

Determination of Venom Components from the Endoparasitoid Wasp *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

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ABSTRACT Venom from the endoparasitoid wasp *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) was isolated in pure form. Total protein determination indicated an average value of 0.04 µg protein per venom sac. The molecular weights of the venom components were estimated with reference to molecular weight markers and reference proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis indicated that venom primarily contains proteins with molecular weights between 20 and 106 kDa. The presence of melittin and apamin in wasp venom was shown by SDS-PAGE and reversed-phase high-performance liquid chromatography (HPLC). Infrared spectroscopic data confirmed the acidic nature of the venom and the presence of amines, peptides, proteins, and enzymes in the venom. Venom noradrenaline was separated using thin-layer chromatography and verified by infrared spectroscopy.

KEY WORDS *Pimpla turionellae*, venom, electrophoresis, chromatography, infrared spectroscopy

THE RECENT STUDIES OF hymenopteran venoms have profoundly affected the advances in modern biochemistry, pharmacology, and medicine (Soldatova et al. 1998, Konno et al. 2000). The characterization of venom is also a rich source of information on the physiological functions (Coudron et al. 2000, Parkinson et al. 2002a, Rivers et al. 2002), ecological interactions, taxonomic, and phylogenetic studies (Leluk et al. 1989, Doury et al. 1997) of parasitoids. The composition of venom from Hymenoptera may vary within groups or even species (Leluk et al. 1989, Skinner et al. 1990, Sanchez et al. 1994). Studies on venom composition and activity have been greatly focused on those of *Apis* and *Vespa* species, which are of great interest to humans (Abreu et al. 2000, Costa and Pala 2000). However, parasitic Hymenoptera venom has recently become a valuable resource of natural substances that have promise in the construction of biological insecticides (Coudron and Brandt 1996). Parasitic wasps, which lay their eggs in or on their hosts, are important regulators of insect pests. Adult female parasitoids possess a stinging apparatus that is used to inject maternally derived secretions into the hemocoel of their hosts during oviposition (Parkinson et al. 2002b). Several physiological traits of the host are altered by female wasp secretions (polydnviruses, ovarian proteins, and venom) injected at oviposition (Doury et al. 1997, Digilio et al. 2000). Venom secretions modify the host physiology in various ways to assist the development of the parasitoid's progeny

(Parkinson et al. 2002b). The presence or absence of proteins in parasitoid venom may even indicate the host stage for oviposition (Leluk et al. 1989). Venoms from koinobiont species are frequently associated with temporary paralysis and involved in the suppression of host movements during oviposition (Nakamatsu et al. 2001). However, most of the idiobiont parasitoids paralyze the host permanently, and thus preserves it for a long time for the feeding and development of the progeny larvae (Wharton 1993, Nakamatsu and Tanaka 2003).

Pimpla turionellae L. (Hymenoptera: Ichneumonidae) is an idiobiont endoparasitoid wasp that uses hosts from an extremely wide range of lepidopteran species (Kansu and Uğur 1984). Little is known about the composition of venom from this endoparasitoid, although functionally its role in inhibiting neuromuscular transmission at the synaptic site (Kilincer 1975) and disabling hemocytes (Osman 1978) in host species has been suggested. The structure of *P. turionellae* venom apparatus and the primary chemical groups present in the venom sac have been demonstrated (Uçkan and Gülel 1990, Uçkan 1999). The current study aims to determine the venom sac components and the molecular weights of peptides and proteins of *P. turionellae* venom.

Materials and Methods

Insects. The solitary, pupal endoparasitoid *P. turionellae* were reared on pupae of greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Adult parasitoids were maintained at 25 ± 2°C, 12:12 h (L:D) photoperiod and fed with a 50% (vol:vol) honey solution. Parasitoid females were also provided with host

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pupae (four pupae for 10 female wasps) once every 3 d to meet their protein requirement (Kansu and Uğur 1984).

Venom Extraction. The venom sacs of 15- to 20-d-old female wasps were removed by grasping the ovipositor and pulling. The venom sac was collected by grasping the duct leading to the ovipositor with finely tipped forceps under a stereoscopic microscope and placed in 100 μ l distilled water. The content of each venom sac was removed by piercing and drained into distilled water. The solution was centrifuged at $3,000 \times g$ for 10 min to remove cell debris. For our experiments, three tubes, each containing the contents of 50 venom sacs and 100 μ l distilled water, were made up to a final volume of 200 μ l by adding distilled water and adjusted to a final concentration of 0.25 venom sac content per microliter (0.01 μ g/ μ l). The tubes were kept at -20°C for further investigations.

Total Protein Assay and Gel Electrophoresis. Total protein determination was performed by the method of Bradford (1976) using bovine serum albumin (BSA; Sigma, St. Louis, MO) as the standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 20% acrylamide) was carried out with the method described by Laemmli (1970). SDS-PAGE (14 and 12.5%) was performed according to Shagger and Von Jagow (1987) using Bio-Rad Mini Protean III apparatus. The gel that contained 20% polyacrylamide was stained with silver stain (Silver-stain kit, cat. no. AG-25; Sigma). Fourteen and 12.5% polyacrylamide gels were stained with Coomassive Blue (G250; Bio-Rad, Richmond, VA). For electrophoresis, BSA (68 kDa), β -lactoglobulin (18.4), lysozyme (14.3), aprotinin (6.5), melittin (2.84), apamin (2.02), and bradykinin (1.06; Sigma) were used as reference proteins. Bio-Rad low range molecular weight standards, phosphorylase B (106), BSA (81), ovalbumin (47.5), carbonic anhydrase (35.3), soybean trypsin inhibitor (28.2), and lysozyme (20.8), were used as molecular weight markers. The molecular weights of the protein bands were estimated with reference to molecular weight markers and reference proteins (Sigma and Bio-Rad).

High-Performance Liquid Chromatography. High-performance liquid chromatography (HPLC) was performed to confirm the presence of pure apamin and melittin determined by SDS-PAGE to be present in venom. Apamin and melittin (Sigma) were used as standards. The contents of 20 venom sacs were placed into 100 μ l distilled water, and the solution was chromatographed on a reverse-phase HPLC column (AceIII C₁₈, 12.5 cm by 4.0 mm i.d.; MAC-MOD Analytical; Chadds Ford, PA). The mobile phases were (A) 0.1% TFA in acetonitrile:water (80:20) and (B) 0.1% TFA in water. Venom was separated by linear gradient (5–80%) using mobile phase A at 40 min. The flow-rate was maintained at 1.0 ml/min, and the elution was monitored at 280 nm.

Thin Layer Chromatography. Thin-layer chromatography (TLC) was used to separate serotonin, dopamine, noradrenaline, and histamine (Sigma). 10 by 10 cm silica gel plates (generated from splitting pre-

parative 20 by 20 Silica gel 60 GF₂₅₄ into four pieces; Merck, Darmstadt, Germany) were used for the development of samples. Each plate was loaded with 5 μ l of venom and each standard solution and developed with the following solvent systems: (A) ethanol-ammonia, 100:12:16 (vol:vol); (B) methanol-chloroform, 1:1 (vol:vol) (Leonard 1972); and (C) trifluoroacetic acid-acetonitrile-water, 1:95:5 (vol:vol). The plates were viewed under UV light (254 nm) and photographed. Retardation factor (R_f) values were estimated.

Infrared Spectroscopy. The presence of peptides and proteins determined by SDS-PAGE led us to verify this result by infrared spectroscopic analysis. For infrared spectroscopy, 10 μ l venom solution was kept on a watch glass at 30°C until its liquid phase (distilled water and ethanol added for facilitating the evaporation of water) was completely removed. The dry material was homogeneously ground with potassium bromide, and the infrared spectrum was shown.

The bands formed in TLC by reference noradrenaline and the venom component that migrated at the same position as standard noradrenaline were scraped off, transferred into eppendorf tubes, and dissolved in 200 μ l ethanol. The solutions were centrifuged at $3,000 \times g$ for 3 min to remove silica gel. The supernatants were removed on to watch glasses and incubated for 30 min at 30°C . After removing the liquid phase (ethanol), the infrared spectrum was recorded at room temperature and compared to verify the presence of noradrenaline. A Perkin-Elmer Spectrum BX-II infrared spectrometer was used for all spectra (Perkin-Elmer, Beaconsfield Buks, England).

Results and Discussion

Parasitoid venoms that cause paralysis of host species are complex mixtures of low- and high-molecular weight compounds such as amines, peptides, proteins, and glycoproteins (Doury et al. 1997, Coudron et al. 2000). UV absorbance comparison of venom sac components with standard protein solutions indicated an average value of 0.04 μ g protein per venom sac. This value is significantly lower than the average value of 180 μ g of protein per venom sac for *P. hypochondriaca* (Retzius) (Parkinson and Weaver 1999). *P. turionellae* females may use their venom for the purpose of reproduction and nutrition. The ability of a single female wasp to paralyze a great number of host pupae to feed or lay eggs indicates that a small fraction of the venom sac contents may be sufficient to produce effective paralysis in the host species.

SDS-PAGE profiles indicated that *P. turionellae* venom is composed of a highly complex mixture of polypeptides (Fig. 1). Venom primarily consists of components with molecular weights between 20 and 106 kDa (Fig. 1). However, there were also protein bands higher or lower than this range. Studies have revealed that social and some parasitoid Hymenoptera venoms constitute low molecular weight proteins (Leluk et al. 1989, Doury et al. 1997). Unlike the venom of the other endoparasitoid species, *Cotesia*

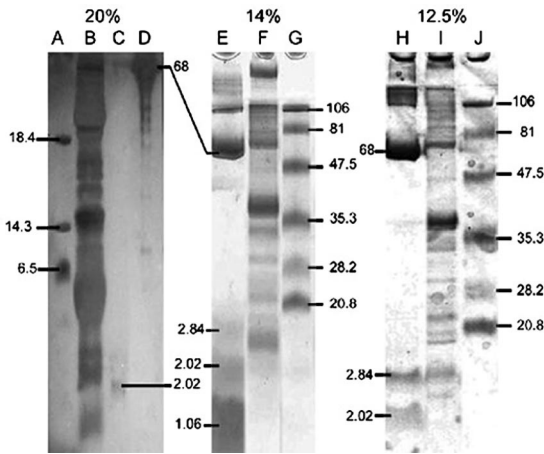


Fig. 1. SDS-PAGE at 20% (A–D), 14% (E–G), and 12.5% (H–J) (wt:vol) gel for standards (1 mg in 1 ml buffer solution) and venom components (5 μ l venom solution). (A) β -lactoglobulin (18.4), lysozyme (14.3), aprotinin (6.5); (B, F, and I) venom; (C) apamin (2.02); (D) BSA (68); (E and H) BSA (68), melittin (2.84), apamin (2.02), bradykinin (1.06); (G and J) Bio-Rad low range molecular weight standards: phosphorylase B (106), BSA (81), ovalbumin (47.5), carbonic anhydrase (35.3), soybean trypsin inhibitor (28.2), lysozyme (20.8).

congregate (Say) (Beckage et al. 1987), *Chelonus nearcurvimaculatus* (Jones and Leluk 1990), and *Microplitis demolitor* Wilkinson (Strand et al. 1994), the venom of *P. turionellae* contains several proteins smaller than 20 kDa (Fig. 1). The venom of *Eupelmus*

orientalis (Crawford) has also been shown to contain several proteins smaller than 15 kDa (Doury et al. 1997). These small peptides may generally be neurotoxins in higher Hymenoptera (Schmidt 1982), which is consistent with the paralyzing function of *P. turionellae* venom (Kilincer 1975). Venom from an endoparasitoid wasp *P. hypochondriaca* was previously investigated, and proteins including phenoloxidases, a laccase, and a serine protease were identified (Parkinson et al. 2002b). Thus, several bands with molecular weights around or higher than 106 kDa demonstrate the presence of high molecular weight proteins and enzymes in *P. turionellae* venom. Protein bands with molecular weights ranging from 21 to 97 kDa have been detected in venoms of other parasitic hymenopterans (Digilio et al. 2000, Parkinson et al. 2002a, Nakamatsu and Tanaka 2003). The results of our SDS-PAGE analysis are also in close agreement with those reported by Parkinson et al. (2002b). Both *P. turionellae* and *P. hypochondriaca* seem to contain a considerably greater number of constituents in their venoms than the venom from the egg parasitoid *C. nearcurvimaculatus* (Jones and Leluk 1990) and the larval endoparasitoid *M. demolitor* (Strand et al. 1994).

SDS-PAGE analysis (Fig. 1) indicated that venom might contain melittin (2.84 kDa) and apamin (2.02 kDa). The presence of paralyzing proteins such as apamin, melittin, and kinin in parasitoid wasp venom has been suggested to occur in those species in which oviposition occurs in an active host stage such as *P. turionellae* (Leluk et al. 1989). However, we could not detect the presence of bradykinin in venom.

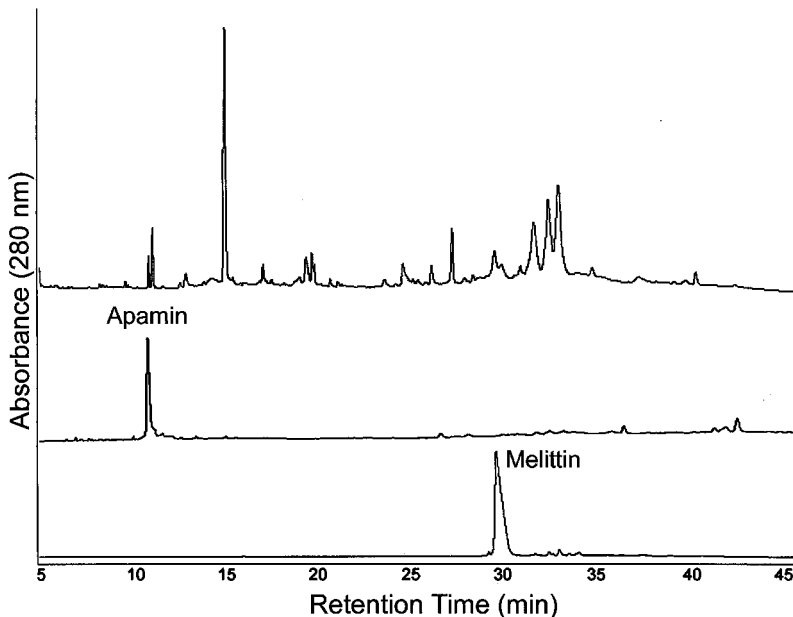


Fig. 2. Fractionation of the venom sac content (4 venom sac equivalents) of *P. turionellae* and determination of the presence of apamin and melittin (1 μ g/ μ l; Sigma) in venom sac content by reversed-phase HPLC using AceIII C₁₈ (12.5 cm by 4.0 mm). Eluent A: 0.1% TFA in acetonitrile:water (80:20); eluent B: 0.1% TFA in water. Linear gradient: 5–80% A at 40 min. Injection volume: 20 μ l. Detection: 280 nm. Flow rate: 1.0 ml/min.

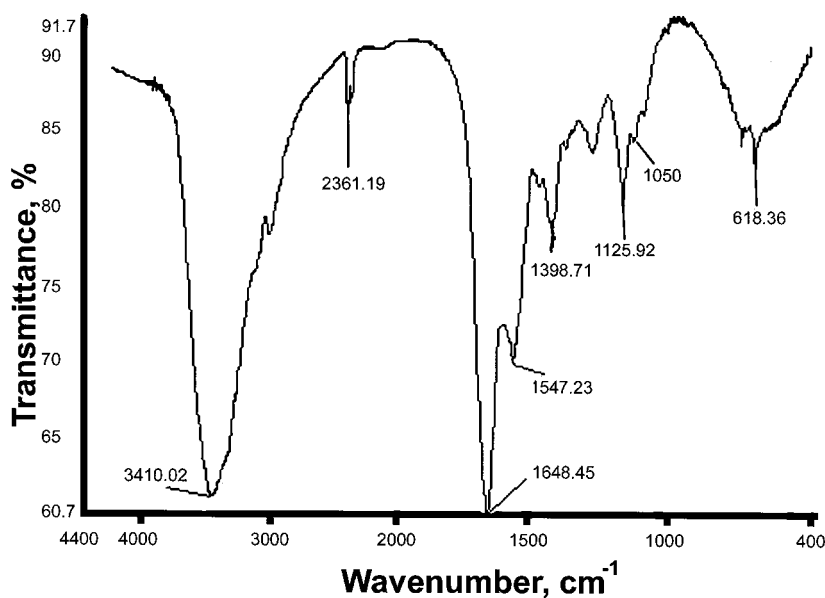


Fig. 3. Infrared spectrum of *P. turionellae* venom (10 μ l venom solution).

HPLC analysis supported the presence of melittin and apamin in the venom (Fig. 2).

Infrared spectrum of the venom is shown in Fig. 3, and characteristic infrared bands are interpreted in Table 1. The infrared absorption bands at 3,410, 1,648, and 1,547 cm^{-1} can be interpreted to indicate the presence of secondary amine and amide groups and to confirm the proteinous nature of the venom. However, the infrared band at 3,410 cm^{-1} is disproportionately large and could be caused in part by traces of ethanol. The vibration at 1,398 cm^{-1} may be associated with the acidic (carboxylic) nature of the venom (Leonard 1972). The C-H in-plane bending at 1,125 cm^{-1} is characteristic of aromatic compounds (Stuart 1997). The absorption band at 1,050 cm^{-1} suggests that there may also be structures containing phosphorous in the venom, possibly enzymes. The C-H bending at 618 cm^{-1} is much more likely to be olefinic C-H rock (characteristic of alkynes) (Stuart 1997). The venom sac components seem to lack a

carbohydrate moiety because there were no absorption bands at 3,600 (OH peak), 2,900 (C-H stretching), and 1,700 (C=O stretching) cm^{-1} , which are basic characteristic infrared spectroscopic bands of carbohydrates (Fig. 3; Table 1) (Fritz and Schenk 1979, Stuart 1997). Our results suggesting the acidic and proteinous nature of the venom are in conformity with those the investigation of Leonard (1972) from sawfly larvae venom. Leluk et al. (1989) also confirmed the presence of acidic proteins in the venom of an ichneumonid parasitoid, *Chelonus sp. near curvumaculatus*, with their isoelectrofocusing study.

The R_f values from TLC analysis of venom and standards on three different solvent systems are given in Table 2. The best separation was achieved on trifluoroacetic acid-acetonitrile-water (1:95:5 by vol.). However, we could only detect the presence of noradrenaline in *P. turionellae* venom (Fig. 4). The very close R_f values obtained for venom and noradrenaline from three different solvent systems (Table 2) is a

Table 1. Characteristic infrared bands and their possible assignments verifying the presence of peptides and proteins in *P. turionellae* venom sac content

Frequency (cm^{-1})	Possible assignment
3410	N-H stretching, secondary amines
2361	CO_2
1648	C=O stretching, amides
1547	N-H bending, secondary amines
1398	C-O-H in-plane bending, carbonyl compound
1125	C-H in-plane bending
1050	P-O-H bending
618	Olefinic C-H rock

Ten microliters of venom solution (2.5 venom sac equivalents) was used for infrared spectroscopic analysis.

Table 2. R_f values from thin-layer chromatographic analysis of venom and standard solutions developed with three different solvent systems: ethanol-water-ammonia, 100:12:16 by vol; methanol-chloroform, 1:1 (v/v); and trifluoroacetic acid-acetonitrile-water, 1:95:5 by vol

R_f values	Development systems		
	Ethanol-water-ammonia	Methanol-chloroform	Trifluoroacetic acid-acetonitrile-water
Venom	0.77	0.79	0.66
Serotonin	0.59	0.63	0.70
Dopamine	0.71	0.72	0.64
Noradrenaline	0.78	0.78	0.66
Histamine	0.00	0.00	0.21

Each plate was loaded with 5 μ l of venom (1.25 venom sac equivalents) and each standard solution.

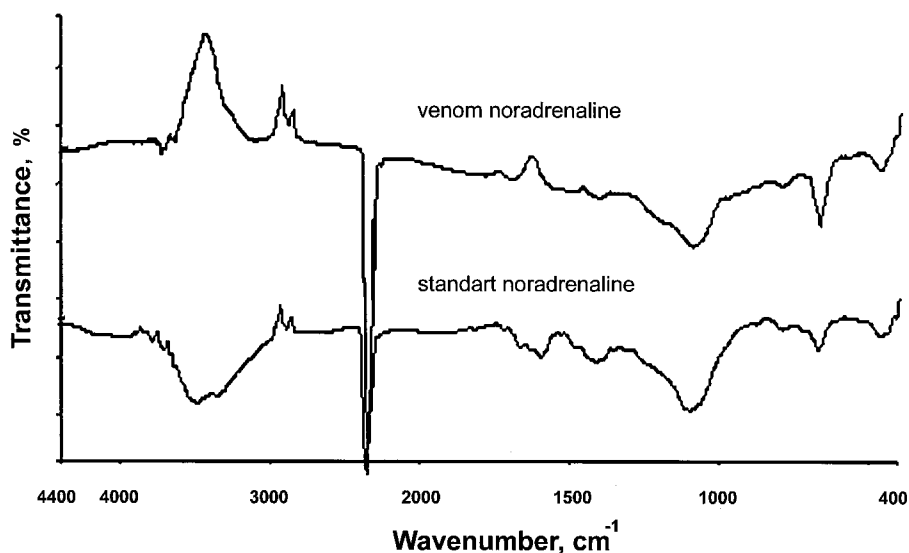


Fig. 4. Comparative infrared spectrum of standard noradrenaline (Sigma) and venom noradrenaline detected in TLC.

strong indication that noradrenaline is actually a component of the venom. The widespread existence of biogenic amines and catecholamines, which we used as standards in our investigation, has also been shown in the venom content of *Apis* and *Vespa* species (Owen and Sloley 1988, Weisel-Eichler et al. 1999). Endoparasitic wasps, such as those from the genus *Chelonus*, which parasitize host eggs, do not possess acutely toxic venomous components (Leluk et al. 1989). However, the significant quantity of noradrenaline detected by TLC in our study indicates that noradrenaline is one of the components of the pupal endoparasitoid *P. turionellae* venom and therefore may be fairly effective in the paralysis of host pupae. Thus, analysis of the parasitoid venom components may indicate the correlation between the venom content of the wasp and its host stage preference. *P. turionellae* can also parasitize and lay eggs in larvae and prepupae of different host species (Kansu and Uğur 1984). Therefore, the presence of noradrenaline in venom is consistent with the polyphagous nature of this wasp. It was also previously reported that the paralysis of host pupae by the parasitoid wasp could be explained by the presence of venomous contents inhibiting neuromuscular transmission at the synaptic site (Kılınçer 1975). The presence and functions of neurotoxic compounds in parasitic wasp venom have also been investigated by other studies (Visser et al. 1976, Skinner et al. 1990).

We also compared the infrared spectrum of standard noradrenaline and noradrenaline developed on TLC (Fig. 4). Comparative infrared spectroscopic analysis revealed that two spectra illustrated similar peaks. The bands between 2,850 and 3,000 cm^{-1} can be attributed to the symmetric and asymmetric C-H stretching bands from aliphatic compounds like noradrenaline. The strong bands observed near 2,360 cm^{-1} might have resulted from atmospheric absorption by CO_2 (Stuart 1997). The NH_2 bending

vibrations occurring near 1,600 cm^{-1} indicate the aliphatic compound of noradrenaline (Stuart 1997). The stretching and bending of C-H and C-H-O between 1,100 and 1,400 cm^{-1} can be attributed to the presence of a benzene ring. The bands representing the out-of-plane bending vibrations of substituted benzene between 650 and 950 cm^{-1} may also be an indicator of a benzene ring in both compounds (Fig. 4) (Fritz and Schenk 1979, Stuart 1997). The presence of noradrenaline in wasp venom was consistent with the infrared spectrum of noradrenaline standards. *P. turionellae* venom has a wide range of proteins in molecular weight and includes neurotoxic components such as apamin, melittin, and noradrenaline. These observations are consistent with the polyphagous nature of *P. turionellae* and thus the requirement for a venom that has paralytic activity in several developmental stages of a wide range of hosts.

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References Cited

- Abreu, R.M.M., R.L.M. Silva de Moraes, and O. Malaspina. 2000. Histological aspects and protein content of *Apis mellifera* L. worker venom glands: the effect of electrical shocks in summer and winter. *J. Venom Anim. Toxins* 6: 87-98.
- Beckage, N. E., T. J. Templeton, B. D. Nielsen, D. I. Cook, and D. B. Stoltz. 1987. Parasitism-induced hemolymph polypeptides in *Manduca sexta* (L.) larvae parasitized by the braconid wasp *Cotesia congregata* (Say). *Insect Biochem.* 17: 439-455.

- Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem.* 72: 248–254.
- Costa, H., and M. S. Pala. 2000. Agelotoxin: a phospholipase A₂ from the venom of the neotropical social wasp *Casununga (Agelais pallipes pallipes)* (Hymenoptera: Vespidae). *Toxicon*. 38: 1367–1379.
- Coudron, T. A., and S. L. Brandt. 1996. Characteristics of a developmental arrestant in the venom of the ectoparasitoid wasp *Euplectrus comstockii*. *Toxicon*. 34: 1431–1441.
- Coudron, T. A., M.M.K. Wright, B. Puttler, S. L. Brandt, and W. C. Rice. 2000. Effect of the ectoparasite *Necremnus breviramulus* (Hymenoptera: Eulophidae) and its venom on natural and factitious hosts. *Ann. Entomol. Soc. Am.* 93: 890–897.
- Digilio, M. C., N. Isidoro, E. Tremblay, and F. Pennacchio. 2000. Host castration by *Aphidius ervi* venom proteins. *J. Insect Physiol.* 46: 1041–1050.
- Doury, G., Y. Bigot, and G. Periguet. 1997. Physiological and biochemical analysis of factors in the female venom gland and larval salivary secretions of the ectoparasitoid wasp *Eupelmus orientalis*. *J. Insect Physiol.* 43: 69–81.
- Fritz, J. S., and G. H. Schenk. 1979. Electronic absorption spectra, fluorescence and infrared spectroscopy, pp. 430–460. In J. S. Fritz and G. H. Schenk (eds.), *Quantitative analytical chemistry*, Allyn and Bacon, Boston, MA.
- Jones, D., and J. Leluk. 1990. Venom proteins of the endoparasitic wasp *Chelonus near curvimaclatus*: characterization of the major components. *Arch. Insect Biochem. Physiol.* 13: 95–106.
- Kansu, İ. A., and A. Uğur. 1984. *Pimpla turionellae* (L.) (Hym.-Ichneumonidae) ile konukçusu bazı lepidopter pupaları arasındaki biyolojik ilişkiler üzerine araştırmalar. *Doğa Bilim Dergisi*. 8: 160–173.
- Kılınçer, N. 1975. Untersuchungen über die hämocytaire Abwehrreaktion der puppe von *Galleria mellonella* L. (Lep.) und über ihre Hemmung durch den puppenparasiten *Pimpla turionellae* L. (Hym.; Ichneumonidae). *Z. Angew. Entomol.* 78: 340–370.
- Konno, K., M. Hisada, H. Naoki, Y. Itagaki, N. Kawai, A. Miwa, T. Yasuhara, Y. Morimoto, and Y. Nakata. 2000. Structure and biological activities of eumenine mastoparan-AF (EMP-AF), a new mast cell degranulating peptide in the venom of the solitary wasp (*Anterhynchium flavomarginatum micado*). *Toxicon*. 38: 1505–1515.
- Laemmlı, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227: 680–685.
- Leluk, J., J. Schmidt, and D. Jones. 1989. Comparative studies on the protein composition of hymenopteran venom reservoirs. *Toxicon*. 27: 105–114.
- Leonard, G. J. 1972. The isolation of a toxic factor from sawfly (*Lophyrotoma interrupta klug*) larvae. *Toxicon*. 10: 597–603.
- Nakamatsu, Y., Y. Gyotoku, and T. Tanaka. 2001. The endoparasitoid *Cotesia kariyai* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* larvae by venom and Ck polydnavirus. *J. Insect Physiol.* 47: 573–584.
- Nakamatsu, Y., and T. Tanaka. 2003. Venom of ectoparasitoid, *Euplectrus* sp. near plathyphenae (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. *J. Insect Physiol.* 49: 149–159.
- Osman, S. E. 1978. Die Wirkung der sekrete der weiblichen genitalanhangsdrüsen von *Pimpla turionellae* L. (Hym., ichneumonidae) auf die hämocyten und die ein-kapselungsreaktion von wirtspuppen. *Z. Parasitenkd.* 57: 89–100.
- Owen, M. D., and B. D. Sloley. 1988. 5-Hydroxytryptamine in the venom of the honey bee (*Apis mellifera*): variation with season and with insect age. *Toxicon*. 26: 577–584.
- Parkinson, N. M., and R. J. Weaver. 1999. Noxious components of venom from pupa-specific parasitoid *Pimpla hypocondriaca*. *J. Invertebr. Pathol.* 73: 74–83.
- Parkinson, N., C. Conyers, and I. Smith. 2002a. A venom protein from the endoparasitoid wasp *Pimpla hypocondriaca* is similar to snake venom reprolysin-type metalloproteases. *J. Invertebr. Pathol.* 79: 129–131.
- Parkinson, N., E. H. Richards, C. Conyers, I. Smith, and J. P. Edwards. 2002b. Analysis of venom constituents from the parasitoid wasp *Pimpla hypocondriaca* and cloning of cDNA encoding a venom protein. *Insect Biochem. Mol. Biol.* 32: 729–735.
- Rivers, D. B., M. M. Rocco, and A. R. Frayha. 2002. Venom from the ectoparasitic wasp *Nasonia vitripennis* increases Na⁺ influx and activates phospholipase C and phospholipase A₂ dependent signal transduction pathways in cultured insect cells. *Toxicon*. 40: 9–21.
- Sanchez, F., M. Blanca, A. Miranda, M. J. Carmona, J. Garcia, J. Fernandez, M. J. Tarres, M. C. Rondon, and C. Juarez. 1994. Comparison of *Vespula germanica* venoms obtained from different sources. *Int. Arch. Allergy Immunol.* 104: 385–389.
- Schmidt, J. O. 1982. Biochemistry of insect venoms. *Annu. Rev. Entomol.* 27: 339–368.
- Shagger, H., and G. Von Jagow. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* 166: 368–379.
- Skinner, W. S., P. A. Dennis, and G. B. Quistad. 1990. Partial characterization of toxins from *Goniozus legneri* (Hymenoptera: Bethyidae). *J. Econ. Entomol.* 83: 733–736.
- Soldatova, L. N., R. Cramerı, M. Grachl, D. M. Kemeny, M. Schmidt, M. Weber, and U. R. Mueller. 1998. Superior biologic activity of the recombinant bee venom allergen hyaluronidase. *J. Allergy Clin. Immunol.* 101: 651–698.
- Strand, M. R., J. A. Johnson, T. Noda, and B. A. Dover. 1994. Development and partial characterization of monoclonal antibodies to venom of the parasitoid *Microplitis demolitor*. *Arch. Insect Biochem. Physiol.* 26: 123–136.
- Stuart, B. 1997. Biological applications of infrared spectroscopy. John Wiley & Sons, New York.
- Uçkan, F. 1999. The morphology of the venom apparatus and histology of venom gland of *Pimpla turionellae* (Hym.; Ichneumonidae) females. *Tr. J. Zool.* 23: 461–466.
- Uçkan, F., and A. Gülel. 1990. Endoparazitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) dişilerinde zehir aparatının yapısı ve zehirin başlıca kimyasal grubunun tayini. X. Ulusal Biyoloji Kongresi, Erzurum.
- Visser, B. J., W. Spanjer, H. De Klonia, T. Piek, C. Van Der Meer, and A.C.M. Van Der Drift. 1976. Isolation and some biochemical properties of a paralyzing toxin from the venom of the wasp *Microbracon hebetor* (Say). *Toxicon*. 14: 357–370.
- Weisel-Eichler, A., G. Haspel, and F. Libersat. 1999. Venom of a parasitoid wasp induces prolonged grooming in the cockroach. *J. Exp. Biol.* 202: 957–964.
- Wharton, R. A. 1993. Bionomics of the braconidae. *Annu. Rev. Entomol.* 38: 121–143.