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Development of mass-rearing techniques for *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae) and *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Neuroptera: Chrysopidae)

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Abstract
The present study aimed to standardize the mass rearing technique for *Corcyra cephalonica* (Stainton) and *Chrysoperla zastrowi sillemi* (Esben-Petersen). To achieve this, six *Corcyra* diets (CD) were evaluated for *C. cephalonica*, four larval diets (LD) were evaluated for *C. z. sillemi* larvae and five adult diets (AD) were evaluated for *C. z. sillemi* adults. Among the six *Corcyra* diets (CD), the diet CD6 which contained Wheat + Rice + Groundnut + Yeast in proportion of 41% + 41% + 5% + 3% exhibited a shorter larval period (32.32 days), pupal period (8.54 days), and longer male longevity (8.43 days), female longevity (9.86 days) and higher fecundity (287 eggs/female) compared to the other diets. Studies on larval diet for *C. z. sillemi* larvae revealed that the larvae reared on LD1 (*C. cephalonica* eggs) showed a shorter larval duration (11.92 days), pupal duration (7.45 days), and adult emergence percentage (88.9%). Similarly, among the adult diets (AD) for *C. z. sillemi* adults, AD5 which comprised Tea pollens + 20g Protein-X + 20g Honey in 250 ml of water showed higher male longevity (56.4 days), female longevity (72.5 days), oviposition period (66.25 days), fecundity (744.8 eggs/female), and fertility percentage (84.55%) compared to other adult diets. Based on the study, CD6 (Wheat + Rice + Groundnut + Yeast), LD1 (*C. cephalonica* eggs) and AD5 (Tea pollens + Proteinx + Honey) were found to be effective and superior diets for mass rearing of *C. cephalonica, C. z. sillemi* larvae and *C. z. sillemi* adults, respectively.

Keywords: Green lacewings, mass rearing, natural diet, rice moth, tea pollens

Introduction

The rice moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae) commonly known as the "flour moth" or "grain moth," is considered a major pest of rice, maize, wheat, groundnut, sorghum, cotton seeds, and millet (Chaudhuri and Senapati, 2017) [5]. In addition to being a pest, it is widely considered a very good host for the mass production of various bio-control agents including about 60 parasitoids and 15 predators worldwide (Manjunath, 2014) [18]. In India, various bio control research organizations, extension units and many private organizations utilizes *C. cephalonica* for the mass production of numerous natural enemies (Kalyanakumar *et al.*, 2013) [13]. To date, plenty of natural and artificial diets have been studied for the mass production of *C. cephalonica* (Kumar and Shenhmar, 2001; Sathpathy *et al.*, 2003) [15, 27]. Additionally, the present study aims to contribute to the mass rearing of *C. cephalonica* by adopting cost-effective diets. For this purpose, six different natural diets with various compositions were selected for the evaluation process and the impact of diets on the biological parameters of *C. cephalonica* was studied.

Among insect predators, green lacewings (Neuroptera: Chrysopidae) are an important group known as significant predators of a large number of soft-bodied insects and mites (Aravind *et al.*, 2013) [1]. The population dynamics of chrysopids naturally vary based on agricultural ecosystems and can be enhanced by inoculative or inundative releases in these ecosystems (Nordlund *et al.*, 2001) [21]. Previous literatures has described numerous protocols for culturing chrysopids for inoculative or inundative releases using natural, sub-artificial and artificial diets (Ridgway *et al.* 1970; Morrison 1985; Yazlovetsky, 2001) [19, 24, 34]. Chrysopids exhibit two different feeding behaviours: predatory larvae feed on a wide range of soft-bodied insects, while adults are non-predatory and feed on pollen, nectar and honeydew (Dean and Satasook, 1983; Principi and Canard, 1984) [7, 23]. Mass rearing predators for inoculative or inundative releases in agricultural ecosystems is a crucial component of biological pest control. Therefore, this study also focuses on mass rearing larvae and adults of *C. z. sillemi* using various diets. For this study, four different larval diets were selected for mass rearing the larval phase of *C. z. sillemi* and five adult diets were selected for mass rearing adult *C. z. sillemi*.

Materials and methods

1. Evaluation of different diets for *Corcyra cephalonica*

The initial culture of the rice moth, *C. cephalonica* was obtained in the form of eggs from the Entomology Division, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. In this study, six different diet compositions were selected and evaluated for the mass rearing of *C. cephalonica* under laboratory conditions. The compositions of the diets are presented in Table 1. Whole grains such as rice and groundnut were cut into small pieces using a domestic mixer grinder. Each diet compositions was mixed in its appropriate proportions and then sterilized in a hot air oven at 100ºC for 60 minutes to eliminate stored grain pests and prevent secondary infestations. After cooling, to prevent mold growth and compensate for grain moisture lost during sterilization, the diets were sprayed with 0.1% formalin. Streptomycin sulfate @ 0.5 g/3 kg was added to prevent bacterial infection in the diet. The required amount (1 kg) of prepared diets was transferred into a plastic tray (45cm in length × 30cm in width) and 3% dry powdered yeast was added before introducing the *C. cephalonica* eggs. About 1 cc of *Corcyra* eggs was added and mixed thoroughly for uniform distribution, then covered with muslin cloth. All trays were maintained under the aforementioned experimental conditions and monitored daily. Each treatment was replicated five times. Biological parameters such as incubation period, total larval period, pupal period, and percentage of adult emergence were recorded daily. Once adults emerged, five pairs of adult *Corcyra* were randomly selected from each diet and fed a diet of 10% honey (Dabur honey) and vitamin-E in a plastic container (30cm in height × 10cm in width) covered with mosquito net cloth. Adults were monitored daily, and male longevity, female longevity, fecundity and fertility rates were recorded throughout the study period.

Table 1: Different diets for *C. cephalonica* and their compositions

Corcyra diet (CD)	Diet composition	Proportion (%)
CD1	Rice + Yeast	97 + 3
CD2	Rice + Groundnut + Yeast	92 + 5 + 3
CD3	Wheat + Yeast	97 + 3
CD4	Wheat + Groundnut + Yeast	92 + 5 + 3
CD5	Wheat + Rice + Yeast	48.5 + 48.5 + 3
CD6	Wheat + Rice + Groundnut + Yeast	41 + 41 + 5 + 3

2. Effect of different larval diets on *C. z. sillemi* larvae

Four different larval diets (LD) were evaluated on the predatory stages of *C. z. sillemi*. The diets included LD1 (*Corcyra* eggs), LD2 (*Corcyra* neonate larvae), LD3 (*Corcyra* advanced larvae) and LD 4 (red spider mites). Newly hatched larvae (<12 h old) were randomly selected from the mass rearing unit and transferred individually to small plastic containers (height 5cm × width 3cm). Each larva was reared individually with various hosts until they became pupae. The containers were then closed with muslin cloth after introducing both predator and prey. All rearing containers were maintained under the laboratory conditions mentioned above. Each treatment was replicated ten times. Daily observations were made on all rearing containers, recording instar-wise duration, pupal duration, survival percentage and adult emerging percentage until the completion of the study.

3. Effect of different adult diets on *C. z. sillemi* adults

Five different adult diets (AD) were evaluated including: AD1 (Tea pollens + 40g honey in 250ml of water), AD2 (30g yeast + 60g protein-X + 40g honey in 250ml of water), AD3 (60g protein-X + 40g honey in 250 ml of water), AD4 (50g honey in 250ml of water) and AD5 (Tea pollen + 20g protein-X + 20g honey in 250ml of water). The aim was to determine the best diet by studying various biological parameters. All diets were mixed with water and provided to adults on cotton wool. Tea pollens were freshly collected from the field and served to the adults. Newly emerged *C. z. sillemi* (<12 h old) adults were randomly selected from the stock culture, sexed based on morphological characteristics, paired, and then individual pairs released into individual plastic containers (30cm in height × 10cm in width) containing artificial diet. Upon release, the tops of the plastic containers were covered with black chart paper to collect eggs and the paper had small holes for aeration purposes. The experimental containers were maintained under laboratory conditions as described above. Diets were changed every two days and adults were continuously reared on the same diet until the death of both males and females. Each treatment was replicated five times. Biological parameters such as pre-oviposition period, oviposition period, post-oviposition period and fecundity of each pair were recorded. All eggs were collected daily and placed in separate plastic containers (30cm in height × 10cm in width) to study hatchability, survivability and adult emergence.

4. Statistical analysis

All data from the experiments were combined and subjected to one-way analysis of variance (one-way ANOVA). Means were compared using Duncan's Multiple Range Test (DMRT) with IBM SPSS®, version 23 software.

Results and discussion

1. Impact of different diets on *Corcyra cephalonica*

The life cycle of *C. cephalonica* consists of nine stages: egg, six larval stages, pupa and adult. The effects of different diets on various biological parameters of *C. cephalonica* are presented in Tables 2 & 3. The results show that *C. cephalonica* developed on all the diets evaluated during the study. However, the different diets significantly influenced the biological parameters of *C. cephalonica.* The incubation period ranged from 3.02 to 4.20 days, with the shortest duration observed in treatment CD3 (3.40 days) and the longest in treatment CD4 (3.84 days). *C. cephalonica* went through five larval stages before reaching the pupal stage. The total larval developmental period of *C. cephalonica* varied from 31.1 to 38.66 days depending on the diet. The longest and shortest larval durations were seen in treatments CD3 and CD6, respectively. The pupal stage of *C. cephalonica* lasted longer in CD1 (12.38 days) and shorter in CD6 (8.54 days). Male and female longevity were highest in CD6 (male: 8.43 days, female: 9.86 days) and lowest in CD1 (male: 6.38 days, female: 7.52 days). *C. cephalonica* showed a higher fecundity rate (287 eggs per female) in treatment CD6.

This comparative study on the impact of different diets specifically CD1, CD2, CD3, CD4, CD5 and CD6 on biological parameters revealed that the diets significantly affect the biological parameters of *C. cephalonica*. These findings were consistent with early reports by Hassan *et al.* (2020) [12], Padhy *et al.* (2020) [20], and Soumya, (2023) [31]. In the present study, the results of the incubation period were similar to those of a study conducted by Arun Kumar *et al.* (2018) [2], who evaluated nine different diets for *C. cephalonica* and found that the incubation period ranged from 3 to 4 days.

Adult longevity and fecundity are two major biological parameters to consider when estimating egg production. The study found that the diet CD6 exhibited higher adult longevity and fecundity followed by CD2, CD4, CD5, CD3 and CD1. The findings showed that adding groundnut to diets significantly impacted adult longevity and fecundity compared to diets without groundnut. A combination of carbohydrates and proteins in the rearing diets resulted in healthy adults with higher longevity and fecundity. Increased fecundity and fertility were observed in *C. cephalonica* when reared on diets incorporating proteins such as pulses, groundnut and yeast (Haritha *et al.*, 2000; Bhandari *et al.*, 2014) [4, 11]. Additionally, diets containing high nutrient sources resulted in the production of high-quality eggs with potentially higher hatchability rates (Nathan *et al.*, 2006) [20]. The present study also experienced a similar phenomenon, where the incorporation of groundnut in the diet significantly increased the various biological parameters of *C. cephalonica*.

Table 2: Developmental durations (in days) of *C. cephalonica* on different *Corcyra* diets

Corcyra diet (CD)	Incubation period	Total larval period	Pupal period	Total developmental period	Male longevity	Female longevity	Fecundity
CD1	3.78 ± 0.18^{bc}	35.68 ± 0.58^{c}	12.38 ± 0.24^{d}	51.48 ± 0.65^{c}	6.38 ± 0.33^{a}	7.52 ± 0.25^{a}	164.4 ± 14.57^{a}
CD2	3.52 ± 0.19^{ab}	34.04 ± 0.21^{b}	9.66 ± 0.22^{b}	47.22 ± 0.36^{b}	7.68 ± 0.19^{b}	9.16 ± 0.47^{c}	254.1 ± 13.69^{b}
CD3	3.40 ± 0.16^{a}	37.67 ± 0.79^{d}	11.52 ± 0.33^{c}	52.59 ± 0.53^{c}	6.48 ± 0.19^{a}	7.8 ± 0.21^{a}	180.2 ± 12.14^{a}
CD4	3.84 ± 0.34^{d}	34.48 ± 0.48^{ab}	9.84 ± 0.27^{b}	48.52 ± 0.69^{b}	7.84 ± 0.51^{b}	9.02 ± 0.42^{c}	252.8 ± 8.98^{b}
CD5	3.60 ± 0.15^{abc}	34.72 ± 0.56^{ab}	9.64 ± 0.25^{b}	47.96 ± 0.52^{b}	7.88 ± 0.19^{b}	8.44 ± 0.11^{b}	240.4 ± 14.29^{b}
CD6	3.62 ± 0.18^{abc}	32.32 ± 0.70^{a}	8.54 ± 0.39^{a}	44.48 ± 0.87^{a}	8.43 ± 0.52^{c}	9.86 ± 0.46^{d}	287.0 ± 7.24^{c}
Mean ±SD (*n*=5) followed by the same letter in the colum is not significant at the 5% level according to Duncan's Multiple Range Test (DMRT)							

Table 3: Biological parameters of *C. cephalonica* on different *Corcyra* diets

Corcyra diet (CD)	Egg hatching %	Pupation %	Adult emergence (%)	Male emergence (%)	Female emergence (%)
CD1	80 ± 1.14^{a}	72 ± 0.44^{a}	70 ± 0.84^{a}	33 ± 3.03^{ns}	37 ± 2.41^{a}
CD2	85 ± 0.83^{c}	81 ± 1.00^{d}	75 ± 0.54^{b}	31 ± 2.8^{ns}	44 ± 3.27^{c}
CD3	82 ± 1.00^{b}	74 ± 0.70^{b}	66 ± 1.34^{c}	29 ± 4.67^{ns}	37 ± 3.67^{a}
CD4	84 ± 1.14^{c}	80 ± 0.83^{d}	74 ± 1.58^{c}	34 ± 2.54^{ns}	40 ± 2.00^{ab}
CD5	81 ± 1.22^{ab}	75 ± 0.84^{c}	73 ± 1.14^{c}	31 ± 0.84^{ns}	42 ± 1.67^{bc}
CD6	89 ± 1.48^{d}	87 ± 0.55^{e}	81 ± 0.70^{d}	32 ± 2.51^{ns}	48 ± 2.07^{d}
Mean ±SD (*n*=5) followed by the same letter in the colum is not significant at the 5% level according to Duncan's Multiple Range Test (DMRT); ns=not significant.					

2. Impact of different larval diets on *C. z. sillemi* Larvae

The study revealed that different larval diets significantly impacted the durations of the first instar ($F_{(3, 36)} = 3.07$; $P = 0.04$), second instar ($F_{(3, 36)} = 8.198$; $P < 0.0001$), third instar ($F_{(3, 36)} = 4.041$; $P = 0.014$), total larval duration ($F_{(3, 36)} = 11.772$; $P < 0.0001$) and pupal duration ($F_{(3, 36)} = 7.233$; $P = 0.001$) (Table 4). Specifically, the total larval developmental duration and pupal duration of *C. z. sillemi* were 11.92 and 7.45 days, 12.48 and 7.95 days, 13.17 and 8.3 days, and 13.8 and 8.35 days in LD1, LD2, LD3 and LD4, respectively.

The larval diets significantly impact the duration of larvae, pupae, survival rate and adult emergence of *C. z. sillemi*. Larval and pupal development durations were significantly shorter when feeding on *C. cephalonica* eggs, and longer on *O. coffeae*. Despite, longer development times, *C. z. sillemi* was able to complete its predatory life stage on all four diets evaluated. *C. z. sillemi* showed a shorter larval duration (11.92 days) and pupal duration (7.45 days), and a higher adult emergence rate (88.9 %) when reared on LD1 compared to the other diets. Figure 1 displays the mass rearing of *C. z. sillemi* larvae using *C. cephalonica* eggs.

The larval developmental duration of the predatory stage of chrysopids generally varies based on their diet, as reported by various authors such as Ghanim and El-Adl, (1987) [10], Shalaby *et al.* (1998) [29], and El-Serafi *et al.* (2000) [8]. The developmental duration is significantly faster when chrysopids are reared on lepidopteran eggs, particularly *C. cephalonica* (Saminathan *et al.*, 1999) [26]. Saminathan *et al.* (1999) [26] evaluated ten different hosts against the developmental parameters of *C. carnea* and found that *C. carnea* showed a higher preference for host eggs over other stages. They also reported that a shorter developmental period was observed when reared on *Helicoverpa armigera* eggs and *C. cephalonica* eggs. These results align with previous studies on *C. carnea* conducted by Sharma and Verma (1991) [30]; Balasubramani and Swamiappan (1994) [3].

Table 4: Larval and pupal durations (in days) of *C. z. sillemi* on different larval diets

Particulars	Larval diets (LD)			
	LD1	LD2	LD3	LD4
First instar duration	3.73 ± 0.20^{a}	3.93 ± 0.13^{ab}	4.09 ± 0.17^{ab}	4.3 ± 0.82^{b}
Second instar duration	3.91 ± 0.18^{a}	4.04 ± 0.12^{ab}	4.33 ± 0.31^{b}	4.7 ± 0.67^{c}
Third instar duration	4.28 ± 0.15^{a}	4.51 ± 0.21^{ab}	4.75 ± 0.32^{b}	4.8 ± 0.63^{b}
Total larval duration	11.92 ± 0.30^{a}	12.48 ± 0.23^{a}	13.17 ± 0.42^{b}	13.8 ± 0.36^{b}
Pupal duration	7.45 ± 0.37^{a}	7.95 ± 0.49^{b}	8.3 ± 0.42^{b}	8.35 ± 0.62^{b}
Mean ±SD (*n*=10) followed by the same letter in the row is not significant at the 5% level according to Duncan's Multiple Range Test (DMRT)				

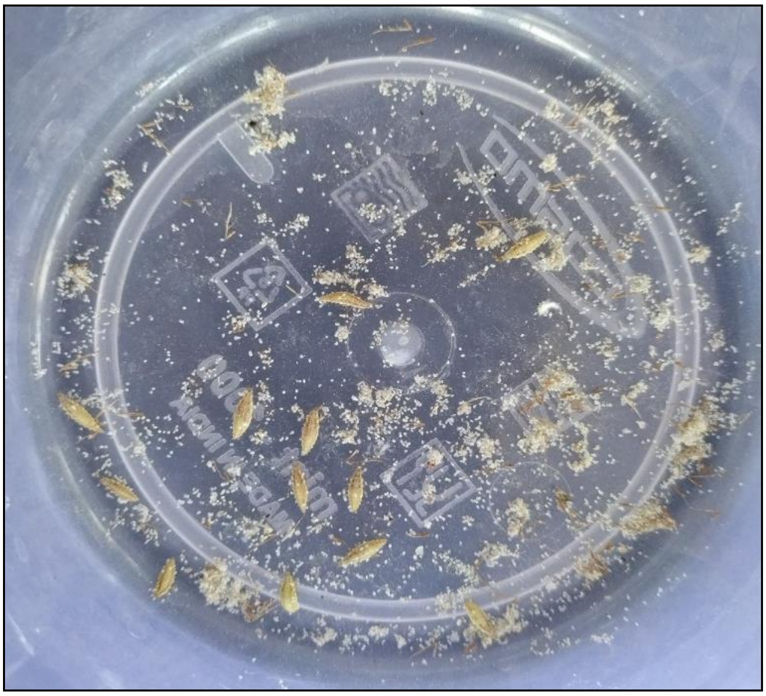

Fig 1: Mass rearing of *C. z. sillemi* larvae using *C. cephalonica* eggs

3. Impact of different adult diets on *C. z. sillemi* adults

Various adult diets (AD) were examined to understand the adult biological parameters of *C. z. sillemi*. The study results showed that different diets did not influences the pre-oviposition period of *C. z. sillemi* ($P > 0.05$). However, other biological parameters such as oviposition period ($F_{(4, 20)} = 46.195$; $P < 0.0001$), post-oviposition period ($F_{(4, 20)} = 7.401$; $P = 0.001$), fecundity rate ($F_{(4, 20)} = 870.439$; $P < 0.0001$), fertility rate ($F_{(4, 20)} = 19.461$; $P < 0.0001$), male longevity ($F_{(4, 20)} = 15.665$; $P < 0.0001$) and female longevity ($F_{(4, 20)} = 43.063$; $P < 0.0001$) were significantly influenced by the adult diets (Table 5).

Among the evaluated adult diets, adults fed with AD5 exhibited a higher oviposition period (66.25 days), fecundity (744.89 eggs/ female), fertility percentage (84.55), female longevity (72.5 days) and male longevity (56.4 days) compared to other diets. The present study found that adult biological parameters were significantly higher when adults were fed a diet containing tea pollens, honey and protein-X. Figure 2 displays an adult *C. z. sillemi* feeding on tea pollen. Kathiar and Jaber, (2020) [14] reported that *C. carnea* exhibited a maximum oviposition period (14.20 days), highest fecundity (217.40 eggs), hatching percentage (88.6%), and highest male (16.40 days) and female longevity (19.40 days) when fed a diet containing water: sugar: pollen (10ml: 7g: 4g). The study results are consistent with the studies of Figueira *et al.* (2000) [9], Costa *et al.* (2002) [6], Sattar and Abro. (2011) [28].

The biological parameters of *C. z. sillemi* such as oviposition period, fecundity, fertility and adult longevity were lower in the diet containing honey alone. These parameters began to increase when the composition of the diet improved by adding yeast, protein-X and tea pollen. Naturally, adult chrysopids require protein and sugar sources to maximize their survival and reproduction rates. Oviposition mostly does not occur when females are fed only sugar (sucrose or honey). However oviposition can occur when they are fed sugar along with pollen (Wäckers and van Rijn, 2012) [33]. Protein sources significantly impact the reproductive success of the chrysopidae and the requirement for protein may vary based on the sex of the adults. Male chrysopids need a small amount of protein for the development of accessory glands and spermatophores, while females need a large amount of protein for the development of oocytes and accessory glands in their reproductive system (Villenave *et al.*, 2005, Li *et al.*, 2009) [32].

According to Roulston *et al.* (2000) [25], many arthropods consume pollen as a protein source due to its protein content ranging from 2.5% to 61%. Pollen naturally contains a wide range of nutrients such as carbohydrates, lipids, vitamins, and inorganic minerals. These nutrients improve the predators' fitness and make them nutritionally stable for many life processes (Lundgren, 2009) [17].

Table 5: Biological parameters (in days) of adult *C. z. sillemi* on different adult diets

Adult diets	Pre-oviposition	Oviposition	Post-oviposition	Fecundity	Fertility %	Female longevity	Male longevity
AD1	3.25 ± 0.43^{ns}	57.75 ± 2.59^{d}	2.5 ± 0.5^{a}	634 ± 12.47^{d}	80.61 ± 4.88^{c}	63.51 ± 2.69^{d}	52.41 ± 3.36^{cd}
AD2	3.25 ± 0.43^{ns}	50.75 ± 4.02^{c}	3.5 ± 0.5^{bc}	476.8 ± 11.79^{c}	53.26 ± 8.13^{ab}	57.5 ± 4.03^{c}	48.2 ± 2.28^{bc}
AD3	3.0 ± 0.0^{ns}	42.25 ± 4.53^{b}	4.25 ± 0.43^{d}	436.6 ± 12.86^{b}	61.59 ± 13.19^{b}	49.5 ± 4.61^{b}	42.4 ± 6.46^{ab}
AD4	3.0 ± 0.0^{ns}	34.75 ± 5.35^{a}	3.5 ± 0.5^{bc}	389.6 ± 9.45^{a}	50.63 ± 4.75^{a}	41.25 ± 5.07^{a}	36.8 ± 3.96^{a}
AD5	3.5 ± 0.5^{ns}	66.25 ± 3.49^{e}	2.75 ± 0.83^{ab}	744.8 ± 9.09^{e}	84.55 ± 5.17^{c}	72.5 ± 3.84^{e}	56.4 ± 4.83^{d}
Mean ±SD (*n*=5) followed by the same letter in the colum is not significant at the 5% level according to Duncan's Multiple Range Test (DMRT); ns=not significant.							

Fig 2: Adult *C. z. sillemi* feeding on tea pollen

Conclusions

The study's findings revealed that there are effective and superior diets for mass rearing both *C. cephalonica* and *C. z. sillemi.* For *Corcyra*, a diet consisting of 41% wheat, 41% rice, 5% groundnut and 3% yeast showed better results compared to other diets. Additionally, among the larval diets for *C. z. sillemi*, using *C. cephalonica* eggs was found to be the most effective and superior option for *C. z. sillemi* larvae. Similarly, a diet containing tea pollen, protein-X and honey (Tea pollen + 20g + 20g in 250ml of water) was also found to be effective and superior for *C. z. sillemi* adults.

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