemicals there is a dire need to develop an alternate measure for the sustainable management of small, soft-bodied, sap sucking insects that cause severe damage to various field crops, fruits and vegetables [8, 9].
Different bio-control agents are available for the management of insect pests. Among these, *C. carnea* is a beneficial predator because of its compatibility with a variety of environmental conditions, diversity of food and ability to hunt 80 species of pest. Under pesticide free conditions numerous species of predators attack mealybugs, (Hemiptera: Pseudococcidae) and can successfully regulate mealybug population [10-12]. The voracious predatory larvae feed on all immature stages of whiteflies and various aphid species, including *Myzus persicae* Sulze [13] and *Aphis glycines* Matsumura [14].
In Malaysia, the potential of Neotropical Chrysopidae predators especially green lace wing, *Chrysoperla nipponensis* in the pest management programs has not been prior evaluated. There is a lack of studies on the functional responses of *C.*

Nipponensis fed on natural and artificial diets that will provide necessary information for mass production and their effectiveness in IPM programs. Therefore, studies were planned to evaluate the functional response of *C. nipponensis* reared on artificial diet and rice moth, *Corcyra cephalonica* eggs against aphid, *Aphis craccivora*, mealybug, *Paracoccus marginatus* and whitefly, *Bemisia tabaci.* The expected results will be helpful to improve practical predictive powers and evaluation of *C. nipponensis* to develop pest management strategies based on biological control.

2. Materials and Methods

2.1 Collection and culture of insect preys

Insects used in this experiment i.e., *A. craccivora*, *P. marginatus* and *B. tabaci* were collected from the agricultural field of Universiti Putra Malaysia, Sri Serdang, Malaysia (3º02 N, 101 42 E, 31 m elevation) during 2014. The cultures of *A. craccivora*, *P. marginatus* and *B. tabaci* were maintained on long beans, *Vigna unguiculata*, pumpkin fruits, *Cucurbita moschata* and egg plants, *Solanum melongena* in an insectary.

2.2 Culture of green lacewing, *C. nipponensis*

The culture of *C. nipponensis* was maintained on eggs collected from the surrounding agricultural fields of Universiti Putra Malaysia. A colony of *C. nipponensis* was established using adults. Adult insects were reared in rectangular cages 37 x 28cm x 22cm in size. Rearing cages were covered with black cloth and adults were fed on artificial diet consisted of yeast + sugar + honey + casein and distilled water. The mixture forms a paste that was provided on plastic strips. After hatching, the larvae were reared separately in order to avoid cannibalism. The eggs laid by the females were collected daily.

2.3 Composition of larval artificial diet

Artificial diet for the larvae of *C. nipponensis* was prepared using different ingredients composed of 100 g ground beef liver, 100 g ground beef (with 25% fat), 15 g sucrose, 100 g hen's eggs, 10 ml distilled water, 20 g honey (5 g dissolved in 15 ml water), 14 g brewer's yeast, 5 ml acetic acid, 0.5 g potassium sorbate, 0.5g ginger, and 5 ml vitamin solution.

2.4 Preparation of larval artificial diet

All the ingredients used were weighed carefully by using Digital Analytical Balance (Sartorious, BT-224S, Germany). The ground beef and ground beef liver were cut in small pieces using knife and kept in a refrigerator for 24 hours. The mixture of meat, honey, water, preservatives (including antibiotics) and yeast were blended in a food processor (Panasonic, MK-5087M, Japan). In a beaker, 20 ml of water was heated at 80-90 °C on hot plate (IKA-COMBIMAG RCT 31197, China) and 15 gm sucrose, 5 ml of acetic acid and antibiotics were added and stirred with magnetic stirrer. Then 100 gm of blended eggs were added. All ingredients were blended (BRAUN, ZK-200, Germany) for 5-6 minutes until the entire mixture was of a stringy paste-like consistency and diet was ready to feed the larvae in trays of ELISA wells.

2.5 Rearing of host insect *C. cephalonica*

The culture of *C. cephalonica* (Stainton) was maintained on mixture of sterilized maize, rice, wheat and semolina (1:1:1:1) placed in plastic cages. Eggs of *C. cephalonica* were spread over the diet inside the cages to develop at adult stage that was collected for mating in a plastic cage. The eggs produced were collected in a glass plate, and placed in the freezer to exhaust egg viability and were provided as food for rearing *C. nipponensis*.

2.6 Experimental Procedures

The functional response of 3rd larval instars of *C. nipponensis* fed on artificial diet and *C. cephalonica* eggs to immature stages of *A. craccivora*, *P. marginatus* and *B. tabaci* were observed at 25±2 °C, 55-85% RH and 12L: 12D photoperiod. The different prey densities were 15, 30, 60, 90, 120 and 150 used in this experiment. The predatory larvae were starved for 12 hours before experiment then transferred to the experimental arena (9 cm diameter plastic Petri dish) for 24 hours using fine camel hair brush. The number of each prey consumed by the predatory larvae was recorded by counting the live preys. The experimental design was a Completely Randomized Design (CRD) with eight replications for each prey densities.

2.7 Data analysis

The functional response of predatory larvae reared on artificial diet and *C. cephalonica* eggs to prey densities was expressed by fitting the data to Holling's disc equation [15]:

$$Na = \acute{a}TN/ (1+aThN)$$

Where; Na is the number of prey consumed by the predator per time unit, á is the search rate of a predator, T is the total exposure time (1day), N is the original number of preys presented to every predatory larvae at the start of experiment, and Th defines handling time for each prey caught (proportion of the exposure time that a predator spends in identifying, pursuing, killing, consuming and digesting prey). The relationship between mean numbers of preys consumed versus the original number of preys presented to predatory larvae at the beginning of the experiment (prey consumed) / (prey density x 100) was also estimated. The obtained results were further fitted with regression lines using Statistical Analysis Software version 9.4 (SAS Institute Inc. 2014).

3. Results and Discussion

Figure 1 shows the functional response of third larval instars of *C. nipponensis* reared on artificial diet and *C. cephalonica* eggs (natural diet). The specifications concurred with the type II functional response that predator appeared towards varied densities of its preys which are determined by consumption of predator and handling time. Type II functional response is also the most commonly observed model in predators including insects [16, 17]. For the type II functional response, consumed prey is not density dependent (i.e. the intensity of consumed prey does not increase with prey density) [18]. The feeding of *Chrysoperla carnae* on *Heliothis virescens* eggs showed similar type of functional response [19]. The predatory larvae showed an increasing trend with increase in prey density, however, the instantly decreased at higher prey density that coincide with the findings of [20, 21]. The parameters recorded for the functional response are not accurate measurement by laboratory testing and could not be directly linked to the field conditions, because of the very low prey density or because most prey are already consumed, it is only useful to compare the effectiveness of natural enemies required as a bio-control agents [22, 23, 24]. In the laboratory conditions the search rate is limited by handling time that time need to capture and adsorb one prey whereas, in field it is limited to searching behavior. Therefore, the response of *C. nipponensis* may be different from its response in nature.

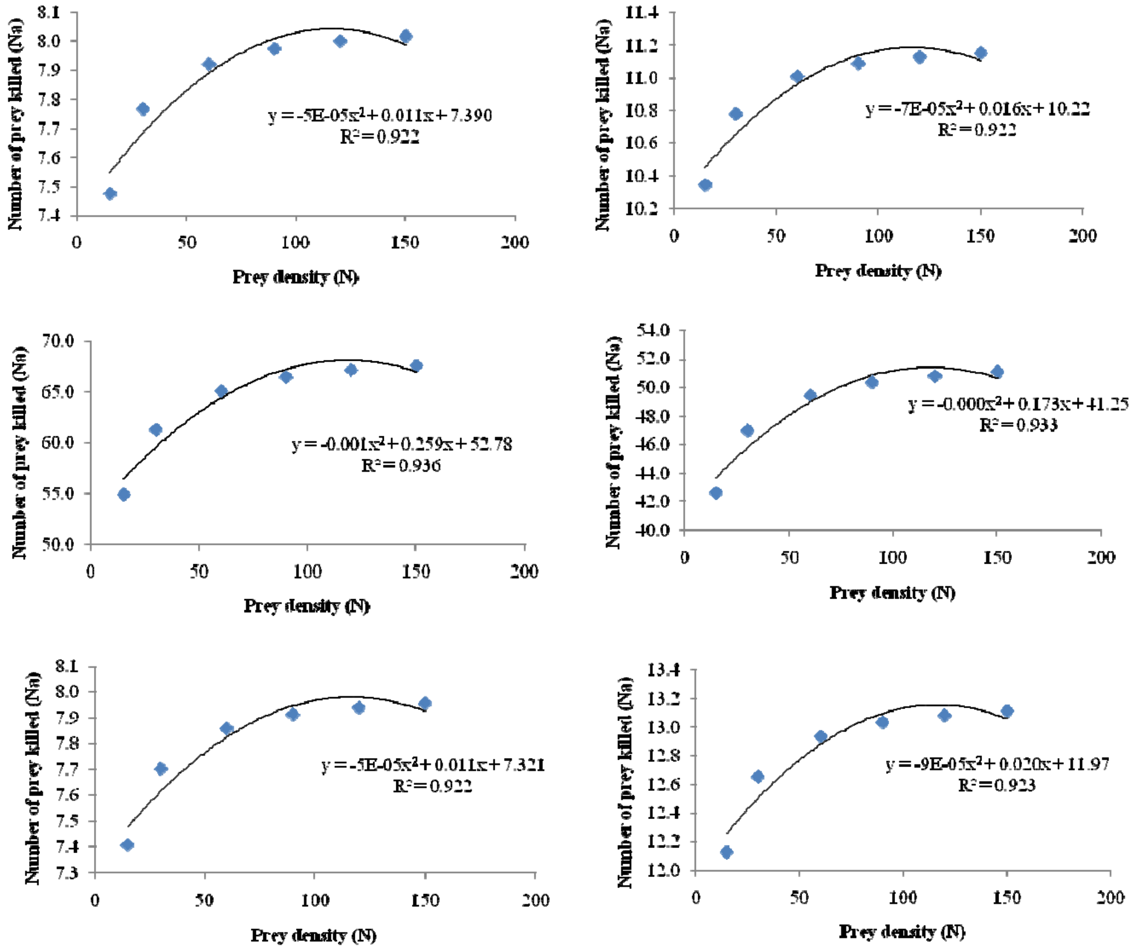

Fig 1: Type II functional response of artificial diet (left) and *C. cephalonica* eggs (right) reared *C. nipponensis* larvae to different densities of aphid *A. craccivora*, papaya mealybug *P. marginatus* and whitefly *B. tabaci* under laboratory conditions.

Results presented in Table 1 shows the functional response of third larval instar of *C. nipponensis* fed on artificial and natural diets at varied prey densities of aphids, mealybugs and whiteflies. Based upon the results, the highest and shortest search rate (á) of artificial diet and *C. cephalonica* eggs reared larvae were recorded on mealybugs (0.688 and 0.644) and aphids (0.370 and 0.358), respectively. The lowest handling time (Th) of 0.41 per prey by larvae reared on artificial diet was on mealybugs and highest of 2.13 when reared on eggs of *C. cephalonica* on aphid. The maximum and minimum predation rate of artificial diet and *C. cephalonica* eggs reared larvae were recorded on aphids (0.48 and 0.46) and mealybugs (2.43 and 2.17), respectively. The results obtained for search rate, handling time and predation rate of artificial diet and *C. cephalonica* reared larvae against aphids, mealybugs and whiteflies were fitted to second degree of polynomial curve. The R^2 values on artificial diet and *C. cephalonica* eggs reared larvae against aphids (0.922 and 0.922), mealybugs (0.934 and 0.933) and for whiteflies (0.922 and 0.923) were recorded respectively. The results revealed that there is no such major difference observed in artificial diet and *C. cephalonica* eggs reared larvae for different parameters to respective prey species. It showed the potential of larval artificial diet equivalent to the eggs of *C. cephalonica*. The change in the starvation level carries on secondary components affects the values of the attack rate and handling time [25]. Furthermore, increased convergence with prey was observed in starved larvae reared on artificial diet as compared to eggs of *C. cephalonica*.

Table 1: The rate of successful search (a), handling time (Th) and the maximum predation rate (1/Th) describing type II functional response parameters of *C. nipponensis* at different densities of preys reared on artificial diet and eggs of *C. cephalonica*.

	Aphid		Mealybug		Whitefly	
Third instar	**Artificial diet**	***C. cephalonica***	**Artificial diet**	***C. cephalonica***	**Artificial diet**	***C. cephalonica***
Search rate (a')	0.370	0.358	0.688	0.644	0.398	0.404
Handling time (Th)	2.051	2.133	0.410	0.459	1.854	1.814
Maximum predation rate (1/Th)	0.487	0.468	2.433	2.177	0.539	0.551
R2	0.922**	0.922**	0.934*	0.933*	0.922**	0.923*

*Significant at 0.05 level of probability
**Significant at 0.001 level of probability

Present study revealed that *C. nipponensis* reared on artificial diet showed potential to be used in efficient implementation of Integrated Pest Management (IPM) for the control soft bodied insect pests. These pests provided a good diet for the growth and development of *C. nipponensis*. It is suggested that interested in the control of soft-bodied insect pests like aphids, mealybugs and whiteflies should pay attention to this predator as bio-control agent in Malaysia and so enhancing the potential of natural enemies.

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