



*Original Article*

## The propensity of different larval stages of lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to control aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) evaluated on Canola *Brassica napus* L.

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### Abstract

Green lacewings (Neuroptera: Chrysopidae) are considered among the most effective generalist predators of aphids. In the present experimentation, the use of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars of the chrysopid *Chrysoperla carnea* (Stephens) larvae against aphids pest was investigated under field conditions in *Brassica napus* L. Four releases of predator's 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae were made from the time of aphid's appearance on canola crop till its maturity at fortnightly intervals. The influences on aphids due to the larvae of *C. carnea* predator were assessed by examining pest incidence and abundance of the natural enemies at plant growth stage, and seed yield recorded at crop harvest in the test field. Results indicated that predators, irrespective of their developmental stage, reacted very positively to their preys' reduction except in untreated control. Of the different larval stages tested, the applications of 1<sup>st</sup> instar followed by 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae were most effective in reducing aphids' population compared with untreated control. In the similar fashion, the releases of 1<sup>st</sup> and 2<sup>nd</sup> instar larvae of *C. carnea* were more effective in increasing crop yields compared with check treatment. Obviously, the applications of 1<sup>st</sup> and 2<sup>nd</sup> instar larvae of *C. carnea* involved efficiently in prey location and consumption, and performed predation activity for longer period (2-3 weeks). On the other hand, the release of 3<sup>rd</sup> instar larvae was too late to play a direct beneficial role in crop protection suggesting that they may have less time (1 week) to remain involved in efficient prey consumption. Further, 1<sup>st</sup> or 2<sup>nd</sup> instar larvae may be much hungrier and eaten more pests in study areas without resting or moving to new location and thus can potentially be used to enhance biological control of aphids.

**Keywords:** aphid, plant protection, insect pest, canola, rape, lacewing

### 1. Introduction

Canola refers to the cultivars of either rapeseed (*Brassica napus* L.) or field mustard (*Brassica campestris* L. or *Brassica rapa* L.) in the family Brassicaceae. Its seeds are used to produce edible oil suitable for consumption by humans and livestock (Dupont *et al.*, 1989). Canola crop is attacked by a number of insect pests of which aphids are very important. The green peach aphids *Myzus persicae*

(Sulzer) (Homoptera: Aphididae) tend to feed on the underside of the canola. The green peach aphids feed by sucking sap from their hosts, and the seedlings of crops can be stunted by the attack of large population of this species. This aphid vectors virus diseases in more than 30 plant families, including *Brassica* sp., (Hill, 1983). It can attain very high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant. Prolonged aphids infestation can cause appreciable reduction in the yield of crop (Sarwar, 2011). Contamination of harvestable plant material with aphids or with aphid's honeydew also causes losses. Aphids are inherently difficult to kill with contact insecticides because they are often under the leaves

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or on new sheltered plant growth. Even systemic insecticides, which can kill aphids feeding under the leaf when applied to the upper surface, are much less effective at cool temperature (McLeod, 1991). Use of chemicals has so far been considered the most effective means of control of the pests. Since the use of pesticides is wrought with several disadvantages, a biological control program based on integrated pest management is a more rational strategy (Ahmad *et al.*, 2011). Biological control is a method of controlling pests through the use of natural enemies in agriculture that is an environmentally sound and effective means of mitigating pest density (Sarwar *et al.*, 2012; Sarwar, 2013 a; Sarwar, 2013 b; Sarwar, 2014).

The species in genus *Chrysoperla* have long been considered the most important naturally occurring predators in many cropping systems, including vegetables, fruits, nuts, fiber and forage crops, ornamentals, greenhouse crops, and forests. Worldwide, they are also ranked as some of the most commonly used and commercially available natural enemies. Their larva, commonly called an aphidlion, is a voracious feeder and can consume up to 200 aphids or other prey per week. In addition to aphids, it can eat mites and insect pests including thrips, mealybugs, immature whiteflies, small caterpillars and insects' eggs (Tauber *et al.*, 2000). Lacewing larvae are also known to feed on a wide variety of other soft-bodied arthropods including a lot of different aphid species and spiders by attacking prey and sucking out their body fluids. It is a voracious feeder on first instar nymphs of mealybug, *Phenacoccus solenopsis* Tinsley (Khan *et al.*, 2012). The use of lacewings to control arthropod pests has been reported for several crops, worldwide (Canard *et al.*, 1984). According to Kannan (1999), natural enemies encountered preying on aphids were chrysopids, coccinellids and syrphids, the first of these being the most important and dominant predators. A number of studies have demonstrated the role of lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) releases to enhance biological control of aphids (Sarwar *et al.*, 2011; Sarwar, 2013 c). Messina and Sorenson (2001) reported that lacewings reduced the aphid population on some plants and their effectiveness was 84%. These studies have shown that feeding and deployment of lacewing for the manipulation of its populations as aphid predator is currently used in integrated management of this pest.

Biological control by the use of predator *C. carnea* has also gained importance for pest management in Pakistan. Some recent studies provide a crucial example of release sites for lacewings against *Bemisia tabaci* (Genn.) in cotton (Zia *et al.*, 2008), to manage the population of aphids on wheat (Iqbal *et al.*, 2008) and to control *Helicoverpa armigera* (Hubner) in tomato (Usman *et al.*, 2012). Results depicted that *C. carnea* can be used as an effective biological control agent for successful implementation of integrated pest management program to reduce the use of insecticides and save foreign exchange spent on pesticides import. The efficiency of lacewing to control pests can be affected by many

factors, including use of different predator instars which may be a crucial factor in the success of augmentative biological control. Evaluation of *C. carnea* releases in the field showed that the releases of its larvae had better survival compared to the releases in egg form. For example, releases of second-instar larvae have proven very successful for the control of the green peach aphid in peppers, tomato and eggplant (Tauber *et al.*, 2000). Lacewings commonly are sold and dispensed as eggs or adults, however, larval releases may sometimes be more effective. Eggs are less reliable for releases and some early releases did not hatch, probably because of poor weather conditions. Further, the eggs may not hatch if weather is extremely cold or hot at faster rates. At this moment, it is crucial to evaluate the biological and economic advantages of releasing one or the other developmental stages of *C. carnea* to devise efficient methods for introducing the role of various larval stages. Keeping in view the above facts, field studies were conducted to evaluate the field efficacy of different instar larvae of *C. carnea* against aphids infesting canola plant.

## 2. Materials and Methods

These experiments were conducted in the research area at Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan, which is an establishment of Pakistan Atomic Energy Commission.

### 2.1 Rearing of *Chrysoperla carnea*

The colony of *C. carnea* was primarily established in laboratory from eggs, larvae or adults of predator collected from the field and placed in the climatic control room at  $27 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  R.H., at Nuclear Institute of Agriculture, Tandojam. Adults of *C. carnea* were reared in plastic rearing cages feeding on semisolid artificial diet containing yeast, honey and water (1: 1: 1). For rearing of larvae, medium sized (500 mg) gelatin capsules were used as test tube for culturing individually to avoid cannibalism and eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) offered as diet for *C. carnea* immatures.

### 2.2 Experimental technique

The crop *Brassica napus* L. variety 'Rainbow' was sown under field conditions in November 2009. Experiment for the release of *C. carnea* larvae was laid out in Randomized Complete Block Design (RCBD). There were four treatments including control and the treatments were replicated three times. The distance from one experimental field to another was 30 m. Each field was divided into four divisions denoting different treatments. The size of each division in each field was  $2.5 \text{ m}^2$ . The fourth division of each field of field grown cultivar was kept as check. All the standard agronomic practices were followed to raise crop (use of nitrogen and phosphorus fertilizers, plant spaced 30 cm apart, irrigation

done thrice, and no use of synthetic or natural pesticides). Meteorological data (temperature and relative humidity) were also recorded during the observation period.

Same age *C. carnea* larvae (mixed populations of males and females) were released in each replication in canola crop, starting from February to March. Four releases of predator's 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae were made from the time of aphid appearance on canola plant till crop maturity at fortnightly intervals. The predator releases were started against pest ravage early in the flowering season when aphids were present on crop (by counting numbers of pest when there was at least one aphid per leaf) to obtain maximum benefits of pest control. Lacewing larvae were shipped to the field in an inert medium carrier (rice hulls) to provide separation, facilitate the proper placement, and uniform field distribution of the predator. For avoiding larval cannibalism, they were kept separately during transit and provided with food comprising eggs of angoumois grain moth *S. cerealella*. Lacewing larvae were released at a rate of 5,000 immatures per acre with a two week intervals to establish a colony in the field. To release 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae of predator, the contents of the container were simply sprinkled over the pest infested crop or where aphids were most likely to make an appearance. One by one, or row by row, the pre-hatched larvae were taped out onto the infested plants' foliage, to insure their dispersal evenly among the infested crops, and avoiding placing predators too close to one another to prevent cannibalism. The influence on aphids due to their *C. carnea* predators was assessed by examining pest incidence and effectiveness of the natural enemies in the test field. The data regarding population monitoring of *C. carnea* were collected by observing and counting its eggs, larvae and adults 7 days after each predator releases. On each replicate of a treatment, five random samples of plants were examined to record predator populations. To examine the performance of predator, five random plants were selected from each treatment, and aphid population was counted on a per plant basis 7 days after each treatment. The data on grain yield were observed at the harvesting time to determine the role of predators in suppressing pests and enhancing yield of crop. The data were then converted into per hectare basis with the formula = Yield per plot  $\times$  10,000/ Plot size.

### 2.3 Statistical technique

The data were analyzed by analysis of variance (ANOVA) and means were separated with least significant difference (LSD) at 0.05% level of significance by using computer software Statistix 8.1.

## 3. Results and Discussion

The green peach aphid *Myzus persicae* was the most abundant pest species in canola at flowering and podding growth stages of crop. There were no other insect pests upon which *C. carnea* can feed on during the experiment beside

aphid pest. Field releases of *C. carnea* showed significant reduction in aphid population in predator-treated areas. The overall analysis of data showed that predator population in all the treatments differed significantly ( $P= 0.05$ ) from one another. The untreated plants were severely damaged by aphids, as evidenced by appearance of sooty mold, leaf yellowing and leaf dropping at the end of the experiment. The mean meteorological data during the whole period of observations were temperature 13.5°C (minimum) and 28.5°C (maximum), and relative humidity 75.5%. Apparently, meteorological conditions under field experiment throughout the study period might be associated with rise and fall in population of aphids and predation potential of *C. carnea*.

### 3.1 Populations of *Chrysoperla carnea* eggs, larvae and adults

Mean populations of *C. carnea* (eggs, larvae and adults) were found to be significant by differences in different treatments (Table 1). Of the different larval stages tested, significantly the best results on predator population occurrence were achieved with biologically managed crop compared with untreated control. On all treatments, population of *C. carnea* developed, but maximum egg abundance was recorded in crop which was inoculated with 1<sup>st</sup> instar practiced (1.66 eggs/plant). It was statistically dissimilar to applications of 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae (1.22 and 0.66 eggs/plant, respectively), while, eggs number was significantly lower in the control, where no release was made (0.11 eggs/plant). Treatments receiving 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars of predator larvae had significantly higher larval population of *C. carnea* (1.55, 1.11 and 0.55, respectively). In contrast, mean population of predator larvae was significantly lower in control canola field (0.11). Maximum numbers of adult *C. carnea* were recorded in application of 1<sup>st</sup> instar larvae (0.88) followed by 2<sup>nd</sup> and 3<sup>rd</sup> instars (0.36 and 0.18, respectively) compared with non released crop differing significantly from one another ( $P= 0.05$ ).

### 3.2 Populations of aphid and grain yield

The data given in Table 2 show aphid populations recorded in different treatments; almost all predator released treatments gave good pest control, but not the untreated crop. The mean population data, however, showed that treatments differed significantly from one another ( $P=0.05$ ). Significantly maximum decline in aphid populations over control (74.16 aphids per plant) was noted where applications of 1<sup>st</sup> and 2<sup>nd</sup> instar larvae followed by inoculation with 3<sup>rd</sup> instar of *C. carnea* were done (34.50, 47.25 and 60.16 aphids per plant, respectively). The pod yield or grain yield parameter showed significant variations in canola treatments ( $P=0.05$ ). Of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars of *C. carnea* released., 1<sup>st</sup> instar proved the most effective treatment resulting in significant increase in grain yield (783.33, 641.67 and 570.00 g per 2.5 m<sup>2</sup>, respectively) over control (503.33 g per 2.5 m<sup>2</sup>). Increase in

Table 1. Populations of different life stages of *Chrysoperla carnea* on Canola *Brassica napus* L.

S. No.	Treatments	<i>C. carnea</i> Eggs/plant	<i>C. carnea</i> Larvae/plant	<i>C. carnea</i> Adults/plant
1.	1 <sup>st</sup> instar <i>C. carnea</i> larvae applied	1.66 a	1.55 a	0.88 a
2.	2 <sup>nd</sup> instar <i>C. carnea</i> larvae applied	1.22 b	1.11 b	0.36 b
3.	3 <sup>rd</sup> instar <i>C. carnea</i> larvae applied	0.66 c	0.55 c	0.18 b
4.	Control	0.11 d	0.11 d	0.00
LSD Value		0.367	0.439	0.494

Means sharing similar letters are not significantly different by LSD at P=0.05

Table 2. The propensity of different larval stages of *Chrysoperla carnea* to control aphids on Canola *Brassica napus* L.

S. No.	Treatments	Aphids population/ plant	Yield per 2.5 m <sup>2</sup> (gm)	Yield per hectare (Kg)
1.	1 <sup>st</sup> instar <i>C. carnea</i> larvae applied	34.50 d	783.33 a	3133.00
2.	2 <sup>nd</sup> instar <i>C. carnea</i> larvae applied	47.25 c	641.67 b	2566.00
3.	3 <sup>rd</sup> instar <i>C. carnea</i> larvae applied	60.16 b	570.00 c	2280.00
4.	Control	74.16 a	503.33 d	2013.00
LSD Value		9.288	49.164	

Means sharing dissimilar letters are significantly different by LSD at P= 0.05

crop yield contained by predator applied areas was due to a direct beneficial role played by larvae of *C. carnea* in pest suppression and crop protection.

In the lacewings released fields, adult predator was recorded in higher numbers than in the non-released check. Likewise, *C. carnea* eggs and larvae were also observed in abundance in the predator released fields. The development of the aphid populations was variable both in the released and non-released fields because *C. carnea* larvae fed to the maximum on pests before they become adults. However, the highest number of aphids per plant was recorded in the non-predator released crop areas. Of the different larval stages tested, the applications of 1<sup>st</sup> instar followed by 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae were effective in reducing aphid populations and increasing yield compared with untreated control. Clearly, 1<sup>st</sup> and 2<sup>nd</sup> instar larvae of *C. carnea* were efficient in prey location and consumption, and performed predation activity well for longer period (2-3 weeks). On the other hand, the release of 3<sup>rd</sup> instar larvae was too late to play a direct beneficial role in crop protection suggesting that they may have less time (1 week) to remain involved in efficient prey consumption. Further, 1<sup>st</sup> or 2<sup>nd</sup> instar larvae may be extra hungrier and thus eaten more pests in treated areas without resting or moving to a new location, so can be potentially used to enhance biological control of aphids as compared to 3<sup>rd</sup> instar.

Undoubtedly, during the current findings, *C. carnea* larvae proved one of the fastest predators available, and their

release on plant foliage enhanced their survival. Also, the lacewing larvae needed less protection than eggs from predation by certain other arthropod predators. Other predators are able to be attracted to the eggs on the card to eat prey and generally interfere with biological control. The reasons that why pre-hatched larvae of *C. carnea* are the best form of green lacewing for pest control may be because unlike adult predators, they cannot fly away, and thus, can walk for long distance looking for pests and pest eggs. Unlike eggs, pre-hatched larvae are big and strong enough to live for some days and can walk less or more to find food. Unlike putting out lacewing eggs, the pre-hatched larvae are further able to fend off other predators and may survive well in bad weather. Hence, larval releases of lacewings may be more effective in comparison to eggs or adults to decrease aphid's intensity. Gautam and Tesfaye (2002) observed that the predatory potential of the predator *C. carnea* was high in the older instars than to the younger ones. The predation efficiency of *C. carnea* from 1<sup>st</sup> to 3<sup>rd</sup> instars increased tremendously (Sattar *et al.*, 2007). But, these evidences are mainly from closed cage laboratory studies. However, the remaining time to pupate, handle prey and feed period were some what lower for the third instar than first instar larvae. On the other hand, there was higher prey consumption duration from the younger stage from hatching to pupae formation resulting huge amount of prey devoured. However, lower or higher temperature may have more negative effect on survival of first instar *C. carnea* compared with the effect on third instar

larvae. Further, second and third instar *C. carnea* larvae may show better survival at fluctuating temperature compared with first instar.

The survey of scientific literature on the use of different larval stages of *C. carnea* has not enough analogous data available; hence, it is hard to relate the results of present work with any other studies carried out elsewhere. However, the present findings can be compared with the previous work conducted by many workers who stated that applications of biological agents have the potential to control aphids. Pari *et al.*, (1993) released *C. carnea* against infestation of aphids (*Macrosiphum euphorbiae* and *Chaetosiphon fragaefolii*) at a density of at least 20 larvae/ linear m of each paired row and achieved satisfactory pest control results. Hemagirish *et al.*, (2001) with four releases of *C. carnea* in the field in equal installments at fortnightly intervals got satisfactory control of aphid. Ashfaq *et al.*, (2007) studied the efficiency of *C. carnea* against aphid *Brevicoryne brassicae* infesting canola oil seed crop using treatments comprising release rates of eggs. The egg release rates showed significant effects on aphid population reduction and the treatments were statistically different from one another. Farooq and Tasawar (2008) evaluated various measures for the control of canola pests, *Brevicoryne brassicae* and *Lipaphis erysimi*. The efficiencies of the predators *Coccinella septempunctata* and *C. carnea* when released singly or released together was increased as the releasing period was prolonged and yields were significantly affected in all the treatments. Similar observations were also made in the present findings where release of *C. carnea* showed some significant control of aphids. However, repeated applications of biological agents can produce significant results rather than single release of predators.

This study further revealed that the release of predator not only showed significant control of aphids, but also increased crop yield as compared to control treatment. The conclusion drawn from the results are that the utilization of biological control agents were proved to be an admirable strategy in an aphids pest management, exerting a direct beneficial role in crop protection ultimately increasing amount of seeds produced. These findings are in consistent with those of Iqbal *et al.* (2008) who also reported similar findings. Releases of *C. carnea* larvae may be more effective and larvae releases were superior to egg release for control of the pest and increasing grain yield. The new advances in insectaries for production and dispensing systems of *C. carnea*, may improve the economics of commercial releases of larvae. The predator can provide better control when the plant's leaves are in contact with adjacent plants; so that contiguous leaf contact improves lacewing dispersal and perhaps might decrease opportunities for cannibalism. It is suggested that 1<sup>st</sup> instar larvae of *C. carnea* may be effective in reduction of aphid density, if this treatment is repeated after every fortnightly interval. Clearly, more field tests using commercially feasible release rates are necessary.

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