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REVIEW

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Ecological mechanisms and prospects for utilization of toxins from parasitic hymenopterans

Abstract Insects in the order Hymenoptera defend themselves, attack prey and regulate hosts using toxins that are effective in small quantities. In this study, advances in the researches on parasitic hymenopteran toxins are summarized in terms of the production, categories, components, properties, ecological functions and mechanisms. The glands that produce venoms derive from the ectoderm tissue and evolve from the accessory glands of the reproductive system. Venoms are excreted by the poison gland or acid gland of mature female wasps and stored in reservoirs. The components of insect toxins are very complicated, and hymenopteran venoms contain alkanes, alcohols, aldehydes, ketones, organic acids, esters, lactones, proteins, polypeptides, enzymes, amines and other compounds. Toxins of parasitic hymenoptera play an important adaptive role. They can increase the probability of successful oviposition by paralyzing hosts, enhancing offspring survival by inhibiting host development and immunoreaction, and improving the nutrition available for their progeny by disturbing the hosts' physiological response. Venoms of the ectoparasitoids often lead to arrested development, permanent paralysis and even death of hosts. These toxins are usually broad-spectrum and act on the central nervous system or at the neuro-muscular junction. While most endoparasitoids are koinobionts, these parasitoids can regulate the physiology and development of the hosts, but no longer paralyze the hosts permanently. Also, they kill the hosts in a concealed but safe position after the hosts cocoon or build their pupal cells. Venoms of koinobiont parasitoids can contain polydnaviruses (PDV) that regulate the growth and development of the hosts by inhibiting the immune system and influencing the metamorphosis of hosts. Thus, PDVs are commensal and mutualistic, but non-pathogenic, with parasitoids at the molecular level. Promising prospects for the utilization of insect toxins, especially as medicines or specific bioinsecticides, are discussed. Because insect toxins are mixtures of complex ingredients and are usually produced in small quantities, isolation and purification of all the ingredients with bioactivity are needed for biochemical and toxicological research and for practical application.

Keywords insect toxins, ecological mechanisms, hymenopteran, parasitoids, prospects for utilization

1 Introduction

The study on insect toxins is a rather popular field around the world. The discovery of the ecological mechanisms of toxins is not only important in understanding the co-evolution history among species, but also promising for the utilization in pharmacy and toxicology. Insect toxins are allomones, and are the products of population evolution, with defending, attacking and regulating the hosts as the basic functions. As the majority of the animal kingdom in species and quantities, insects should produce maximum classes of toxins. At present, many species in Hymenoptera, Lepidoptera, Hemiptera, Coleoptera and some species in Diptera, Orthoptera, Dermaptera, Neuroptera, Trichoptera, Blattodea, and Isoptera are known to produce toxins (Habermehl, 1981; Ke, 1988). However, compared with the insect species, the known insect toxins are really limited, especially in those presocial insects and nonsocial insects or solitary insects. Kawamoto and Kumada (1984) and Aplin and Rothschild (1972) studied lepidopterous toxins. In hymenopterans, studies on toxins have mostly focused on social insects such as bees, ants and vespids, because it is easy to obtain large numbers of experimental materials by collecting from the field or rearing in the laboratory. Generally speaking, parasitic wasps are solitary hymenopterans. Here, the progress of

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insect toxins concerning parasitic wasps is mainly summarized.

2 Production of insect toxins

The ovipositor is specialized in all the groups of Aculeata, Hymenoptera. It is not used to lay eggs, but is only an apparatus for injecting venoms, so its poison gland is usually highly developed. Except for Aculeata (including Chrysidoidea, Vespoidea and Apoidea), all the other superfamilies in Apocrita have this dual-purpose ovipositor for both depositing eggs and injecting venoms. The accessory glands of Apocrita are specialized and can produce toxins. Many species of Apocrita envenom hosts prior to oviposition (Gauld and Bolton, 1988). These venoms can disable hosts. Some venoms paralyze the hosts instead of killing them immediately, so that the hosts can stay fresh when parasite progeny consumes them.

The poison gland is one of the optimum morphological structures reflecting the evolutionary relationships among parasitoids (Chen et al., 2000). Generally speaking, the venom apparatus of hymenopterans derives from the ectoderm, and evolves from the accessory glands of the reproductive system, jointing with the ovipositor or aculeus on the end. Venoms are secreted by the poison glands or acid glands of mature female wasps, and stored in venom reservoirs (Piek, 1986; Pan and Chen, 2003). Newly hatched larvae of some parasitoids also can produce toxins. Toxins and venoms mentioned here are different, toxins are the main contents of venoms, and venoms consist of many other substances besides toxins. For instance, venoms from female adults of Braconidae and Ichneumonidae contain polydnavirus (PDV). Usually, immature wasps do not produce venoms. For example, venom reservoirs of newly emerging *Euplectrus kuwanae* females are small and contain no venomous fluid. However, they grow rapidly, and the venomous fluid gradually accumulates in the reservoirs after four days of emergence (Uematsu and Sakanoshita, 1987).

3 Classes, components and properties of toxins

Insect toxins are mixtures with complicated components. The known toxins of hymenopterans include hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones and enzymes, etc (Ke, 1988). So far, scientists in many countries have conducted a great number of studies on the chemical constituents of bee venom. Now, it is known that bee venom contains many polypeptides, enzymes, amines and other compositions, in which the melittin is the main active ingredient (Fang et al., 1988). Gas chromatography-mass spectrometry analyses show that the major volatiles in the venom gland of *Polybioides raphigastra* are alkanes, monounsaturated alkenes and 2-alcohols. Several pyrazines, a spiroacetal and aromatics were also identified as trace compounds (Sledge et al., 1999). Many proteins, polypeptides, putrescine, dopamine, amino acids and other organic acids have been isolated from the venoms of various parasitic wasps (Pan et al., 2004). Analyses on the toxic proteins of *Chelonus* sp. near *curvimaculatus* reveal that the principal active ingredient is an isomerous protein with a relative molecular weight of 3.3×10^4. Its molecular structure is composed of 12 particular repeated sequences in series, and every repeated sequence contains 14 residues (Jones et al., 1992). Leluk et al. (1989) found that proteins and polypeptides of low molecular weight are typical for the venoms of ants (Formicidae), social wasps (Vespidae) and bees (Apidae). Six species of ichneumonoid parasitoids they examined lack these low molecular weight proteins. However, some other studies indicated that the venom of *Aphidius ervi* contained small molecular proteins (Tremblay et al., 1998).

The venom of *Pimpla hypochondriaca* can resist insect hemocytes and disturb the immune reaction of hosts. A series of 4–22 kD proteins are contained in the venom, and most proteins include more than six residues of cysteine, in which many proteins have similar sequences (Parkinson et al., 2004). Parkinson et al. (2002a) identified more than 20 venom constituents ranging approximately from 5 to 100 kD. Using random 5′ end sequencing of a *P. hypochondriaca* venom gland from a cDNA library, they isolated a cDNA encoding from a 25.3 kD protein containing a signal peptide and having sequence similarity to serine proteases. The venom of *P. hypochondriaca* also contained abundant phenoloxidases, and three genes from a cDNA library made from venom-producing glands were isolated (Parkinson et al., 2001). *P. hypochondriaca* venom also contains antibacterial and proteolytic activity. Antibacterial activity was detected against the Gram-negative bacteria, *Escherichia coli* and *Xanthomonas campestris*, but not against *Pseudomonas syringae*, nor against two Gram-positive bacteria, *Bacillus cereus* and *B. subtilis*. Endopeptidase and aminopeptidase activities were also detected in the venom (Dani et al., 2003). An aspartylglucosaminidase (AGA)-like protein was found in the venom of the endoparasitic wasp *Asobara tabida*, which had a polymeric conformation and was formed of 30 and 18 kD subunits. The enzyme usually had the analogous activity of lysosomal enzymes, stored in the reservoir of a parasitoid as a precursor molecule (Moreau et al., 2004). Baker et al. (2005) identified a homologous series of five chemical classes from the mixture in the Dufour glands and venoms of *Bracon cephi* and *B. lissogaster*, and parasitoids of the wheat stem sawfly, *Cephus cinctus*. The major components included acetate esters of saturated

and unsaturated primary alcohols with present chain lengths from C_{12} to C_{20} (such as hexadecanyl acetate, octadecanyl acetate and octadecenyl acetate), a homologous series of monoenes from C_{23} to C_{35}, and a homologous series of *n*-alkanes from C_{19} to C_{31}, as principal of *n*-Tricosane. Minor components in both species included a homologous series of both mono- and dimethyl branched alkanes.

4 Ecological functions of toxins

Toxins of parasitic hymenopterans greatly enhance their self fitness, for instance, increasing the probability of successful oviposition by paralyzing the hosts, increasing the progeny survival by inhibiting the growth and immunity of the hosts, and improving the nutritional needs for the offspring by regulating the physiological actions of the hosts. Toxins of *Tetrastichus* sp. help wasp progeny to survive inside the pupae of the host *Ostrinia furnacalis* (Ren et al., 2004). Toxins also influence the interspecific competition between parasitic wasps. Toxins of some parasitoids can interrupt the successful development of other wasps' progeny. For example, the wasp *Copidosomopsis tanytmema* cannot survive in *Anagastra kühniella* envenomated by another parasitoid *Microbracon hebetor* (Katzner and Cruz, 1998).

4.1 Toxins from ectoparasitoids

Toxins of ectoparasitoids often lead to arrested development, permanent paralysis and even death of hosts (Beard, 1978; Bocchino and Sullivan, 1981). Most ectoparasitoids are idiobiont; their hosts usually live in concealed refuge locations such as wood borers, and the nutritional resource of the hosts are enough for the development of wasp offspring. Idiobiont parasitoids sting to permanently paralyze or kill the hosts before laying eggs; after hatching, the wasp progeny consumes the hosts which have lost defensive ability. The wasp progeny would never be banished or hurt in the revival of the hosts. Few ectoparasitoids are koinobiont yet, and then the oviposition sites are rather important for these ectoparasitic species. Some of them sting the hosts to paralyze them temporarily so that they can deposit eggs precisely on certain sites of the bodies of the hosts, where eggs do not drop easily, such as the conjunctivae.

Many ectoparasitoids sting and envenomate hosts before oviposition. If they do not lay eggs after paralyzing the hosts, the attacked hosts can stay in narcosis for several months, and then die (Askew, 1971). Idiobiont endoparasitoids avoid or restrict the disadvantageous responses to their offspring larvae from hosts through various approaches, including injecting venoms for paralyzing hosts (Vinson and Iwantsch, 1980b). Fuhrer and Willers (1986) found that the idiobiont endoparasitoids *Pimpla turionellae* larvae excrete a substance via the anus, which can resist blackening, accompanied against bacteria and fungi. This component is evidently secreted by two increscent Malpighian tubes and then enters into the hindgut.

The physiological functions of toxins from some parasitoids are host-specific. Toxins of *Pteromalus puparum* can significantly reduce the spreading and encapsulation capacity of host hemocytes, and even kill these hemocytes. However, the venom from *Nasonia vitripennis* shows no impact on the hemocytes of the unnatural host *Pieris rapae* under the same conditions (Zhang et al., 2004). Contrarily, Rivers et al. (1993) found that the venom from *N. vitripennis* revealed high activity not only to its host, but also to non-host *Trichoplusia ni*. The injection of calyx fluid and the venom of *Microplitis croceipes* could differentially affect the growth and development of its natural host *Helicoverpa zea*, and atypical host, *Galleria mellonella*, but only a minimal effect was observed in another atypical host, *Spodoptera exigua* (Ferkovich and Gupta, 1998). The stinging adult female and the biting newly-hatched larva of the solitary ectoparasitoid *Eupelmus orientalis* can both permanently paralyze and stop the development of host *Callosobruchus maculatus* larvae. These two processes of host envenomation appeared to be independent and complementary in primary parasitism. The host larvae paralyzed by the injection of venom were still alive, despite inactivity. Protein electrophoretic profiles show the significant differences between the two venoms from wasp adults and first instar larvae. Phospholipase activity was found in both the female venoms and the larval secretions of *E. orientalis*, whereas hyaluronidase was specific to the adult venom (Periquet et al., 1997). Actually, phospholipase and hyaluronidase are two common inclusions of insect venoms. In addition, venoms of some insects additionally contain acid phosphatases, esterases, lipases, α-glucuronidases, and β-galactosidases, etc (Ke, 1988). Newly hatched larvae of *E. orientalis* could paralyze host larvae through biting hosts and secreting toxins. In contrast, later larval stages of *E. orientalis* were subjected to high mortality rates when confronted with non-stung hosts. The secretions injected by an ovipositing female only contributed to a reduction in egg mortality, but did not seem to have any further influence either on the survival of the developing parasitoids once they had hatched, or on their mean developmental time (Doury et al., 1995). It may be caused by the behavioral but not physiological changes of parasitoid larvae.

Recently, during our studies on the braconid, *Spathius agrili* Yang, a solitary larval ectoparasitoid of *Agrilus planipennis* Fairmarire, we found the wasp females paralyzed host larvae before oviposition (Yang et al., 2005, 2008). Apparently, the amount of venom injected

inside the host's body was very small, but the efficiency was remarkable, rapid and permanent. Some remaining unrotten host larval tissues were often observed inside the galleries after the pupation of wasp progeny. Some other host larvae were envenomated without eggs, but the hosts were permanently paralyzed and lost the ability of feeding and never recovered again. The paralysis status usually lasted around one month before decomposition. Thus, isolation of these toxins would have promising utilization prospects.

4.2 Toxins from endoparasitoids

Most endoparasitoids are koinobiont, attacking exposed, active and immature host larvae. These parasites allow hosts to develop further for a period of time after laying eggs. Generally, host larvae are not killed until they complete their development and cocoon or build pupal cells in safe locations (Gauld and Bolton, 1988). Some authors suggested that koinobiont parasitoids should be evolved from idiobiont parasitoids, in which some species did not permanently paralyze hosts any longer, but subtly regulated the physiological activities and development of hosts (Vinson and Iwantsch, 1980a).

The offspring of many endoparasitoid develop fully only when the hosts build safe chambers for pupating (Gauld, 1984). They also inject toxins to make host larvae cocoon ahead of maturity (Shaw, 1983; Buhler et al., 1985). Although many endoparasitoids penetrate the ovipositors of the hosts only once, there is distinct evidence showing that venom can enter the body of host along with eggs (van Veen, 1981). Despite the seemingly inappreciable impacts of venoms from koinobiont parasitoids on hosts at the beginning, the following significances are remarkable (Vinson and Iwantsch, 1980a). Shaw (1981) found that the venoms from a koinobiont endoparasitic bracinid and a koinobiont ectoparasitic eulophid significantly inhibited the epidermal histolysis of host larvae when they exuviate. This might be an ecological adaptation for preventing being dropped along with host exuvia. Jones et al. (1986) found an egg-larval parasitic wasp *Chelonus* sp. secreted toxins into host eggs before oviposition, and subsequently caused the host larvae to metamorphose prematurely before the last larval stage. Strand et al. (1986) pointed out that the venom from *Telenomus* spp. contained an inhibitory factor arresting host embryogenesis. Some authors believed that some species in the superfamily of Ichneumonoidea injected a compound into the host larvae of the last instar, which stopped the prothoracic gland from producing ecdysone and thus prevented the hosts from pupating (Iwantsch and Smilowitz, 1975; Dover et al., 1987). The venom of *Chelonus inanitus* alone was ineffective in causing developmental arrest, and calyx fluid alone was effective only at high doses; but they together showed significantly synergistic effects. The SDS-gel electrophoretic analyses of calyx fluid and venom showed that both consist of a great number of polypeptides of various sizes (Soller and Lanzrein, 1996). As mentioned above, the early instar larvae of some parasitoids can also secrete toxins with various functions. Some are the same as adult toxins, paralyzing hosts and preventing putrescence, while others are used for the reduction of intraspecific competition through inhibiting the development of other conspecific individuals by toxins secreted by earlier hatched larvae. For instance, when an egg of *Campoletis sonorensis* deposited on the host larvae of *Heliothis virescens* hatched first, the other eggs would not hatch successfully (Hegazi and Vinson, 1998).

5 Mechanisms of toxins

5.1 Idiobiont parasitism

Toxins of idiobiont parasitoids often lead to arrested development, permanent paralysis and even death of hosts. Generally, these toxins act on the central nervous system (CNS) or neuro-muscular junction, and most of them are of broad spectrum (Piek, 1986). The direct envenomation by a predatory wasp, *Ampulex compressa*, into the ganglia of a cockroach's brain induced a long term paralysis of the prey through the actions of nervous toxins on the neuro-muscular junction (Haspel et al., 2003). The venom from the *Nasonia vitripennis* caused the localization of intracellular calcium release in the injured cells of hosts through the activation of phospholipase C (Rivers et al., 2005). Both female adults and newly hatched larvae of ectoparasitoid *E. orientalis* can produce venoms and permanently deactivate host *Callosobruchus maculatus* larvae. The two venoms have similar effects on host cell metabolism, exhibiting a block of protein synthesis. However, hosts are still alive evidently. Both of the two venoms contain phospholipase, whereas hyaluronidase only consisted in the venom of female adults (Periquet et al., 1997). Ectoparasitoid *N. vitripennis* regulated host cellular immune responses of *Sarcophaga bullata* by affecting host hemocytes. Hemocytes from envenomated hosts were altered in the nearly identical fashion to that observed for natural parasitism. The total number of circulating hemocytes declined sharply by 60 min post-envenomation, the number of plasmatocytes reduced but not granular cells, and the ability of plasmatocytes and granular cells to spread when cultured in vitro was abolished within 1 h. As with parasitized hosts, the decrease in plasmatocytes was due to cell death and inhibition of spreading. Isolated crude toxins also blocked the adhesion and spreading of these hemocyte types in vitro (Rivers et al., 2002). Thus, it appears that

maternally derived venom disrupts host immune responses almost immediately following oviposition and the inhibition is permanent.

5.2 Koinobiont parasitism

Koinobiont parasitoids can regulate host development by envenomating the hosts during oviposition. Subsequently, many studies confirm that their venoms contain a virus, called polydnavirus (PDV). The PDV exists in the calyx fluid of the oviduct of braconids and ichneumonids, with their genomes integrated with the genomes of parasitoids, and are injected into host larvae during parasitization. The PDV can regulate host development and influence host metamorphism through the inhibition of the host immune system for the successful growth of parasitoid progeny, e.g. delaying host pupation (Tanaka and Vinson, 1991b) and improving food consumption in host larvae (Nakamatsu et al., 2001). The injection of the combined venom and ovary extract from *Aphidius ervi* revealed an increase in the titre of various proteins, particularly in the 43–47 kD interval, in the haemolymph of host *Acyrthosiphon pisum* after 48 h following injection (Tremblay et al., 1998). The venom of *Catolaccus grandis* significantly affects the concentration and proportion of free amino acids in the haemolymph of the host *Anthonomus grandis* (Morales-Ramos et al., 1995). The venom of ectoparasitoid *Euplectrus separatae* increases the lipid content in the haemolymph of host *Pseudaletia separate* to satisfy more suitable nutritional requirements for parasitoid offspring (Nakamatsu and Tanaka, 2004).

The calyx fluid from *Cardiochiles nigriceps* requires the fluid from the venom gland and reservoir to inhibit the pupation of host *Heliothis virescens*. The pupation of all hosts injected with *C. nigriceps* calyx fluid mixed with *C. nigripes* venom was either inhibited or became larval-pupae intermediates. In contrast, the calyx fluid of *C. nigriceps* mixed with the venom of either *Microplitis croceipes* or *Campoletis sonorensis* was unable to inhibit host pupation, although all the three species successfully parasitize the same host. The synergistic component of *C. nigriceps* venom was found to be a 66-kD protein (Tanaka and Vinson, 1991b). Thus, the relationship between PDVs and parasitoids is a mutualism at the molecular level. Fleming (1992) has reviewed the studies on PDVs in detail. Polydnaviruses are nonpathogenic themselves, and usually are commensal or mutualistic with parasitoids (Renault et al., 2005). Toxins may only cause host paralysis for successful oviposition, but some studies reveal the significant synergistic actions of toxins on PDVs (Soller and Lanzrein, 1996). The venom from *Microplitis demolitor* does not affect host development, but appears to synergize the activity of calyx fluid (Strand and Dover, 1991). The requirement for venom in parasitism may differ between host species, and that dosage plays an important role in interpreting the interaction between calyx and venom components.

In fact, a large number of undoubted researches indicate that toxins from many koinobiont parasitoids restrain the immunity of host hemocytes. *Eulophus pennicornis* can regulate the haemolymph ecdysteroid titre of host *Lacanobia oleracea* larvae and thus affect host development (Weaver et al., 1997). Marris et al. (2001) found that attacks by the ectoparasitoid *E. pennicornis* prevented the larvae of *L. oleracea* from moulting. *E. pennicornis* arrested host development through indirect effects on their hosts' prothoracic glands (PGs), and the interruption of ecdysone production. The PDV and venom of *Chelonus inanitus* affect the endocrine system of the host, *Spodoptera littoralis*, by inhibiting the activity of the prothoracic gland and arresting host larval development (Lanzrein et al., 1998). Studies on *E. pennicornis* and its host *L. oleracea* showed that the hemocytes of parasitized larvae were evidently damaged and disintegrated, but wasp venom itself had no significant effect on host larval hemocytes. The *L. oleracea* larva has five main hemocyte types, namely, plasmatocytes, granular cells, spherule cells, oenocytoids and pro-hemocytes, representing 56%, 30%, 10%, 2% and 2% of the population, respectively. Parasitization by *E. pennicornis* resulted in an increase in the number of circulating hemocytes up to the third day, followed by a decrease towards the eighth day, along with changes to the morphology and viability of the cells. On the ninth day, extensive hemocyte damage and disintegration was evident. These changes were not observed when larvae were injected with *E. pennicornis* venom, or when hemocytes were exposed directly to venom in vitro (Richards and Edwards, 1999). Richards and Edwards (2000) further confirmed that the suppression of host hemocyte-mediated recognition and phagocytosis was not a secondary effect of nutritional deprivation and was not due to venom components, but rather it was a direct result of the parasitization of *L. oleracea* by *E. pennicornis*.

The activation of the prothoracic gland of the host *Pseudaletia separata* is apparently disturbed and suppressed by the calyx and venom fluids of parasitic wasp *Apanteles kariyai* (Tanaka, 1987). The braconid *Cardiochiles nigriceps* successfully parasitizes the larva of the host *Heliothis virescens* by inhibiting host pupation. The calyx and venom of wasp fluids are directly able to depress the function of the prothoracic gland of the host and the competence of the prothoracic gland to respond to prothoracicotropic hormone (PTTH), resulting in the reduction of the release of ecdysteroid (Tanaka and Vinson, 1991a). Endoparasitoid *Cotesia kariyai* regulates the development and metabolic efficiency of host *P. separate* via toxins and PDVs (Nakamatsu et al., 2001). *Chelonus* sp. near *curvimaculatus* parasitized the egg of host *Trichoplusia ni*. *Chelonus*

venom proteins are very stable in the host egg during the first two days of egg development. Then, on the last day before hatching, they are rapidly degraded by the proteolytic enzymes and contribute to the regulation of host growth (Leluk and Jones, 1989). The injection of the venom from ectoparasitoid *Euplectrus* sp. near *plathypenae* increases the lipid and protein content in host *P. separata* hemolymph to support the growth and development of the offspring larvae of the wasp (Nakamatsu and Tanaka, 2003).

6 Utilization prospects of insect toxins

Insect toxins can cause pain and local damage in large vertebrates, which may be lethal on small animals, including insects. Hymenoptera toxins act on the central nervous system (CNS) as most of animal-origin toxins. The studied toxins from solitary hymenopterans are much less than those from social insects. The efficiency of toxins from idiobiont parasitoids is terribly amazing, and tiny doses often lead to permanent paralysis and even death of hosts. Furthermore, paralyzed or dead hosts' bodies can stay fresh for a long time (more than ten days to one month) without rotting even under natural conditions. Therefore, the utilization prospects of these toxins are very promising when isolated.

In general, koinobiont parasitoids paralyze free-living hosts and they are not, or only very temporarily, paralyzed, but the toxins act very rapidly. Hosts are often paralyzed immediately after being stung, but come to develop continuously several minutes to half an hour later. However, idiobiont parasitoids often result in a complete and permanent or long-term paralysis of hosts, but with a delay of about 10 min to two days (Piek, 1986). Because the toxins from idiobiont parasitoids can make hosts stop feeding, these toxins are more practical for the development of biological insecticides.

6.1 Application on medicines

As is known, toxins from hymenopterans can cure human beings of some diseases. The Chinese used bee-sting to heal rheumatoid arthritis a hundred years ago. Cantharis toxins are commonly used to cure cancers in clinics (Ke, 1988). Proteins are the major active ingredients of insect toxins, including various sizes of polypeptides, enzymes, amines (histamine, tryptamine, acetylcholine) and some other substances such as aldehydes and benzoquinones. Currently, most investigations on insect toxins focus on the application in human medicines (Kawamoto and Kumada, 1984; Piek, 1986), in which bee toxins are studied in the most detail (Cherubini et al., 1987). Now, the bee venom is known for its curative effects on many human diseases, including rheumatism, cardiovascular diseases, asthma, arthritis and arrhythmia, etc (Fang et al., 1988). Isolation of allergens in insect venoms and research on their properties are the fundamentals for healing human anaphylaxis and poisoning. However, this is not the emphasis of this study, so unnecessary details are not given here.

6.2 Development of bio-insecticides

Actually, toxins from hymenopterans can also be used as specific insecticides (Wang and Yang, 2004). As mentioned above, toxins are so effective and apparently harmless to natural enemies, because the offspring larvae of parasitoids can consume the paralyzed hosts by toxins without poisoning. If these active components are isolated and purified respectively, some clues may be discovered for the development of novel insecticides (Piek and Sommeijer, 1991). Contrary to the typical action of most animal venoms, both social wasp venoms and solitary wasp venoms seem to paralyze the prey. This suggests a specific action on parts of the nervous system. However, the solitary wasp venoms contain many agonists and antagonists of synaptic transmission, and their paralyzing action is very complicated. Besides a contribution of agonists to the initiation and enhancement of the action of antagonists, one of the venoms contains two antagonists with different actions, and one of these antagonists enhances the effect of the others. These compounds may be used to develop pesticide synergists with high efficiency (Piek, 1987).

Parkinson et al. (2002b) have detected a paralytic factor in the venom from wasp, *Pimpla hypochondriaca*. This factor was further purified and called pimplin, with a molecular mass of approximately 22 kD. Pimplin was effective against the fly *Musca domestica*. A 40 ng dose of pimplin administered to adult *M. domestica* by intrahaemocoelic injection was sufficient to kill all the flies tested. Quistad et al. (1988) determined the injection toxicities of toxins from some hymenopterans such as bees, vespids and bumblebees, on the larvae of *Manduca sexta*. Results showed that the bee venom was the most toxic, with the major active ingredients as polypeptides, phosphatidases and amines. Rivers et al. (1993) examined the bioassay of venom from *Nasonia vitripennis* on host *Sarcophaga bullata* and several non-host insects. The venom was found to be very effective on natural hosts, as well as some non-host insects such as *Trichoplusia ni*. Venom from ectoparasitoid *N. vitripennis* suppressed development in *Sarcophaga bullata*, *Phormia regina* and *Sarcodexia sternodontus*, but *Musca domestica* died quickly in response to envenomation (Rivers and Denlinger, 1995). These toxins show promising developmental prospects as biological insecticides with high efficacy.

7 Discussion

At present, researches on the biochemistry of insect toxins from parasitic hymenopterans are still in a very early stage. As a branch of entomology or biochemistry, very few studies about insect toxins have been reported in China, especially on toxins of those solitary hymenopterans. In fact, more toxins should be found by entomologists first, because entomologists often observe insect habits, and are familiar with the phenomena of parasitization and predation. A prerequisite to studies on the nature of the toxins is the development of methods for their collection. It is not easy to collect insect toxins because of the tiny content. The earliest collection of toxins is performed by means of mechanical stimulation, also called the "milking" method. A wasp is held between two fingers with forceps and the clear drop of venom that appears at the tip of the extended sting is collected. Venoms produced in this way are highly pure, but this method is not suitable for wasps of small sizes. Another mass collection for venom is the electrical stimulation method, which uses electric shocks to make wasp adults sting and eject venoms. This method will not hurt wasps severely, but is mostly apt for the venom collection of social wasps, especially honey-bees. To the solitary wasps, toxins usually are collected with extraction methods, i.e. extracting toxins through the homogenization of batches of whole insects or venom apparatus. Although this method is easy and convenient, the toxins are contaminated with intestinal tract or materials from other tissues. Moreover, it is not certain that the venom produced by wasps when they sting normally is completely identical with the contents of the venom reservoirs (Piek, 1986).

However, investigations on the toxins cannot only be limited to autecology and population ecology, it also should be further studied in microcosmic aspects. Most insect toxins are complex mixtures, and the contents of active components usually are very little. Thus, the isolation and purification of every active ingredient of insect toxins are necessary in the research of biochemistry and toxicology, which is the foundation for application.

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