First Attempt to Develop a Rearing Method for the Native Green Lacewing *Chrysoperla lucasina* in Tunisia

Wejdène Karouia, LR / Bioagresseurs et Protection Intégrée en Agriculture (LR14AGR02), INAT, 43 Av. Charles Nicolle, Cité Mahrajène, 1082, Tunis, Université de Carthage, Tunisia, **Faten Hamdi,** ControlMed, Rue Ibn Batouta, Résidence La Colombe, 2015, Le Kram, Tunis, Tunisia, **Synda Boulahia-Kheder,** LR / Bioagresseurs et Protection Intégrée en Agriculture (LR14AGR02), INAT, 43 Av. Charles Nicolle, Cité Mahrajène, 1082, Tunis, Université de Carthage, Tunisia

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ABSTRACT

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Green lacewing (*Chrysoperla lucasina*) is well known for its polyphagous predator larvae which are commonly used in pest's biological control worldwide. In Tunisia, this auxiliary occurs in the nature in association with several pests but it has never been mass reared and released in biological control programs. Hence, the chrysopid species *C. lucasina* was chosen for an attempt of rearing in order to strengthen its natural populations. The study was carried out in the insectarium of the company ControlMed specialized in *Trichogramma* parasitoid production. All equipment used for green lacewing larvae and adult rearing was newly designed for this study. Two diets were given to larvae and adults to assess their biological performances. The first diet was based on pollen for adults and eggs of *Ephestia kuehniella* for larvae. The second diet consisting of a pasty mixture of honey, yeast and eggs of *E. kuehniella*, was supplied to adults and larvae. The laboratory rearing conditions were kept constant with 26 ± 2°C, 65 ± 5% RH and a photoperiod of 16L: 8D. The diets based on pollen and *E. kuehniella* eggs gave the best biological parameters for both adults and larvae. This study which allowed to design a simple technique to rear green lacewing based on affordable material, is a first step for developing a mass rearing of chrysopids in Tunisia. However, further improvements are required for adult and larvae diets to increase reproductive and developmental performance, as well as an economic feasibility study.

Keywords: Artificial diets, biological performances, *Chrysoperla lucasina*, green lacewing, low-cost material, insect rearing

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In agroecosystems of temperate regions, the larvae of lacewings *Chrysoperla* spp. are well-known as effective biological control agents of a wide variety of pests such as aphids, and other small soft-bodied arthropods as well as lepidopteran eggs and larvae (Devetak and Klokokovnic 2016; Huang and Enkegaard 2010; Loru et al. 2014; Rosenheim et al. 1999; Villenave-Chasset 2007). Adults of the genus *Chrysoperla* Steinman (1964) are palyno-glycophagous feeding on sugar-rich food such as honeydew, nectar and plant fluids combined with pollen from a wide range

Corresponding author: Wejdène Karouia
Email: karouia.wejdene@gmail.com

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of plant families (Devetak and Klokokovnic 2016). Among the most common species of the *Chrysoperla* complex occurring in the vicinity of crops, are *Chrysoperla carnea,* and *Chrysoperla lucasina* (Villenave-Chasset et al. 2006).

Identification and genetic structure of lacewing species are important issues for mass rearing and biological control purpose (Lavagnini et al. 2015). However, identification of the cryptic species of the *carnea* complex is sometimes problematic based on the morphology (Canard et al. 2007; Henry et al. 2002; Tauber et al. 2000).

The species *C. lucasina* (Lacroix) (Neuroptera: Chrysopidae), green lacewing, is often dominant in Mediterranean region (Thierry et al. 1996). In Tunisia, it is relevant in fruit and citrus orchards and as predator of several pests such as mealybugs, citrus leafminer and whiteflies (Boukhris-Bouhachem 2011). Fortunately, *C. lucasina* has characteristics making it reliably identifiable (Henry et al. 1996). It remains green during winter diapause while the other species change to brownish or reddish (Henry et al. 2002).

The green lacewing is a good candidate as biological control agent and its predation performance could be improved by several approaches. For example, biological conservation control by supplying supplemental food (Messelink et al. 2016) or introduction of certain plants could enhance chrysopid regulation action. Indeed, *C. lucasina* adults were mostly attracted by the Brassicaceae *Capsella bursapastoris* and *Biscutella auriculata* as well as the Asteraceae *Anthemis arvensis,* which could be introduced to enhance predation (Alcala Herrera et al. 2020).

The second approach is the classical biological control by mass rearing and releasing of biological control agents (Pappas et al. 2011). *C. lucasina*, *C. carnea* and *C. externa* are among the most studied species for this use (Bezerra et al. 2016; Pappas et al. 2011). Several mass rearing approaches of chrysopids have been developed mostly based on factitious preys such as *Ephestia kuhniella* or *Sitotroga cerealella* (Albuquerque et al. 1994; Haramboure et al. 2016). However, this type of rearing is very costly due to the production cost of factitious preys, that makes it difficult to sustain for a wide agricultural use. Several artificial diets were tested in combination or not with factitious preys to reduce the cost of prey production with various results (Haramboure et al. 2016; Jokar and Zarabi 2014; Polak et al. 1998; Ye et al. 2017). The genus *Chrysoperla* became commercially available since the 90's in the USA, Europe and China (Loru et al. 2014) with *C. carnea* and *C. externa* sold in Europe, Latin America and Asia (Tauber et al. 2000). However, in the Maghreb region (North Africa), Chrysopidae have been little studied for mass rearing to control crop pests (Limem-Sellami and Chermiti 2015). In Tunisia, so far, no specific action has been conducted to enhance local lacewing populations. Mass rearing of lacewings has never been achieved, while in the citrus agroecosystems of Cap Bon, several species were identified as autochthon natural enemies. The species *C. lucasina*, *C. carnea* and *C. affinis* were the most frequent in Tunisian citrus orchards with 53%, 24% and 18% respectively. However, lacewings were generally present with low frequency (Limem-Sellami 2017). This can be explained by the overuse of broad-spectrum insecticides against citrus pests. For example, neonicotinoid insecticides which are very commonly sprayed against aphids, are very likely to affect populations of *C. carnea* (Rogers et al. 2007).

In order to reduce pesticide use to control crop pests, various environmentally friendly methods are increasingly used or are under investigation in fruit orchards in Tunisia (Bachrouch et al. 2008; Boulahia-Kheder et al. 2012). In a framework of promoting conservation biological control, our study aimed to develop for the first time in Tunisia, an affordable and easy approach to rear the native green lacewing *C. lucasina* using a factitious prey and other nutritional supplements, to enhance its natural populations. This could be a first step for a mass rearing of green lacewings in Tunisia. In this paper we describe (1) the device designed to rear larvae and adults as an outcome, and report (2) the biological performances of two diets supplied to larvae and adults.

MATERIALS AND METHODS

Study site.

The study was carried out in the insectarium of a private company (ControlMed) specialized in the mass-rearing of beneficial insects for biological control of crop pests. Currently, this company which is located at Sidi-Thabet region (36º 54′ 31″ North, 10º 02′ 33″ East) (Governorate of Ariana) mainly produces the parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) used for the biological control of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) in pomegranate orchards. In this study, we aimed to optimize the exploitation of the eggs of *E. kuehniella* as food already produced for *T. cacoeciae* rearing.

Collection and identification of green lacewing adults.

The first step to start the rearing process, was the collection of adults of chrysopids. Adults have been collected using an aerial net and a small aspirator, mainly from pear, peach and citrus in six orchards located in Sidi-Thabet (36° 54′ 31″ N, 10° 02′ 33″ E) during the period 23/05- to 05/07-2017. The summer season was favorable for the availability of green lacewing associated with *Myzus persicae* aphids and citrus leafminer *Phyllocnistis citrella* populations. Taxonomic identification of collected adults was achieved using a binocular microscope based on the keys of Henry et al. (1996) and Mazel et al. (2006).

Rearing conditions and diets.

After being identified, the adults were maintained in a climatic chamber at 26 ± 2°C temperature, 65 ± 5% relative humidity and 16:8 h light:dark photoperiod, as recommended in Loru et al. (2014).

Six adult units were performed according to Loru et al. (2014) with some modifications. A 20 cm high and 9 cm diameter cylinder made from a plastic water bottle was used. The upper opening was closed with tulle mesh and secured with rubber bands. The unit was kept vertically fixed on the closed end (Fig. 1A). Dark Bristol paper or Kraft paper (10 cm/10 cm) cardboard were accordion fold lined in the inner part of the cylinder and fixed by clips to play the role of a laying substrate. Food was presented in a half Petri dish (90 cm diameter × 1.5 cm height) and water was supplied by damping cotton in distilled water with making sure that water did not drip down (Fig. 1A). The sex ratio was 1:1 as recommended in Mudassar et al. (2013) so each unit hosted 3 couples of green lacewings. The selection of males and females for mating is not recommended for a mass-rearing as it could affect genetic variability. However, we did it because our goal was mainly to test the experimental device.

The eggs laid on Bristol paper in each adult unit were transferred in 6 larval units daily. Each larval unit contained eggs laid on the same day from the same adult unit. Then the newborn larvae were placed in appropriate units, until adult stage. Each larval stage (1st, 2nd, or 3rd) was placed in separated rearing units to avoid cannibalism. During larval development, each stage duration (days) was recorded. The larval rearing unit was a 16.4 cm × 6.5 cm rectangular transparent plastic food box with a plastic lid, available in supermarkets (Fig. 1B). Tiny holes have been made for the ventilation of rearing units. After emergence, the new adults were sexed based on the genitalia and put into the adult's containers.

In this study, the rearing units used for adults and larvae were very small for mass-rearing (Fig. 1). However, they could give the first guidelines for a larger rearing.

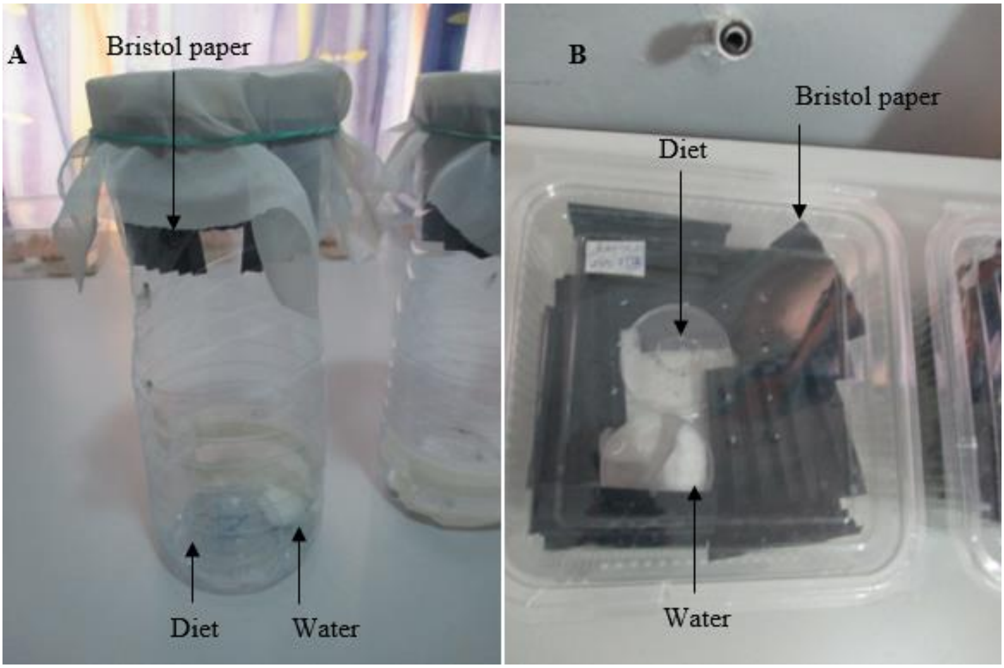

Fig. 1. Rearing units of green lacewing (*Chrysoperla lucasina*): For adults (A) and for larvae (B).

Rearing units for adults and larvae, Petri dishes and the climatic chamber have been cleaned regularly to avoid any type of contamination (mites, fungi, bacteria). Rearing units were serviced three times a week while Petri dishes and the climatic chamber were cleaned every day. The paper towels were changed daily and the pollen was renewed twice a week. The cotton was refilled with distilled water or renewed every day.

Two diets were given to adults and two to larvae (Table 1). Adults collected from orchards (F_0) were divided into two groups, one fed with the diet D_1 which was pure multi-floral honeybee pollen (Royaume des abeilles®, origin: Tunisia) and the second group was fed with the diet D_2 which is a pasty mixture of multi-flowered honey + beer yeast (Phytothera®) + *E. kuehniella*'s eggs (Table 1). This diet was obtained by mixing the 3 ingredients manually with a spoon and was preserved 7 days in a refrigerator at 4°C. The *E. kuehniella*'s eggs were provided by the hosting company which produces them throughout the year to rear *T. cacoeciae.* The larvae originated from the adult green lacewing fed with pollen, were supplied with eggs of *E. kuehniella* (diet D_3) until they completed their development (Table 1). The adults (F_1) were supplied with pollen and their progeny with eggs of *E. kuehniella* until they reached the adult stage (F_2).

The larvae originated from the adults fed with the diet D_2, were supplied with the same diet (D_2) until they finished their development. The obtained adults (F_1) were fed again with D_2.

For each diet, 3 replicates were carried out. Then, performances of the adults and larvae were compared for each diet in order to determine the best one for both stages. The evaluated parameters are those of the generation (F_1).

Table 1. Composition of the 3 diets (D_1, D_2, and D_3) used for rearing of *Chrysoperla lucasina*

Adults		**Larvae**
D_1	**D_2**	**D_3**
Honeybee pollen (2 g)	• 0.03 g beer yeast • 0.03 g *Ephestia kuehniella*'s eggs • 0.3 g honey	*Ephestia kuehniella*'s eggs (0.5 g)

Biological parameters assessed.

For all stages of development of the green lacewing, some biological parameters were evaluated. The performances resulted from each diet for both adults and larvae, were evaluated and compared. For adults (F_1), the parameters recorded were: longevity (days), mortality rate ((number of dead adults/total number of adults) × 100) (per week), duration of development time from eggs to adults (days), without distinguishing sexes, and daily fecundity per female (mean number of eggs laid/day), total fecundity per female (average daily fecundity × longevity).

Egg fertility was estimated each day by collecting and counting eggs laid from the adult units, and then transferring them to the larval units. On each unit, a label indicated the origin of eggs and their laying date. Thus operating, we followed the development of each larvae and evaluated the biological parameters. The parameters recorded for eggs and larvae were the fertility, the pupation rate (percentage of larvae reaching the pupal stage) and the adult emergence rate (percentage of pupae reaching the adult stage). The statistical analysis was performed by using the XLSTAT software of Microsoft Excel. This program was adopted for analysis of variance (ANOVA) between the tested diets.

Predation test.

Preliminary tests were carried out to assess the predatory capacity of green lacewing larvae reared under controlled conditions. The objective was to determine whether larvae fed on factitious diets alone (eggs of *E. kuehniella)* would easily accept natural preys. For this, first and third instars larvae of green lacewing were exposed to one kind of prey in a Petri dish (90 cm diameter × 1.5 cm height): aphid *M. persicae* adults or citrus leafminer *P. citrella* larvae. Both of these preys were available in the orchards during the study period. The test was carried out on 4 young larvae (2 replicates) and 6 older ones (3 replicates) and the total number of preys consumed by larvae was counted.

RESULTS

Design of the green lacewing rearing device.

The green lacewing species collected for rearing was identified as *C. lucasina*. In our study, the experimental device setup for rearing green lacewing was designed using inexpensive equipment available in Tunisia (see Material and methods). In our rearing device, some strengths and weaknesses can be highlighted.

The most interesting findings are the ease of design and low cost of adult and larva rearing units. The major difficulty was avoiding larval cannibalism which increased with lack of food, number of larvae per unit and temperature. Thus, it would be more appropriate to use larger equipment for mass rearing. This is also suggested for adult units to help feed insects and facilitate the cleaning of units, while adults are flying.

Regarding the diets for adults, the diet D_1 composed of honeybee pollen was appreciated and did not pose issues of cleanness. However, the diet D_2 where one of the ingredients is honey, required frequent cleaning. It caused fouling of adult’s wings, legs and abdomen. So, insects became unable to move and this can lead to adult death. For larvae, the same problem was faced with the diet D_2.

Biological performance according to diet.

Based on the biological parameters evaluated for one generation of green lacewing, the diets that produce the best results were D_1 for adults and D_3 for larvae (Tables 2, 3). Indeed, for adults the diet D_1 allowed a significantly higher fecundity and longevity than D_2 and the shortest duration of development from egg to adult (Table 2). The consumption of food provided to adults was estimated at about 0.1 g per day for D_1 and D_2 and per rearing unit housing 6 individuals.

For larvae, the diet D_3 allowed a significantly better pupation and adult emergence rates, and the shortest larval development (Table 3).

Table 2. Biological parameters of *Chrysoperla lucasina* adults fed on two diet

Biological parameter	Diet		*p* value	Degree of freedom	F value
	D_1	D_2			
Fecundity (eggs/female/day)	9.08 ± 6.13 a	3.64 ± 4.13(b	0.00	120	29.78
Total fecundity (eggs/female)	236.19 a	69.13 b	0.00	120	47.70
Longevity (days)	26	19	-	-	-
Mortality (%)	32.72 ± 0.15 a	24.07 ± 0.18 a	0.29	16	1.19
Duration of develop-ment time (days)	24.25 ± 7.80 a	39.67 ± 12.22(b	0.03	5	4.23
Egg hatching rate (%)	24.49 ± 0.20 a	20.82 ± 0.12 a	0.40	59	0.71

Data are mean values ± SD (standard deviation). Values within each line followed by the same letter are not significantly different ($p < 0.05$).

Table 3. Biological parameters of *Chrysoperla lucasina* larvae fed on two diet

Biological parameter	Diet		*p* value	Degree of freedom	F value
	D_2	D_3			
Pupation rate (%)	4.30 ± 0.11 b	23.64 ± 0.17 a	0.000002	59	24.45
Adult emergence (%)	30.36 ± 0.48 b	60.95 ± 0.32 a	0.05	58	16.58
Duration of larval development (days)	9.00 ± 4.42 a	7.71 ± 2.27 a	0.34	27	0.95

Data are mean values ± SD (standard deviation). Values within each line followed by the same letter are not significantly different ($p < 0.05$).

Preliminary test for predation ability.

The predation behavior of larvae of *C. lucasina* from our rearing against natural preys was tested. The average consumption of a young green lacewing larva (L_1) was 3 adults of *M. persicae* aphids per hour. Third instar larvae were more voracious than young ones, with an average consumption of 9.67 aphids/hour (Table 4). Regarding the citrus leafminer *P. citrella*, the consumption of green lacewing larvae was reduced. It was 3 larvae/hour for the third instar of *C. lucasina* larvae. Moreover, a very young larva of green lacewing (L_1) was not able to even consume a whole old leafminer larva.

Table 4. Predation ability of 1st and 3rd instars larvae of *Chrysoperla lucasina*

Prey species (number consumed/hour)	Predator stage	
	1st instar larva	3rd instar larva
Phyllocnistis citrella larvae	0.2±0.28	2±3
Myzus persicae adults	3±2.83	9.67±2.08

Data are mean values of three replicates ± SD (standard deviation).

DISCUSSION

Currently in Tunisia, the biological control of crop pests is little applied at the farmer scale due to the unavailability of entomophagous with an affordable cost. This study aimed to develop a simple and low-cost device to rear green lacewing, as a first step that could be replicated in a bigger scale for a mass rearing. Specifically, the objective was to take benefit of *E. kuhniella*'s eggs as ingredients of the diets for larvae and adults. Especially that Lepidopteran eggs are one of the preferred foods to green lacewing larvae (Legaspi et al. 1994) but their production is relatively costly (Tauber et al. 2000). The availability of an egg production should optimize the very costly larvae production of green lacewing because the three stages are predators (Tauber et al. 2000).

The species *C. lucasina* was chosen for a rearing attempt in order to be released in fields to enhance its natural populations. This species was selected because it was the most abundant one among the specimens collected and it seems well adapted to Tunisian climatic conditions. Our observations agree with Limem-Sellami and Chermiti (2015). Such experiment was carried out by Polak et al. (1998) for *C. chaquensis* collected from cotton fields in Argentina, mass-reared and released to increase their regulation action.

The major results of this study are the design of a simple and inexpensive rearing device for *C. lucasina* and the selection of two diets that ensure the development or larvae and adults. However, for mass rearing purpose, development and reproduction parameters need to be improved.

Rearing device and diets.

The rearing conditions were a constant temperature of 26 ± 2 °C, a relative humidity of 65 ± 5% and a photoperiod maintained on 16:8. These conditions are close to those used in most rearings of chrysopid species and allowed the best performance (Albuquerque et al. 1994; Atlihan et al. 2004; Huang and Enkegaard 2010; Jokar and Zarabi 2012; Liu and Chen 2001).

The rearing units designed for larvae and adults were made with cheap equipment locally available. It consisted of a cylinder (20/9 cm) cut from a plastic water bottle covered with a tulle mesh. The height of the cylinder (20 cm) was to increase the space flight for adults and the only one opening at the top of the bottles (instead of 2) was to reduce mesh tulle handling and fastening. In each unit, an accordion-like strip of Kraft or Bristol paper was placed as an egg-laying site. Both papers were equally used by females to lay eggs; so Kraft paper can be adopted because it's less expensive than Bristol.

Then eggs laid were separated daily by cutting Kraft paper to isolate newly emerged larvae individually. The rearing unit for larvae was a rectangular transparent plastic food box with a plastic lid (16.4 cm / 6.5 cm). Each day, the eggs laid were placed in the same box. The three larval stages were separated in order to avoid cannibalism. At the end of the larval development, the obtained adults were sexed and put into the adult's unit.

Four diets were proposed, two for adults and two for larvae, (1) a multi-floral honeybee pollen and (2) a mixture of eggs of *E. kuehniella* + honey + beer yeast, to adults and (3) this same mixed diet and (4) eggs of *E. kuehniella* to larvae. The amount of food consumed by adults per day for both diets was about 0.1 g/unit.

Biological performance.

All diets have ensured surviving of *C. lucasina*, but did not provide the same biological performance. The pollen for adults and *E. kuehniella* eggs for larvae, gave the best biological parameters. This result matches the objective of this study that was to take advantage of the egg production of *E. kuehniella*, as this factitious prey is known to be very expensive: around 500-600 euros/kg in 2008 (Vandekerkhove et al. 2008).

The pollen-based diet for adults and *E. kuehniella* eggs for their offspring allowed the significantly highest fecundity, the highest hatchability, the highest longevity and the significantly shortest development duration time: around 236 eggs, 24%, 26 days and 24 days respectively. However, the mortality was higher than with the mixed diet: 33% vs. 24%. Compared to other studies on chrysopid rearing, but with other species than *C. lucasina*, some adult parameters are considered encouraging: longevity, mortality and development duration. However, fecundity and hatching ability particularly need to be improved. For instance, the fecundity of females fed with pollen (236 eggs in total) is considered low compared to other findings. Indeed, the total fecundity reached was in average of 400-500 to 1000 eggs depending on the species and the diet (Albuquerque et al. 1994; Alghamdi and Sayed 2017; Hagen and Tassan 1970 in Tauber et al. 2000; Haramboure et al. 2016; Jokar and Zarabi 2014; Polak et al. 1998; Ye et al. 2017). The parameter “fecundity” is very important as it is a start-reproduction parameter that will influence the productivity of the mass rearing. Our findings were close to that of Jokar and Zarabi (2012) which varied from 7 to 10 eggs/female/day. The diet that ensured this fecundity contains a high amount of vitamin E (vitamins and chicken egg yolk) which is appropriate for rearing of adults of *C. carnea* (Balouch et al. 2016)

The egg hatchability is also a critical reproduction parameter for the mass rearing of *C. lucasina* as a biocontrol agent. In our study, this parameter was around 24% which is considered very low compared to 85% for *Dichochrysa tacta* fed by *E. kuhniella eggs* (Alghamdi and Sayed 2017) and to 70% at least, depending on diets for *C. externa* (Haramboure et al. 2016) and also to 92% for *C. chaquensis* fed with a mixture of honey, yeast, wheat germ and water (Polak et al. 1998).

Hence, honeybee pollen alone did not allow a satisfactory reproduction potential for a mass rearing. Consequently, adults should be supplied with vitamins in addition to pollen in order to increase fecundity and fertility. Our choice of a diet based on honeybee pollen was motivated by its nutritive value, as it is rich in carbohydrates, proteins and amino acids (Villenave-Chasset 2007). Furthermore, it is the essential diet for green lacewing, ladybugs and hoverflies (Leroy et al. 2008). The slight disadvantage of using pollen is the availability of various kinds of pollen derived from different plants (Loru et al. 2014).

The longevity of adults fed with pollen obtained in our rearing (26 days) was close to that recorded by Balouch et al. (2016) for *C. carnea* with a diet based on water, sugar and yeast (30 days) and that achieved by Alghamdi and Sayed (2017) for the species *Dichochrysa tacta* where larvae were fed on *E. kuhniella* eggs (27.7 days in average). However, 26 days is very short compared to that obtained with *Mallada basalis* (45 days) on an artificial diet (Ye et al. 2017).

Regarding the mortality of adults, in our rearing we obtained 32% on average without distinguishing between sexes, which is too high. The survival of adults should be studied according to age and sex of adults as it is known that in chrysopids, females live more than males, in the *C. carnea* group.

For larvae, the diet based on *E. kuhniella* eggs allowed the shortest larval development and significantly the highest pupation and adult emergence rates: respectively around 7.7 days, 23.6% and 61%. A short larval development is a relevant factor for a species to be mass reared and the value found in our rearing is considered interesting compared to other studies in close conditions but with other species and different diets: 12.2 days for *C. externa* (Albuquerque et al. 1994); 12.83 days for *M. basalis* (Ye et al. 2017); 13.5 days for *C. paraguaria*, 11 days for *C. externa* and 9 days for *C. chaquensis* (Polak et al. 1998).

However, the parameters “pupation rate” and “adult emergence rate” obtained with the retained diets, respectively 23.64% and 60.95%, were not enough high to ensure an efficient mass rearing. The pupation rate was 45% with minerals and vitamins (Balouch et al. 2016) but only 10.1 % with diet based on *E. kuehniella* (Alghamdi and Sayed 2017). Hence, it is important to add vitamins and minerals to larvae diet to improve pupation rate. In the research result of Balouch et al. (2016), the adult emergence rate did not exceed 30% with a diet containing water, sugar and yeast. Moreover, a cost-analysis should be conducted for *C. lucasina* reared on pollen for adults and *E. kuhniella*’s eggs for larvae, in order to estimate the economic feasibility as achieved by Mudassar et al., (2013) who found that *C. carnea* mass production was more economically efficient on *Sitotroga cerealella* eggs than on *Phenacoccus solenopsis* crawlers.

Predation ability.

The average consumption of a young *C. lucasina* larva was 2 adults of *M. persicae*/hour, while the third instar larvae were more voracious with an average consumption of 9.67 aphids/hour. This same instar consumed 3 larvae of *P. citrella*/hour, while a younger *C. lucasina* larva cannot even consume an entire old leafminer larva. These preliminary results only gave an idea about acceptation of the natural prey for larvae reared on *E. kuhniella* eggs. They cannot indicate the real predatory capacity because of the low number of replicates and also because *C. lucasina* larvae were not starved. Polak et al. (1998) reported that the third instar of green lacewing was the most voracious, suggesting a high number of preys being consumed during the larval development. This characteristics of Chrysopidae, with their polyphagy and great mobility (Polak et al. 1998), make their interest as predators.

In summary, the device designed in our study is quite satisfactory for a small rearing for experimental purpose. However, this device as well as the diets based on pollen for adults and *E. kuhniella*’s eggs for larvae, have to be improved to produce a higher yield. Indeed, for a mass rearing, more space is recommended that means a whole room dedicated to rearing with spacious units. Regarding diets for adults, honeybee pollen must be complemented by vitamins and amino acids to improve fecundity and fertility of eggs. The preoviposition period should also be checked. For *C. lucasina* larvae, eggs of *E. kuhniella* are a good diet, but should be complemented with other ingredients to increase pupation rate. With these improvements, *C. lucasina* should have the potential to be a candidate for mass rearing and release as a biocontrol agent against several crop pests in Tunisia. This work is nevertheless a first step for an application at larger scale.

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RESUME

Karouia W., Hamdi F. et Boulahia-Kheder S. 2023. Première tentative de développement d'une méthode d'élevage de la chrysope verte autochtone *Chrysoperla lucasina* en Tunisie. Tunisian Journal of Plant Protection 18 (1): 15-27.

La chrysope verte (*Chrysoperla lucasina*) est bien connue pour ses larves prédatrices polyphages qui sont communément utilisées dans la lutte biologique contre les ravageurs dans le monde entier. En Tunisie, cet auxiliaire est présent dans la nature en association avec divers ravageurs mais il n'a jamais été élevé en masse et lâché dans le cadre de programmes de lutte biologique. Ainsi, l'espèce de chrysope *C. lucasina* a été choisie pour une tentative d'élevage afin de renforcer ses populations naturelles. L'étude a été réalisée dans l'insectarium de la société ControlMed spécialisée dans la production des parasitoïdes du genre *Trichogramma*. Tout le matériel utilisé pour l'élevage des larves et des adultes de la chrysope verte a été nouvellement conçu pour cette étude. Deux régimes alimentaires ont été donnés aux larves et adultes pour évaluer leurs performances biologiques. Le premier régime était à base de pollen pour les adultes et d'œufs d'*Ephestia kuehniella* pour les larves. Le deuxième régime fourni aux adultes ainsi qu'aux larves, était un mélange pâteux de miel, levures et œufs d'*E. kuehniella*. Les conditions d'élevage au laboratoire ont été maintenues constantes avec 26 ± 2°C, 65 ± 5% HR et une photopériode de 16L:8D. Les régimes à base de pollen et d'œufs d'*E. kuehniella* ont donné les meilleurs paramètres biologiques respectivement pour les adultes et les larves. Cette étude ayant permis de concevoir une technique d'élevage simple de *C. lucasina* basé sur du matériel à prix abordable, constitue une première étape pour le développement d'un élevage de masse des chrysopes en Tunisie. Cependant, des améliorations des régimes alimentaires des adultes et des larves sont nécessaires pour augmenter les performances de reproduction et de développement, ainsi qu'une étude de faisabilité économique.

Mots clés: Chrysope verte, *Chrysoperla lucasina*, élevage d'insectes, matériel à bas coût, performances biologiques, régimes artificiels

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ملخص

قروية، وجدان وفاتن حمدي وسندة بولحية-خذر. 2023. أول محاولة لتطوير طريقة تربية الحشرة الخضراء شبكية الأجنحة *Chrysoperla lucasina* المحلية في تونس.
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تُعرف الحشرة الخضراء شبكية الأجنحة أو أسد المنّ (*Chrysoperla lucasina*) بيرقاتها المفترسة المستخدمة عبر العالم في المكافحة البيولوجية. في تونس، توجد هذه الحشرة طبيعيا مقترنة بعديد الآفات، لكن لم يتم مطلقاً اكثارها ونشرها في في إطار المكافحة البيولوجية. لذلك، تم اختيار هذه الحشرة لمحاولة إكثارها كميا من أجل تعزيز مجتمعاتها الطبيعية. احتضنت مخابر شركة "كنترولماد ControlMed" المختصة في إكثار شبه الطفيليمن جنس *Trichogramma*. في هذه الدراسة، تم اختبار نظامين غذائيين للبالغين واليرقات لحشرة أسد المنّ. يستند النظام الغذائي الأول بالنسبة إلى البالغين على حبوب اللقاح. ويتكون النظام الغذائي الثاني من خليط من بيض عثّة الطحين (*Ephestia kuehniella*) والعسل والخميرة. وبالنسبة إلى اليرقات، كان النظام الغذائي الأول هو بيض عثّة الطحين والنظام الغذائي الثاني هو خليط من بيض عثّة الطحين والعسل والخميرة .تم الحفاظ على ظروف ثابتة للتكاثر، تتلخص في درجة حرارة 26 ± 2 ºس، نسبة رطوبة 65 ±5 %، 16 ساعة إضاءة / 8 ظلام في اليوم. وفقا للنتائج التي تم الحصول عليها، تم اختيار حبوب اللقاح للبالغين وبيض عثّة الطحين لليرقات كأفضل أنظمة غذائية. هذه الدراسة التي مكنت من تصميم تقنية إكثارأسد المنّ، تعد خطوة أولى يمكن تبنيها لتطوير إنتاج هذه الحشرة في تونس لكن يبقى ضروري إدخال تحسينات على النظام الغذائي للرفع من كفاءة الحشرة في التكاثر والتطور.

كلمات مفتاحية: تربية الحشرات، تغذية صناعية، تكلفة منخفضة، كفاءات بيولوجية، حشرة أسد المنّ (الحشرة الخضراء شبكية الأجنحة)، *Chrysoperla lucasina*

LITERATURE CITED

Albuquerque, G.S., Tauber, C.A, and Tauber, M.J. 1994. *Chrysoperla externa* (Neuroptera: Chrysopidae): life history and potential for biological control in Central and South America. Biological Control 4:8–13.

Alcalá Herrera, R., Fernández Sierra, M.L., and Ruano, F. 2020. Attraction of green lacewings (Neuroptera: Chrysopidae) to native plants used as ground cover in woody Mediterranean agroecosystems. Plos One 15(10) e0239847. https://doi.org/10.1371/journal.pone.0239847

Alghamdi, A., and Sayed, S. 2017. Biological Characteristics of indigenous *Chrysoperl acarnea* (Neuroptera: Chrysopidae) fed on a natural and an alternative prey. Asian Journal of Biology 2 (2): 1-6.

Atlihan, R., Kaydan, B., and Ozgokçe, M.S. 2004. Feeding activity and life history characteristics of the generalist predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae) at different prey densities. Journal of Pest Science 77: 17-21.

Balouch, S., Bukero, A., Nizamani, I.A., Kumbhar, M.I., Rajput, L.B., Buriro, R.A., Shah, A.R., Qureshi, N.A., Sheikh, J.A., Nahiyoon, S.A., and Rajput, Z. 2016. Rearing of adult green lacewing, *Chrysoperla carnea* (Stephens) on different artificial diets in the laboratory. Journal of Basic and Applied Sciences 12: 289-292.

Bachrouch, O., Mediouni-Ben Jemâa, J., Alimi, E., Skillman, S., Kabadou, T., and Kerber, E. 2008. Efficacy of the Lufenuron Bait Station Technique to Control Mediterranean Fruit Fly (Medfly) *Ceratitis capitata* in Citrus Orchards in Northern Tunisia. Tunisian Journal of Plant Protection 3: 35-45.

Bezerra, C.E.S., Amaral, B.B., and Souza, B. 2016. Rearing *Chrysoperla externa* larvae on artificial diets. Neotropical Entomology 46: 93-99.

Boukhris-Bouhachem, S. 2011. Aphid Enemies Reported from Tunisian Citrus Orchards. Tunisian Journal of Plant Protection 6: 21-27.

Boulahia-Kheder, S., Trabelsi, I., and Aouadi, N. 2012. From chemicals to IPM against the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae). Pages 301-320. In: Integrated Pest Management and Pest Control Current and Future Tactics. Marcelo L., M. L. Larramendy and S. Soloneski, Eds. In TechOpen, 668 pp. DOI: 10.5772/32686.

Canard, M., Letardi, A., and Thierry, D. 2007. The rare Chrysopidae (Neuroptera) of southwestern Europe. Acta Oecologica 31: 290-298.

Devetak, D., and Klokokovnic, V.2016. The feeding biology of adult lacewings (Neuroptera): a review. Trends in Entomology 12: 29-42.

Haramboure, M., Mirande, L., and Schneider, M.I. 2016. Improvement of the mass rearing of larvae of the neotropical lacewing *Chrysoperla externa* through the incorporation of a new semiliquid artificial diet. BioControl 61: 69-78. DOI 10.1007/s10526-015-9699-7.

Henry, C.H., Brooks, S.J., Johnson, J.B., and Duelli, P. 1996. *Chrysoperla lucasina* (Lacroix): a distinct species of green lacewing, confirmed by acoustical analysis (Neuroptera: Chrysopidae). Systematic Entomology 21: 205-218.

Henry, C.H., Brooks, S.J., Duelli, P., and Johnson, J.B. 2002. Discovering the True *Chrysoperla carnea* (Insecta: Neuroptera: Chrysopidae) Using Song Analysis, Morphology, and Ecology. Annals of the Entomological Society of America 95 (2): 172-191.

Huang, N., and Enkegaard, A. 2010. Predation capacity and prey preference of *Chrysoperla carnea* on *Pieris brassicae*. BioControl 55: 379-385.

Jokar, M., and Zarabi, M. 2012. Surveying effect kind of food on Biological parameters on *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory Conditions. Egyptian Academic Journal of Biological Sciences 5 (1): 99-106.

Jokar, M., and Zarabi, M. 2014. Comparative Study of Different Diets Efficiency on Some Biological Parameters of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). Molecular Entomology 5 (1): 1-9.

Lavagnini, T.C., Morales, A.C., and Freitas, S. 2015. Population genetics of *Chrysoperla externa* (Neuroptera: Chrysopidae) and implications for biological control. Brazilian Journal of Biology 75 (4): 878-885.

Legaspi, J.C., Carruthers, R.I., and Nordlund, D.A. 1994. Life history of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) provided Sweetpotato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) and other food. Biological Control 4: 178-184.

Leroy, P., Francis, F., Verheggen, F.,Capella, Q., Fagel, Q., and Haubruge, E. 2008. La coccinelle à deux points (*Adalia bipunctata*), le chrysope commun (*Chrysoperla carnea*) et le syrphe ceinturé (*Episyrphus balteatus*), nos principaux prédateurs indigènes plutôt que la coccinelle asiatique (*Harmonia axyridis*) exotique et invasive dans nos écosystèmes. Cercle des Naturalistes de Belgique, L'érable 1: 7-10.

Limem-Sellami, E. 2017. Contribution à l'étude de l'entomofaune auxiliaire en vergersd'agrumes : Biodiversité, Bioécologie et Interactions environnementales. Thèse de Doctorat, Institut Supérieur Agronomique de Chott Mariem, Université de Sousse, Tunisie, 154 pp.

Limem-Sellami, E., and Chermiti, B. 2015. Preliminary Survey of the Green Lacewings (Neuroptera: Chrysopidae) in Citrus Orchards in Northern East of Tunisia (Cap Bon). Acta Horticulturae 1065: 1181-1186. DOI:10.17660/ActaHortic.2015.1065.150

Liu, T.X., and Chen, T.Y. 2001. Effects of three aphid species (Homoptera: Aphididae) on development, survival and predation of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Applied Entomology and Zoology 36 (3): 361-366.

Loru, L., Fois, X., Ramasani, S.V., Fadda, L.M., and Pantaleoni, R.A. 2014. An innovative, lowcost, small-scale rearing method for green lacewings. Biodiversity Journal 5 (2) : 221-224.

Mazel, R., Canard, M., and Thierry, M. 2006. Clés synoptiques des Chrysopidae de France (Neuroptera). Revue de l'Association Roussillonnaise d'Entomologie, Tome XV (1): 29-45.

Messelink, G.J., Vijverberg, R., Leman, A., and Janssen, A. 2016. Biological control of mealybugs with lacewing larvae is affected by the presence and type of supplemental prey. BioControl 61: 555-565.

Mudassar, M.T., Haq, E., Naeemullah, M., Shakeel, M., Ashfaque, M., Nisa, A. and Khan, J. 2013. Economic rearing of *Chrysoperla carnea* Stephens) (Neuroptera: Chrysopidae) in insectary. Pakistan Journal of Agricultural Research 26 (4): 1-8.

Pappas, M.L., Broufas, G.D. and Koveos, D.S. 2011. Chrysopid predators and their role in biological control. Journal of Entomology 8 (3): 301-326.

Polak M.G.A., Contreras G.B., Marinich M.J., Royo O.M., Simonella M.A. and Poisson J.A.F. 1998. Mass Rearing and Use of a New Species of Chrysoperla (Neuroptera : Chrysopidae) in Cotton Crops in Argentina. Pages 672-674. In: Proceedings of the World Cotton Research Conference-2. September 6-12, 1998, Athens, Greece.

Rogers, M. A., Krischik, V.A., and Martin, L.A. 2007. Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouse. Biological Control (42): 172–177.

Rojht, H., Budija, F., and Tradan, S. 2009. Effect of temperature on cannibalism rate between green lacewings larvae (*Chrysoperla carnea* [Stephens], Neuroptera, Chrysopidae). Acta Agriculturae Slovenica 93: 5-9.

Rosenheim, J.A., Limburg, D.D., and Colfer, R.G. 1999. Impact of Generalist Predators on a Biological Control Agent, *Chrysoperla carnea*: Direct Observations. Ecological Applications 9 (2): 409-417. https://doi.org/10.2307/2641128

Tauber, M. J., Tauber, C. A., Daane, K. M., and Hagen, K.S. 2000. Commercialization of Predators: Recent Lessons from Green Lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). American Entomologist 46 (1): 26-38.

Thierry, D., Cloupeau, R., and Jarry, M. 1996. Distribution of the sibling species of the common green lacewing *Chrysoperla carnea* (Stephens) in Europe (Insecta: Neuroptera: Chrysopidae). Pages 233-240. In: Symposium on Pure and Applied Research in Neuropterology, May 2-6, 1994, Cairo, Egypt. Ed. Canard, M., Aspöck, H. and Mansell, M.W. Toulouse, France.

Vandekerkhove, B., Parmentier, L., Van Stappen, G., Grenier, S., Febvay, G., Rey, M., and De Clercq, P. 2008. Artemia cysts as an alternative food for the predatory bug *Macrolophus pygmaeus*. Journal of Applied Entomology 133 (2):133–142.

Villenave-Chasset, J. 2007. Étude de la Bio-écologie des Névroptères dans une perspective de lutte biologique par conservation. Relations entre l'environnement des cultures de choux (végétation et structure paysagère) et l'abondance, la distribution et le comportement. Thèse de Doctorat, Université d'Angers, France, 241 pp.

Villenave-Chasset, J., Deutch, B., Lodé, B., and Rat-Morris, E. 2006. Pollen preference of the *Chrysoperla* species (Neuroptera: Chrysopidae) occurring in the crop environment in western France. European Journal of Entomology 106 (4): 771–777. DOI:10.14411/eje.2006.104.

Ye, J., Li, J., Li, Z. and Han, S. 2017. Rearing of *Mallada basalis* (Neuroptera: Chrysopidae) on modified artificial diets. Plos One 12(9): 1-9. e0185223. https://doi.org/10.1371/journal.pone.0185223.
