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# Impact of organophosphorus insecticide, malathion on the progeny sex ratio of *Pimpla turionellae* L.

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***Abstract:*** *The effects of 0.001, 0.01, 0.1 and 0.5 ppm concentrations of malathion, an organophosphorus insecticide, viewed over the course of the experiment, on adult emergence and sex ratios of emerging adults of the parasitic wasp, Pimpla turionellae L. (Hymenoptera: Ichneumonidae) were investigated. Diets which contain 0.001 and 0.01 ppm malathion significantly increased the total adult emergence on day 13, 28 and 31 when compared to control diet. Although 0.01 and 0.1 ppm malathion increased the female emergence, higher concentration of malathion (0.5 ppm) significantly decreased the female emergence. Adult female emergence significantly decreased on day 10, 16 and 25 in the group which was fed with the meridic diet containing 0.5 ppm malathion. This study indicated that orally administrated high concentration of malathion (0.5 ppm) significantly decreased female emergence of P. turionellae.*

***Key words:*** *Pimpla turionellae, Insecticide, Malathion, Sex ratio*
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## Introduction

Despite the fact that chemicals have good efficiency in controlling the pest population in field conditions, they also cause the destruction of insects that are beneficial to the ecosystem. In addition to this, many pest species gain resistance to these types of chemicals due to the fact that these chemicals are being used in excessive amounts and senselessly (Ozparlak, 2003; Joshi *et al.,* 2007; Srivastava *et al.,* 2008). Some proportions of these types of chemicals accumulated either directly or as composed more toxic metabolites, in the soil, in the body of the plant or animal cannot be eliminated for a long time. These substances can easily pass on to birds, mammals and humans. Because of this, the importance of biological control agents that do not have harmful effects to the nature and do not target organisms have been increasing (Ekebas *et al*., 2000; Choudhary *et al.,* 2008).

Sublethal doses of insecticides basically effect insect populations in three ways. These are: by effecting their survival durations, by effecting individuals' reproduction capabilities and by effecting genetic materials of future generations (Moriarty, 1969). In accordance with that, they change the activities of enzymes, cause abnormalities about reproduction, may cause abnormalities about nutrition and nutrition habits, behavioral malfunctions, metabolism abnormalities, abnormalities in parasitation and in parasite emergence (Haynes, 1988).

Malathion is an organic insecticide that is registered in 1956 in United States of America (Chamber, 1992) and it was being widely used to control the adult mosquitoes around the world (Pant, 1983). Compared to other insecticides in the same class it is a non-recyclable inhibitor of cholinesterase (Golz and Shaffer, 1955) and by affecting the nervous system of pests, cause their deaths (Gallo and Lawryk, 1991).

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For the protection of natural environment, biological control plays an important role and the production of natural control agents for this purpose in a high quality and in rich quantities is important for the success of this type of control. Singh (1977) reported that over 750 types of insects, spiders and acars could be produced with artificial foods in laboratory conditions, which shows the importance that is given to this subject. It is important for the success of biological control producing parasitoids and predators in biological control programs, in addition to knowing the subjects between placing out these to the environment and their ability to parasite the host, their susceptibility to the harmful chemicals, especially to pesticides.

The research studies with the insecticides have been about the resistance that the insects show against the insecticides (Bansal and Singh, 2004; Du *et al*., 2005; Johal *et al*., 2007), the effects of insecticides on enzyme activities (Bughio and Wilkins, 2004) and morphological and physiological effects of lethal and sublethal doses (Overmyer and Noblet, 2003; Bondarenko *et al*., 2004), however the studies that are made on the effects of orally taken insecticides on the sex ratio of beneficial insects which are used for biological control have been insufficient. Therefore the study on effects of the malathion on *Pimpla turionellae* L. when give in different concentrations was undertaken.

## Materials and Methods

*Galleria mellonella* was utilized as host for *P. turionellae* in the study. Insects were obtained from Cukurova University, Science and Letters Faculty, Department of Biology, Animal Physiology Laboratory where they have been reared for several years. In the experiment, newly emerged, unmated and unfed *P. turionellae* individuals were used.

In each repetition, 10 female and 5 male *P. turionellae* were located in a cage with dimensions 20x20x20 cm. Insects were fed by dropping equal amount of synthetic diet on to aluminum foil. The diets were given daily for 1 hr at the same time each day during the experiments (Coskun *et al*., 2005). Malathion [0,0-dimethyl S-1,2di (carboethoxy ethyl phosporodithioate)],(Hektas Company, Turkey) at 96% purity was put in the nutrient, while it was being prepared as microliters and as 0.001, 0.01, 0.1, and 0.5 ppm per 100 ml nutrient, nutrients of experiment groups have been prepared.

The insects were allowed to parasitize their host species beginning 10 days after the start of the experiment and every 3 days thereafter through day 31. Ten *G. mellonella* pupae were given to the *P. turionellae* for 1 hr. The pupae were wrapped with double layers of netting to prevent feeding on hemolymph. After each egg deposition period, parasited pupae were put into tightly closed beakers and kept in the same laboratory conditions, for the adult emergence. Adult emergence in each series were calculated by calculating, emerging individual number ratio compared to the total pupae number that is left in the cages to get parasited, female emergence ratio by calculating, the male and female ratios compared to the total emerging adult number.

All experiments have been repeated at least three times at different dates. Data from the different concentration of malathion feeding experiment were compared with each other and with the control. Statistical analysis was done with "Student-Newman Keuls Test (SNK)" (Sokal and Rohlf, 1969). Differences between groups were considered to be significant at the probability level of 0.05%.

## Results and Discussion

In the study, the impact of different malathion concentrations (0.001, 0.01, 0.1 and 0.5 ppm ) on the progeny sex ratio of adult *P. turionellae*, viewed over the course of the experiment, were investigated with used to meridic diet. Effects of different malathion concentrations on the total adult emergence of *P. turionellae*, over the days, are given on Table 1.

During the 10$^{th}$ day after the parasitation procedure started, total adult emergence among the insects feeding only with the nutrient containing 0.5 ppm malathion has significantly decreased. Minimum total emergence with 66.67% percent has been gained within the insects which are feeding with the aforementioned nutrient. In the 13$^{th}$ day of the experiment period, maximum adult emergence of 93.33% happened in the group that had been feeding with the 0.01 ppm malathion containing nutrient.

In the 19$^{th}$ day, within concentrations that were tried out only the value of 0.1 ppm has effected and decreased the total adult emergence significantly compared to other concentrations that were tried out. In the 22$^{nd}$ day, minimum adult emergence was obtained from the group which consisted of the insects that were feeding with the food containing 0.5 ppm malathion and this value is significant compared to other groups. Aforementioned maximum adult emergence in a day is obtained from the 0.01 ppm value.

The main concerns in mass production of *P. turionellae,* which is an endoparasitoid hymenopter species, is to develop the techniques which result in maximum number of productive female individuals and it is also very important to have information on mass production of parasitoids and predators, susceptibility to harmful substances and pesticides for a successful biological control program Singh (1977) mentioned that biological control plays an important role to protect the nature and natural control agents used for that purpose must have been in high quality and quantity.

In the 25$^{th}$ day of experiment period, minimum adult emergence was obtained from the insects feeding with 0.001 ppm malathion containing nutrients in 63.33% ratio. This obtained value is only at a significant level for the 0.01 ppm malathion group which has a maximum adult emergence of 86.67%. In 31$^{st}$ day, which is the last day of the experiment period, minimum adult emergence was observed in the control group as 46.67% followed by 0.1 ppm malathion as 56.67%. In the aforementioned day, maximum adult emergences that were observed in 0.001 ppm values as 86.67% (Table 1).

Insecticides on sublethal doses by effecting the death rate, reproduction abilities and new generation's genetic material ( Assie *et al*., 2007), might effect their development, emergence rate and sex ratio (Ergin *et al*., 2007). Nadda *et al*. (2005) reported that sublethal concentration of a synthetic pyrethroid decreased the number of adult emerged and especially malathion decreases the fertility among insects significantly.

When the nutrient involved 0.001 ppm malathion, it significantly increased adult individual emergence in the 13$^{th}$, 28$^{th}$ and 31$^{st}$ day. This increase continued for 0.01 ppm in the 13$^{th}$, 25$^{th}$, 28$^{th}$ and 31$^{st}$ day. In another study it was shown that 0.001 ppm malathion had increased *P. turionellae*'s egg production by 50% and the cause of this had been evaluated as the stimulation of insect's reproduction processes (Ozkan and Emre, 1997). Reproduction potential of insects might show a diversification depending on the gradual working of nervous and endocrine systems. Because the tried out concentrations might cause secretion of juvenile hormone by stimulating the neuroendocrine system (Campion, 1972), it gives the idea that the adult individual increase observed among *P. turionellae* is based on the increased egg reproduction. The effects of malathion that is taken with nutrient to *P. turionellae*'s female adult emergence ratios,over the days, are given in Table 2.

In the 10$^{th}$ day, female individual emergence had increased in the 0.1 ppm malathion containing nutrient compared to other concentrations those were tried out. In the aforementioned day, female individual emergence significantly decreased in the insects group which were feeding with the nutrient that contained 0.5 ppm malathion reached a value such as 34.72%. In the 13$^{th}$ day of experiment period, female emergence ratio that happened as 41.11% in the control group significantly decreased to 30.36% in the highest concentration of 0.5 ppm malathion feeding group. In the aforementioned day, maximum female emergence was observed within the insects that were feeding on the 0.1 ppm malathion nutrient as 69.64%.

**Table - 1:** Effects of malathion on the adult emergence (%) of *P. turionellae*

| | Total emergence adult | | | | |
|---|---|---|---|---|---|
| | Malathion concentrations (ppm) | | | | |
| | (Control) | 0.001 | 0.01 | 0.1 | 0.5 |
| Day | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * |
| 10 | 93.33±6.66$^{a}$ | 93.33±3.33$^{a}$ | 93.33±3.33$^{a}$ | 80.00±0.00$^{ab}$ | 66.67±6.66$^{b}$ |
| 13 | 50.00±5.77$^{c}$ | 70.00±0.00$^{b}$ | 93.33±3.33$^{a}$ | 76.67±3.33$^{b}$ | 76.67±3.33$^{b}$ |
| 16 | 73.33±6.66$^{a}$ | 66.67±6.66$^{a}$ | 86.67±3.33$^{a}$ | 66.67±3.33$^{a}$ | 83.33±3.33$^{a}$ |
| 19 | 86.67±3.33$^{a}$ | 93.33±3.33$^{a}$ | 86.67±3.33$^{a}$ | 56.67±3.33$^{b}$ | 83.33±3.33$^{a}$ |
| 22 | 86.67±3.33$^{ab}$ | 80.00±5.77$^{b}$ | 96.67±3.33$^{a}$ | 76.67±3.33$^{b}$ | 56.67±3.33$^{c}$ |
| 25 | 73.33±3.33$^{ab}$ | 63.33±3.33$^{b}$ | 86.67±3.33$^{a}$ | 76.67±3.33$^{ab}$ | 73.33±3.33$^{ab}$ |
| 28 | 53.33±3.33$^{c}$ | 73.33±3.33$^{b}$ | 73.33±3.33$^{b}$ | 90.00±0.00$^{a}$ | 83.33±3.33$^{ab}$ |
| 31 | 46.67±3.33$^{d}$ | 86.67±3.33$^{a}$ | 66.67±3.33$^{bc}$ | 56.67±3.33$^{cd}$ | 73.33±3.33$^{b}$ |

No statistical difference at $p<0.05$ level for the data with the same letter in the same line. $\overline{X} \pm s\overline{x}$ : Arithmetic mean ± Standard deviation

**Table - 2:** Effects of malathion on the female emergence (%) of *P. turionellae*

| | Female emergence (%) | | | | |
|---|---|---|---|---|---|
| | Malathion concentrations (ppm) | | | | |
| | (Control) | 0.001 | 0.01 | 0.1 | 0.5 |
| Day | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * | $\overline{X} \pm s\overline{x}$ * |
| 10 | 46.67±3.33$^{bc}$ | 53.33±4.62$^{b}$ | 53.33±4.62$^{b}$ | 70.83±4.16$^{a}$ | 34.72±1.38$^{c}$ |
| 13 | 41.11±4.84$^{d}$ | 52.38±4.76$^{bc}$ | 57.04±1.48$^{b}$ | 69.64±3.71$^{a}$ | 30.36±3.71$^{d}$ |
| 16 | 72.22±2.77$^{a}$ | 65.28±1.38$^{a}$ | 61.57±3.24$^{a}$ | 65.08±4.20$^{a}$ | 36.11±1.38$^{b}$ |
| 19 | 57.41±4.89$^{b}$ | 68.15±5.18$^{b}$ | 53.70±1.85$^{b}$ | 35.56±2.22$^{c}$ | 80.10±3.78$^{a}$ |
| 22 | 65.28±1.38$^{ab}$ | 49.47±3.67$^{b}$ | 82.96±2.96$^{a}$ | 73.81±1.20$^{a}$ | 53.33±3.10$^{b}$ |
| 25 | 72.62±1.20$^{a}$ | 42.06±4.82$^{b}$ | 61.57±3.24$^{a}$ | 65.48±2.97$^{a}$ | 32.14±5.45$^{b}$ |
| 28 | 37.78±2.22$^{b}$ | 45.83±5.86$^{ab}$ | 58.93±1.78$^{a}$ | 59.26±3.70$^{a}$ | 44.44±5.55$^{ab}$ |
| 31 | 21.67±1.66$^{c}$ | 23.15±0.92$^{c}$ | 45.24±2.38$^{a}$ | 35.56±2.22$^{b}$ | 27.38±1.20$^{c}$ |

No statistical difference at $p<0.05$ level for the data with the same letter in the same line, Arithmetic mean ± Standard deviation

In the 16$^{th}$ day, within the groups feeding control, 0.001, 0.01 and 0.1 ppm containing nutrients, no significant difference was observed; in the group feeding on the nutrients containing 0.5 ppm malathion concentration, female emergence significantly decreased and happened as 36.11%. In the 19$^{th}$ day, compared to the control whereas only the female individual emergence among insects feeding on 0.1 malathion containing nutrients significantly dropped, in the group feeding on 0.5 ppm it increased. Other concentrations those were tried out did not have a significant effect.

In the 22$^{nd}$ day, in the control group female emergence ratio was 65.28%. None of the malathion concentration which was tested did not show any effect on the female emergence when compared to the control group. In the 25$^{th}$ day of experiment period, while compared to the control 0.01 and 0.1 values did not have significant effects and other concentrations those were tried out had decreased the female individual emergence significantly. In the aforementioned day, minimum female individual emergence was obtained from 0.5 ppm concentration as 32.14%.

In the data gathered from the study, it is seen that female adult emergence has increased significantly when the nutrient contained 0.01 and 0.1 ppm malathion compared to the other concentrations. The increase in female individual emergence after the 25$^{th}$ day of experiment period is more remarkable. Among the insects with arrhenotoky diploid female individuals come out from fertilized eggs and haploid male individuals come out from unfertilized eggs (Godfray, 1994). These types of insects can store the sperms in their spermatheca which they earned during copulation (Chevrier and Bressac, 2002). It is clearly seen that the effect which is observed in *P. turionellae* in specific concentrations is a very important mechanism for the insect to survive in negative environment conditions. Because of this, they can create productive female individuals even in late periods of their life cycle.

In the 28$^{th}$ day, compared to the control only within the insects feeding on 0.01 and 0.1 ppm containing malathion female emergence increased. In the 31$^{st}$ day, which is the last day of the experiment period, according to data that was gathered maximum female individual emergence was obtained from the insects feeding on 0.01 ppm malathion containing nutrients as 45.24%, followed by 0.1 ppm nutrient as 45.25%. Other concentrations those were tried out did not have a significant effect on female individual emergence (Table 2).

Like other many endoparasitoid hymenopter species, the continuity of *P. turionellae* population depends on the hatching of one of the many eggs that are left on the host pupa by the female insect. Because of this advantage from the biological control agents that are aimed to be produced for using both in natural environment and in biological control, it is important to hold the female individual number in a high level in the population.

Malathion, is an acetylcholine esterase inhibitor. It shows its effect on nervous system by combining with acetylcholine esterase, which prevents the accumulation of acetylcholine in toxic levels (Yang *et al*., 2008). These types of chemicals are known to not only affect the nervous system, but also the other systems those are under the control of this system (Ware, 1989).

The increase of malathion in the nutrient effected the female emergence negatively and decreased it. This decrease significantly appeared in diet which included 0.5 ppm malathion. The demonstration of among Propoxur applied *Anopheles albimonus* because of these application females are eliminated in eggs (Bailey *et al.*, 1980) is a supportive fact to the data that we gathered in high concentrations. The causes of this negative effect other than the cholinesterase inhibition of the insecticide, depending on the negative effects on the insect's feeding behavior, may be the decrease of sperm formation in the male insect due to the insufficient synthesis as a result of abnormalities in protein and glycogen synthesis.

It is inevitable that these types of insecticides that are used in the control against pests affect the beneficial species by direct contacts or in nutritional ways. Because of this, it reveals the importance of extending biological control more and taking the necessary steps to make the chemical control consciously to protect the natural environment.

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