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Orijinal araştırma (Original article)

New records of canthariphily among beetles (Coleoptera) from Iran

Iran ISLAMI[1] Mahmood Reza NIKBAKHTZADEH[1*]

Summary

Four coleopteran species of *Cantharis livida* Linnaeus, 1758 (Cantharidae), *Gastrophysa polygoni* Linnaeus, 1758 (Chrysomelidae), *Malachius bipustulatus* Linnaeus, 1758 (Melyridae) and *Certallum ebulinum* (Linnaeus, 1767) (Cerambycidae) from different regions of Iran exhibited an obvious resemblance to the predominant meloid species occurring where they were collected. Tiny amounts of cantharidin- far below the average content for meloids was detected in them using GC-MS analysis. Because of the evident similarity (approximate size, general colouration, elytral pattern, habitat and seasonal activity) between each of these cantharidin-bearing mimics and their meloid models, it is assumed that a Müllerian mimicry exists reciprocally in which some non-producer beetles obtain cantharidin from external sources to be chemically protected against their natural enemies and enhance their natural fitness.

Key words: Coleoptera, Meloidae, cantharidin, canthariphily, pharmacophagy, mimicry

Anahtar sözcükler: Coleoptera, Meloidae, kantharidin, kantharifil, pharmacophagy, mimikri

Introduction

Cantharidin is highly toxic to a wide variety of animals, including birds, amphibians and mammals (Dettner, 1997). This compound has been known to humans for about 2000 years due to its physiological activities such as blistering (McCormick & Carrel, 1987; Wang, 1989; Juanjie et al., 1995; Pemberton, 1999). Autogenous producers of cantharidin occur exclusively (Dettner, 1997; Hemp et al., 1999 a; b) within the coleopteran families of Meloidae (Dixon et al., 1963; Capinera et al., 1985; Blodgett et al., 1991; Carrel et al., 1993) and Oedemeridae (Carrel et al., 1986; Nicholls et al., 1990; Holz et al., 1994; Frenzel & Dettner, 1994) in which it occurs in the haemolymph and other tissues to chemically protect them and their immature stages (Carrel & Eisner, 1974). A considerable number of insects (mostly Coleoptera and Diptera), so-called canthariphilous

[1] Department of Medical Parasitology & Entomology, College of Medical Sciences Tarbiat Modares University, P.O. Box 14115-331, Tehran, Iran
* Sorumlu yazar (Corresponding author) e-mail: nikbakht_m@excite.com
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(unable to synthesize cantharidin *de novo*), are attracted towards the natural sources of cantharidin or even the synthetic compound (Holz et al, 1994; Eisner et al., 1996a; Frank & Dettner, 2001). They feed on it without any obvious ill-effects (Hemp & Dettner, 2001) and presumably utilize the compound against their natural enemies (Dettner, 1997). A few studies have by-far quantified cantharidin of the canthariphilous insects (Frenzel & Dettner, 1994; Holz et al., 1994; Eisner et al., 1996 a;b), whereas most reports have been concentrated on the identification, distribution, morphology and behaviour of this group of insects. Six families of beetles are known to be canthariphilous (Hemp & Dettner, 2001).

In this study four beetles from the families Cerambycidae, Melyridae, Chrysomelidae and Cantharidae, were analyzed for cantharidin. They were quantitatively compared to co-occurring meloid producers in order to present more detailed evidence relating to their canthariphily. There was obvious mimicry occurring between the cantharidin producer and the canthariphilous beetle of each locality.

Material and Methods

Field collection

Meloid and non-meloid coleopterans co-occurring on wild flowers (mostly Family Astraceae) of the same natural habitat were manually collected. In this way, 15 specimens of *Cantharis livida* Linnaeus, 1758 (Coleoptera: Cantharidae), 7 specimens of *Gastrophysa polygoni* Linnaeus, 1758 (Coleoptera: Chrysomelidae), 26 specimens of *Malachius bipustulatus* Linnaeus, 1758 (Coleoptera: Melyridae) and 5 specimens of *Certallum ebulinum* (Linnaeus, 1767) (Coleoptera: Cerambycidae), were respectively collected from southern, central and western Iran (Table 1). Data concerning the collected species of meloid beetles in each locality are also summarized in Table 2.

Table 1. Non-meloid species and their collecting data

Species	Collecting locality	Reference grid	Collecting date	Number of specimens
CL [1]	Hamedan Province (Western Iran)	N' 44° 34 E' 31° 48	May 2006	15
GP [2]	Fars Province (Southern Iran)	N' 36° 29 E' 39° 51	June 2006	7
MB [3]	Isfahan Province (Central Iran)	N' 02° 34 E' 03° 51	April 2007	26
CE [4]	Chaharmahal Province (Central Iran)	N' 19° 32 E' 51° 50	June 2006	5

[1] *Cantharis livida* Linnaeus, 1758 (Coleoptera: Cantharidae);
[2] *Gastrophysa polygoni* Linnaeus, 1758 (Coleoptera: Chrysomelidae);
[3] *Malachius bipustulatus* Linnaeus, 1758 (Coleoptera: Melyridae);
[4] *Certallum ebulinum* (Linnaeus, 1767) (Coleoptera: Cerambycidae).

Table 2. Predominant species of meloid beetles (Coleoptera: Meloidae), co-occurred with the collected non-meliod species

Species	Collecting locality	Reference grid	Collecting date	Number of specimens
C G [1]	Hamedan Province (Western Iran)	N' 44° 34 E' 31° 48	May 2006	12
AS [2]	Fars Province (Southern Iran)	N' 36° 29 E' 39° 51	June 2006	18
AS [2]	Isfahan Province (Central Iran)	N' 02° 34 E' 03° 51	April 2007	21
MI [3]	Chaharmahal Province (Central Iran)	N' 19° 32 E' 51° 50	June 2006	17

[1] *Croscherichia goryi* (Marseul, 1870);
[2] *Alosimus smyrnensis* (Maran, 1942);
[3] *Muzimes iranicus* (Maran, 1942).

Specimens were stored in cold boxes in the field and later on transferred to the laboratory where they were kept frozen at -30°C. Voucher specimens are deposited in the laboratory of Medical Entomology at Tarbiat Modares University, Tehran, Iran.

Extraction and chemical analysis

Freeze-dried specimens were placed in silanized test tubes (6 mm ID). Specimens were treated with 100-200 microliter of 6 N HCl (Extra Pure, Merck, Germany) and stored at 120°C for 4 hours in order to liberate the bound cantharidin from the whole body tissues. An equal amount of chloroform (Extra Pure, Merck, Darmstadt, Germany) was added to the hydrolysed specimens right after cooling. Samples were agitated on a mixer for one minute and subsequently centrifuged for 5 minutes at 3000 r/m to split the organic and mineral phases. The lower organic phase was filtered and transferred into the silanized glass vials and little amount of deionised water was added to avoid evaporation.

Quantitative analysis and verification of trace detection

One microlitre of each extract was injected into a GC-MS Varian 3800 instrument, equipped with a CP-Sil 8cb (5 % Phenyl and 95 % dimethyl polysiloxane, non-polar) bounded phase fused silica capillary column (25 m, 32 mm ID, 0.25 µm FT). Helium was used as a carrier gas at 2 mm/min velocity. Injector and detector temperature were set at 250 and 300°C respectively. The temperature programme was increased from 60°C to 120°C at 10°C/min and then maintained at 120°C for 5 min. Thereafter, the temperature was raised at 10°C/min to 275°C, maintained at 275°C for 5 min. The detector was offset 5.00 min; mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 scan/sec from *m/z* 50–250. Cantharidin, recognisable by characteristic MS fragments of *m/z* 128, 96 and 67, eluted after 18.50 min. Autointegration was achieved by Saturn® Workstation package, Saturn view™ version 5.2.1, 1989-1998, Varian

Associates, Inc. Authentic cantharidin (purity 98%, SIGMA, Steinham, Germany) was used as external standard. Vials of insect extracts and external standards were vigorously shaken before any injection. Chloroform blanks were injected between all samples, but an extract from *Tenebrio molitor* Linneaus, 1758 (Coleoptera: Tenebrionidae), prepared in the same manner as the other beetle samples, was additionally injected between runs of non-meloid beetles as the negative control.

Statistical analysis

Cantharidin mass in each sample was calculated using a standard regression curve, calculated using seven 1 µl injections of ascending concentrations of authentic cantharidin from 5-5000 ng/µl. The repeatability of chemical experiments was tested over 4 samples and each cantharidin-bearing specimen was compared with its co-occurred meloid species (model) of the same habitat by the two-tailed Mann-Whitney test in 95% confidence level. All statistical analyses were carried out using SPSS statistical package ver.11.5.0, USA.

Results and Discussion

Quantitative GC-MS analyses of the whole body extracts demonstrated the presence of a very small amount of cantharidin in analysed non-meloid samples which was usually lower than 3 µg/beetle, except for *C. livida* with a mean value of 4.92 µg (Table 3). Thus *C. livida*, *G. polygoni*, *M. bipustulatus* and *C. ebulinum* are hereby reported as cantharidin-bearing species. However, comparison of the concentration of cantharidin in these beetles with those of both sexes of the meloid producers at the same collecting locations (Table 3) indicated that they possessed significantly less cantharidin (Mann-Whitney test, $P < 0.05$, $n = 4$, see Fig. 1). No cantharidin was found in the alternate runs of *T. molitor*, which used as negative control.

Table 3. The detected volume of total cantharidin in studied meloid and non-meloid beetles[1]

Species	Cantharidin titre[2] (Mean ± SD)
C. livida (np)[3]	4.92 ± 0.56
C. goryi (p)[4]	0.18 ± 0.11 ♂
	0.11 ± 0.03 ♀
G. polygoni (np)	0.7 ± 0.56
M. bipustulatus (np)	1.88 ± 0.32
A. smyrnensis (p)	2.03 ± 2.07 ♂
	0.52 ± 0.29 ♀
C. ebulinum (np)	2.74 ± 0.13
M. iranicus (p)	5.21 ± 3.75 ♂
	4.52 ± 2.52 ♀

[1] Both sexes were analysed for meloid specimens, but since no difference exists between the cantharidin titre of the two sexes of non-producers, they were not accordingly separated.
[2] Total cantharidin (µg/beetle), n= 4.
[3] (np)= non-producer of cantharidin.
[4] (p)= cantharidin producer.

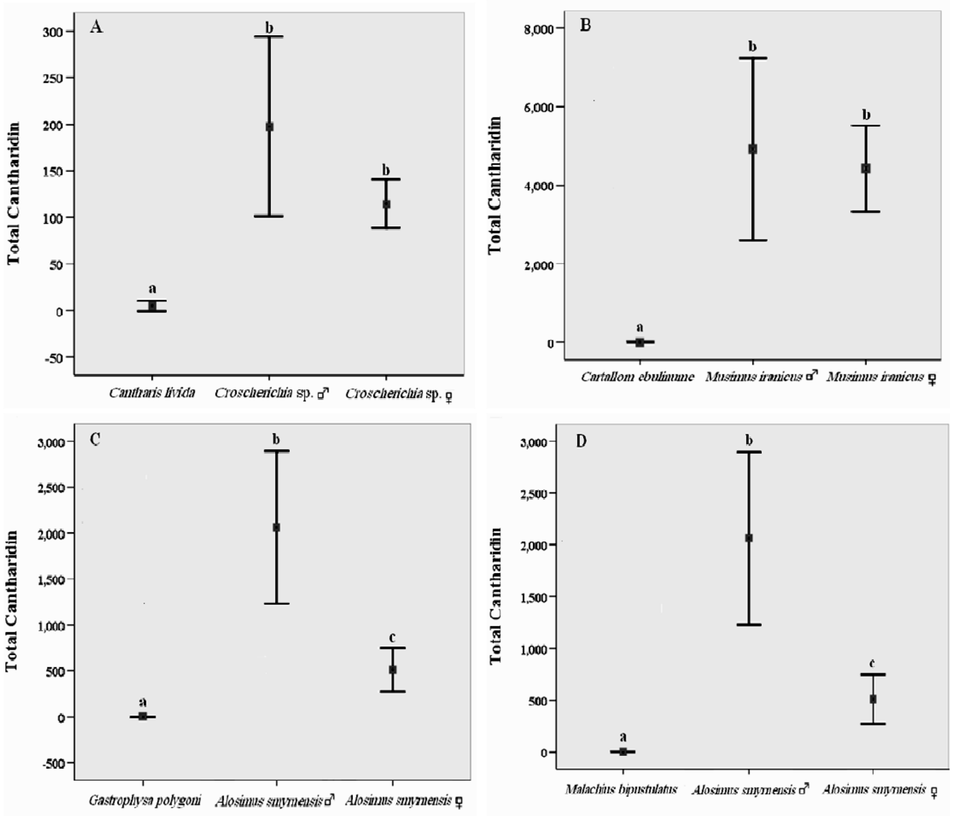

Figure 1. Comparison of average contents (mean ± SD, n= 4) of cantharidin (µg/beetle) between A: *Cantharis livida* (Coleoptera: Cantharidae) & *Croscherichia goryi* (Coleoptera: Meloidae), B: *Certallum ebulinum* (Coleoptera: Cerambycidae) *& Muzimes iranicus* (Coleoptera: Meloidae), C: *Gastrophysa polygoni* (Coleoptera: Chrysomelidae) & *Alosimus smyrnensis* (Coleoptera: Meloidae), D: *Malachius bipustulatus* (Coleoptera: Melyridae) & *Alosimus smyrnensis* (Coleoptera: Meloidae), Mann-Whitney test, P= 0.029, d_f = 1. Comparisons were made with the both sexes of meloid species. Rectangles with the same letter are not statistically different from each other.

Experiments on the non-meloid species were not arranged on a sex-discriminative basis, since previous studies indicated no statistically significant difference between total cantharidin content of sexes (Frenzel & Dettner, 1994; Holz et al., 1994).

Canthariphilous taxa which are unable to synthesize cantharidin *de novo* (Dettner, 1997), obtain it from an external source. Thus their total volume of accumulated compound is considerably lower than any meloid species (Schütz & Dettner, 1992; Nikbakhtzadeh & Tirgari, 2002). The maximum cantharidin titre of the collected non-meloid species of this study is 100-1000-fold lower than that of either sex of the studied meloids (Figure 1). It was similarly indicated that the cantharidin volume in the canthariphile pyrochroid *Schizotus pectinicornis* (Linneaus, 1758) (Coleoptera: Pyrochroidae) was 30-fold lower than in the oedemerid producer, *Oedemera femorata* (Scopoli, 1763) (Coleoptera: Oedemeridae) (Holz et al., 1994). The highest volume of cantharidin in a canthariphile Diptera (1.5 µg) was also recorded for the anthomyiid *Anthomyia pluvialis* (Linneaus, 1758) (Diptera: Anthomyiidae) which is several to thousand folds less than any studied producer (Frenzel & Dettner, 1994). In our work not more than 3 µg of cantharidin could be found in each specimen of *C. ebulinum*, *G. polygoni* and *M. bipustulatus*, but content for the cantharid *C. livida* was measured up to 5.47 µg/beetle. This is the highest content of total cantharidin which is so-far recorded in a canthariphile (consumer) species. The family Chrysomelidae contains two canthariphilous species, *Aristobrotica angulicollis* (Erichson) (Chrysomelidae: Galerucinae) (Mafra-Neto & Jolivet, 1994) and *Oulema melanopus* (Linnaeus 1758) (Nikbakhtzadeh & Tirgari, 2002). A population of *A. angulicollis* was discovered in Brazil, feeding on an adult meloid, *Epicauta aterrima* Klug, 1825 (Mafra-Neto & Jolivet, 1994). Cantharidin is also found in *O. melanopus* which co-occurrs with the meloid, *Alosimus smyrnensis* (Maran, 1942) (Nikbakhtzadeh & Tirgari, 2002). This work introduces *G. polygoni*, the third canthariphilous chrysomelid.

In this work we report the first confirmed record of canthariphily in Family Cantharidae which was observed in *C. livida*. It showed a clear similarity to the meloid, *Cantharis goryi* (Marseul, 1870). We also record the only canthariphile species within the Family Melyridae, *M. bipustulatus* which bears a small amount of cantharidin.

Cantharidin was previously known from a single species of cerambycid, *Phymatodes testaceus* (Linnaeus 1758) of western Iran (Nikbakhtzadeh & Tirgari, 2002), which was co-occurs with *A. smyrnensis*. *Certallum ebulinum* is the second species in Family Cerambycidae in which cantharidin has been so-far detected and this time shows an obvious pattern of mimicry to its meloid model, *Muzimes iranicus* (Maran 1942) (Coleoptera: Meloidae).

Both *M. bipustulatus* and *G. polygoni* were similar to *A. smyrnensis* in size, general and elytral colouration, habitat and seasonal activity. We assume that the Müllerian mimicry phenomenon may explain such similar characteristics between each of the canthariphile mimic species and the mentioned meloid models in which these non-producing species deceive the enemies by not only apparent similarity to an unpalatable species, but by up-taking the chemical defence secretion, cantharidin. It is a keystone towards a higher natural fitness. We exactly do not know how these cantharidin-consumers obtain their cantharidin loads, but it seems very probable that they supply it by feeding on their co-occurred meloid species. Our hypothesis is supported first by the fact that no plant source is known for cantharidin (Dettner, 1997) and secondly by the attraction of some other canthariphile insects to the living adult blister beetles for the purpose of feeding (Pinto, 1978; LeSage & Bousquet, 1983; Butler, 1984; Williams & Young, 1999; Hemp & Dettner, 2000).

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Özet

İran'da Coleoptera takımına bağlı kantharifil böceklere ait yeni kayıtlar

İran'ın farklı bölgelerindeki Coleoptera takımına bağlı dört tür; *Cantharis livida* Linnaeus, 1758 (Cantharidae), *Gastrophysa polygoni* Linnaeus, 1758 (Chrysomelidae), *Malachius bipustulatus* Linnaeus, 1758 (Melyridae) ve *Certallum ebulinum* (Linnaeus, 1767) (Cerambycidae), toplandıkları yerlerdeki baskın Meloid türlerine çok açık bir benzerlik göstermişlerdir. Küçük miktarlarda - Meloidler için ortalama değerin çok altındaki miktarlarda - cantharidin GC-MS analizi kullanılarak tespit edilmiştir. Kantharidin taşıyan taklitlerin her birinin, kendi meloid modelleri ile aralarındaki açık benzerlikten dolayı (yaklaşık büyüklük, genel renklenme, elytra yapısı, habitat ve mevsimsel aktivite), bu maddelerin üreticisi olmayan böceklerin, doğal düşmanlarına karşı kimyasal olarak korunmak ve kendi doğal sağlıklarını arttırmak için, dış kaynaklardan cantharidin elde ettiği birbirinin yerine geçen bir Müllerian taklitçiliğinin var olduğu kabul edilmektedir.

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