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Effects of Various Diets on the Oviposition and Sex Ratio of *Pimpla turionellae* L.

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ABSTRACT

Adult female *Pimpla turionellae* were reared on chemically defined synthetic diets and the effects of various diet compositions on the egg production, emergence, sex ratio, protein and glycogen levels were investigated. The diet containing 50% honey had no significant effect on all the parameters and also on prolonged development time for egg production. Egg production, fertility and sex ratio increased in animals fed with diet having reduced glycogen. The adult *P. turionellae* could tolerate different diets without any serious problem during their development. An important concept in developing control diet was that components presented were digestible and were highly utilizable for adult *P. turionellae*.

Key Words: *Pimpla turionellae;* Sex ratio; Synthetic diets; Parasitoid rearing

INTRODUCTION

Many species can be reared on artificial diets to the adult stage (Grenier, 1997). In recent years, interest in artificial rearing of parasitoids has increased. Artificial rearing may not only prove useful for investigation insect biology and behavior but also be suitable for mass producing parasitoids more easily and less expensively (Greany *et al.,* 1984; Thompson, 1986, 1999; Vinson, 1986, 1994). Advantage of mass rearing on artificial diet is that ingredients can be selected that are available all year round and are easy to prepare and to store (Vanderzant, 1974; Singh, 1977).

Species of parasitic hymenoptera are used extensively in biological control as agent for permanent suppression of a pest or as insectary reared, for instance *Pimpla turionellae* L. is an Ichneumonid endoparasitoid that attack many lepidopterous species, such as larvae or pupae of *Aporia crataegi* (L) (Pieridae), *Lymantria dispar* (L) (Lasiocampidae), *Ephestia kuehniella Zell*. (Pyralidae) (Thompson, 1957). It is the first representative of the parasitic hymenopterous who can easily be reared (Yazgan, 1981).

The metabolic and physiologic needs of *P. turionellae,* which can attack parasitically on many kinds of lepidoptera and a few coleoptera even some hymenoptera species, can be obtained by the synthetic diet which is improved in both larval and adult period (Yazgan, 1981; Emre, 1988). It is seen that the synthetic diets that are produced by this way can generally investigate the nutrient requirements in larval period (Dadd, 1985; Thompson, 1999; Thompson & Hagen, 1999). Hymenoptera insects need glycogen as the main energy source besides protein and especially the amino acids just like the other animals (Dadd, 1985; Zografou *et al.,* 1988).

Although many nutrition requirements were carried out for *in vitro* rearing of *P. turionellae* (Yazgan, 1981; Emre, 1988; Sulanç, 1991; Sulanç & Emre, 2000; Ozalp & Emre, 2001), much more researches need to be done about the synthetic diets without insect materials in order to rear *P. turionellae* continue its reproduction throughout its whole life period.

In the present work the diets are developed in order to increase the egg production and emergence of the adult female *P. turionellae* this development is made by comparing the results that are found with the synthetic or the natural diet (only 50% honey).

MATERIALS AND METHODS

The stock species of *P. turionellae* were reared 50% honey and the haemolymph of the greater wax moth *Galleria mellonella* (L) pupae and maintained at, 23±2°C and 75±5% relative moisture subjected to 12 h light photoperiod.

The insects that are used in this experiment are selected from the not feed and mated individuals who appeared when the stock insects parasitized the *G. mellonella* pupae in the same laboratory conditions.

In this work, a synthetic nutrient with known chemical structure (Emre, 1988; Table I) was used as the control diet. Diet 3 was prepared by changing some of the ingredients in the control diet. Diet 1 was consisting of only 50% honey.

The experiments were being continued with cages that had 20x20x20 dimensions. The insects were fed by equal amounts of food (0.1 mL) dropped on to aluminum foil. The feeding process was made at exactly the same time through each day for 1 h. The cages were cleaned to prevent the reproduction of microorganisms because of the insect remnants.

Table I. Composition of diet for rearing adult *P. Turionallae*

Constituent	Diet 1	Control (Diet 2)	Test (Diet 3)
		mg 100 mL^{-1} diet	
L-Amino acid mixture	-	3000.00	4000.00
Lipid mixture	-	540.96	1081.92
Water-soluble vitamins	-	284.38	142.19
Inorganic salt mixture	-	75.00	75.00
RNA	-	75.00	75.00
Sucrose	-	14000.00	7000.00
2N KOH	-	280.00	280.00
50% Honey solution	+	-	-

In the experiment series, the *G. mellonella* pupae which were surrounded with two layers of metal cages were kept in the experiment cages in order to make the *P. turionellae* females lay. This process was reported on the 10th day of the experiment's beginning until the 37th day once in three days.

The effect of different nutrients on the fecundity (the number of eggs produced per female in the life span) and fertility (the percentage of eggs hatched produced during the life span) was made by method described previously (Emre, 1988). To calculate the sex ratios 10 females and five male insects were put at each cage. Ten *G. mellonella* pupae were given to experimental insects to make them leave their eggs. At the end of incubation period, male and female individuals emerged from pupae were recorded. The results of the experiment were examined in two different periods. First one was between day 10 and 22, and the other one was between day 25 and 37.

To analyze proteins and glycogen, 10 female individuals were homogenized in 10% TCA after the wet weights were measured at the end of experiment as described by Plummer (1971) and Roe *et al.* (1961). The total amount of glycogen was estimated according to Roe *et al.* (1961) using glycogen as standard. Total protein content was determined calorimetrically using the Quantitative Biuret Assay (Plummer, 1971). Bovine serum was used as a standard. The effects of the tested diets on the total protein and glycogen content of insect were measured by determining the average amount and percentage of the total protein and glycogen according to wet weight of adult *P. turionellae*.

All experiments were repeated at least for three times. The statistical analyses of the outcomes of the experiment were made by "Student-Newman Keul's Test (SNK)'' (Sokal & Pohlf, 1969). The difference between the averages was expected when it was bigger than the F value in 0, 05 probability level.

RESULTS

The effect of different diets on the egg production, egg hatch and total sex ratio values are given in Table II. In between day 10 and 22; the control diet increased the egg production and made it a value as 28.67. The highest egg hatching value was seen in diet 3 (82.55%); whereas, the lowest value in egg hatching (76.03%) and egg production (14.78) occurred in diet 1. It was interesting that the emergence of the adult kept the same value for insects that had diet 2 and 3. But the adult female emergence percentage was higher in diet 3 (64.48%) than the control diet (49. 50%).

In between day 25 and 37, a decrease in all values is observed. The egg production decreased approximately 50% with diet 2 and 3. Adult emergence had the lowest value in diet 1 (27.33%); diet 3 had a positive effect in all parameters when it was compared with the control diets.

The effects of the different diets on the amount of protein and glycogen that synthesized by the insects at the end of the experiment period are given in Table III. The synthesized protein amount of the adult *P. turionellae* individuals was decreased in diet 1 (2.96%). There were no differences in the protein amount between diet 3 and 2. Diet 1 increased the glycogen amount with a ratio of 70% compared with the other diets.

Table II. Effects of diet on fecundity, fertility and sex ratio of *P. turionellae.* Means±S.E.

Parameters	10thday-22thday			25thday-37thday		
	Diet 1	Control (Diet 2)	Test (Diet 3)	Diet 1	Control (Diet 2)	Test (Diet 3)
Fecundity	14.78±0.52 a	28.67±.0.22 c	26.19±0.60 b	15.69±0.57 b	13.00±0.25 a	16.75±0.43 b
Fertility (%)	76.03±0.14 a	78.67±0.10 b	82.55±0.95 c	77.46±0.81 b	71.67±0.27 a	82.95±0.42 c
Emergence adult (%)	56.00±1.15 a	71.33±2.40 b	71.33±2.40 b	27.33±0.67 a	48.00±2.31 b	55.33±0.67 c
Emergence female (%)	46.38±1.11 a	49.50±1.28 a	64.48±2.30 b	58.42±3.08 c	25.11±1.21 a	49.42±1.59 b

Table III. Effects of diet on total protein and glycogen content of adult *P. turionallae*

	Average wet weight (mg)	Total % protein (w.w)	Total % glycogen (w.w)
Diet 1	21.05	2.96±0.75 a	0.16±0.01 c
Diet 2	24.08	4.14±0.08 b	0.07±0.01 b
Diet 3	22.17	3.96±0.18 b	0.03±0.02 a

Statistical analyses were carried out separately for each group; Values followed by the same letter in each column are not significantly different from each other. P>0, 05

DISCUSSION

Many kinds of parasitoid species such as *P. turionellae* which can be used as biologic agents can be raised with diets that do not contain insect materials (Bratti & Coulibaly, 1995; Cohen, 2000).

It was observed that diet 1 had a negative effect on *P. turionellae's* egg production and hatching. Emre and Yazgan (1990) reported that the nutrient that was used as the control diet didn't have any kind of negative effect on the egg production of adult female *P. turionellae*. Nettles (1990) suggested that insect metabolism members have a significant effect on some insects that are produced in laboratories. Although *Muscidifurax zaraptor* is produced successfully in the diet without insect materials, the success is appeared to be in low percentages (Fanti & Vinson, 2000). The results have similarity only with the 50% honey-water diet. The outcomes that are found in the experiment proved that in *P. turionellae,* 50% honey-water mixture needs to be given with *G. mellonella* pupae in order to have optimum development. Diet 3 has some advantages when it is compared with diet 2. Although there is a decrease in glycogen and protein amounts when diet 3 is used, especially egg production and female outcome has an increase. Just like for *P. turionellae* for many endoparasitoid hymenoptera species it is important to have one egg developed for this reason it is very important to find out new techniques to increase female individual emergence. The differences between diet 3 and the control diet can be connected to the differences between the nutrients. It is obvious that the decrease in the glycogen amount occurred because of the decrease in the sucrose amount. Although sucrose is an important energy source, the decrease in sucrose did not have a negative effect on the life functions. In *P. turionellae* diets the sucrose ratio needs to be 7% in order to have an optimum development (Emre, 1988). It is told that sucrose is needed for the life functions of *Dacus oleae* in Tsiropoulos' (1980) work. The other important variation is the difference in the ratios of the vitamin mixtures.

As a result diet 1 negatively affected the egg production and sex ratio. This is because; although diet 1 is a good carbohydrate source, it does not contain enough lipid, amino acid, source and vitamin mixtures like diet 2 and diet 3. Few researches have been done on this subject, differences in diets resulted in an increase in the number of eggs of tested insects such as *D. olea* (Tsiropoulos, 1980, 1992), *A. domesticus* (McFarlane, 1988) and *M. desertus* (Bashan & Balci, 1994) and this clearly suggest that balance of nutrient is the important factor on *P. turionellae's* reproduction

The sex ratio in parasitic hymenoptera can be affected by different factors. The size of the host (Sandland, 1979a, 1979b, 1980; Kazmer & Luck, 1995), number of the intercourse (Allen *et al.,* 1994), temperature (Kfir & Luck, 1979) and photoperiod (Hoelscher & Vinson, 1971) are the factors. Nutrition factors are important for the sex ratio (Thompson & Hagen, 1999). Yazgan (1972) told that the increase in amino acids increases the number of female individuals in the diet which is made for *Itoplectic conquisition*. It is obvious that differences in the diets of the adults affect the reproduction activity (Susan *et al.,* 1997; Martin *et al.,* 2000) and behaviors of the males (Idris & Grafus, 1997; Patt *et al.,* 1997). The results in the experiment are interesting because it shows that the differences in *P. turionellae* sex ratio can be affected by these factors.

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