Orjinal araştırma (Original article)

Effects of diazinon on antioxidant enzymes and adult emergence of the parasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)[1]

Diazinonun parasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nin antioksidan enzimleri ve ergin birey çıkışına etkileri

Tamer KAYIŞ[2*]   İskender EMRE[3]   Mustafa COŞKUN[2]

Summary

In this study, the effects of the organophosphorus insecticide diazinon on antioxidant enzyme activities, and female and total adult emergence ratios of *Pimpla turionellae* L., were investigated when using a synthetic diet containing 0.01, 0.10, 0.25, 0.50 and 0.75 ppm of the insecticide. Sublethal concentrations of diazinon were supplied to *P. turionellae* for 24, 48, 72 and 96 hours for enzyme activity and thirty days for determining emergence ratios.

P. turionellae gave different antioxidant responses to insecticidal stress, depending on the concentrations and diazinon exposure time. When compared to the control and especially in high concentrations (0.50 and 0.75 ppm), diazinon increased SOD activity. Although diazinon also caused increases in CAT activity, these increases were not consistent.This study found that the CAT activity didn't fall below the control level.

 Diazinon significantly reduced female and total adult emergence of *P. turionellae* and SOD played a more significant protective role against diazinon toxicity.

Key words: *Pimpla turionellae*, antioxidant enzymes, insecticidal stress, fecundity, diazinon

Özet

Organofosforlu bir insektisitin *Pimpla turionellae*'nin antioksidan enzim aktivitelerine ve toplam ve dişi birey çıkışına olan etkileri 0.01, 0.10, 0.25, 0.50 ve 0.75 ppm diazinon içeren sentetik besin kullanılarak araştırıldı. Subletal diazinon konsantrasyonları, antioksidan enzim aktivitelerini belirlemek için 24, 48, 72 ve 96 saat süreyle, ergin birey çıkışını belirlemek için ise 31 gün boyunca *P. turionellae*'ya verildi.

P. turionellae insektisit stresi altında diazinonun dozuna ve maruz kalma süresine bağlı olarak farklı tepkiler gösterdi. Diazinon özellikle yüksek konsantrasyonlarda (0.50 ve 0.75 ppm) SOD aktivitesinin kontrole göre önemli ölçüde artmasına neden oldu. Bununla beraber CAT aktivitesinde düzensiz artışlara neden oldu. Çalışmadaki önemli bir bulgu, CAT aktivitesi hiçbir konsantrasyonda kontrol seviyesinin altına düşmedi.

Toplam ergin ve dişi çıkışı diazinondan önemli ölçüde etkilenerek azaldı. Sonuç olarak, SOD'nin *P. turionellae*'de diazinon toksisitesine karşı çok önemli bir koruyucu rol oynadığı ve diazinonun toplam ve dişi birey çıkışını önemli ölçüde azalttığı belirlendi.

Anahtar sözcükler: *Pimpla turionellae*, antioksidan enzimler, insektisit stresi, verimlilik, diazinon

Introduction

Organophosphorus insecticides (OPs) are one of the most used classes of pesticides for their low toxicity and low persistence when compared to organochlorine insecticides (Pournourmohammadi et al. 2005). Uncontrolled and extensive usage of OPS chemicals negatively affects many non-target organisms, such as parasitoid and predator insects that are used for biological control (Das & Mukherjee, 2000; He et al., 2002). These insecticides irreversibly inhibit the enzyme acetylcholine esterase (AChE) and lead to excessive accumulation of acetylcholine which prevents the transmission of nerve impulses in the central nervous system (Howard & Pope, 2002).

It is known that many xenobiotics like insecticides may cause oxidative stress by generating reactive oxygen species (ROS) and alterations in ROS scavenging enzymes (Dettbarn et al. 2006; Milatovic et al. 2006). As a result, they can damage cellular biomolecules such as proteins, lipids and nucleic acids (Le Bourg, 2001). Insects, like other organisms, have antioxidant defense mechanisms, such as antioxidant enzymes (SOD, CAT, and GPX) and non-enzyme antioxidants (ascorbic acid, thiols, alpha tocopherol) to protect their cells from oxidative damages (Felton & Summers, 1995).

Superoxide dismutase (SOD) converts the superoxide radical to peroxide and oxygen (Fridovich, 1978). Catalase (CAT) targets hydrogen peroxide and quickly converts it to water and oxygen. Additionally, insects have ascorbate peroxidase (APOX) also targeting hydrogen peroxide but only at low concentrations (Clavaron-Mathews et al., 1997) and glutathione S-transferase with peroxidase-like activity (GSTpx) which is effective in targeting hydroperoxides but is not effective in targeting hydrogen peroxide (Ahmad et al., 1991; Ahmad, 1992)

The endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) is widely used for biological control.. It needs a host to complete part of its life cycle (Coskun et al. 2005). *P. turionellae* can use as host many species of Lepidoptera, such as larva or pupae of the ''Black Veined White'' *Aporia crataegi* (L) (Pieridae), the ''Gypsy moth'' *Lymantria dispar* (L) (Lasiocampidae) and the ''Mediterranean flour moth'' *Ephestia kuehniella* Zell. (Pyralidae) (Thompson, 1957). Adults feed on plant nectar, pollen and host pupae in nature. Therefore, it is possible to be exposed to insecticides through nutrition (Sak et al. 2006), respiration and contact.

Diazinon is an organophosphate insecticide that is used widely to control pest insects in agriculture for its high activity and board spectrum against pests (Pekhonen & Zhang, 2002; Yamamato et al. 2010).Insecticides like diazinon may cause serious side effects such as decreasing parasitism ability, hatching ratio and emergence ratio (Baykal et al. 2005; Desneux et al. 2007; Gulfer et al. 2009) of non-target beneficial organisms such as parasitoids and predators which are used for biological control. It is reported that they are more sensitive to toxicants than their prey (Kazmirowa & Ortel, 2000; Büyükgüzel, 2006; Sak et al. 2006) which is a negative factor in biological control programs. The aim of biological control is to keep pest populations at minimum level, and to reduce side effects on ecosystems. Therefore an optimum number of biological control agents should be in the areas where pests are situated (Coşkun et al., 2009).

Female hymenopteran parasitoids can determine gender by controlling sperm entrance to eggs by their haplo-diploid sex determination system (Flanders, 1956); therefore they are the ideal organisms for sex ratio studies (Godfray, 1994). In arrhenotokous insects, such as *P. turionellae*, the sex ratio is regulated by females via the neuroendocrine system. Virgin females produce only male offspring, while mated females can produce female offspring (Cook, 1993).

Some research on insecticides has been about the insect resistance (Bansal & Singh, 2004; Du et al. 2005) and morphological and physiological effects of lethal and sublethal doses (Overmyer & Noblet, 2003; Bondarenko et al., 2004). There are a limited number of studies about the effects of insecticides on antioxidant enzymes, and female and total adult emergence of parasitoids (Delpuech & Meyet, 2003; Bughio & Wilkins, 2004; Buyukguzel, 2006; Gulfer et al. 2009).

One of the aims of this study was to determine the activity of some antioxidant enzymes under diazinon induced oxidative stress and the other was to determine the effects of diazinon on the female and total adult emergence ratio of the biological control agent, *P. turionellae*.

Material and Methods

Insects

Pimpla turionellae L. (Hymenoptera: Ichneumonidae) individuals were used in all experiments. They were reared on pupae of greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). The stock *P. turionellae* culture was maintained at 25±2ºC, 75±5% humidity, 12:12h (L: D) photoperiod and fed with 50% honey solution and *G. mellonella* hemolymph.

Experimental procedure

The synthetic diet which was developed by Emre (1988) (Table 1) was used in all experiments as the control diet, and diazinon (Hektas 99,5% purity) was added to the diet at 0.01, 0.1, 0.25, 0.50 and 0.75 ppm.

Table 1. Composition of the chemically-defined synthetic diet for *P. turionellae* adults (Emre, 1988)

Constituent	mg/100 ml diet	Constituent	mg/100 ml diet
L-Amino acid mixture	3000.00	Water soluble vitamin mixture	284.38
Alanine	210.00	Ascorbic acid	10.6105
Arginine-HCl	150.00	Biotin	0.0379
Aspartic acid	195.00	Ca-Panthothenate	2.8042
Cysteine	39.00	Choline chloride	246.3158
Glutamic acid	315.00	Folic acid	0.1137
Glycine	192.00	Inositol	17.0526
Histidine	120.00	Nicotinic acid	5.6842
Hydroxproline	57.00	Pyridoxine-HCl	0.2842
Isoleucine	156.00	Riboflavin	1.3263
Leucine	231.00	Thiamine-HCl	0.1516
Lysine	159.00		
Methionine	90.00	Inorganic salt mixture	75.00
Phenylalanine	165.00	$FeCl_3$ $6H_20$	2.1583
Proline	246.00	K_2HPO_4	45.0129
Serine	195.00	Na_2HPO_4 $12H_2O$	6.2201
Threonine	165.00	$MgSO_4$ $7H_2O$	15.7853
Tryptophane	60.00	$MnSO_4$ H_2O	0.0479
Tyrosine	120.00	$CoCl_3$ $6H_2O$	0.5798
Valine	135.00	$CuSO_4$ $5H_2O$	0.6721
		$CaCl_2$	3.6684
Lipid mixture	540.96	$ZnCl_2$	0.8552
Cholesterol	138.8430		
Linoleic acid	8.0331	Miscellaneous	
Linolenic acid	25.5537	Ribonucleic acid	75.00
Oleic acid	10.5950	Sucrose	14000.00
Palmitic acid	0.6777	2N KOH	280.00
Stearic acid	0.2314	2N K_2HPO_4*	14.03
Tween 80	357.0248	Distilled water to 100 ml	

 * : Added into the water soluble mixture solution.

Four females and two males newly hatched were placed in a 1000 cc-glass beaker that was tightly covered with a mesh, in order to determine the effects of diazinon on antioxidant enzyme activity in each repetition, unfed and unmated. Insects were fed with the above mentioned diet for 24, 48, 72 and 96 hours. At the end of these periods, insects were weighed and stored at -80ºC until biochemical analysis.

Biochemical analysis

Homogenization

Insects were homogenized to 1/20 (w/v) ratio in ice-cold 50 mM phosphate buffer (pH 7.4), then centrifuged at 15000 x g for 30 minutes at 4ºC. The supernatant was used for biochemical analysis.

Total protein amount

Protein amount in the supernatant was determined according to Lowry et al. (1951), by using bovine serum albumin as standard.

SOD activity

Total SOD activity was determined with the xanthine/xanthine-oxidase/nitroblue tetrazolium (NBT) method of Sun et al. (1988). Xanthine/xanthine oxidase-produced O^{2-} reduced NBT to formazan, which was assessed spectrophotometrically at 560 nm. SOD competed with NBT for dismutation of O^{2-} and inhibited its reduction. The level of this reduction was used as a measure of SOD activity. Total SOD activity was expressed in units per mg of protein; one unit of SOD was defined as the amount of enzyme that inhibited the rate of nitroblue tetrazolium reduction by 50%.

CAT activity

Catalase activity was determined according to Aebi (1984). Briefly, hydrogen peroxide (20 mM) in a phosphate buffer (50 mM, pH 7.0) was used as hydrogen peroxide substrate and decrease of the substrate in H_2O_2 concentration at 25ºC was followed spectrophotometrically at 240 nm for 1min. Activity of the enzyme was expressed in units per mg of protein, with 1 unit equaling the amount of enzyme that degrades 1µM H_2O_2 in a minute.

Determination of female and total adult emergence ratio

To determine the effects of diazinon on sex ratio, ten females and five males that matured on the same day were transferred one by one to experimental cages (25x25x25 cm). Insects were fed with the above mentioned diet during the experiment for 1 hour each day at the same time. Ten *G. mellonella* pupae were provided for the wasps to lay their eggs 10 days after the beginning of experiment and every 3 days until the 31st day. After oviposition, all pupae were placed in a beaker, and held until the adults' emergence. Numbers of emerging male and female wasps were recorded. Progeny emergence number was compared to total number of emerging adults, and sex ratio of progeny was calculated. This experiment was repeated three times and data were collected for statistical analysis.

Adult emergence was determined as a percentage and the number of emerged individuals was compared to the total number of pupae placed in the cages to be parasitized.

Statistical analyses

All experiments were repeated three times. Values from the different diazinon concentrations were evaluated by comparisons with the control and other groups. Statistical analyses were done by using the software package SPSS 12.00 for Windows Basic and Advanced Models. Differences between groups were considered to be significant at the probability level of 0.05%.

Results

Table 2 shows the effects of diazinon on SOD activity of treated females. At 24th h., diazinon, except for lowest concentration (0.01 ppm), caused an increase in SOD activity compared to control, while it caused a reduction in SOD activity at 48th h at 0.01, 0.10 and 0.25 ppm. Other concentrations did not alter SOD activity compared to control at 48th h. SOD activity significantly changed at 72nd h. Low concentrations of diazinon (0.01 and 0.10 ppm) caused a decrease in SOD activity, while other concentrations (0.25, 0.50 and 0.75 ppm) caused an increase in SOD activity. The activity of SOD increased in all diazinon concentrations at 96th h. compared to control.

Catalase activity was less affected by diazinon than SOD activity (Table 3). CAT activity increased in the diet which included 0.10 and 0.25 ppm diazinon at 24th h., while this increase occurred only at highest diazinon concentration (0.75 ppm) at 48th h. CAT activity did not alter significantly at 72nd h. compared to control. At 96th h., 0.25 ppm diazinon caused a significant increase in CAT activity.

Table 2. Effects of different diazinon concentrations on SOD activity of *P. turionellae*

Diazinon (ppm)	SOD Activity (U/mg Protein)			
	Time (hour)			
	24 (Mean±S.D)*	48 (Mean±S.D)*	72 (Mean±S.D)*	96 (Mean±S.D)*
0.00**	0.5918±0.042 d	0.8395±0.009 a	0.6683±0.014 c	0.4075±0.005 e
0.01	0.6945±0.018 cd	0.6435±0.027 c	0.5300±0.010 d	0.6346±0.006 c
0.10	0.8061±0.041b	0.5621±0.011 d	0.4064±0.007 e	0.7913±0.016 a
0.25	0.9237±0.040 a	0.6988±0.021 b	0.7712±0.008 b	0.6687±0.002 b
0.50	0.6496±0.002 c	0.8451±0.008 a	0.7723±0.012 b	0.7768±0.003 a
0.75	0.7881±0.015 b	0.8647±0.015 a	0.8711±0.006 a	0.5921±0.005 d

** Control.
*Values followed by the same letter are not significantly different from each other ($P > 0.05$, T test).

Table 3. Effects of different diazinon concentrations on CAT activity of *P. turionellae*

Diazinon (ppm)	CAT Activity (U/mg Protein)			
	Time (hour)			
	24 (Mean±S.D)*	48 (Mean±S.D)*	72 (Mean±S.D)*	96 (Mean±S.D)*
0.00**	21.21±0.200 c	20.57±1.040 b	21.78±0.550 a	21.29±0.653 b
0.01	20.95±0.430 c	22.68±0.529 b	22.42±0.901 a	21.44±0.628 b
0.10	23.37±0.691 b	20.45±0.354 b	20.96±0.940 a	21.30±0.702 b
0.25	27.90±0.904 a	22.75±0.551 b	20.62±0.849 a	24.33±0.270 a
0.50	20.76±0.371 c	21.62±0.456 b	18.55±1.030 a	20.96±0.777 b
0.75	20.58±0.537 c	26.04±0.618 a	20.18±0.918 a	23.43±0.735 ab

** Control.
*Values followed by the same letter are not significantly different from each other ($P > 0.05$, T test).

Effects of diazinon on female and total adult emergence ratio of *P. turionellae* are given in Table 4. Highest (0.75 ppm) and lowest (0.01 ppm) diazinon concentrations caused a decrease in total adult emergence ratio compared to control, while other diazinon concentrations did not significantly affect total adult emergence ratio. The female emergence ratio significantly decreased in almost all diazinon concentrations; however, the changes that occurred at 0.10 ppm were not statistically significant compared to control.

Table 4. Effects of diazinon on total adult and female emergence of *P. turionellae*

Diazinon (ppm)	Emergence Ratio (%)	
	Total (Mean±S.D)*	Female (Mean±S.D)*
0.00**	76.25 ± 2.16 a	53.75 ± 2.60 a
0.01	65.00 ± 1.90 b	35.42 ± 3.97 b
0.10	67.92 ± 3.25 ab	45.83 ± 1.67 ab
0.25	70.42 ± 2.53 ab	43.33 ± 2.92 b
0.50	67.08 ± 3.25 ab	40.42 ± 3.00 b
0.75	62.08 ± 1.10 b	41.25 ± 0.72 b

*Values followed by the same letter are not significantly different from each other. (P >0.05, T test).
** Control.

Discussion

Activity of antioxidant enzymes may increase or decrease under chemical stress. These changes depend on the strength and duration of stress factors. Increasing antioxidant enzyme activity is not a general rule (Cheung, 2001).

The results presented in this study show that SOD plays a very important role against the damaging effects of diazinon-induced oxidative stress. SOD activity increased especially in high diazinon concentrations (0.50 and 0.75 ppm) almost every day, but these changes were not significant when compared to control at 48th h. Adamski et al. (2003) reported that induction of SOD activity is the main response to OPs toxicity. A similar result was obtained by Buyukguzel (2006). Malathion (lower than 1.0 ppm) caused a significant increase in total SOD activity compared to control in *P. turionellae*; increase in SOD activity indicates an increase in superoxide anion production (Zhang et al. 2004). On the other hand, contrary to expectations, our study demonstrated that the activity of SOD was reduced in low diazinon concentrations (0.01 and 0.10 ppm) at the 48th and 72nd hours. Similar findings were obtained under insecticidal stress in various organisms by Gultekin et al. (2000) and Durmaz et al. (2006). Achudume et al. (2010) suggested that reduction of SOD activity protects organisms against oxidative stress. In some cases, the superoxide radical, by itself or after its transformation to H_2O_2, causes a strong oxidation of the cysteine in the enzyme and decreases SOD activity (Dimitrova et al. 1994). We assume that these reductions may be an effort to regulate oxidative balance by the insect.

In this study, CAT activity was less affected than SOD activity by diazinon. An important finding in this study was that diazinon did not cause a decrease in CAT activity compared to control. It is known that CAT activity can be inhibited by the accumulation of superoxide anion (Kono & Fridovich, 1982); but in this study CAT activity was not inhibited by the superoxide anion which may result from SOD activity. On the other hand, CAT activity showed little increase regardless of concentrations and periods of diazinon treatment. It is known that CAT activity is directly regulated by the concentration of hydrogen peroxide (Fornazier et al. 2002). Insects have higher CAT activity level than mammals and this activity is rarely altered by exogenous sources of oxidative stress (Ahmad & Pardini, 1990). In our study, CAT activity rarely increased in response to diazinon-induced oxidative stress compared to the controls. Elevated activity of CAT is an adaptive response to enhanced generation of hydrogen peroxide. After 72nd h. of treatment, CAT activity did not differ compared to controls. This finding is comparable with a study of *Bombix mori* (Yamamoto et al. 2010). Although responses to oxidative stress vary according to species, CAT is inefficient at low hydrogen peroxide levels due to its high Km values (Ahmad et al. 1991). Some

reports suggest that insects possess different antioxidant enzymes, such as, ascorbate peroxidase (APOX) and dehydroascorbate reductase (DHAR) for removing low levels of hydrogen peroxide (Summers & Felton, 1993; Mathews et al. 1997). Also, *P. turionellae* may have used one of these enzymes against hydrogen peroxide at 72nd h. However, more detailed studies are needed to understand the actual mechanisms.

Parasitoids are very important for biological control. In endoparasitoid hymenopter species, like *P. turionellae*, continuity of population depends on hatching of one of the eggs that is left on the host pupa by the female. Therefore the female rate in the population is very important for controlling pest numbers.

Insecticides can affect sex ratio (Buyukguzel, 2006; Gulfer et al. 2009), egg production and hatchability (Baykal et al. 2005), survival rate (Desneux et al. 2007), cause behavioral and metabolic abnormalities in parasitoids (Haynes, 1988), sperm production and quality, and can induce deformation in ovaries (George & Ambrose, 2004).

In our study, total adult and female emergence was significantly affected by diazinon treatment. These numbers decreased depending on diazinon concentrations which is an undesirable situation for biological control. Similar results were obtained under insecticide stress by other researchers (Lee et al. 1998; Delpuech & Meyet, 2003). These changes can be explained by the aforementioned reasons.

In conclusion, this study showed that diazinon significantly altered antioxidant enzyme activity and decreased the female and total adult emergence ratis of the parasitoid *P. turionellae.* We suggest that *P. turionellae* can be used as a model organism in pesticide-induced stress studies and that SOD can be used as a bioindicator in oxidative stress in parasitoid hymenoptera. This research also provides further information about the oxidative toxicity of OPs in parasitoid insects.

Acknowledgements

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