Journal of Biological Control, 38(2): 214-219, 2024, DOI: 10.18311/jbc/2024/36445

Research Article

Impact of diet composition of *Corcyra cephalonica* (Lepidoptera: Pyralidae) on the development and reproduction of *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae)

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ABSTRACT: Utilizing parasitoids for augmentative biological control frequently depends on the physiological state of the parasitoids and the pest population density at the time of release. *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is commonly used as an egg parasitoid for biological insect pest control via augmentation and field release. A crucial component of mass production programmes is the host's nutritional compatibility. So, the investigations were carried out to study the effect of diet composition of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) on developmental and reproductive parameters of *Trichogramma chilonis* Ishii. The *T. chilonis* was reared on *Corcyra cephalonica* fed on different diets (D_1-D_7) in the laboratory at 25°C±1°C with 75±5% RH. Based on pooled data of two generations *T. chilonis* had shown significantly highest parasitization of 85.80%, adult emergence percentage 75.00% and sex-ratio 1.62:1 on rice moth eggs obtained from Maize (48.5%) + Sorghum (48.5%) + Groundnut (3%) diet (D_5). *Trichogramma* adult's emergence was observed earlier (3.10 days) on the host's eggs reared on the Bajra (100%) diet (D_1). In case of adult longevity, the maximum longevity of 7.70 days was recorded on the host's egg obtained from the D_5 diet over the others. These findings may help to improve the developmental and reproductive performance of *T. chilonis* and an optimized D_5 diet may be useful for mass rearing of this egg parasitoid.

KEYWORDS: *Corcyra cephalonica*, emergence, longevity, parasitization, sex ratio*, Trichogramma chilonis*

(Article chronicle: Received: 02-02-2024; Revised: 18-04-2024; Accepted: 23-04-2024)

INTRODUCTION

Biocontrol agents are a significant component of IPM and its demand is growing day by day. There is potential to increase the production and use of these bioagents (Barratt *et al.*, 2018). Biological control techniques can help to mitigate the harmful effects of chemical pesticides on the environment while slowing the spread of insecticide resistance in pest insects (Frank, 2010; Liang *et al.,* 2018; Tang *et al.*, 2010). Insects' natural enemies as biological control agents are more environmentally friendly alternatives to chemical pesticides that might potentially reduce or eliminate their uses (Lou *et al.*, 2013). Egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are the most widely utilised and extensively researched biological control agents for agricultural and forest lepidopterous pests via augmentation and field release (Funde *et al.*, 2020; Murali-Baskaran *et al.*, 2021; Tian *et al.*, 2017). The parasitoid wasp Trichogramma is found worldwide, particularly in lepidopteran hosts which are infesting diverse crops They are minute endoparasites of insects' eggs, sometimes giving nearly 100% parasitism depending upon the availability of favourable conditions. They can destroy the pest in its early stages and be employed in conjunction with other pest control approaches, and they can easily be reared in huge quantities on alternate hosts (Cherif *et al.*, 2021; Li, 1994; Smith, 1996). To ensure the regular large-scale release of beneficial insects their continuous mass-rearing is necessary, which is a commercial activity in numerous countries, Cherif *et al.* (2021) have evaluated the importance of laboratory mass-rearing settings, which has a big impact on the success of released egg parasitoids in the field. (Pak & Van Lenterene, 1988) reported that the Trichogramma strains performed well in the laboratory and also adapted to field conditions.

Trichogrammatids are one of the important parasitoids amenable for mass production, which can be accomplished by mass culturing of its factitious host, either *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) or *Sitotroga cerealella* (Olivier). The quality of the Trichogrammatids in the laboratory mostly depends on the quality of the host eggs, which ultimately depends on the host's nourishment.

Consequently, host food may have an impact on the nutritional value of host eggs as well as the survival of Trichogramma and other egg parasitoids disseminated throughout the environment as biological control agents (Finney 1964; Fisher 1958; Hunter, 2003). The stored grain pest rice meal moth, *C. cephalonica*is an excellent surrogate host for the production of various bio-control agents such as egg parasitoids- *Trichogramma* spp., egg larval parasitoids- *Chelonus blackburni*, larval parasitoids- *Bracon* spp., *Goniozus nephantidis*, *Apanteles angaleti* and insect predators- *Chrysoperla carnea*, *Mallanda boniensis*, *Crytorhinus feltiae*, *Neoplectana carpocapsea* and *Rhynocoris* sp. (Kumar & Murthy, 2000). Therefore, the present study was conducted to assess the developmental and reproductive parameters of *T. chilonis* on eggs of *C. cephalonica* which were reared on different diets.

MATERIALS AND METHODS

Setting up of experiment and source of insect culture

The present investigation was conducted at the Biocontrol Laboratory, Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar (29.15ºN, 75.70ºE) from 16 October 2020 to 30 April 2021 at 25°C±1°C with 75±5% RH. We obtained nucleus culture for the rearing of *C. cephalonica* and developmental, reproductive studies of *Trichogramma chilonis* from an existing culture in the Bio-control Laboratory, Department of Entomology, CCSHAU, Hisar. *T. chilonis* was collected from Til hawk moth *Acherontia styx* eggs collected from sesame fields of the experimental area in Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. These parasitoids were maintained on *C. cephalonica* eggs reared on a dietary mixture of bajra, sorghum, and maize so that they could adapt in the lab before the experiment.

Preparation of artificial diets

We formulated 7 diets (D_1-D_7) prepared in different proportions from the milled grains (3-4 pieces) of bajra (*Pennisetum typhoides* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*) alone or in a mixture. The composition of different diets *viz*. D_1-Bajra (100%), D_2-Maize (100%), D_3-Wheat (100%), D_4-Bajra (48.5%) + Sorghum (48.5%) + Groundnut (3%), D_5-Maize (48.5%) + Sorghum (48.5%) + Groundnut (3%), D_6-Wheat (48.5%) + sorghum (48.5%) + groundnut (3%), D_7-Mix Bajra (32.33%) + Maize (32.33%) + Wheat (32.33%) + Sugar (3%). The rice moths were reared in wooden rearing cages (41 x 22 x 12 cm^3). The rearing cages were sterilized for 2 hours in a hot air oven at 80°C to eliminate hibernating macro and some microorganisms. After that, the oven-exposed feeding material was added at 1.5 kg per rearing enclosure, along with 5 g yeast powder per cage. To check the growth of microbial pathogens, each rearing cage was sprayed with 0.1% formalin and 0.05% streptomycin sulphate solution. 5 g sulphur powder/cage was also dispersed to avoid storage mite infestation.

Maintenance of insect culture

Freshly laid *C. cephalonica* eggs from the different diets were collected and we maintained *T. chilonis* culture for the experiment by sterilizing them under UV light (30 watts) for 20 minutes. 100 freshly laid eggs of rice moth from each diet after sterilization were pasted on the strip (7 x 2 cm^2) of tricho card with *Acacia* gum. Small drops of 100% honey were streaked on the upper end of the strip for feeding newly emerging parasitoids. The strip containing fresh eggs was placed with a Trichogramma-infested strip (2 x 1 cm) in the same glass vial (15 x 2.5 cm^2) and sealed with a cotton plug (6:1) with 5 replications by using a Completely Randomized Design to conduct the various observations. This study was carried out for 2 generations.

Determination of parameters

To determine how different diet-fed *C. cephalonica* eggs affected parasitization percentage, adult emergence, sex ratio, adult longevity and adult emergence percentage of *T. chilonis* the vials containing strips of tricho cards were observed daily under stereo zoom binocular microscope (Zeiss Stemi 508 Carl Zeiss AG Company, Germany). Exposed host eggs were held for 3-4 days in the vials. We started observing them daily and parasitized eggs turned into black colour.

Per cent parasitization, time of adult parasitoids emergence and emergence percentage

The parasitization percentage was estimated as several parasitized eggs out of a total (100 eggs) pasted on a strip of tricho card based on the colour of the eggs. For the next 3-4 days' daily observation was taken into account about the number of eggs that got parasitized.

$$\text{Per cent parasitization}\left(\%\right)=\frac{\text{Number of parasitized eggs}}{\text{Total number of pasted eggs}}\text{ x }100$$

The duration between exposures of eggs for parasitization to the first emergence of *Trichogramma chilonis* adult was recorded to calculate the time of emergence. To avoid variability average emergence time of 40 individuals was taken into account**.** Also, the total number of emerged parasitoids was calculated from the parasitized eggs to estimate the emergence percentage.

Adult longevity and sex ratio

To determine the life expectancy, a time interval from the date of emergence of *T. chilonis* adults till the death of

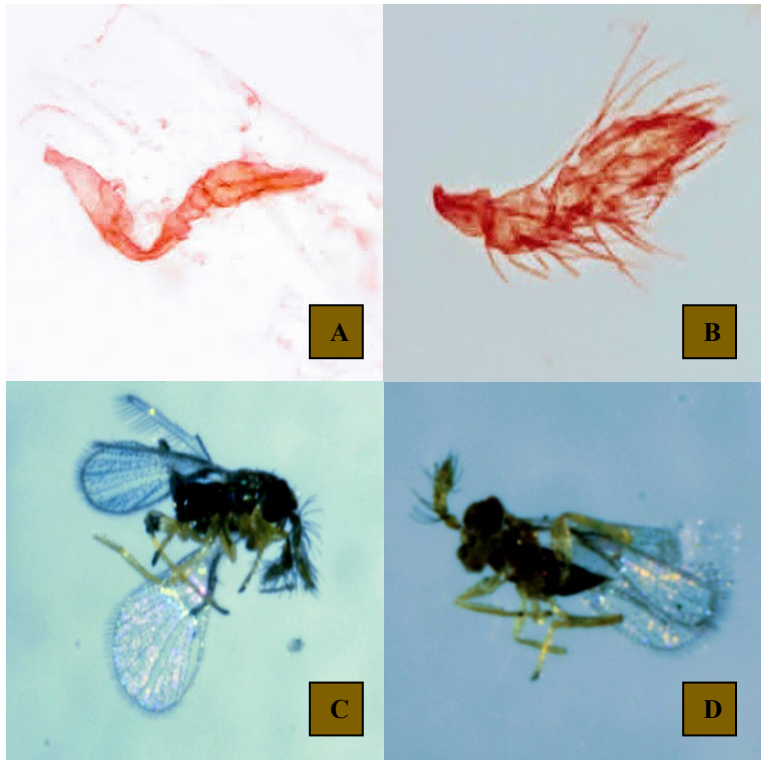


Figure 1. (A). ♀ antenna; **(B).** ♂ antenna; **(C).** Female parasitoid; **(D).** Male parasitoid.

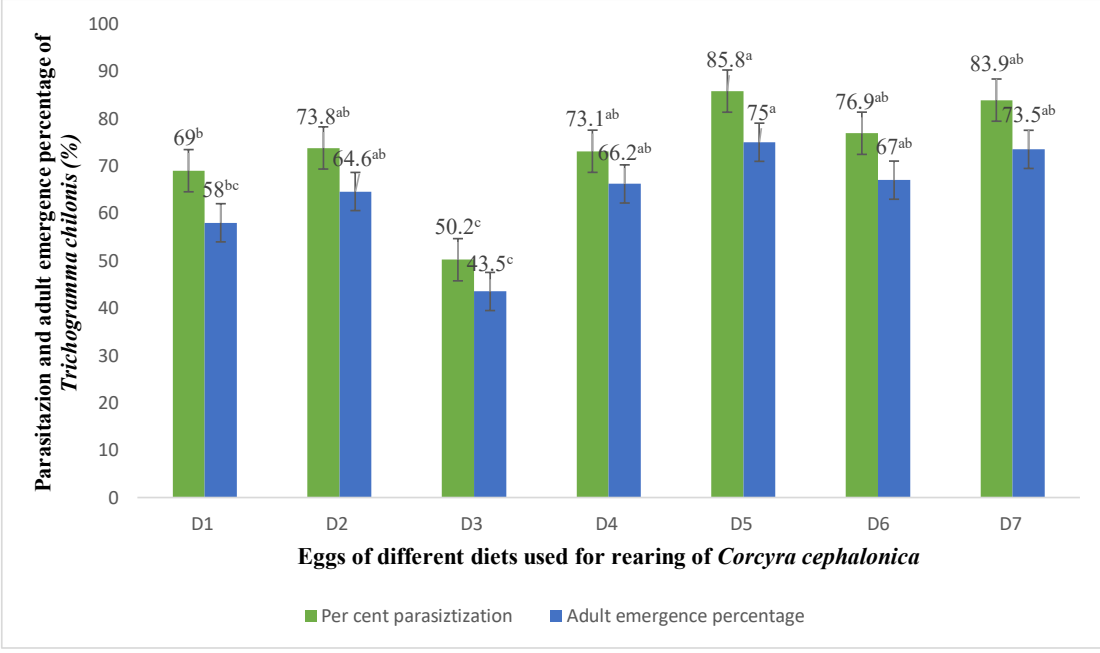
these wasps was noted. Newly emerged *T. chilonis* adults were fed with honey and they were separated in individual vials. The emerged adults are allowed to die and the sex ratio was worked out based on morphological characters. The female is pale in colour and larger than the Male with a yellowish-orange body and tapering abdomen. The antenna is short, not distinct, was less plumose with short bristles. The male is darker and usually smaller than the female, with a dark round abdomen (Figure 1). The antenna is distinct, long and plumose, with black long bristles (Nagarkatti & Nagaraja, 1979).

Statistical analysis

Statistical analysis of data was carried out using SPSS software version 20.0 (Statistical Package for the Social Sciences) for Windows. Homogeneity of variances was ensured before executing the Analysis of Variance in Completely Randomized Design. One-way analysis of variance was used for multiple comparative analyses and treatment means were separated by the Honest Significant Differences (HSD) method, when applicable. Subsequently, the overall means were compared using Tukey's HSD Test at a 95% significance level.

RESULTS AND DISCUSSION

The current study provided information on the impact of different diets of *Corcyra cephalonica* on developmental and reproductive traits of *Trichogramma chilonis* like parasitization, emergence percentage, time of adult parasitoids emergence, sex ratio and adult longevity. Our results from pooled data of both generations (first and second) revealed that host eggs reared on different diets had a significant effect on the developmental and reproductive parameters of *T. chilonis*. Among the 7 evaluated diets, out of 100 eggs taken initially, significantly highest parasitization of 85.80% was obtained on D_5 diet eggs but the lowest parasitization was 50.20% on D_3 diet eggs (Figure 2). Parasitization percentage of D_1, D_2, D_4, D_6 and D_7 diets eggs as 69.00%, 73.80%, 73.10%, 76.90% and 83.90%, respectively were not statistically different. Millets' nutritional indices, as well as the larger size of eggs of rice moths, reared on the D_5 diet are the factors that favoured more parasitism. This study supports the previous findings by (Tiwari & Khan, 2003; Kamble *et al.*, 2006) who reported the highest per cent parasitization 51.96% and 88.40% by *T. chilonis* on eggs deposited by rice moths, reared on a maize + yeast diet and sorghum, respectively. The time taken by adult parasitoids to emerge from parasitized eggs was significantly longer 5.90 days on D_3 diet eggs meanwhile shortest time taken to emerge was 3.10 days on D_1 diet eggs which was on par 3.30 days with D_4 diet (Table 1). No significant variation was observed in the parasitoids emergence period from D_2, D_5 and D_7 eggs. This might be due to the nutritional composition of the diet on which rice moths were reared. The eggs with the highest



Where, D_1 - Bajra (100%), D_2 - Maize (100%), D_3 - Wheat (100%), D_4 - Bajra (48.5%) + Sorghum (48.5%) + Groundnut (3%), D_5 - Maize (48.5%) + Sorghum (48.5%) + Groundnut (3%), D_6 - Wheat (48.5%) + sorghum (48.5%) + groundnut (3%), D_7 - Bajra (32.33%) + Maize (32.33%) + Wheat (32.33%) + Sugar (3%)

Figure 2. Effect of different diets on parasitization percentage and parasitoids emergence percentage of *Trichogramma chilonis* reared on the eggs of *Corcyra cephalonica*.

degree of parasitization showed the greatest emergence. This could be due to the carbohydrate, fat and protein along with vitamin content supplied by maize and sorghum during their developmental period inside the eggs. Present findings are in confirmation with Mehendale (2009) where maximum *T. chilonis* emergence (94.67%) was observed from parasitized eggs of *C. cephalonica* grown on millet mixtures. Similarly, the emergence percentage of adult parasitoid *T. chilonis* was found significantly highest at 75.00% from parasitized eggs of *C. cephalonica* fed on D_5 diet eggs and the lowest at 43.50% parasitoid emergence was recorded on D_3 diet eggs (Figure 2). The higher nutritive value of eggs facilitates faster development and to emerge earlier.

Adult *T. chilonis* parasitoid survived significantly longer than 7.70 days on rice moth eggs of D_5 diet eggs but a shorter life span of 5.00 days was observed on diet D_1 diet eggs (Table 1). Present findings are in confirmation with Mehendale (2009) where female parasitoid longevity was found at 4.47 days and 1.73 days when host eggs were fed on sorghum + groundnut + yeast powder and sorghum alone, respectively. Patel (2011) also recorded the longevity of adult parasitoids reared on rice moth eggs. The longest life expectancy for male (4.61 days) and female (6.89 days) parasitoids was noted from the eggs of rice moth which were fed on a mixture of sorghum with 10 per cent groundnut seed, 2 per cent yeast powder and 3 per cent sugar powder whereas, lowest longevity of male (2.79 days) and female (3.92 days) parasitoid was noted from the eggs of rice moth fed with bajra alone. The longest life span of 9.1 days of *T. chilonis* was found in maize (Mamung & Kumar, 2016).

The emergence of adults from parasitized host eggs revealed that the sex ratio was female-biased, among which a substantially higher sex ratio was 1.67:1 on D_5 diet eggs and the lowest 1.03:1 on D_3 diet eggs (Table 1). There was no significant difference in the sex ratio of D_1, D_2, D_4 and D_6 diet eggs. Our results about the sex ratio of *T. chilonis on C. cephalonica* eggs reared on various diets were also in line with (Mehendale, 2009; Naik *et al.*, 2015; Patel, 2011) who recorded that the highest female-aligned sex ratio on millets and millets mixture. Nathan *et al.* (2006) discovered a link between carbohydrate, protein, and fat content of the diet and survival of *T. chilonis*.

Table 1. Effect of different diets on adult emergence, adult longevity and sex ratio of *Trichogramma chilonis* reared on the eggs of *Corcyra cephalonica*

Treatments	Adult emergence (days)			Adult longevity (days)			Sex Ratio (F: M)		
	First Generation	Second Generation	Pooled	First Generation	Second Generation	Pooled	First Generation	Second Generation	Pooled
D_1 - Bajra (100%)	3.00^{a}	3.20^{a}	3.10^{a}	4.80^{b*}	5.20^{c}	5.00^{b}	1.16:1^{b}	1.08:1^{ab}	1.11:1^{bc}
D_2 - Maize (100%)	4.20^{ab}	4.00^{abc}	4.10^{ab}	6.20^{ab}	7.00^{abc}	6.60^{ab}	1.08:1^{b}	1.16:1^{ab}	1.12:1^{bc}
D_3 - Wheat (100%)	6.00^{b}	5.80^{c}	5.90^{c}	5.60^{ab}	5.80^{abc}	5.70^{b}	1.07:1^{b}	0.98:1^{b}	1.03:1^{c}
D_4 - Bajra (48.5%) + Sorghum (48.5%) + Groundnut (3%)	3.20^{a}	3.40^{ab}	3.30^{a}	4.80^{b}	5.40^{bc}	5.10^{b}	1.21:1^{b}	1.21:1^{ab}	1.21:1^{bc}
D_5 - Maize (48.5%) + Sorghum (48.5%) + Groundnut (3%)	4.00^{ab}	4.60^{abc}	4.30^{ab}	7.60^{a}	7.80^{a}	7.70^{a}	1.90:1^{a}	1.44:1^{a}	1.67:1^{a}
D_6 - Wheat (48.5%) + Sorghum (48.5%) + Groundnut (3%)	5.40^{b}	5.40^{bc}	5.40^{bc}	6.20^{ab}	6.60^{abc}	6.40^{ab}	1.25:1^{b}	1.28:1^{ab}	1.26:1^{bc}
D_7 - Bajra (32.33%) + Maize (32.33%) + Wheat (32.33%) + Sugar (3%)	4.40^{ab}	4.00^{abc}	4.20^{ab}	7.20^{a}	7.60^{ab}	7.40^{a}	1.49:1^{ab}	1.30:1^{ab}	1.40:1^{ab}
SE m (±)	0.24	0.22	0.20	0.24	0.25	0.21	0.63	0.39	0.41
MSE	1.14	1.18	0.52	1.16	1.43	0.71	0.75	0.42	0.22

*Different letters denote significantly different values, at a significance level $p<0.05$ according to Tukey's HSD Test.

CONCLUSION

The present studies exhibited the impact of different diets on certain life history traits of *T. chilonis* and revealed the best dietary composition for mass production of quality egg parasitoids. Optimizing and improving parasitoid mass rearing is important for biological control efficacy. Among the seven diets tested, D_5 [maize (48.5%) + sorghum (48.5%) + groundnut (3%)] was found as the most preferred diet for the development of *T. chilonis* on the eggs of *C. cephalonica* which resulted in high per cent parasitization, sex ratio, adult longevity and emergence percentage.

ACKNOWLEDGEMENT

The authors are grateful to Professor and Head, Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University Hisar, India, for providing the required facilities for this study. This experiment was conducted as part of the M.Sc. thesis of the first author.

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