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Role of vitamins on various biological aspects of an egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymanoptera: Trichogrammatidae) on its laboratory host, *Corcyra cephalonica*

Kapinder[1], Tarkeshwar[1] and Ashok Kumar Singh[2]
[1, 1, 2] (Department of Zoology, University of Delhi, India)

Abstract: *Parasitic wasps play major role in sustainable agriculture through their ability to regulate population of herbivorous insect pests and nutrition is the key component to the success of the parasitoids. In the present study, the effect of nutrition was revealed by a significantly higher survival of both male and female parasitoids when fed on sucrose and other vitamins as compared with water fed and unfed. The survival of adult wasps was also not much affected when different concentration of vitamins was tested except for pyridoxine in which survival of adult wasp was inversely related to concentration. Female parasitoids lived longer than the male in all treatments as well as in control. Parasitisation by female T. chilonis showed no significant difference except pyridoxine which showed lowest parasitisation percentage. The sex ratio in all treatments showed female biased populations except pyridoxine which showed male biased population. The present study may be helpful to select the best artificial food source which can be artificially sprayed in the field to increase the longevity and parasitisation potential of T. chilonis*

Keywords*: Survival, Parasitisation, Trichogramma, Sex-ratio, vitamin, sucrose*

I. Introduction

Host plant, its pest and parasitoid of pest form a triangle of interacting forces in which nutrition play a decisive role in influencing the pest parasitoid relationship. The overall performance of a parasitoid is largely dictated by their energy resources. Among all the insects, parasitoids are unique in their nutritional requirements as some adult parasitoids not only depend on the host for feeding, but may also require sugar solutions, such as nectar or honeydew, as a source of energy (Jervis and Kidd, 1986). Nutritional deficiency in parasitoids causes poor parasitization, emergence and poor sex ratio, resulting in their reduced efficiency as biological control agents.

Carbohydrates provide an important source of energy to parasitoids as in other animals. Sugar feeding has been reported to increase parasitoids longevity (Wäckers, 2001; Siekmann *et al.*, 2001) as well as its fecundity (Schmale *et al.,* 2001). In addition, well fed parasitoids are usually more active and more focused in seeking out their herbivores hosts (Wäckers, 1994). Therefore, the availability of sugar source is a key factor in the population dynamics of a parasitoid-host system in biological control program.

Although the sugars are likely to be the most important nutrients in promoting longevity, there is also the possibility that micronutrients such as vitamins may also benefit parasitoid. There is very little knowledge on the effects of dietary components, especially vitamins, on the longevity and reproduction of hymenopteran parasitoid especially *Trichogramma* spp. which prevent us from making any generalizations on these species. It is the most important and has received the maximum attention, because of its importance in biological control. *Trichogramma* has been attempted to control against at least 28 different phytophagous pest species on 20 different crops. In India, 12 indigenous species of *Trichogramma* have been used, besides a few introduced species in release programmes. Among these, *Trichogramma chilonis* Ishii, is widely distributed in the Indian subcontinent, which has been widely used for the control of many lepidopterous pests.

Present study was done to understand the role of some water soluble vitamins on the parasitisation, adult emergence, and sex-ratio of *Trichogramma chilonis.*

II. Materials And Methods

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) was reared on rice meal moth, *Corcyra cephalonica* Stainton (Lepidoptera: Galleriidae). Initial culture of the host insect and the egg parasitoid was started from the insects obtained from the Biological control laboratory of the Division of Entomology, I.A.R.I., New Delhi.

A. Host insect culture

Larvae of *C. cephalonica* were reared on broken grains of maize (*Zea mays*), Jowar (*sorghum vulgare)* and Bajra (*Pennisetum typhoides*) that were sterilized in oven at a temperature of 100^{o} C for 1 h to eliminate the possibility of infestation by other stored grain pests. All the grains were mixed thoroughly and put in 3 L glass jars and inoculated with freshly laid eggs of *C. cephalonica* at 0.5cc per jar. The culture was maintained at a temperature of 27±2^{o}C, 55 ± 5 % RH and a photoperiod of 12L: 12D. Freshly emerged adult moths were collected every morning and transferred to mating cum oviposition cages. It consisted of an inverted funnel (21 cm dia X 23 cm ht) fitted over a sieve (22 cm dia) having a mesh size of 10mesh/cm. The mouth of the stem of the funnel was covered with a cotton plug soaked with 10% sucrose solution as an adult feed. The adults were released from the stem of the funnel. The whole set up was placed over a tray lined with filter paper to collect the eggs that passed through the sieves. Use of sieve at the open end of the funnel ensured collection of clean eggs in the tray, devoid of any scales or broken parts of the adult moths. Eggs were collected daily and were used for further inoculation for maintaining the host culture, parasitoids multiplication and other studies.

For multiplication of *T. chilonis* and other studies, 0-24 h old host eggs were exposed to ultra violet radiation in a closed wooden chamber at a distance of 15 cm from the UV-lamp, for about 30min., in order to kill the developing host embryo.

B. Egg parasitoid culture

Culture of the egg parasitoid, *T. chilonis,* was maintained in the glass vials (2.5 cm dia X 10 cm ht). The adult parasitoids were offered an egg card (8 cm X 2.5 cm), made of Ivory paper, containing approximately 1000 sterilized healthy eggs of *C. cephalonica.* They were allowed to parasitize the eggs for 24 h so as to prevent superparasitism. Each vial was provided with 50% honey solution as adult food in the form of fine streak on the inner wall of the glass vial and secured tightly with cotton plug. After 24 h the egg cards were removed and kept for development in a fresh tube after clearing the parasitoids. Parasitized eggs turned black on the fourth day after parasitisation. These parasitised egg cards were used for further maintenance of culture as well as for the experiment. The parasitoid culture was maintained in a B.O.D. incubator at 27±1^{o} C, at 65 ± 5 % R.H and a photoperiod of 12:12 (L: D).

C. Preparation of the vitamin solutions

The different concentrations of the vitamins were prepared by dissolving the required amount of the vitamin in 20% sucrose solution. The vitamins selected for the bioassay were ascorbic acid, riboflavin, pyridoxine, thiamine and cyanocobalamin. To prepare 0.1% test solution 10 mg of the vitamin was dissolved in 10 ml of 20% sucrose solution and 0.001% test solution was prepared by serial dilution of 0.1% concentration in 20% sucrose solution.

D. Effect on longevity

The adult male and female parasitoids were separated on the basis of antennal variation. Diet was provided in the form of fine streak along the glass wall twice a day. Longevity of the parasitoid was recorded on the basis of mortality of the parasitoid in each vial, after an interval of every 12 h. The replicates were maintained at 26±1^{o}C temperature and 70±5% relative humidity with 12:12 of L: D photoperiod. The bioassays were replicated five times, each replicate consisting of responses of 10 wasps.

E. Effect on parasitisation and sex-ratio

One pair of adult *T. chilonis* was released in each homeopathic vial and provided different vitamin diets for two hour prior to placement of egg cards. Thereafter single egg card (2.5cm X 0.5 cm) containing 100 UV sterilized eggs of *C. cephalonica* (pasted with diluted acacia gum), kept in each vial and females were allowed to parasitize the eggs for 24 h. then the egg cards were removed from the vials and kept in B.O.D. incubator. The number of eggs parasitized was recorded by counting the blackened eggs. Total number of parasitoids emerged out of the parasitized eggs and sex ratio were recorded.

F. Statistical analysis:

Data were subjected to one-way ANOVA. Means were separated by Tukey's test to analyze the significant difference between the control and different concentrations and also differences among different concentrations. All statistical analysis was performed on computer software program SigmaStat 2.0 (Jandel Scientific, 1995). Whereas, sex-ratio was analyzed by student t-test.

III. Results

A. Effect on longevity

The longevity of male and female parasitoid, *T. chilonis,* fed on 20% sucrose solution (9.5 days and 10.9 days respectively) was significantly longer as compared to those fed on water (1.1 days) or unfed (0.8 days) parasitoids. Also, the survival of both male and female parasitoids on different vitamins was significantly higher than on water fed or starved parasitoids. However, no significant difference was observed in the survival of the male parasitoids fed on 0.001% concentration of different vitamins and 20% sucrose. Similar observations were also recorded in female parasitoids that had statistically equal survival on different vitamins at 0.001% concentration and 20% sucrose, except for pyridoxine (13.4 days) which showed significantly (P<0.05) longer survival than on all other vitamin treatments (Table.1).

The longevity of male parasitoids fed on 0.1% concentration of different treatments was statistically similar with 20% sucrose solution. The survival of male wasps was significantly lower on pyridoxine (7.4 days) as compare to ascorbic acid (10.9 days). There was no significant difference was observed in the survival of the female parasitoids fed on different vitamins (0.1% concentration) and control (20% sucrose) (Table.1).

Table 1. Survival of *T. chilonis* on 20% sucrose solution mixed with different vitamins

Sex	Type of food	Concentration (%) Mean ± S.D.	
		0.1%	0.001%
Male	No food	0.8 ± 0.274^{b}	0.8 ± 0.274^{b}
	Water	1.1 ± 0.42^{b}	1.1 ± 0.42^{b}
	Sucrose (20%)	9.5 ± 1.06^{ac}	9.2 ± 2.52^{a}
	Ascorbic acid	10.9 ± 0.55^{a}	7.1 ± 1.25^{a}
	Riboflavin	10.3 ± 0.97^{ac}	8.4 ± 0.89^{a}
	Pyridoxine	7.4 ± 0.65^{c}	9.9 ± 2.10^{a}
	Thiamine	8.9 ± 2.65^{ac}	8.5 ± 1.54^{a}
	Cyanocobalamin	8.4 ± 2.966^{ac}	7.8 ± 0.57^{a}
Female	No food	0.9 ± 0.42^{b}	0.9 ± 0.42^{b}
	Water	1.4 ± 0.42^{b}	1.4 ± 0.42^{b}
	Sucrose (20%)	10.9 ± 1.34^{a}	10.9 ± 1.34^{ac}
	Ascorbic acid	13.2 ± 4.56^{a}	9.38 ± 1.89^{a}
	Riboflavin	12.4 ± 1.71^{a}	10.4 ± 0.96^{a}
	Pyridoxine	10.5 ± 3.72^{a}	13.4 ± 2.41^{c}
	Thiamine	12.0 ± 3.66^{a}	10.4 ± 1.43^{a}
	Cyanocobalamin	11.2 ± 1.82^{a}	9.8 ± 0.45^{a}

Note: Means followed by different superscripts in the particular column differ significantly with in the sex (P<0.05)

A comparison was done between two concentrations (0.1% and 0.001%) of tested vitamins, where the longevity of male parasitoids fed on 0.1% ascorbic acid and riboflavin was significantly higher than the wasps fed on 0.001%. Whereas, survival on pyridoxine at 0.1% was statistically lower than 0.001% concentration. However, no significant difference was observed in the survival of female wasps when compared between 0.1% and 0.001% concentration of vitamins (Table 2).

Table 2. Mean survival of male and female *T. chilonis* on different concentrations of vitamin

Sex	Conc. (%)	Survival (days) Mean ± S.D.				
		Ascorbic acid	Riboflavin	Pyridoxine	Thiamine	Cyanocobalamin
Male	0.001	7.1 ± 1.25^{a}	8.4 ± 0.89^{a}	9.9 ± 2.1^{a}	8.5 ± 1.54^{a}	7.8 ± 0.57^{a}
	0.1	10.9 ± 0.55^{b}	10.3 ± 0.97^{b}	7.4 ± 0.65^{b}	8.9 ± 2.65^{a}	8.4 ± 2.97^{a}
Female	0.001	9.38 ± 1.89^{a}	10.4 ± 0.96^{a}	13.4 ± 2.41^{a}	10.4 ± 1.43^{a}	9.8 ± 0.45^{a}
	0.1	13.2 ± 4.56^{a}	12.4 ± 1.71^{a}	0.5 ± 3.72^{a}	12.0 ± 3.66^{a}	11.2 ± 1.82^{a}

Note: Means followed by different superscripts in the a particular column of individual vitamin differ significantly (P<0.05)

B. Effect on parasitisation and sex-ratio

The parasitisation percentage was not significantly affected by any vitamins except pyridoxine. The parasitisation on ascorbic acid (68%), riboflavin (68.4%), thiamine (67.8%) and cyanocobalamin (66.5%) was statistically similar, whereas, pyridoxine showed significantly lower parasitisation of 33.6% when compare to rest of the vitamins.

The emergence trend of male and female parasitoids reared on different diets showed significant correlation between the type of food and sex of the progeny. There was no significant difference in male-female sex ratio was observed when parent parasitoids were fed pyridoxine (male 51.22% and female 41.47%). However, when parent wasps were fed on other tested food, significantly higher numbers of female progeny was produced as compared to males.

Table 3. Parasitisation percentage and sex-ratio of *T. chilonis* on 0.1% concentration of vitamins

Food	Parasitisation (%)	Mean ± S.D. Sex-ratio	
		Male	Female
20% Sucrose	58.4 ± 9.18[a]	20.00 ± 6.11[a]	63.35 ± 12.32[b]
Ascorbic acid	68 ± 4.69[a]	25.29 ± 12.58[a]	63.30 ± 5.57[b]
Riboflavin	68.4 ± 4.93[a]	33.22 ± 6.63[a]	59.80 ± 10.13[b]
Pyridoxine	33.6 ± 9.762[b]	51.22 ± 16.50[a]	41.47 ± 23.97[a]
Thiamine	67.8 ± 8.38[a]	28.02 ± 4.97[a]	58.74 ± 8.71[b]
Cyanocobalamin	66.5 ± 7.31[a]	34 ± 8.84[a]	53.72 ± 9.03[b]

Note: Means followed by different superscripts in the column of parasitisation and along the row in sex-ratio differ significantly (P<0.05, t-test).

IV. Discussion

The provision of supplemental food to parasitoid, *Trichogramma chilonis* appears to be an important factor for increasing longevity of the parasitoid. Both the male and female *T. chilonis,* fed on sucrose solution survived for longer duration as compared to those fed on water or starved wasp. Earlier studies have also shown that starvation significantly reduces the longevity of male and female parasitoids (Jervis and Kidd, 1986), whereas provisioning of food increases the longevity of *T. carverae* and *T.* nr *brassicae* (Gurr and Nichol, 2000). In the present study, *T. chilonis* has been observed to survive less than one day (0.9 days) without food. However another species of this parasitoid, *T. brassicae* and *T. carverae* have reported to survive without food for 5 days and 6 days respectively without food (Gurr and Nichol, 2000).

The longevity of the parasitoids on different vitamin treatments and control sucrose solution was statistically same. This indicates that the tested concentrations equally support survivorship of both male and female parasitoids. Individual sugars and mixture have been also earlier observed to significantly affect the longevity of parasitoid, *Bathyplectes curculionis* (Jacob and Evans, 2004). The survivorship of the parasitoids on different vitamins, except pyridoxine, was comparable with that of sucrose solution. Pyridoxine has concentration dependent effect on survival period of parasitoids. The observation clearly indicates that dose of vitamin is critical for the overall fitness of the parasitoids, and requirement of pyridoxine by *T. chilonis* is far less than other vitamins tested. Higher quantity of vitamin in diet has been reported to decrease the feeding of insects (Friend, 1958). Etebari and Matindoost (2004) observed that high dose of vitamin B_3 in the diet interrupt feeding and normal growth of silkworm larvae. The observed High mortality was recorded during molting of larvae. Significant decrease in larval weight was observed when silkworm larvae were fed on 3% vitamin C incorporated diet. Incorporation of high level of ascorbic acid in house cricket diet has been observed also to inhibit spermatogenesis and decrease the viability of eggs (Mcfarlane, 1992).

Vitamins do not appear to influence parasitization percentage of insects, except pyridoxine that has been observed to reduce rate of parasitization. This may be because pyridoxine adversely affects egg production of parasitoids. However, there are certain reports that some vitamins such as Vitamin E enhance the egg production in the insects (House, 1966).

All vitamins, except pyridoxine, appear to cause female biased populations. This is contrary to the earlier observation of Leatemia *et al.* (1995), who found male biased population in long lived females of *T. minutum* when fed on different concentrations of honey solution. In the present study, the sex ratio was studied with the eggs, laid in 24 hr of emergence, and not for the whole life of the parasitoid.

V. Conclusion

Vitamins play important role in survival, longevity and parasitization of *T. chilonis*. However, effect of pyridoxine on the fitness of this parasitoid is dose dependent. Lower dose of pyridoxine has positive effect but at higher dose it cause lower longevity, lower parasitization and male biased populations. This means that higher concentration of pyridoxine adversely affect fitness of *T. chilonis*. The reversed has been reported for riboflavin, which inhibits growth of several insect species at lower dose but this ill effect is not observed at higher dose (Akov, 1962; Vanderzant, 1963). However, to understand the actual effect of any component of food on the fitness of the parasitoids, studies 2 or 3 generations are required (Ouye and Vanderzant, 1964; Kasting and McGinnis, 1967; Dadd, 1973).

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