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Investigation of Abundance and Diversity of Cantharidin Compounds in the *Hycleus colligatus, Mylabris schrenki, Mylabris cincta,* and *Hycleus schah* Species from Kerman Province, Iran

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ABSTRACT

Cantharidin and cantharidin-related compounds (CRCs) are synthesized by Meloidae to defend against predators. Also, Cantharidin and CRCs are known as biopesticides. Because biopesticides are increasingly used to replace chemical pesticides in pest control, it is necessary to investigate the amount of cantharidin and CRCs (palasonin and palasoninimide) as known biopesticides. In this research, cantharidin content was determined in four species of Meloidae (*Hycleus colligatus, Mylabris schrenki, Mylabris cincta,* and *Hycleus schah*) which were abundant in Kerman province. Each species was divided into three groups including males, mated females, and virgin females. The results showed that *Hycleus colligatus* had the highest level of cantharidin (Mean± SE: 20.38± 0.49 µg/mg). The levels of cantharidin for *Mylabris schrenki*, *Mylabris cincta*, *Hycleus schah* were 12.44± 0.17, 5.24± 0.06, and 3.49± 0.03µg/mg, respectively. The level of cantharidin in mated females and virgin females in all species, had the highest and lowest levels, respectively. The results showed that only males can synthesize cantharidin and transfer it to females during mating. Mated females get this toxin from males during mating and use it for defending eggs from attacking predators and inducing feeding versions in predators exposed to the toxin. Also, in this research, palasonin and palasoninimide content were determined in these four species. Palasonin and palasoninimide were found in mated females but not in males indicating that CRCs do not have the same transfer pattern as cantharidin and thus, unlike cantharidin, could not have been transferred from males to females. The study showed that in mated females of four species, the percentage of cantharidin was more than palasonin and palasoninimide.

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Introduction

The Meloidae family with about 125 genera and 3000 species (Bologna & Pinto, 2002; Arnett, 2002), distributed in the temperate steppe, warm and dry regions, the sub-tropical and tropical savannas (Bologna & Pinto 2002). The Meloidae are one of the most interesting families in Coleoptera because of their biology and important toxins (Bologna & Pinto, 2002). Cantharidin, $C_{10}H_{12}O_4$, a monoterpene anhydride (Fig. 1), is a natural and high toxin that is produced by only Meloidae and Oedemeridae (Dettner, 1997). Cantharidin biosynthesis happens during the immature stages of both sexes in Meloid beetles. Adult females of Meloidae have cantharidin, but they cannot synthesize it, and this ability is lost in them (Sierra *et al*., 1976). They get this toxin from males during mating and use it for defending eggs from attacking predators and inducing feeding versions in predators exposed to the toxin (Sierra *et al*., 1976; Carrel & Eisner, 1974).

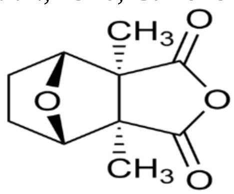

Fig. 1. Chemical structure of Cantharidin ($C_{10}H_{12}O_4$)

Cantharidin bleeds from the leg joints of Meloidae (reflexive bleeding) when they are aroused from other organisms (Carrel & Eisner, 1974). This compound can cause skin blisters when it contacts the skin of the body as its defensive system (Mizota, 2001; Arnet *et al*., 2002; Nikbakhtzadeh & Tirgari, 2008). In addition, Cantharidin is known as a biopesticide because of its high insecticidal activity (Wu *et al.*, 2015). However, the function of these biopesticides is to experiment on some pests such as *Plutella xylostella* (Lepidoptera: Plutellidae) (Zhiyong *et al*., 1998). It has low toxicities to vegetables, non-target organisms, earthworms, and soil microorganisms (Feng, 2007). Some compounds that are similar to cantharidin, known as cantharidin-related compounds (CRCs), have been discovered in Meloid beetles. The synthesis and function of CRCs are not clarified yet. Cantharidin-related compounds, such as cantharidin, are toxin but their toxicity and volume are much less than cantharidin. It has been observed that they are only in mated females. Males do not produce these compounds (Nikbakhtzadeh and Tirgari, 2002; Nikbakhtzadeh *et al*., 2012). Palasonin (demethylcantharidin) ($C_9H_{10}O_4$), which lacks one of the angular methyl groups of cantharidin.

Palasonin is the first cantharidin-related compound that has been discovered from blister beetles. It was reported for the first time in *Hycleus lunatus* (Meloidae) from Southern Africa by Dettner *et al*. (1997). After that, Nikbakhtzadeh *et al*. (2002) reported palasonin in *Cynaeolytta sp* from Nairobi`s suburbs in East Africa and *Hycleus polymorphus* and *Mylabris quadripunctata* from Southern Africa. Then Mebs *et al*. (2009) detected palasonin in two other species of Meloidae (*Hycleus tinctus* and *H. oculatus*) in Southern Africa. The second CRCs, palasoninimide (C9H10O3N) was reported in *Hycleus lunatus* from Southern Africa by Dettner *et al*. (1997).

Palasonin was isolated in 1967 from seeds of Butea monosperma and has good repellent activity against insects (Fietz *et al*., 2002; Nikbakhtzadeh *et al*., 2012). In terms of chemical structure, palasonin and palasoninimide are similar to Cantharidin, a natural toxin produced by Meloidae insects. Cantharidin and CRCs have been reported to work by inhibiting serine/threonine protein phosphatases (PSPs). They are known as biopesticides and have been widely studied and found to have the toxicity to control many important pests.

All over the world, agriculture is always adversely affected by pests (insects, weeds, and plant pathogens), which cause up to 45% of crops to be lost annually. Application of pesticides is therefore a common and critical method to improve crop yield. The chemical amounts of pesticides used persist and accumulate in the environment (more than 99.7%). These pesticides undergo various processes *e.g.*, catabolism, physicochemical, and biodegradation, which are closely related to the composition and activity of soil microbial community. Considering the deleterious effects of chemical pesticides, and biopesticides, with a new mode of action, higher effectiveness and eco-friendliness should be individually, sequentially, and simultaneously applied to maintain crop production. Since biopesticides are increasingly used to replace synthetic pesticides in pest control, it is necessary to investigate the amount of cantharidin and CRCs (palasonin and palasoninimide) as known biopesticides. In this research, the cantharidin and two Cantharidin-Related Compounds' content were investigated in four abundant blister beetle species from Kerman province and the difference in the amount of cantharidin was compared.

Materials and Methods

Materials

The chemical materials used in this research including HCl 37%, Chloroform, Dimethyldichlorosilane, and Cantharidin (purity 98%) were purchased from MERCK, Germany, and Sigma, Germany.

Beetle collection

Adults of four species of Meloidae (*Hycleus colligatus, Mylabris schrenki, Mylabris cincta,* and *Hycleus schah*) (Fig. 2) were collected by sweep net and hand on their various host plants from different regions of Kerman province, Iran (Nezhad-Ghaderi *et al.,* 2021) (Table 1).

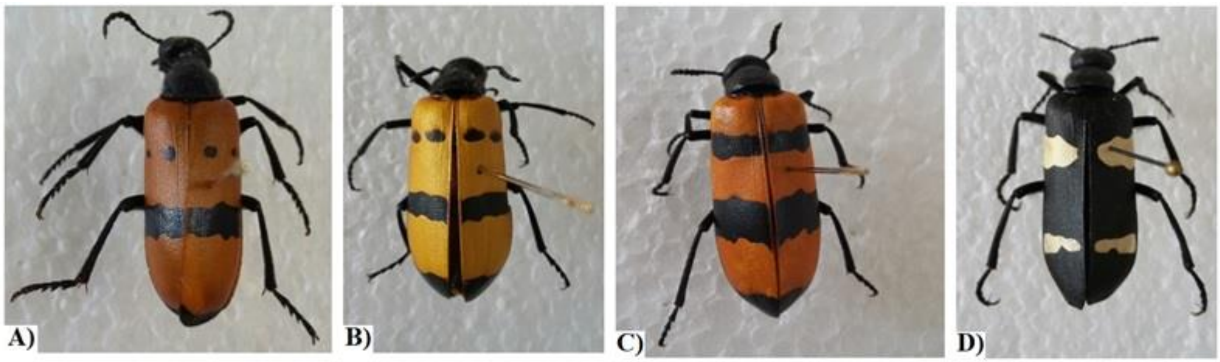

Fig. 2. Four species of Meloidae: A) *Hycleus colligatus*; B) *Mylabris schrenki*; C) *Mylabris cincta*; D) *Hycleus schah*.

Table 1. Sampling regions, host plants and the latitude & longitude of four studied species

Species (Meloidae)	Sampling regions	Host plants	Latitude & longitude
Hycleus colligatus	Kerman	Asteraceae,*Matricaria chamomi*	30°16'51.7"N 57°03'58.5"E
Mylabris schrenki	Rafsanjan	Malvaceae, *Alcea*	30°03'46.9"N 57°06'21.2"E
Mylabris cincta	Zarand	Asteraceae,*Cynara scolymus*	30°16'24.5"N 57°05'10.2"E
Hycleus schah	Bardsir	Asteraceae, *Anthemis*	30°03'05.6"N 57°06'45.4"E

Then they were placed in the small cool box. Specimens were transported alive to the biochemistry laboratory and maintained in the freezer at -20 °C. The beetles were identified using a key of the old-world genera of Meloidae. Subsequently, selected species were identified and confirmed at the species level by Professor Marco A. Bologna at the University of Rome, Italy. The external genitalia of females were additionally examined for fresh spermatophores, which indicate recent copulation. Also, the virginity of specimens was diagnosed by investigation of the internal reproductive system. The virgin and mated females were diagnosed with the existence of eggs in their bodies. The mated females had some eggs in their bodies. Then the specimens were divided into three groups: males, mated females, and virgin females.

Extraction

Cantharidin content was determined from four species of Meloidae. Each species is divided into three groups (male, mated females, and virgin females). Five repetition**s** were considered for each group of four species. For this purpose, specimens were dried at -55°C and 9× 10^{-2} mbr pressure using a Freeze dryer (CHRIST, ALPHA 1-2 LD, Germany) for 24 hours, and dry weight (DW) was measured for all beetles. Then, they were crushed to powder in a test tube for 20 minutes. Whole dried and powdered bodies were hydrolyzed in small test tubes with 600 µl of 6N, HCl. Then, test tubes in the oven at 120°C for 4 hours. An equivalent volume of chloroform was added and each sample was vigorously shaken on a vortex mixer for 60 seconds. Next, all of the samples were placed in the Centrifuge machine and centrifuged at 3000 rpm for 5 minutes. The organic phase was removed and transferred into a new test tube (Holz *et al*., 1994; Mebs *et al*., 2009).

All test glassware tubes and vials were silanized for 24 hours with dimethyldichlorosilane solution in chloroform 5%. (Silanization is the attachment of an organosilyl group to some chemical species. Almost always, silanization is the conversion of a silanol-terminated surface to an alkylsiloxy-terminated surface. This conversion confers hydrophobicity to a previously hydrophilic surface. This process is often used to modify the surface properties of glass, silicon, alumina, quartz, and metal oxide substrates, which all have an abundance of hydroxyl groups).

Cantharidin Standards

Authentic cantharidin was used as an external standard. Five ascending concentrations of cantharidin (50, 200, 500, 800, and 1000 ng/µl) were used to create a correlation cure (Y= 26436x+ 418880, R^2= 0.9857) for cantharidin quantification (Fig. 3).

Quantitative gas chromatography-mass spectrometry

Cantharidin and CRCs (palasonin and palasoninimide) of each sample were measured by quantitative GC-MS (Gas Chromatography-Mass). One microliter of each extract was injected in non-split mode into an Agilent 6890 N GC instrument, connected to a 5973 N mass detector (Agilent Technologies, USA, 2004). Regular negative controls (chloroform blanks) were injected between runs to ensure that none of the detected records was due to prior contamination. Vials of insect extracts and external standards were vortexed for 30 seconds before any injection. The gas chromatograph was equipped with an HP5 (5% phenyl and 95% methylsiloxane, non-polar) bonded-phase fused-silica capillary column (60 m, 0.25 mm ID, 0.25 µm FT). Helium was used as the carrier gas at 2 mm/min velocity. The injector, ion source, and transfer line temperatures were set at 230, 150, and 275°C, respectively. A post-run, during which the temperature was increased to and maintained at 280°C for five minutes, was used to clean the injector, inlet, and column from any non-degraded particle. The detector was set for 5.00 min, mass range from 10 to 650 amu, and mass spectra were taken at 70 eV (in EI mode). The electron Impact Ionization (EI 70 ev) provided mass spectra with a characteristic fragmentation of cantharidin, palasonin, and palasoninimide. The total mass Spectra analyzed by MSD chemstation software and base peaks were compared by the NIST MS Library V.2.0.1 of mass spectral data bank.

Statistical Analyses

The data of cantharidin were analyzed by considering variance (two-way ANOVA; sex and species) for cantharidin (dry-weight), and mg cantharidin per beetle. Significant means were separated with the student's t-test at the P= 0.05 level. All accomplished analyses were done with SPSS ver. 24.00 (USA).

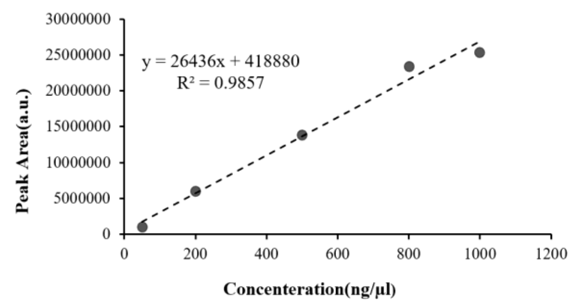

Fig. 3. Linear regression Cantharidin standards.

Results

Cantharidin

Cantharidin content was measured and compared in three groups including males, mated females, and virgin females of four species of Meloidae (*Hycleus colligatus*, *Mylabris schrenki*, *Mylabris cincta*, and *Hycleus schah*) that are abundant in Kerman province (Table 2).

Table 2. Titre of cantharidin (µg/mg) in four species of Meloidae according to sex in Kerman proviance, Iran

Meloidae spp.	n	Mean	SE	Mean±SE	SD	Range	Variance
Hycleus colligatus(Male)	5	19.202612	0.3321993	19.20±0.33	0.7428203	18.23-20.16	0.552
Hycleus colligatus(Mated female)	5	20.382000	0.342296	20.38±0.49	0.7513561	20.24-20.53	0.561
Hycleus colligatus(Virgin female)	5	15.675752	0.3298150	15.67±0.32	0.7374887	14.71-16.63	0.544
Mylabris schrenki (Male)	5	11.522537	0.1743558	11.52±0.17	0.3898713	11.03-12.06	0.152
Mylabris schrenki (Mated female)	5	12.446820	0.1732665	12.44±0.17	0.3874252	12.35-12.55	0.154
Mylabris schrenki (Virgin female)	5	7.990377	0.1769297	7.99±0.17	0.3956268	7.50-8.54	0.157
Mylabris cincta (Male)	5	4.947266	0.0620027	4.94±0.06	0.1386423	4.75-5.11	0.019
Mylabris cincta (Mated female)	5	5.241680	0.0675690	5.24±0.06	0.1392317	5.23-5.26	0.020
Mylabris cincta (Virgin female)	5	4.423740	0.0608366	4.42±0.06	0.1360348	4.24-4.57	0.018
Hycleus shah (Male)	5	3.328485	0.0332139	3.32±0.03	0.0742686	3.22-3.42	0.006
Hycleus shah (Mated female)	5	3.495220	0.0335781	3.49±0.03	0.0745924	3.46-3.51	0.008
Hycleus shah (Virgin female)	5	2.897700	0.0335554	2.89±0.03	0.0750322	2.79-2.99	0.003

The average cantharidin concentration in three groups of *Hycleus colligatus* (males, mated females, and virgin females) were 19.20± 0.33, 20.38± 0.49 and 15.67± 0.32 µg/mg, respectively. The average cantharidin concentration in three groups of *Mylabris schrenki* (males, mated females, and virgin females) were 11.52± 0.17, 12.44± 0.17 and 7.99± 0.17 µg/mg, respectively. The average cantharidin concentration in three groups of *Mylabris cincta* (males, mated females, and virgin females) were 4.94± 0.06, 5.24± 0.06, and 4.42± 0.06 µg/mg, respectively. The average cantharidin concentration in three groups of *Hycleus schah* (males, mated females, and virgin females) were 3.32± 0.03, 3.49± 0.03, and 2.89±0.03 µg/mg, respectively. Among the studied species, the three groups of *Hycleus colligatus* had the highest amount of cantharidin than the other species. In *Hycleus colligatus*, mated females had more cantharidin than males and virgin females. They had 1.06 folds more cantharidin than males and 1.30 folds more than virgin females. The three groups of *Hycleus schah* had the least cantharidin among the studied species. In *Hycleus schah*, mated females had 1.05 folds more cantharidin than males and 1.20 folds more cantharidin than virgin females (Table 2). The data showed that the total mean. Data showed that the total mean concentration of cantharidin for all studied species was the highest in mated females and a little lower in males. It was lowest in virgin females (Fig. 4A). These concentrations in males and mated females were significantly higher than in virgin females (two-way ANOVA test, ($P < 0.05$). There was a significant difference between the cantharidin concentrations of both sexes. This comparison showed that mated females had more cantharidin in their bodies than males and virgin females. Also, the results showed that the total mean of cantharidin concentration *in Hycleus colligatus* with 18.42 µg/mg was the highest in the studied species. The total mean of cantharidin concentration in four species (*Hycleus colligatus*, *Mylabris schrenki*, *Mylabris cincta*, and *Hycleus schah*) were 18.42, 10.56, 4.87, and 3.24 µg/mg per beetle, respectively (Fig. 4B).

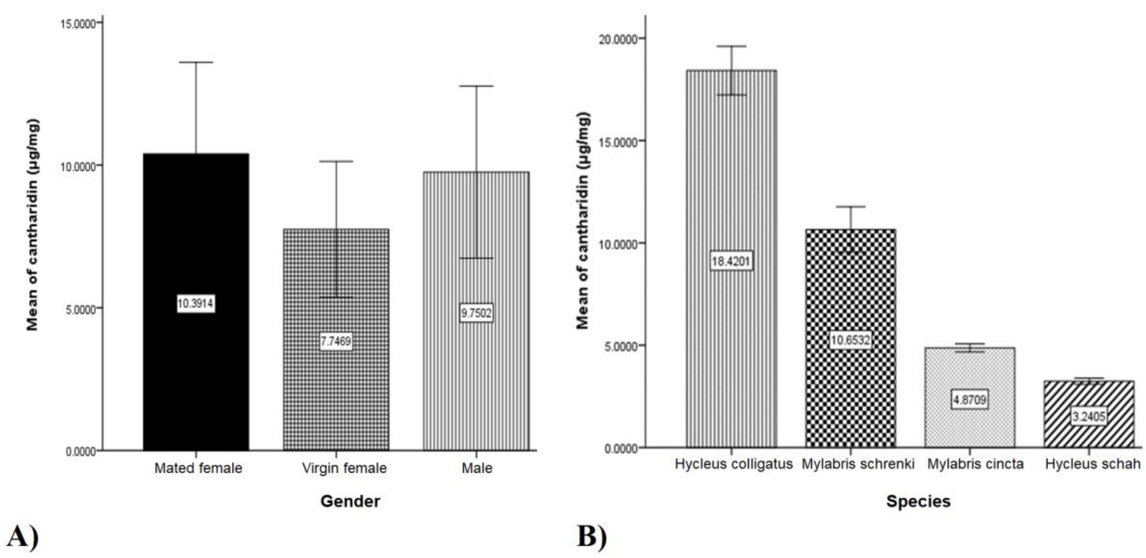

Fig. 4. Comparison of cantharidin in gender and species: A) Comparison of cantharidin values in three groups of four studied species (mated female, virgin female, male); B) Comparison of cantharidin concentration in four studied species (The test was calculated by two-way ANOVA test with $P < 0.05$, Error bars in all chart was %95 CI).

According to (Table 3), the 95% confidence limit and the significance level of 0.05 of the P-value calculated in the two-way ANOVA test and considering that $P < 0.05$ is the result of a significant difference between the amount of cantharidin present in each case. The study was observed in this research as well as between different groups of these species. According to the two-way ANOVA test and measuring the P-value between different groups (males, mated, and virgin females), there is a significant difference between all the pairs compared to groups and the value of P< 0.05 for all of them is smaller than 0.05. Therefore, there was a significant difference among the three groups of the four studied species (Fig. 5).

Table 3. Comparison of cantharidin levels in males, mated females and virgin females of the four studied species, ($P < 0.05$), from two-way ANOVA test

Species	Compared groups	P
Hycleus colligatus	Male - Mated female	0.042
Hycleus colligatus	Male - Virgin female	0.035
Hycleus colligatus	Mated female -Virgin female	0.030
Mylabris schrenki	Male - Mated female	0.040
Mylabris schrenki	Male - Virgin female	0.033
Mylabris schrenki	Mated female - Virgin female	0.031
Mylabris cincta	Male - Mated female	0.043
Mylabris cincta	Male - Virgin female	0.028
Mylabris cincta	Mated female - Virgin female	0.025
Hycleus shah	Male - Mated female	0.045
Hycleus shah	Male - Virgin female	0.032
Hycleus shah	Mated female -Virgin female	0.027

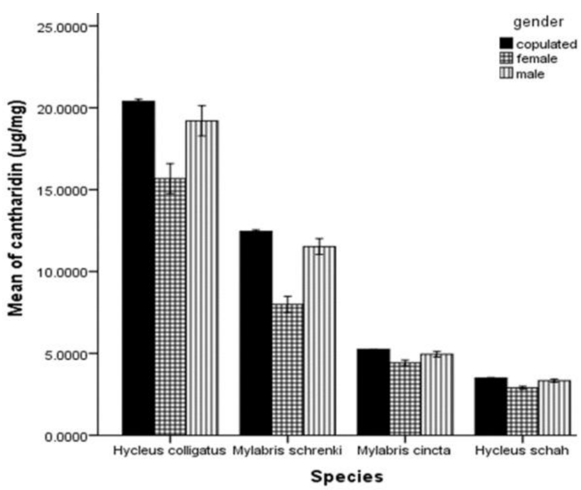

Fig. 5. Comparison of cantharidin values in male, mated and virgin females for four studied species, (The test was calculated by two-way ANOVA test with $P < 0.05$, Error bars in all chart was %95 CI)

CRCs (Palasonin and Palasoninimide)

On the other hand, Cantharidin and Cantharidin-Related Compounds (palasonin and palasoninimide) were investigated on mated females of four studied species. It investigated the percentage of CRCs (Cantharidin-related compounds) and Cantharidin, by gas chromatography-mass. According to Table 4, the percentage of CRCs and Cantharidin was reported based on quantitative gas chromatography/electron impact mass spectrometry. Ionization provided mass spectra with characteristic fragments of cantharidin at 96 m/z and 128 m/z (Fig. 6A), palasonin at 82 m/z and 114 m/z (Fig. 6B), and palasoninimide at 67 m/z, 81 m/z and 83 m/z (Fig. 6C).

In all mated females of the species, the percentage of cantharidin was higher than palasonin and palasoninimide. The percentages of all three chemical compounds in *Hycleus colligatus* were the highest in the studied species. *Hycleus colligatus* had the highest percentage of palasonin and *Hycleus schah* had the lowest percentage of palasonin, about 2.69 times less than *Hycleus colligatus.* In the case of palasoninimide, the percentage of palasoninimide in *Hycleus colligatus* was about 2.36 times more than *Hycleus schah* and had the highest percentage of palasoninimide (Table 4).

Discussion

Many things (biological, physiological, genetic, gender, climate, and environmental) can cause the difference in cantharidin contents among the blister beetles. Mebs *et al*. (2009) assayed cantharidin in two species of Meloidae, *Hycleus oculatus* and *H. tinctus*, from southern Africa (Mebs *et al*., 2009). The mean value per specimen for *H. oculatus* was 1 mg and for *H. tinctus* was 0.2 mg. In our study, it was found that the cantharidin concentration of four species ranged from 0.002 to 0.020 mg per individual. The amount of cantharidin in our study was lower than the amount of cantharidin that they reported. Dettner *et al*. (2003) reported cantharidin concentration (0.012-0.166 mg/mg) in *H. lunatus.* Our results were different from them. Capinera *et al*. (1985) studied cantharidin levels in some other species of blister beetles from Colorado (Capinera *et al*., 1985). They reported cantharidin levels in six species of Meloidae including *Epicauta fabricii, E. murina, E. pennsylvanica, E. maculate, E. sericans, E. immaculate, Z. atripennis L. sphaericollis,* and *M. laevis*. Males and females of *Epicauta immaculate* (mean: 4.25 and 5.21 mg/mg) had the highest levels of cantharidin than other species.

Table 4. The percentage of Cantharidin, Palasonin and Palasoninimide for four mated females studied species.

Mated females	Cantharidin %	Palasonin %	Palasoninimide %
Hycleus colligatus	2.045%	0.078%	0.026%
Mylabris schrenki	1.241%	0.059%	0.021%
Mylabris cincta	0.525%	0.038%	0.014%
Hycleus schah	0.351%	0.029%	0.011%

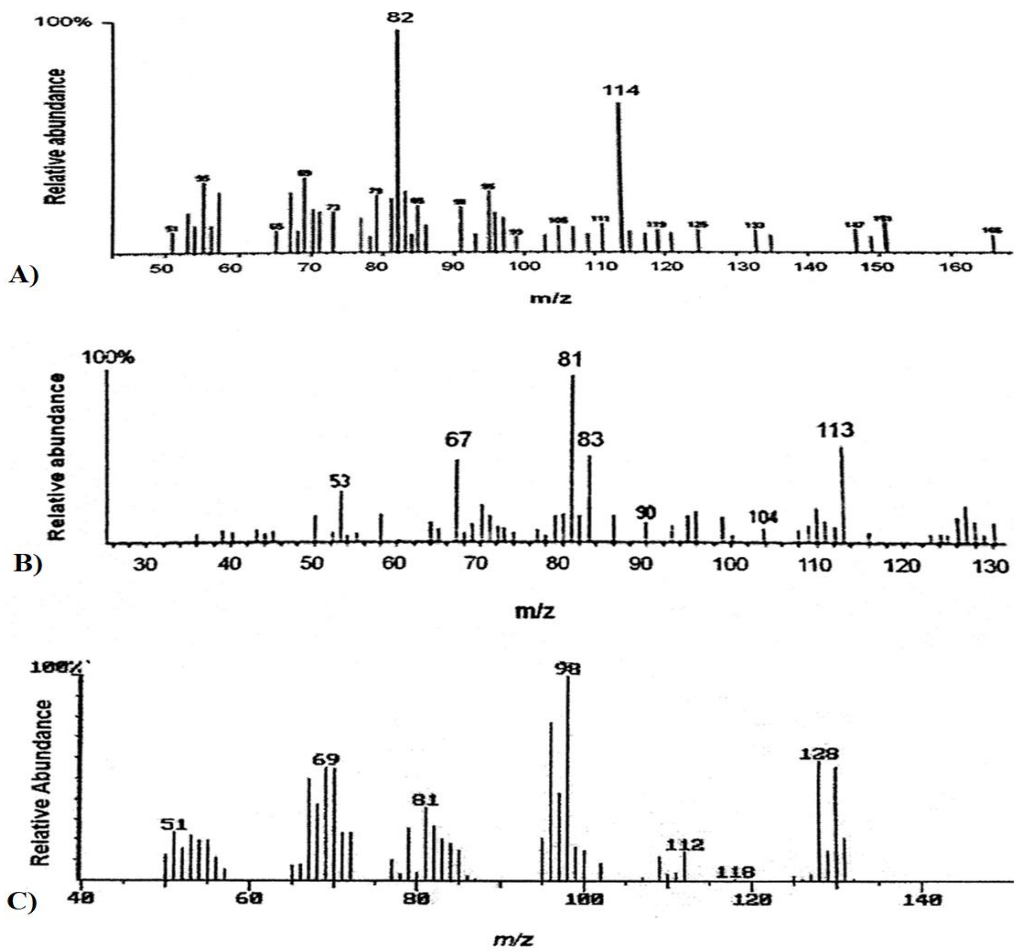

Fig. 6. Mass spectra of Cantharidin, Palasonin, and Palasoninimide: A) Mass spectra of cantharidin with base peaks at 96 m/z and 128 m/z according to a gas chromatograph; B) Mass spectra of palasonin with base peaks at 82 m/z and 114 m/z according to a gas chromatograph; C) Mass spectra of palasoninimide with base peaks at 67m/z, 81m/z and 83 m/z according to a gas chromatograph.

The mean cantharidin concentration in these species was much higher than the cantharidin concentration of species in our study. Nikbakhtzadeh *et al*. (2002) reported range of cantharidin content for some species of Meloidae (Nikbakhtzadeh *et al*., 2002). For example, the range of cantharidin content for *Mylabris schereibersi* and *Mylabris impressa* were 38.073-39.704 and 0.798-29.53 µg/mg, respectively. The cantharidin content of our study were placed in this range of that they reported. Nikbakhtzadeh and Tirgari (2002) investigated the content of cantharidin in nine species from Nahavand county, Hamedan province, Iran (Nikbakhtzadeh & Tirgari, 2002). The amount of cantharidin that they obtained, was more than in our study. The species included*: Mylabris impressa, M. guerini, M. variabilis, M. shreibersi, Alosimus smyrnensis, Muzimes iranicus, Callydos alloushei, Croscherichia sp, and Lydoceras bilineatus.* Males and females of *Muzimes iranicus* had the highest cantharidin concentration than other species (mean: 4.52 and 5.21mg/mg). Males and females of *Callydos alloushei* had the lowest content of cantharidin among all species (mean: 0.05 and 0.06 mg/mg). Nikbakhtzadeh *et al*. (2012) demonstrated the cantharidin concentration in *Hycleus scabiosae* and the concentration of cantharidin was highest in mated females (mean= 11 µg/mg), for males (mean= 10 µg/mg, and for virgin females (mean =5 µg/mg) (Nikbakhtzadeh *et al*., 2012). Therefore, cantharidin concentration in mated females was higher than in males and virgin females. Their results were similar to our study. The cantharidin concentration that they obtained was near to our cantharidin content that was investigated.

These results showed that males produce cantharidin and give it to the females during mating. They use this chemical to defend the survival of their progeny (Mullen and Durden, 2009). Males transfer high amounts of cantharidin to females during mating. This considerably increases the cantharidin demonstrated in mated females which is the reason for the significant difference shown between cantharidin content in three groups (males, mated females, and virgin females). On the other hand, virgin females have significantly lower cantharidin content than fellow males and mated females because they do not produce any cantharidin after the larval stage (Dettner, 1987). Males before copulation have a high volume of cantharidin. It decreases after copulation when most of the cantharidin transfers to the females' bodies (Carrel *et al.,* 1986). Because of that, virgin females that had a low amount of cantharidin, get it in a large amount after copulation and they had the highest titer of cantharidin. Larvae can synthesize cantharidin before pupation. After eclosion, males continue to produce cantharidin, but females cannot synthesize cantharidin (Dettner, 1987; Ghoneim, 2013). During copulation, males transfer nearly all their cantharidin to females and a high content of cantharidin will mass in the eggs during oviposition (Sierra *et al*., 1976; Carrel *et al*., 1993; Eisner *et al*., 1996).

The results showed that males of four species did not show CRCs, but chemical analysis showed the percentage of palasonin and palasoninimide in mated females. The percentage of the two chemically characterized CRCs and cantharidin are present in (Table 4). Palasonin and Palasoninimide exist in mated females, but the percentages of their CRCs were different. In all mated females of the species, the percentage of cantharidin was higher than palasonin and palasoninimide. The percentages of all three chemical compounds in *Hycleus colligatus* were the highest in the studied species but *Hycleus schah* had the lowest percentage of them. Nikbakhtzadeh and Tirgari (2002) investigated palasonin for three species of *Hycleus* (Meloidae) from different countries. They reported 1224.5704 ng/mg DW for *Hycleus polymorphos* from Northern Italy, 1220.8541 ng/mg DW for *H.polymorphos* from southern France, and 2067.0972ng/mg DW for *H.nata* from southern Africa. The amount of palasonin that was investigated was very much low and the results were similar to our study. They also considered C/P as the indication for comparing and categorizing species for different countries. Nikbakhtzadeh and Ebrahimi (2007) reported cantharidin and two CRCs, palasonin and cantharidinimide from *Mylabris impressa* that were collected from Toyserkan county, Hamedan province, Iran. Mebs *et al*. (2009) assayed the contents of cantharidin and palasonin for two species of *Hycleus* (*H. oculatus* and *H. tinctus*) from southern Africa. The concentration of palasonin was reported to range from 6 to 48 µg per beetle for *H. tinctus* and 5 to 45 µg per beetle for *H. oculatus*. Also, they demonstrated the ratio of cantharidin to palasonin. It is about 20:1 for *H.oculatus* and 12:1 for *H.tinctus.* The number of CRCs in our study was lower than the amount of CRCs that they reported. The results showed that the concentration of palasonin was much lower than the cantharidin content. The µg/mg (dry weight) contents of cantharidin in these specimens were much higher than those assayed in *H. lunatus* (0.012-0.166 µg/mg) by Dettner *et al*. (2003). Nikbakhtzadeh *et al*. (2012) was studied cantharidin and CRCs concentration in *Hycleus scabiosae*. The concentration palasonin 17.49 ng/mg and concentration palasoninimide was 9.17 ng/mg in mated females. The CRC amount in these species was similar to the species that was reported. The contents of all CRCs were much lower than the cantharidin content. These studies showed males do not have CRCs and these compounds cannot be transferred from males to females. CRCs are less toxic than cantharidin. They are very safe for embryo and protect them from any harm. Mated females have these compounds and protect eggs (Yong *et al.,* 2009).

Our observation that palasonin and palasoninimide were found in mated females but not in males indicates that CRCs do not have the same transfer pattern as cantharidin and thus, unlike the cantharidin, could not have been transferred from males to females. Two alternative routes can be envisaged for the origin of palasonin and palasoninimide in mated females. Palasonin might be originated directly from the oxidative demethylation of cantharidin or derived from the cyclization of a previously demethylated 12-nor-farnesol, the sesquiterpene precursor of the cantharidin. Since chemical analysis of male extracts did not detect any CRCs. Palasonin and palasoninimide must be directly synthesized in the mated female body from the demethylation of D2C. This provides further evidence that mated females can actively synthesize palasonin and palasoninimide (Dettner *et al.*, 2003)

An ideal pesticide should not adversely affect organisms other than its targeted pests. Due to their natural origin (from natural plants, animal toxins, and living microbes), biopesticides are considered to be safer or to have evolved to have low non-target toxicity (Shao & Zhang, 2017). Because biopesticides are increasingly used to replace synthetic pesticides in pest control, it is necessary to assess the amount of cantharidin and CRCs (palasonin and palasoninimide) as known biopesticides. Due to the deleterious effects of chemical pesticides, biopesticides, with a new mode of action, higher effectiveness and eco-friendliness should be individually, sequentially, and simultaneously applied to maintain crop production (Zhiyong *et al.,* 1998). Three insect-derived compounds, cantharidin, palasonin, and palasoninimide exhibit a similar action mode: they can inhibit protein serine/threonine phosphatases (PSPs) in pests. Protein phosphorylation and dephosphorylation act on living cells to participate in cellular metabolic activities in response to changing circumstances, internal developmental cues, or external environmental stimuli in eukaryotes. Current advances in the development of biopesticides provide the potential to reduce the use of chemical pesticides while maintaining acceptably low crop damage levels (Mayanglambam *et al*., 2021). Many biopesticides have been developed as insecticides. Owing to their advantages including high selectivity, low toxicity, easy degradation, and slow production of resistance, biopesticides are gaining a growing interest as natural pesticides suitable for integrated pest management programs (Li *et al*., 2021).

Conclusion

In this study, cantharidin concentration was investigated in four abundant species of Meloidae from Kerman province. The results showed *Hycleus colligatus* had more cantharidin concentration than other studied species. Cantharidin content was the highest in mated females in all species. Also, the percentage of cantharidin, palasonin, and palasoninimide for four studied species was reported. The percentage of palasonin and palasoninimide was much lower than the percentage of cantharidin. This study aimed to investigate Cantharidin and CRCs (palasonin and palasoninimide) of four common blister beetle species in Kerman province because they are known as biopesticides that can control important pests. Current advances in the development of biopesticides provide the potential to alleviate and reduce the use of chemical pesticides while maintaining acceptably low crop damage levels. Many biopesticides have been developed as insecticides. Owing to their advantages including high selectivity, low toxicity, easy degradation, and slow production of resistance, biopesticides are gaining a growing interest as natural pesticides suitable for integrated pest management programs.

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Conflict of interests

The authors declare no conflict of interest.

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