www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 6/1 (2013) 62-70

*Research article/Araştırma makalesi*

# Effects of parasitization and envenomation by the endoparasitic Wasp *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) on hemolymph protein profile of its host *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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**Abstract**

The effects of dose-dependent envenomation and by parasitization of *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) on the hemolymph protein profile of its host *Galleria mellonella* L. (Lepidoptera: Pyralidae) were investigated. Hemolymph proteins were analyzed using spectrophotometry and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The gel was subsequently scanned and the optical densities (OD) of the bands were analyzed. The quantities of proteins detected 4, 8, and 24 h post-treatments in hemolymph of parasitized and envenomated host pupae did not differ much when compared with those of controls. Of the seventeen different protein bands detected at a range of 19.6-181.12 kDa in the hemolymph, there were only changes in OD values of bands at 23.418, 24.714, 32.434, 34.811, and 45.385 kDa following envenomation and parasitism. The electrophoretic pattern of hemolymph proteins from venom injected and control groups of larvae did not differ much from that of pupae except for new protein bands detected at 33.823 and 41.553 kDa. However, three bands with 45.385, 99.000, and 126.850 kDa were not detected in larvae. Hemolymph protein quantity remained steady at all time points tested except for increases for some bands at 8 h following envenomation. The amount of 34.811 kDa protein decreased immediately at 8 h post-injection of 0.02 and 0.05 VRE of venom whereas injection all venom doses except 0.1 VRE resulted in an increase in the amount for 41.553 and 43.412 kDa proteins. There were no qualitative changes in term of novel protein bands in the hemolymph of hosts. Therefore, we suggest that host regulation of *G. mellonella* by parasitism or envenomation of *P. turionellae* involves quantitative changes in the host plasma proteins but does not lead to the up-regulation of novel proteins.

**Key words:** Hemolymph proteins, *Galleria mellonella*, *Pimpla turionellae*, Venom, Parasitism

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# Endoparazitik arı *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın parazitleme ve zehirinin konağı *Galleria mellonella* L. (Lepidoptera: Pyralidae)'nın hemolenf proteinleri üzerine etkisi

**Özet**

*Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın farklı dozlardaki zehrinin ve parazitlemesinin konağı *Galleria mellonella* L. (Lepidoptera: Pyralidae)'nın hemolenf proteinleri üzerine olan etkileri araştırıldı. Hemolenf proteinleri spektrofotometrik ve Sodyum Dodesil Sülfat Poliakrilamid Jel Elektroforez (SDS-PAGE) teknikleri kullanılarak analiz edildi. Jeller tarandıktan sonra, bantların optik densitometrik değerleri analiz edildi. Parazitlenmiş ve zehirle muamele edilen konak pup hemolenfindeki protein miktarları kontrol gruplarıyla karşılaştırıldığında, muameleden 4, 8 ve 24 saat sonrasında değişiklik göstermedi. Hemolenfte belirlenen 19.6-181.12 kDa aralığındaki on yedi farklı proteinden 23.418, 24.714, 32.434, 34.811 ve 45.385 kDa olanların miktarları parazitleme ve zehir etkisine bağlı olarak değişiklik gösterdi. Larvaların hemolenfindeki proteinlerin elektroforetik dağılımlarında hem kontrol grubunda hem de zehir enjekte edilen gruplarda pupa hemolenfinden farklı olarak 33.823 ve 41.553 kDa büyüklüğünde iki yeni protein belirlendi. Bununla birlikte, larvalarda 45.385, 99.000 ve 126.850 kDa

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 BioDiCon. 274-0912

büyüklüğündeki üç band belirlenmedi. Larvalarda hemolenf proteinlerinden bazıları enjeksiyondan 8 saat sonrasında artış gösterirken, diğer proteinler tüm zaman noktalarında değişiklik göstermedi. 34.811 kDa'luk proteinin miktarı 0.02 ve 0.05 kese eşdeğeri zehir (KEZ) dozlarının enjeksiyonundan 8 saat sonra azalma gösterirken 41.553 ve 43.412 kDa büyüklüğündeki proteinler 0.1 kese eşdeğeri zehir (KEZ) dozu haricindeki tüm dozların enjeksiyonundan sonra artış gösterdi. Konak hemolenfinde zehirleme ve zehir enjeksiyonuna bağlı olarak proteinlerde kalitatif bir değişiklik yani yeni bir protein belirlenmedi. Bu nedenle, *P. turionellae* parazitleme veya zehirleme yoluyla konak *G. mellonella*'yı düzenlemesinde konağın plazma proteinlerinde kantitatif değişikliklere neden olduğunu ancak yeni proteinlerin salgılanmasının düzenlenmesinde etkili olmadığını ileri sürmekteyiz.

**Anahtar kelimeler:** Hemolenf Proteinleri, *Galleria mellonella*, *Pimpla turionellae*, Zehir, Parazitlik

## 1. Introduction

Parasitoid species regulate the nutritional and physiological states of their hosts to ensure their eggs and larvae successfully develop inside the host (Vinson, 1980; Beckage, 1993; Thompson, 1993). Parasitism-induced manipulations of host physiology, biochemistry and endocrinology include host conditioning by injection of maternal factors derived from ovarian secretions [polydnaviruses (PDVs), virus-like particles (VLPs)] (Bae and Kim, 2004; Kaeslin et al., 2005; Li et al., 2007), and/or venom glands (Nakamatsu and Tanaka, 2003; Rivers et al., 2004; Keenan et al., 2007). Parasitism-associated changes induced by the paralyzing toxin and other ovarian secretions during ovipositing by the adult koinobiont endoparasitoids in host hemolymph protein composition have been extensively documented, and can both alter the expression of normal host proteins and cause the synthesis of parasitism specific novel proteins (Beckage, 1993). Koinobiont species temporarily paralyze their hosts and allow them to grow and develop even after parasitization (Gauld, 1988). However, most idiobiont parasitoids paralyze their hosts permanently, and thus preserve the hosts while the parasitoid progeny feed and develop (Wharton, 1993). Such differences in the action of venoms from koinobiont and idiobiont wasps argue that changes in the nutritional content of the hosts (i.e, hemolymph proteins and amino acids) are more likely to be associated with koinobionts.

*Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) is a solitary idiobiont endoparasitoid wasp species which uses prepupae and pupae of hosts from an extremely wide range of lepidopteran species (Kansu and Uğur, 1984) and is devoid of symbiotic viruses. Venom from *P. turionellae* has previously been shown to contain a number of biologically active components including melittin, apamin, noradrenaline, serotonin, and phospholipase B. The venom also displays potent paralytic, cytotoxic, and cytolytic effects towards lepidopteran and dipteran hosts (Uçkan et al., 2004; Uçkan et al., 2006; Ergin et al., 2007). The role of venom and/or parasitism in suppressing host immune defense has also been studied (Er et al., 2010; Uçkan et al., 2010; Er et al., 2011). Here, we investigated the effects of dose-dependent envenomation and parasitization of *P. turionellae* on the hemolymph protein profile of its host *Galleria mellonella* L. (Lepidoptera: Pyralidae).

## 2. Materials and methods

### *2.1 Parasitoid and host rearing*

*P. turionellae* were reared on pupae (1- or 2-day-old) of *G. mellonella* at 25 ± 1°C, 60 ± 5% RH, and with a photoperiod of 12: 12 h, L: D. Adult parasitoids were fed a 30% (v/v) honey solution and provided with host pupae (four pupae for every 10 female wasps once every three days). Host colony was maintained by feeding the insects with natural blackened comb (Uçkan and Ergin, 2002) to maintain similarity to their natural media in bee hives.

### *2.2 Preparation of P. turionellae venom and injection into G. mellonella*

Venom reservoir contents were isolated from honey- and host-fed 15 to 20-day-old females by dissecting out the venom sacs as described previously (Uçkan et al., 2004). Following centrifugation (3,000 g for 10 min at 25 ± 1°C) to remove cell debris, final venom concentrations were adjusted to 0.05, 0.02, 0.01, and 0.005 venom reservoir equivalents (VREs) for pupae and 0.5, 0.1, 0.05, and 0.02 VREs for larvae with PBS (0.138 M NaCl and 0.0027 M KCl in 0.01 M PBS, pH 7.4). These venom concentrations represent doses previously determined to yield host responses yet fall below the calculated $LD_{99}$ for pupae and larvae (Ergin et al. 2006), respectively. A 5 µl solution of the venom preparation was injected between the last two lateral abdominal segments of 1 to 2-day-old pupae (140 ± 20 mg) and on the first hind leg of last instars (260 ± 10 mg) of the host, previously chilled on ice for 10 min, by using a 10 µl Hamilton microsyringe (Hamilton, Reno, NV). Vaseline was applied to the injection area to prevent hemolymph loss. Controls consisted of pupae and larvae untreated, null-injected, and those injected with only 5 µl PBS.

*2.3 Parasitization of G. mellonella pupae*

Parasitization was performed on 1- or 2-day-old host pupae by exposing an individual host pupa (140 ± 20 mg) to an individual 15 to 20-day-old wasp female. Parasitized pupae were held at 25 ± 2°C, 60 ± 5% RH under a photoperiod of 12: 12 h LD, as were the controls and venom-treated pupae, until hemolymph collection. *P. turionellae* females normally parasitize host prepupae and pupae in nature (Kansu and Uğur, 1984), therefore parasitization was not used as an experimental assay for larvae of *G. mellonella*.

*2.4 Hemolymph collection, SDS-PAGE and densitometric analyses*

Hemolymph collection was performed at 4, 8 and 24 h post-treatments from venom-injected, parasitized and control host pupae and larvae. Pupae were bled by piercing the cuticle at the abdomen and larvae on the first hind leg with a sterile 19-gauge needle. Four microliters of hemolymph from each individual pupa and larva were collected at each time period and for each treatment with a glass microcapillary tube (Sigma Chemical Co., St. Louis, MO) and injected into an ice cold eppendorf tube containing 1 mg phenylthiourea (Sigma Chemical, St. Louis, MO, USA) to prevent melanization (Zupko et al., 1993). The hemolymph was spun at 3,000 rpm for 10 min at 4°C to remove hemocytes. The supernatant was transferred to a clean eppendorf tube and vortexed with a pipette. Hemolymph proteins were analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE (10% acrylamide) was carried out with the method of Laemmli (1970). The gel was subsequently scanned using an Uvitec Gel Documentation (BioLab) system and the optical densities (OD) of the bands were analyzed by Gel-Pro Analyzer. Each assay was repeated three times for each time point.

*2.5 Statistical analysis*

Means were compared using one- or two-way analysis of variance (ANOVA) and subsequently, means were separated using Tukey's Honestly Significant Difference (HSD) post hoc tests. SPSS software program (version 15.0 for Windows, SPSS Science, Chicago, IL) was used for data analysis. Results were considered statistically significant when $P < 0.05$.

## 3. Results

*3.1 Effects of parasitization and venom injection on the protein profile of pupae*

The quantities of proteins from parasitized and envenomated host pupae did not differ much when compared with those of unparasitized, null- or PBS-injected controls. Analyses using two-way ANOVAs indicated that the effect of venom injection and parasitization on the protein quantity of host pupae was not significant ($P>0.05$), except for protein bands at 19.600, 23.418, 24.714, 32.434, 40.675, 45.385, and 48.846 kDa ($P<0.05$), but time dependent ($P<0.05$). The relationship between treatment and protein quantity was also not influenced by time ($P>0.05$) except for protein bands at 23.418 and 32.434 kDa. Of the seventeen different protein bands detected at a range of 19.600-181.120 kDa in the hemolymph (Table 1), there were only changes in OD values of bands at 23.418, 24.714, 32.434, 34.811, 40.765, 45.385 and 181.120 kDa following envenomation and parasitism at 4, 8 and 24 h post-treatments. Parasitization and envenomation lead up- or down- regulation of only a few proteins. The amount of 23.418 kDa protein increased immediately at 4 h post-injection of 0.05 VRE of venom whereas parasitism resulted in a significant decrease in amount when compared with controls. However, this tendency did not last at later time points. The trend was also alike for the protein band detected at 24.714 kDa with a drastic increase at the highest dose of venom injection (0.05 VRE) and decrease post-parasitization. A significant increase in the amount of 32.434 kDa protein was only observed at 0.01 VRE injection at 4 h post-treatment. At 24 h post-parasitization, the amount also increased significantly when compared to 4 and 8 h. The same trend was also observed for 34.811 and 40.765 kDa proteins with a time-dependent increase in amount at 24 h post-parasitization and at 0.01 VRE injection of venom for the latter. The intensity of 45.385 and 181.120 kDa proteins also increased with all venom dose injections and parasitization at 24 h post-treatments except for 0.01 VRE injection of venom for the latter band. There appeared no qualitative changes in term of novel protein bands in the hemolymph of parasitized or venom injected pupae (Fig. 1).

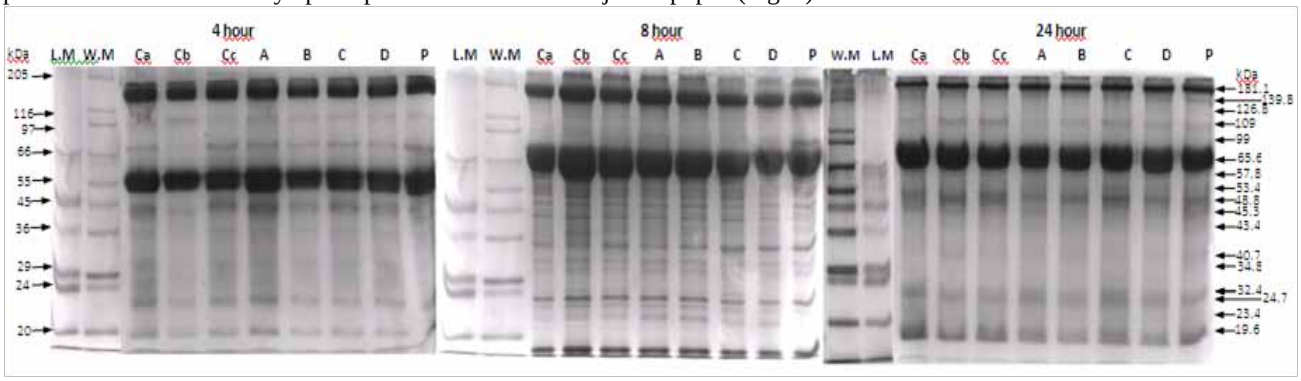


Figure 1. Protein profiles of hemolymph identified by SDS/PAGE using 10% gel from envenomated and parasitized *G. mellonella* pupae at different hours. L.W: Low range molecular weight protein marker (6.5 – 66 kDa); W.M.: Wide range molecular weight protein marker (6.5 – 205 kDa); Ca: Untreated, Cb: Null injected; Cc: PBS injected; A: 0,005 VRE-injected, B: 0.01 VRE-injected; C: 0.02 VRE-injected; D: 0.05 VRE-injected, P: Parasitized

*3.2 Effects of venom injection on the protein profile of larvae*

The electrophoretic pattern of hemolymph proteins from venom injected and control groups of larvae did not differ much from that of pupae with the exception of new protein bands detected at 33.823 and 41.553 kDa. However, three bands with 45.385, 99.000, and 126.850 kDa were not detected in larvae (Fig. 2). Analyses using two-way ANOVAs indicated that the effect of venom injection and parasitization on the protein quantity of host pupae was not significant ($P>0.05$), except for protein bands at 23.418, 34.811, and 65.686 kDa ($P<0.05$), but time dependent ($P<0.05$) except for protein bands at 40.765, 48.846, 139.880, and 181.120 kDa. The relationship between treatment and protein quantity was also not influenced by time ($P>0.05$) except for protein bands at 23.418, 34.811, 41.553, and 43.412 kDa. Hemolymph protein quantity remained relatively steady at all-time points tested, regardless of the venom concentration injected into *G. mellonella* larvae except for significant increases in quantity at 8 h following envenomation for bands 4, 5, 8, 9, 11, 13, and 14 (Table 2). Another exception to this trend was the significant decrease in the quantity of 23.418 kDa protein of venom injected host larvae at 24 h post injection of all venom doses. Similar to that observed in pupae, envenomation led up- or down-regulation of only a few proteins. The amount of 34.811 kDa protein decreased immediately at 8 h post-injection of 0.02 and 0.05 VRE of venom, whereas injection all venom doses except 0.1 VRE resulted in a significant increase in amount for 41.553 and 43.412 kDa proteins when compared with controls (Table 2).

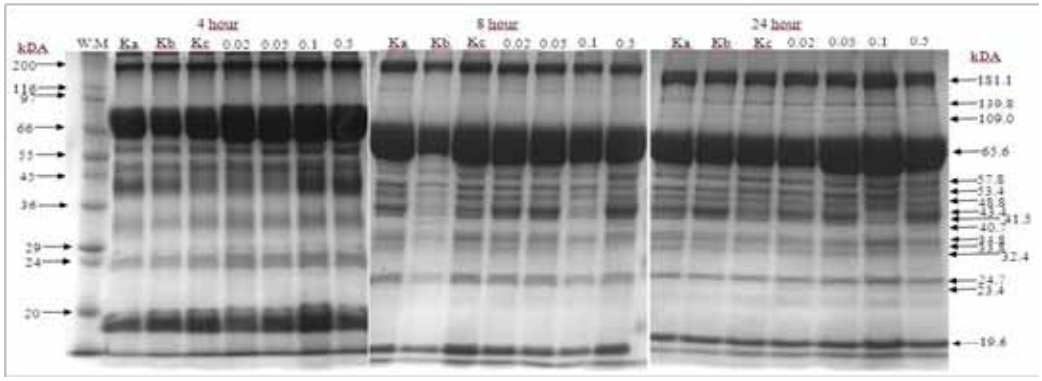


Figure 2. Protein profiles of hemolymph from envenomated *G. mellonella* larvae at different hours were identified by SDS/PAGE using 10% gel. W.M.: Wide range molecular weight protein marker (6.5 – 205 kDa); Ka: Untreated, Kb: Null injected, Kc: PBS injected; 0.02 VRE-injected, 0.1 VRE-injected, 0.5 VRE-injected.

## 4. Conclusions

Many koinobiont endoparasitoids have been shown to interfere with the changes in their hosts' hemolymph milieu and parasitoid-associated factors such as teratocytes, venom, polidnaviruses, and virus like particles appear to play role in this respect (Jervis and Kidd, 1996). However, our results once more support the concept that altering the host nutritional condition for the benefit of wasp offspring is generally thought to be most common for koinobionts, and would presumably not be expected for a solitary idiobiont parasitoid species like *P. turionellae*. (Sak et al., 2011). Consistent with this prediction are the observations in this study that electrophoretic pattern and O.D. values of proteins of hemolymph from *G. mellonella* pupae and larvae did not differ among controls, parasitized or those injected with isolated venom. Neither parasitism nor envenomation caused a complex array of changes in the hemolymph protein profile; there were only a few changes in the amount of some proteins at certain time points. Venom from *P. turionellae* contains several low molecular weight peptides, catecholamines and biogenic amines (Uçkan et al., 2004). It is likely that the sudden increase in the amount of 23.418 and 24.714 kDa proteins at 4 h post-injection of the highest dose of 0.05 VRE might be induced by these paralyzing toxins in venom. Beckage and Kanost (1993) reported that the effects of parasitism on host hemolymph proteins were protein-dependent and the levels of insecticyanin and two subunits of lipophorin (ApoLp-I and II) were similar to those detected in nonparasitized larvae while arylphorin and serpin-like proteins decreased in parasitized larvae of *Manduca sexta*. Among the latter was ApoLp-III (~22 kDa) which has approximately the same molecular weight with these bands. Stress responses in insects are known to be energetically demanding events and the organisms may redirect energy to repair mechanisms, and pathological effects may deplete energy reserves (Korsloot et al., 2004). The increase in the amount of 23.418 and 24.714 kDa proteins at 4 h post-injection may also be attributed to the envenomation-induced increase in energy demand of the pupae, resulting in an increase in lipid transport in hemolymph by these proteins. However, the question why the same proteins decreased at 4 h post-parasitization still requires an explanation, possibly related with the rapid paralysis of pupae upon oviposition. On the other hand, the up-regulation of several proteins for pupae and larvae following envenomation and parasitism for the latter stage may simply be the result of stress induced by parasitoid related secretions. We favor the possibility that defensive proteins may play a role in this up-regulation. Further analysis is required to identify which of these scenarios is correct.

Table 1. Densitometric analysis of hemolymph proteins of *G. mellonella* pupae envenomated and parasitized by *P. turionellae* at different hours.

| **Treatment**[#] | **O. D. Values** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Band 1 (19.600)** | | | **Band 2 (23.418)** | | | **Band 3 (24.714)** | | | **Band4 (32.434)** | | |
| | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** |
| | 0.109 | 0.069 | 0.350 | 0.218 | 0.220 | 0.236 | 0.150 | 0.083 | 0.179 | 0.096 | 0.122 | 0.124 |
| **Untreated** | a x | ab x | a y | ab x | a x | a x | bcd x | a x | a x | a x | a x | a x |
| | 0.079 | 0.080 | 0.289 | 0.220 | 0.212 | 0.161 | 0.133 | 0.096 | 0.164 | 0.117 | 0.145 | 0.117 |
| **Null** | a x | b x | a y | ab x | a x | a x | abc x | a x | a x | a x | a x | a x |
| | 0.075 | 0.059 | 0.292 | 0.228 | 0.190 | 0.179 | 0.162 | 0.071 | 0.157 | 0.120 | 0.113 | 0.143 |
| **PBS** | a x | ab x | a y | ab x | ab x | a x | cd x | a x | a x | a x | a x | ab x |
| | 0.089 | 0.04 | 0.225 | 0.190 | 0.094 | 0.199 | 0.117 | 0.044 | 0.181 | 0.145 | 0.079 | 0.166 |
| **0.005 VRE** | a x | ab x | a y | a y | b x | a y | ab xy | a x | a y | a x | a y | abc x |
| | 0.075 | 0.061 | 0.271 | 0.248 | 0.126 | 0.221 | 0.228 | 0.062 | 0.205 | 0.221 | 0.091 | 0.15 |
| **0.01 VRE** | a x | ab x | a y | ab y | ab x | a x | e x | a y | a x | b x | a y | abc z |
| | 0.088 | 0.063 | 0.219 | 0.230 | 0.162 | 0.209 | 0.177 | 0.080 | 0.196 | 0.138 | 0.091 | 0.186 |
| **0.02 VRE** | a x | ab x | a y | ab x | ab x | a x | d x | a y | a x | a xy | a x | ac y |
| | 0.078 | 0.062 | 0.245 | 0.356 | 0.178 | 0.225 | 0.303 | 0.100 | 0.199 | 0.086 | 0.102 | 0.157 |
| **0.05 VRE** | a x | ab x | a y | c x | ab y | a y | f x | a y | a z | a x | a x | abc x |
| | 0.071 | 0.020 | 0.243 | 0.099 | 0.110 | 0.183 | 0.101 | 0.033 | 0.159 | 0.082 | 0.054 | 0.178 |
| **Parasitized** | a x | a x | a y | d x | ab x | a y | a xy | a x | a y | a x | a x | ac y |

| **Treatment**[#] | **O. D. Values** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Band 5 (34.811)** | | | **Band 6 (40.765)** | | | **Band 7 (43.412)** | | | **Band 8 (45.385)** | | |
| | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** |
| | 0.136 | 0.184 | 0.171 | 0.173 | 0.192 | 0.215 | 0.178 | 0.198 | 0.250 | 0.210 | 0.197 | 0.319 |
| **Untreated** | a x | a x | ab x | a x | a x | a x | a x | a x | a x | a x | abc x | ab x |
| | 0.104 | 0.178 | 0.149 | 0.132 | 0.201 | 0.217 | 0.139 | 0.244 | 0.248 | 0.164 | 0.239 | 0.343 |
| **Null** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.121 | 0.164 | 0.175 | 0.131 | 0.211 | 0.211 | 0.141 | 0.201 | 0.239 | 0.157 | 0.212 | 0.313 |
| **PBS** | a x | a x | ab x | a x | a y | a y | a x | a x | a x | a x | ac x | abc y |
| | 0.116 | 0.124 | 0.187 | 0.144 | 0.111 | 0.199 | 0.151 | 0.131 | 0.218 | 0.176 | 0.137 | 0.269 |
| **0.005 VRE** | a x | a x | ab x | a x | a x | a x | a x | a x | a x | a xy | bc x | bc y |
| | 0.145 | 0.160 | 0.173 | 0.120 | 0.145 | 0.237 | 0.131 | 0.193 | 0.269 | 0.145 | 0.210 | 0.305 |
| **0.01 VRE** | a x | a x | ab x | a x | a x | a y | a x | a x | a y | a x | ac x | abc y |
| | 0.137 | 0.129 | 0.206 | 0.132 | 0.142 | 0.229 | 0.142 | 0.168 | 0.245 | 0.159 | 0.174 | 0.308 |
| **0.02 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | abc x | abc y |
| | 0.134 | 0.131 | 0.178 | 0.116 | 0.163 | 0.193 | 0.123 | 0.182 | 0.224 | 0.140 | 0.196 | 0.250 |
| **0.05 VRE** | a x | a x | ab x | a x | a x | a x | a x | a x | a x | a x | abcxy | c y |
| | 0.096 | 0.096 | 0.217 | 0.091 | 0.087 | 0.226 | 0.080 | 0.100 | 0.252 | 0.103 | 0.118 b | 0.288 |
| **Parasitized** | a x | a x | b y | a x | a x | a y | a x | a x | a y | a x | x | abc y |

| Treatment[#] | O. D. Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Band 9 (48.846)** | | | **Band 10 (53.462)** | | | **Band 11 (57.829)** | | | **Band 12 (65.686)** | | |
| | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** |
| | 0.207 | 0.213 | 0.339 | 0.221 | 0.224 | 0.325 | 0.239 | 0.164 | 0.290 | 0.836 | 0.659 | 0.959 |
| **Untreated** | a x | a x | ab x | a x | a x | a x | a x | a xy | a y | a xy | a x | a y |
| | 0.182 | 0.267 | 0.404 | 0.193 | 0.292 | 0.303 | 0.198 | 0.258 | 0.269 | 0.881 | 0.734 | 0.883 |
| **Null** | a x | a xy | b y | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.171 | 0.261 | 0.395 | 0.179 | 0.259 | 0.353 | 0.223 | 0.255 | 0.276 | 0.794 | 0.747 | 0.878 |
| **PBS** | a x | a x | b y | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.208 | 0.192 | 0.32 | 0.198 | 0.179 | 0.306 | 0.213 | 0.190 | 0.278 | 0.749 | 0.653 | 0.799 |
| **0.005 VRE** | a x | a x | ab x | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.151 | 0.235 | 0.407 | 0.166 | 0.249 | 0.334 | 0.179 | 0.256 | 0.292 | 0.771 | 0.679 | 0.884 |
| **0.01 VRE** | a x | a y | b z | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.171 | 0.215 | 0.321 | 0.181 | 0.216 | 0.348 | 0.193 | 0.215 | 0.295 | 0.802 | 0.689 | 0.828 |
| **0.02 VRE** | a x | a xy | ab y | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.151 | 0.235 | 0.271 | 0.153 | 0.227 | 0.301 | 0.197 | 0.231 | 0.283 | 0.792 | 0.703 | 0.835 |
| **0.05 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.117 | 0.060 | 0.337 | 0.119 | 0.160 | 0.331 | 0.138 | 0.198 | 0.285 | 0.670 | 0.669 | 0.857 |
| **Parasitized** | a x | a x | ab y | a x | a x | a y | a x | a xy | a y | a x | a x | a x |

| Treatment[#] | O. D. Values | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Band 13 (99.000)** | | | **Band 14 (109.000)** | | | **Band 15 (126.850)** | | | **Band 16 (139.880)** | | | **Band 17 (181.120)** | | |
| | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** | **4 h** | **8 h** | **24 h** |
| | 0.230 | 0.130 | 0.283 | 0.204 | 0.113 | 0.216 | 0.203 | 0.103 | 0.206 | 0.451 | 0.099 | 0.302 | 0.736 | 0.550 | 0.848 |
| **Untreated** | a x | a y | a x | a x | a x | a x | a x | a x | a x | a x | a y | a xy | a x | a y | a x |
| | 0.188 | 0.192 | 0.255 | 0.197 | 0.155 | 0.220 | 0.201 | 0.143 | 0.172 | 0.528 | 0.131 | 0.266 | 0.732 | 0.709 | 0.843 |
| **Null** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a y | a y | a x | a x | a x |
| | 0.181 | 0.156 | 0.232 | 0.166 | 0.163 | 0.229 | 0.166 | 0.143 | 0.188 | 0.278 | 0.136 | 0.266 | 0.715 | 0.683 | 0.893 |
| **PBS** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a xy | a x | a y |
| | 0.399 | 0.145 | 0.212 | 0.144 | 0.133 | 0.196 | 0.178 | 0.121 | 0.195 | 0.335 | 0.141 | 0.271 | 0.698 | 0.567 | 0.917 |
| **0.005 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a y | a xy | a xy | a x | a y |
| | 0.389 | 0.150 | 0.228 | 0.177 | 0.176 | 0.206 | 0.174 | 0.138 | 0.194 | 0.313 | 0.158 | 0.283 | 0.709 | 0.626 | 0.923 |
| **0.01 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x |
| | 0.432 | 0.149 | 0.237 | 0.165 | 0.159 | 0.193 | 0.191 | 0.143 | 0.180 | 0.280 | 0.139 | 0.235 | 0.746 | 0.643 | 0.864 |
| **0.02 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a xy | a x | a y |
| | 0.406 | 0.168 | 0.236 | 0.174 | 0.155 | 0.192 | 0.180 | 0.148 | 0.197 | 0.321 | 0.143 | 0.275 | 0.716 | 0.655 | 0.886 |
| **0.05 VRE** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a y | a xy | a x | a x | a y |
| | 0.311 | 0.213 | 0.213 | 0.145 | 0.152 | 0.199 | 0.139 | 0.135 | 0.200 | 0.308 | 0.147 | 0.328 | 0.503 | 0.535 | 0.913 |
| **Parasitized** | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a x | a y |

Table 2. Densitometric analysis of hemolymph proteins of *G. mellonella* larvae envenomated by *P. turionellae* at different hours.

| Treatment[#] | O. D. Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Band 1 (19.600) | | | Band 2 (23.418) | | | Band 3 (24.714) | | | Band4 (32.434) | | |
| | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h |
| **Untreated** | 0,542 a x | 0,433 a x | 0,431 a x | 0,074 a x | 0,285 a y | 0,052 a x | 0,174 a xy | 0,246 a x | 0,077 a y | 0,130 a xy | 0,181 a x | 0,106 a y |
| **Null** | 0,573 a x | 0,440 a xy | 0,425 a y | 0,085 a x | 0,172 a y | 0,042 a x | 0,160 a x | 0,190 a x | 0,080 a y | 0,146 a x | 0,195 a x | 0,196 a x |
| **PBS** | 0,555 a x | 0,376 a y | 0,376 a y | 0,082 a x | 0,207 a y | 0,064 a x | 0,150 a x | 0,228 a x | 0,091 a x | 0,136 a x | 0,160 a x | 0,150 a x |
| **0.02 VRE** | 0,462 a x | 0,442 a x | 0,334 a x | 0,175 a x | 0,279 a x | 0,054 a y | 0,168 a x | 0,241 a x | 0,106 a x | 0,145 a x | 0,192 a x | 0,161 a x |
| **0.05 VRE** | 0,568 a x | 0,463 a x | 0,369 a x | 0,179 a x | 0,170 a x | 0,040 a y | 0,156 a x | 0,189 a x | 0,109 a x | 0,118 a x | 0,169 a x | 0,162 a x |
| **0.1 VRE** | 0,593 a x | 0,478 a x | 0,488 a x | 0,174 a xy | 0,216 a x | 0,070 a y | 0,156 a x | 0,148 a x | 0,080 a x | 0,123 a x | 0,243 a y | 0,150 a xy |
| **0.5 VRE** | 0,592 a x | 0,484 a x | 0,342 a x | 0,161 a x | 0,208 a x | 0,047 a y | 0,155 a x | 0,162 a x | 0,057 a y | 0,125 a x | 0,199 a y | 0,160 a xy |

| Treatment[#] | O. D. Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Band 5 (33.823) | | | Band 6 (34.811) | | | Band 7 (40.765) | | | Band 8 (41.553) | | |
| | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h |
| **Untreated** | 0,216 a x | 0,328 a x | 0,202 a x | 0,202 a xy | 0,315 ab y | 0,159 a x | 0,323 a x | 0,194 a x | 0,269 a x | 0,462 a x | 0,366 a x | 0,353 a x |
| **Null** | 0,205 a x | 0,194 a x | 0,249 a x | 0,172 a x | 0,345 b y | 0,216 a x | 0,261a x | 0,198 ab x | 0,323 a x | 0,401 a x | 0,423 a x | 0,489 a x |
| **PBS** | 0,204 a x | 0,234 a x | 0,232 a x | 0,154 a x | 0,363 b y | 0,194 a x | 0,282 a x | 0,180 a x | 0,192 a x | 0,412 a x | 0,465 ab x | 0,349 a x |
| **0.02 VRE** | 0,200 a xy | 0,246 a y | 0,177 a x | 0,159 a x | 0,172 c x | 0,151 a x | 0,240 a x | 0,379 c y | 0,289 a xy | 0,308 a x | 0,585 b y | 0,410 a x |
| **0.05 VRE** | 0,188 a x | 0,227 a x | 0,196 a x | 0,133 a x | 0,162 c x | 0,173 a x | 0,229 a x | 0,235 ab x | 0,230 a x | 0,316 a x | 0,582 b y | 0,358 a xy |
| **0.1 VRE** | 0,198 a x | 0,259 a x | 0,251 a x | 0,167 a x | 0,214 ac x | 0,228 a x | 0,302 a x | 0,294 abc x | 0,240 a x | 0,433 a x | 0,331 a x | 0,420 a x |
| **0.5 VRE** | 0,198 a x | 0,288 a y | 0,195 a x | 0,153 a x | 0,207 ac x | 0,205 a x | 0,247 a x | 0,334 bc x | 0,237 a x | 0,452 a x | 0,577 b x | 0,411 a x |

| Treatment[#] | O. D. Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Band 9 (43.412) | | | Band 10 (48.846) | | | Band 11 (53.462) | | | Band 12 (57.829) | | |
| | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h |
| **Untreated** | 0,395 a x | 0,332 a x | 0,303 a x | 0,342 a x | 0,437 a x | 0,331 a x | 0,345 a x | 0,505 a x | 0,366 a x | 0,439 a x | 0,394 a x | 0,339 a x |
| **Null** | 0,384 a x | 0,339 a x | 0,387 a x | 0,352 a x | 0,353 a x | 0,392 a x | 0,379 a x | 0,349 a x | 0,448 a x | 0,442 a x | 0,378 a x | 0,396 a x |
| **PBS** | 0,362 a x | 0,364 ab x | 0,353 a x | 0,378 a x | 0,356 a x | 0,401 a x | 0,379 a x | 0,450 a x | 0,438 a x | 0,450 a x | 0,473 a x | 0,398 a x |
| **0.02 VRE** | 0,304 a x | 0,490 c y | 0,310 a x | 0,351 a x | 0,496 a x | 0,371 a x | 0,348 a x | 0,488 a y | 0,370 a x | 0,457 a x | 0,505 a x | 0,401 a x |
| **0.05 VRE** | 0,314 a x | 0,460 bc xy | 0,334 a y | 0,384 a x | 0,397 a x | 0,424 a x | 0,370 a x | 0,470 a x | 0,395 a x | 0,473 a x | 0,441 a x | 0,392 a x |
| **0.1 VRE** | 0,352 a x | 0,440 abc x | 0,449 a x | 0,389 a x | 0,382 a x | 0,456 a x | 0,381 a x | 0,426 a x | 0,474 a | 0,473 a x | 0,447 a x | 0,450 a x |
| **0.5 VRE** | 0,408 a x | 0,457 bc x | 0,346 a x | 0,367 a x | 0,399 a x | 0,337 a x | 0,353 a x | 0,392 a x | 0,337 a x | 0,444 a x | 0,485 a x | 0,412 a x |

| Treatment[#] | O. D. Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Band 13 (65.686) | | | Band 14 (109.000) | | | Band 15 (139.880) | | | Band 16 (181.120) | | |
| | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h | 4 h | 8 h | 24 h |
| **Untreated** | 0,831 a x | 0,861 a x | 0,796 a x | 0,187 a x | 0,214 a x | 0,201 a x | 0,353 a x | 0,291 a x | 0,272 a x | 0,727 a x | 0,757 a x | 0,731 a x |
| **Null** | 0,876 a x | 0,906 a x | 0,888 a x | 0,173 a x | 0,213 a x | 0,223 a x | 0,250 a x | 0,246 a x | 0,302 a x | 0,744 a x | 0,721 a x | 0,791 a x |
| **PBS** | 0,903 a x | 0,968 a x | 0,895 a x | 0,175 a x | 0,214 a x | 0,217 a x | 0,286 a x | 0,301 a x | 0,331 a x | 0,760 a x | 0,787 a x | 0,762 a x |
| **0.02 VRE** | 0,907 a xy | 0,971 a y | 0,838 a x | 0,165 a x | 0,208 a x | 0,214 a x | 0,302 a x | 0,292 a x | 0,309 a x | 0,752 a x | 0,805 a x | 0,757 a x |
| **0.05 VRE** | 0,899 a x | 0,946 a x | 0,877 a x | 0,171 a x | 0,205 a x | 0,203 a x | 0,295 a x | 0,292 a x | 0,295 a x | 0,782 a x | 0,779 a x | 0,705 a x |
| **0.1 VRE** | 0,895 a x | 0,944 a x | 0,873 a x | 0,163 a x | 0,240 a x | 0,240 a x | 0,238 a x | 0,282 a x | 0,342 a x | 0,748 a x | 0,763 a x | 0,772 a x |
| **0.5 VRE** | 0,859 a x | 0,892 a x | 0,812 a x | 0,188 a x | 0,254 a y | 0,195 a x | 0,208 a x | 0,234 a x | 0,276 a x | 0,775 a x | 0,739 a x | 0,683 a x |

**Acknowledgements**

This research was supported by grants (2006-106T255) from the Scientific and Technological Research Council of Turkey (TÜBİTAK).

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*(Received for publicatio September 25 2012; The date of publication 01 April 2013)*