

Review

Enhancement of research interests in physiology and biochemistry of blister beetles (Coleoptera: Meloidae): A review

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The blister beetles (Coleoptera: Meloidae) are global distributed insects except for New Zealand and Antarctic region. They are characterized by some attentive phenomena such as hypermetamorphosis, diapause and cantharidin production. Some meloid genera and species are reported as agronomic pests, depending on both the beetle population and the sensitivity of the crop. So many institutions and researchers are engaged in the systematics, ecology, biology of blister beetles while few only are interesting in their physiology and biochemistry. Therefore, the present work aims to enhance the research attention for investigating several physiological and biochemical aspects of these beetles. Herein, the available reported works in literature were reviewed focusing on food metabolism, reproductive physiology, meloid responses to the environmental factors and physiological nature of diapause, as well as cantharidin production, mode of action, natural utilization, and uses of the compound in the field of pest control and plant growth regulation.

Keywords: Cantharidin, canthariphilous, diapause, endothal, metabolism, glycogen, klinotaxis, osmoregulation, pesticide, phytotoxin.

INTRODUCTION

Family Meloidae is widespread throughout the world except for New Zealand and Antarctic and most Polynesian islands (Dettner et al., 1997; Arnett et al., 2002; Bologna and Di Giulio, 2011). As reported few years ago, Meloidae contains more than 3000 species in 120 genera. They primarily occur in temperate steppic and arid regions, and in sub-tropical and tropical savannas or other open habitats (Bologna, 1991 a, b). The family Meloidae was revised cladistically (Bologna and Pinto, 2001) and four subfamilies were recognized: Eleticinae, Meloinae, Tetraonycinae and Nemognathinae (Pinto and Bologna, 1999; Bologna and Pinto, 2002). In their comprehensive work, Bologna and Pinto (2002) studied the faunistic distribution of family Meloidae in the Old World. In general, faunistic studies on Meloidae of North America had been carried out (Pinto and Bologna, 1999). In Central and South Americas, several zoogeographic and faunistic studies were carried out (Selander and Bouseman, 1960; Barrows, 1980; Peck et al., 2002; Peck, 2006 a, b, 2010; Bologna and Pinto,

2007; Quintino and Monné, 2009; Campos-Soldini, 2011).

Adult beetles can be recognized by morphological characteristics such as soft body, bright coloration, rather elongate, head deflexed with narrow neck, pronotum not carinate at sides, heteromerous tarsi, smooth integument (Borror et al., 1989; Bologna, 1991a,b; Arnett et al., 2002). Postembryonic development includes 7 larval instars and 5 distinctive phases. The first instar larva has usually received the name of triangulin (more recently planidium) because of the peculiar shape of pretarsus, specialized in some genera for climbing and holding the host (Di Giulio and Bologna, 2007). Ghoneim (2013 a) reviewed various embryonic and postembryonic developmental processes.

The bodily fluids of blister beetles contain the skin irritant cantharadin, giving the family its common name (Borror et al., 1989). It is possible that cantharadin acts as a protection against accidental beetle consumption by large herbivores, as some animals will avoid grazing on

vegetation supporting large numbers of orange, red, or otherwise brightly colored blister beetles (Evans and Hogue, 2006). However, some insects are attracted to cantharidin, and this compound is involved in the chemical communication among blister beetles (Young, 1984; Klahn, 1987). Because of the poisonous nature of cantharidin, these beetles periodically are inadvertently eaten (with feed such as hay) by the domestic livestock and horses causing severe illness or death. Also, human health can be affected when come in close contact with the beetle adults or accidentally eating their bodies (Beasley et al., 1983; Capinera et al., 1985; Blodgett et al., 1991).

Several ecological phenomena of the blister beetles, such as population dynamics, seasonal abundance and activity, behavioral ecology, and the effect of various biotic and abiotic environmental factors, had been studied (Ruiz et al., 1994; Nikbakhtzadeh and Targari, 2002; Zhu et al., 2005). Also, various bionomic activities and interrelationships of the family had been described in different parts of the world (Selander, 1986; Pinto and Selander, 1970; Pinto, 1972; Selander, 1988; Dombia, 1992; Bologna and Alsoisi, 1994; Bologna and Pinto, 1995; Senthil-Kumar et al., 1996; Bologna and Di Giulia, 2002). More ecological information, in general, can be found in the literature (Schowaller, 2006; Prakah, 2008; Matthews and Matthews, 2010; Kaptan et al., 2011; Ananthakrishnan and Sivaramakrishnan, 2012). However, several behavioural characteristics were intensively reviewed by Ghoneim (2013 b).

Mostly in the New World, all economic pests belonging to family Meloidae are species of vittata group (Meloinae: Epicautini) which are known as pests of garden and field crops, (Adams and Selander, 1979) and cause economic damages to potato, tomato, alfalfa, soybeans, sugar beet, cotton and a variety of crops and vegetables (Towsend, 2000). Some meloid species had been recorded as agricultural pests in the Old World (Erickson et al., 1976; Ali et al., 2005a, b). Although the presence of blister beetles in different crops is usually not considered to be a serious constraint (Zhu et al., 2005), infestations of crops grown in small-holder plots may cause considerable damage because of the gregarious nature of adult beetles (Hall, 1984; Nikbakhtzadeh, 2004). Certain blister beetles, on account of their moderate size and swarming behavior, can cause considerable damage to crops by feeding on the foliage or flower heads (Booth et al., 1990; Lebesa et al., 2012). On the other hand, the earlier larval instars (triungulins) of some meloid beetles are predaceous on the egg pods of grasshoppers (Orthoptera) and consequently provide a degree of natural (biological) control measure against these grasshoppers. Triungulins of some other meloid beetles are parasitoids or predators on the solitary wild, or even the social, bees (Hymenoptera) suppressing their populations and subsequently prevent important pollinator measure for several crops (Hiller and Wittmann,

1994; Gaglianone, 2005; Zhu et al., 2008 a; Shanklin et al., 2010). It is to be noted that Ghoneim (2013 c) reviewed several agronomic and biodiversity impacts of blister beetles.

Different metabolic and energetic aspects in insects were reviewed, such as: physiological and environmental considerations in bioenergetics (Watanabe et al., 2002; Gade, 2004), energy metabolism during flight (Strang, 1981; Wiegert and Petersen, 1983, Beenackers et al., 1984; Suarez, 2000), energy metabolism during diapause (Hahn and Denlinger, 2007), endocrine or hormonal regulation on the energy metabolism (Candy, 1981; Gade et al., 1997; Van der Horst, 2003; Gade and Auerswald, 2003; Socha et al., 2004), endocrine regulation of energy homeostasis (Schooley et al., 2005; Lorenz and Gäde, 2009), regulation of fat metabolism (Beenackers et al., 1985; Canavoso et al., 2001; Arrese et al., 2001; Athenstaedt and Daum, 2006), Chitin metabolism in insects (Merzendorfer and Zimoch, 2003), reproductive physiology in insects (Engelmann, 1970; Raabe, 1982; Wall, 1993; Hayes and Wall, 1998, 1999; Simonet et al., 2004) and environmental physiology (Wu, 1995; Brown et al., 2004; Chown and Nicolson, 2004; Catalán et al., 2012; Harrison et al., 2012; Zhao and Jones, 2012).

As previously shown, the available literature contains a lot of reported research works about the geographic distribution, systematics, morphology, ecology, bionomics, rearing of different meloid species, as well as cantharidin synthesis and application in veterinary and medical fields while it has little research works on the physiology and biochemistry. Therefore, the present review was achieved primarily for enhancing the enthusiasm of researchers all over the world to be interested in several physiological and biochemical aspects of blister beetles.

FOOD UTILIZATION AND ENERGY METABOLISM

The quantity and quality of food available to insect nymphs and larvae have important consequences for growth and final size of pupae and adults. Also, the quantity and quality of adult food can influence significantly at least some aspects of reproductive performance. Knowledge of food consumption and utilization is of great importance in managing problem pests and the regulation of insect feeding habits is very important in this context (Slansky and Rodriguez, 1987; Stamp and Casey, 1993; Chapman and de Boer, 1995; Schoonhoven et al., 1998; Chown and Nicolson, 2004; Nespolo et al., 2005; Chown and Terblanche, 2007; Speight et al., 2008).

With regard to the blister beetles, the effects of different host plants, pink morning glory *Ipomoea cornea* Jace and Hawaiian Woodrose *Ipomoea tuberosa* Linnaeus (Convolvulaceae), were assessed on the food consumption of the orange blister beetle *Mylabris*

pustulata (Thunberg) (Manoharan et al., 1987). Carrel et al. (1990) examined the gut contents of the bronzed blister beetle *Lytta polita* Say adults which revealed an abundance of pollen, exclusively from the genus *Pinus*. The feeding preference and body water content in wild *L. polita* and laboratory-reared margined blister beetle *Epicauta pestifera* Werner were investigated to ascertain the source of drinking water. In the laboratory, Gorz et al. (1972) studied the feeding deterrence on the ashgray blister beetle, *Epicauta fabricii* (LeConte); the striped blister beetle, *Epicauta vittata* (Fabricius); Pennsylvania black blister beetle *Epicauta pennsylvanica* (De Geer), and *E. pestifera*. Their results showed that both *cis-o*-hydroxycinnamic acid (*cis-o*-HCA) glucoside and coumarin (the lactone form of *cis-o*-HCA) were strong feeding deterrents but *trans-o*-HCA glucoside had no significant deterring effect. The blister beetle *Mylabris indica* Herbst (or *Mylabris balteata* Pallas) was reared on 4 different types of flowering plants by Haniffa and Sekar (1993). They recorded the highest and lowest feeding, assimilation and conversion rates with Asian oil tree *Pongamia glabra* Vent. (Leguminosae) and the ornamental plant *Durandthus repens* Linnaeus (Verbenaceae).

The nourishment components in adults of the blister beetle *Epicauta chinensis* Laporte were determined by Li and Ren (2009). It contained 7 kinds of trace elements, and Zn and Fe reached 0.038 28% and 0.041 47%, respectively in the experimentation sample. The proportion of unsaturated fatty acid and saturated fatty acid was 2.78, the content of amino acid in the experimentation sample was 50.15%, the content of Glu is the highest, reached 7.19%. Analyzed results showed that *E. chinensis* contained high protein, low fat and abundant trace elements. Recently, Ghoneim et al. (2012 a) investigated the effect of food quality on the food preference of the black blister beetle *Meloe proscarabaeus* Linnaeus adults. Also, the effects of food quality on the food utilization efficiencies of the same species were determined. The adult females exhibited the highest approximate digestibility (AD) by feeding on the Egyptian clover *Trifolium alexandrinum* Linnaeus (Fabaceae) but the lowest AD by feeding on the pea *Pisum sativum* Linnaeus (Fabaceae). The efficiency of conversion of ingested food into the biomass (efficiency of conversion of ingested food) was the highest by feeding on *T. alexandrinum* but lowest by feeding on *P. sativum*. The efficiency of conversion of digested food into the biomass (efficiency of conversion of digested food) run in a parallel trend (Ghoneim et al., 2013a). Also, effects of food quality on the the general body metabolites (carbohydrates, proteins and lipidas) in haemolymph and fat body of *M. proscarabaeus* had been determined (Ghoneim et al., 2013b). Effects of cantharidin, the principal substance in bodily fluids of blister beetles, on the utilization of alfalfa and smooth bromegrass (*Bromus inermis*) in ruminants were

determined. A decrease in the feed utilization efficiency may be due to the decrease in the microbial digestion of fibrous feeds (Lenssen et al., 1990).

The distribution and innervations of neurosecretory cells in the brain, a pair of corpora cardiaca and a pair of corpora allata in the cephalic region, as neuroendocrine organs, in *M. pustulata* had been described (Sidhra et al., 1983). Two peptides had been isolated from methanolic extracts of corpora cardiaca of the lunate blister beetle *Decapotoma lunata* Pallas. They were found as the same peptides of two other blister beetles, *Cyaneolytta pectoralis* Gerstaecker and *Mylabris coeca* Thunberg. The corpus cardiacum extract of *D. lunata* was not active in mobilizing carbohydrates or lipids in the beetle (Gade, 1995). The energy substrates were measured in haemolymph and flight muscles of the large bean blister beetle *Mylabris oculata* Thunberg at rest and after tethered, lift-generating flight. Also, two neuropeptides were isolated from the corpora cardiaca which are hypertrehalosaemic and hyperprolinaemic, thus very likely regulating the mobilization of the two important flight substrates of blister beetles, namely carbohydrates and proline (Gade and Auerswald, 1999).

Verma and Prasad (1974) studied the hydrogen-ion concentration and the digestive enzymes in *M. pustulata*. On the same beetle, the distribution of free amino acids in certain tissues was estimated (Dhillon and Sidhu, 1977), the protein content in the haemolymph and Malpighian tubules and the mucoprotein content in Malpighian tubules were determined (Mohamed and Murad, 1979) and the neutral lipids in certain tissues were identified (Dhillon et al., 1983a,b). The protein spectra were comparatively studied in *Mylabris frolovi* Germar, four-spotted blister beetle *Mylabris quadripunctata* (Motschulsky), *Mylabris quadrisignata* Fischer-Waldheim and red-headed blister beetle *Epicauta erythrocephala* Pallas from Xinjiang, China (Hou et al., 1996). The activity levels of aminotransferases were measured in the mid gut of the blister beetle; *M. pustulata* exposed to sublethal doses of carbaryl. The insecticide markedly inhibited the activity levels of all aminotransferases exposed for either short or prolonged treatments (Bharathi, 2008). The histopathological effects of sublethal concentration of Vijay neem on the fat body tissue of male *M. indica* were evaluated. The results revealed a characteristic changes in the fat body cells of treated insects than control insects. Thus, the fat body is the important organ that synthesize and stores energy reserve, in addition to regulate the metabolic activities and reproduction (Vivekananthan et al., 2010). The digestive cellulose hydrolyzing activity in the gut and salivary glands of *M. pustulata* was estimated. The gel filtration, preparative native PAGE, and the maximum activity against carboxy methyl cellulose were determined (Sami et al., 2011).

REPRODUCTIVE PHYSIOLOGY

The egg formation and maturation in Meloidae had been studied (e.g., Margaritis, 1985; Zissler, 1992; Margaritis and Mazzini, 1998; Waring, 2000; Woods and Bonneau, 2006). The electron microscopy was used to investigate the vitellogenesis and egg membrane formation in the terminal ovarian follicles of the Nuttall's blister beetle *Lytta nuttalli* Say. Three kinds of yolk globules were apparently composed predominantly of carbohydrates, lipids, and proteins, respectively (Sweeny et al., 1970). The sequence of events of spermatophore formation in the blister beetle *L. nuttalli* and the roles of the materials from the second and third pairs of male accessory glands had been described. Also, the histochemical composition of the male and female secretions and the components of the spermatophore, as well as the cytology and secretory cycles of the glands were investigated (Gerber et al., 1971). As reported by Verma and Mohanty (1976), three categories of compound yolks (CY) are synthesized during vitellogenesis of *M. pustulata*: CY1 bodies originate *de novo* and are composed of protein-carbohydrate (glycogen)-RNA complex, CY2 bodies originate from precursors in follicular epithelium and are composed of protein-carbohydrate (glycogen) complex and CY3 bodies originate from extraovarian precursors and are composed of protein-acid mucopolysaccharide-glycogen-phospholipid complex. Depending on the results of Manoharan et al. (1987), the host plants affected the fecundity of *M. pustulata*. They presumed that the food rich in protein concentrations is among the principal factors influencing fecundity of the beetle. Bharathi and Govindappa (1990) assessed the effect of carbaryl on the oviposition, hatchability and organic constituents of egg mass of *M. pustulata*. Recently, Ghoneim et al. (2012 b) evaluated the effects of food types on the reproductive potential of *M. proscarabaeus*. No female fecundity could be measured after feeding on *Pisum sativum* because all females died just before oviposition. Adult feeding on *T. alexandrinum* induced the females to be more fecund but feeding on *L. sativa* deleteriously reduced the fecundity. The lethal and sub-lethal concentrations of cantharidin were assessed on the reproductive potential of the cotton bollworm, *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae). Severely affected fecundity and fertility were observed (Khan et al., 2013a).

ENVIRONMENTAL PHYSIOLOGY

A study on the grey blister beetle *Epicauta aethiops* (Latreille) in the Sudan was achieved to determine the reactions to humidity. The beetle species was hygronegative and the water balance was restored by direct drinking or by taking water in with food and probably through the retention of metabolic water. The

orientation mechanisms were mainly orthokinesis, Klinokinesis and Klinotaxis. Also, reactions to light, temperature, and hunger were investigated (El-Amin, 1969). The xeric adaptiveness of several blister beetle species was determined. Rates of water loss and oxygen consumption were generally higher in all species than that reported for other xeric arthropods. All tested meloid species showed strong ability to regulate Na, K, and Ca as well as total osmotic concentration and a high temperature tolerance (Cohen and Pinto (1977). The dietary water intake and loss *via* defecation, absorption, retention and transpiration in *M. balteata* were estimated under the laboratory conditions. Its very poor dietary water retention capacity and very great transpiration of dietary water were thought to be an adaptive mechanism for the high level of flight activity in this beetle (Chockalingam and Manohara, 1980). Several parameters of xeric adaptiveness of the white bladder-bodied blister beetle *Cystodemus armatus* LeConte were studied in the field and laboratory. Under stress with no food available the beetles have higher rates of water loss than any other desert species tested (Cohen et al., 1981). The effects of water stress and rehydration on haemolymph volumes and amino acid content in the same desert blister beetle were determined (Cohen et al., 1986). The attraction of the blister beetle *Hycleus apicicornis* (Guerin de Meneville) to host plants may be partly mediated by visual stimuli, as reported by Lebesa et al. (2011). They compared the responses of beetle adults to plant models of different colors, different combinations of two colours, or three hues of blue of different shapes. Sky blue was the most attractive, followed by white, which reflects light over a broader range. On landing on sky blue targets, beetles exhibit feeding behaviour immediately.

DIAPAUSE AS A PHYSIOLOGICAL PHENOMENON

Under unfavorable environmental conditions, an insect would not survive. Many species initiate a series of metabolic changes well before the unfavorable conditions actually occur. Unlike migration, which is an escape in space, diapause is an escape in time, allowing the insect to withstand the unfavorable conditions while remaining in place (Ghoneim, 2013 a). Insect diapause is a dynamic process consisting of several distinct phases (Hegdekar, 1979; Chapman, 1998; Kostal, 2006). Energetics of insect diapause had been reviewed by Hahn and Denlinger (2011). Also, the neuroendocrine regulation of insect diapause was studied including several key hormones: juvenile hormone, diapause hormone, and prothoracicotropic hormone (Brown and Chippendale, 1978; Yin and Chippendale, 1979; Pener, 1992; Horie et al., 2000; Denlinger, 2002). Concerning the blister beetles, energy substrates for flight in *D. lunata* was investigated. It was suggested that diapausing larvae

accumulated trehalose and some polyol, but not glycogen. The content of glycogen decreased with the development of diapause. Thus glycogen may offer carbon source for producing trehalose, sorbitol, or glycerol (Auerswald and Gade, 1995). Zhu et al. (2008 b) obtained similar results for the Chinese blister beetle *Mylabris phalerata* Pall.

CANTHARIDIN PHYSIOLOGY

The blister beetles are commonly known as "oil beetles" because they release yellow oily droplets of haemolymph from their leg joints (and may be from the antennal joints) when disturbed. This exudation contains toxic material "cantharidin" or "cantharid" (derived from the Greek word 'Kantharos') which acts naturally as an aphrodisiac for adult males and females (for review, see Ghoneim, 2013 c). The adult male biosynthesizes about 17 mg of the toxin, representing 10% of his live weight, whereas the adult female actually loses of her defensive reserves but compensates it from male (Carrel et al., 1993). It is surprising, to us at least, that the larvae manufacture and accumulate cantharidin as they feed and grow in size. Carrel et al. (1993) observed ten life stages of the ebony margined blister beetle *Epicauta funebris* Horn. The first five larval instars produce cantharidin and when disturbed they exude it, as a defensive agent, in a milky oral fluid, not in haemolymph which adult beetles reflexively discharge from leg joints. Moreover, the quantitative analysis of cantharidin in juvenile blister beetles had been carried out by Haskins et al. (1987).

It is worthy to mention that cantharidin analogues, cantharidin-related compounds, cantharidin poisoning to animal and human, cantharidin uses in the traditional and modern medicine are outside of our interest in the present article. In a few exceptional cases only, cantharidin analogues may be cited. Also, the present review stresses on the cantharidin biosynthesized by adults, not by immatures.

Cantharidin of Meloidae: nature, functions and chemical structure

Cantharidin is among the most widely known insect natural products (McCormick and Carrel, 1987; Dettner, 1997) and mainly found in blister beetles (Coleoptera: Meloidae) (Arnett et al., 2002; Dettner et al., 1997; Prestwich and Blomquist, 1987). It is one of the oldest-known toxins from insects (Eisner et al., 1990) and highly toxic to a wide variety of animals, including birds, amphibians and mammals (Dettner, 1997). Cantharidin has been known to humans for about 2000 years due to its physiological activities such as blistering (McCormick and Carrel, 1987; Wang, 1989; Pemberton, 1999).

Cantharidin (haemolymph exudation through leg joints

or/and antennal pores) was discovered in haemolymph and gonads of the Spanish fly *Lytta vesicatoria* (Linnaeus) in quantity larger than any other member of the family (Bohac and Winkler, 1964). The function and intrinsic role of cantharidin in the courtship behaviour of Meloidae has been never fully established. It was suggested that cantharidin might be used by female when selecting a mate at close range (McCormick and Carrel, 1987). Pinto (1974, 1975b) was, in fact, the first to consider male cuticular pores on antennae as being involved in the courtship behaviour of species from the genera *Linsleya* and *Tegrodera*. As pointed out in the iron cross blister beetle *Tegrodera aloga* Skinner, individual males donated a large cantharidin-containing spermatophore to their mates, and body size was correlated with the size of the cantharidin-producing accessory glands (Alcock and Hadley, 1987). For some details, adult males of the blister beetle manufacture quantities of cantharidin larger than those of adult females who compensate it by receiving some quantities from their mates during mating as a nuptial or copulatory gift (Dettner et al., 1997; Pemberton, 1999; Nikbakhzadeh, 2004). A further study was carried out by Nikbakhzadeh et al. (2007) aiming to clarify the transferring mechanism of some quantity of cantharidin from adult male into the genitalia of female as a nuptial gift for mating. Ghoneim (2013 c) comprehensively reviewed the sexual function and other functions of cantharidin in family Meloidae.

Meloid beetles have been attacked by some insects and other natural enemies. It was reported that some blister beetles such as *Meloe* spp. are attacked by *Pedilus* (Coleoptera: Pedilidae) (LeSage and Bousquet, 1983) and/or by some species of Miridae (Heteroptera) (Pinto, 1978). Also, the dark blister beetle *Epicauta murina* (LeConte) and *E. fabricii* are attacked by the fire-coloured beetle *Pedilus lugubris* Say (Williams and Young, 1999). When attacked or disturbed, adults of blister beetles release haemolymph droplets in so called "reflex bleeding". The highly toxic material, "cantharidin" in the haemolymph, is a well defensive reaction against the aggressive creatures. As reviewed by Ghoneim (2013 c), cantharidin is considered responsible for the repellent properties of meloid haemolymph against a wide variety of predators. Although cantharidin serves these beetles effectively in defense against ants and carabid beetles (Carrel and Eisner, 1974), some meloid enemies are undeterred by cantharidin. Among these are frogs, which apparently consume meloids with impunity (Korschgen and Moyle, 1955; Kelling et al., 1990). Acquired cantharidin could convey a protective advantage on frogs *vis à vis* any number of natural enemies, and it could also make frogs poisonous to humans (Eisner et al., 1990). As pointed out by numerous authors (Carrel et al., 1973; Sierra et al., 1976; Carrel et al., 1993; Arnett et al., 2002), adult female of blister beetle uses her own cantharidin and transferred cantharidin for herself and also to cover

or coat her eggs for protecting them against the potential predators.

As reported in one of the oldest published books: Bartholow (1882), the important substance that we can find in cantharides is a neutral, crystallizable principle, cantharidin. We can find also oil, fatty matter, and an odorous material. Oils and fats act as synergists for cantharidin because they increase the solubility and favor the absorption of cantharidin. The chemical structure and properties of cantharidin had been reported by many authors as shown in the literature (e.g., Dauben et al., 1980; Young, 1984; Davidson and Williams, 1987; Tagwireyi and Ball, 2000; Schmidt, 2002). It is a bicyclic terpenoid or anhydride of cantharidic acid with chemical formula $C_{10}H_{12}O_4$. It is an achiral compound. It has a plane of symmetry that goes through the middle of the bonds C2C3 and C5C6. It's a meso compound (2S, 3R) (Verma et al., 2013). The molecular weight (molar mass) is 196.2024 g/mol. At the usual temperature, it is an odorless and colorless solid; melting point is 212 °C., white to light yellow crystal powder, readily soluble in various organic solvents, such as ether, chloroform and acetone, as well as in fixed oils, but only slightly soluble in water or almost insoluble in it. Cantharidin is incompatible with acids, strong oxidizing agents, alcohols, amines, and bases. Although cantharidin was purified and crystallized in 1810 by Robiquet from Spanish fly *Lytta vesicatoria* (Dixon et al., 1963; Prestwitt and Blomquist, 1987), it took 150 years of study to be fully synthesized (Prestwitt and Blomquist, 1987; Carrel et al., 1993). It was isolated and crystallized from the blister beetle *Epicauta hirticornis* (Verma et al., 2013).

Cantharidin is classified as super toxic to animals and humans. Probable oral lethal dose in humans is less than 5 mg/kg or a taste of less than 7 drops for a 70 kg (150 lb.) person. It is very toxic by absorption through skin. When heated to decomposition, cantharidin emits acrid smoke and irritating fumes. Biological activity of cantharidin is described as natural toxin inhibitor of protein phosphatases 1 and 2A (K_i values are 1.1 and 0.19 μM respectively); similar to okadaic acid (9,10-Deepithio-9,10-didehydroacanthifolicin) (Craven and Polak, 1954; Ewart et al., 1978; Aiello, 1998; Honkanen, 1993; Wang et al., 2000; McCluskey and Sakoff, 2001; Huh et al., 2004; Thaqi et al., 2010; Bajsa et al., 2011).

The cantharidin biosynthesis was a debatable issue along several decades ago because the complete mechanism *in vivo* was not known (McCormick et al., 1986). However, it is quite sure that it implicates reactions with as first compound a terpenic alcohol. A proof can be given with mass spectroscopy using the ¹⁴C, ³H, ¹⁸O isotopes. The farnesol represented on the picture is the (E,E)-3, 7, 11-triméthyl-dodeca-2, 6, 10-triene-1-ol. This is a sesquiterpenic alcohol and is an intermediate in the biosynthesis of isoprenoids. The farnesol is quite abundant in the nature. A lot of natural essences, like

Ylang-ylang ones, contain this molecule. Concerning the biosynthesis sites, the highest concentration of cantharidin was observed in sexual organs, particularly in ovary (Nikbakhtzadeh and Tirgari, 2002; Nikbakhtzadeh et al., 2007). In order to clarify the synthesis mechanism of cantharidin in *Mylabris calida* Palla, Jia et al. (2009) studied the protein expression profile during the early and advanced stages of the process. The results revealed that synthesis of cantharidin is a complicate process involved a variety of proteins that the molecular weight ranged from 20 kDa to 45 kDa. The relationship between cantharidin biosynthesis and the mevalonate pathway was investigated (Jiang et al., 2012).

In a related matter, there are few compounds structurally similar to cantharidin, known as cantharidin-related compounds (CRCs), have been found in blister beetles. It is unknown whether this series of compounds are cantharidin metabolites or if they are synthesized *via* an independent biochemical pathway. There are also many questions concerning the method of CRC synthesis and transfer in male and female blister beetles (Nikbakhtzadeh et al., 2012). Nikbakhtzadeh and Ebrahimi (2007) succeeded to extract cantharidin and two CRCs, palasonin and cantharidinimide, from the blister beetle *Mylabris impressa stillata* (Baudi). In the blister beetle *Hycleus scabiosae* (Olivier), an inseminated female incorporates about 38.5 ng of cantharidin, 196.35 ng of palasonin and 269.15 ng of palasoninimide into each egg mass during oviposition (Nikbakhtzadeh et al., 2012).

Cantharidin production by canthariphilous insects

Generally, autogenous producers of cantharidin occur exclusively within the coleopteran families of Meloidae (Dixon et al., 1963; Capinera et al., 1985; Blodgett et al., 1991; Carrel et al., 1993; Dettner, 1997; Hemp et al., 1999 a; b) and false blister beetles Oedemeridae (Carrel et al., 1986; Nicholis et al., 1990; Holz et al., 1994; Frenzel and Dettner, 1994) in which it occurs in the haemolymph and other tissues to chemically protect them and their immature stages (Carrel and Eisner, 1974; Young, 1984; Bologna, 1991; Dettner et al., 1997). In spite of the occurrence of about 1,000 oedemerid species in the Pacific basin and Caribbean, only a few species are known to produce cantharidin (Samlaska et al., 1992).

A considerable number of insects (mostly Coleoptera and Diptera), so-called canthariphilous (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 and Dettner, 2001). They feed on it without any obvious ill-effects (Hemp and 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 and 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 and Dettner, 2001).

In China, Feng et al. (1988) detected and estimated the cantharidin in *Xanthochroa waterhouslet* Harold (Coleoptera: Oedemeridae). Female oedemerid beetles have higher levels than the hopper *Lycorma delicatula* White (Hemiptera: Fulgoridae). The hemipteran *Huechys sanguinea* (De Geer) (Hemiptera: Cicadidae) was reported to contain cantharidin in "Pharmaceutical Fauna of China" and other books, the analytical results indicate that this species does not contain cantharidin. The absence of cantharidin in *H. sanguinea* was confirmed by Li et al. (2007). In China, also, more 9 species in 7 genera, outside family Meloidae, contained cantharidin. They include *Zanna*, *Fulgora* and *Lycorma* (Homoptera: Fulgoridae), *Oxocopsis*, *Heliocis*, *Xanthochroa* and *Oedemera* (Coleoptera: Oedemeridae) (Zhang et al., 2009). Recent studies on the chemical ecology of the fire-colored beetle *Neopyrochroa flabellata* (Coleoptera: Pyrochroidae) show that males use cantharidin both to entice prospective mates and as a mid-copulatory gift (Norton, 2002). Four beetles, belonging to the families Cerambycidae, Melyridae, Chrysomelidae and Cantharidae, were collected from different regions of Iran. Using GC-MS analysis, tiny amounts of cantharidin- far below the average content for Meloidae- were detected in the orange beetle *Cantharis livida* Linnaeus (Cantharidae), *Gastrophysa polygoni* Linnaeus (Chrysomelidae), malachite beetle *Malachius bipustulatus* Linnaeus (Melyridae) and capricorn beetle *Certallum ebulinum* (Linnaeus) (Cerambycidae) (Nikbakhtzadeh, 2009). For more information about canthariphily in insects, see: Young (1984), Frenzel et al. (1992), Hemp et al. (1999), Nardi and Bologna (2000), Shockley et al. (2009).

In plants, cantharidin has not been discovered, however, a seed extract with insecticidal activity, palasonin (demethylcantharidin) is another molecule with a similar structure of cantharidin. This compound is produced by an Indian tree known as Flame of the forest *Butea frondosa* Roxb. and Koen. (Leguminosea: Fabaceae) that grows in the Himalaya. Contrary to the cantharidin, the palasonin is chiral and exists with two enantiomeric forms where the 3-methyl group of cantharidin is missing (Bochis, 1960; Raj and Kurup, 1966; Rydberg and Meinwald, 1996; Rydberg and Meinwald, 1996; Dettner et al., 1997; Fietz et al., 2002; Dettner et al., 2003; Mebs et al., 2009).

Cantharidin isolation and determination

Cantharidin biosynthesis in the blister beetles had been

studied by Carrel et al. (1986, 1987, 1988, 1990). Cantharidin was chemically isolated from *M. proscarabaeus* (Dixon et al., 1963). Employing standard practices of analysis, which involved infrared and NMR spectroscopy, thin-layer chromatography, elemental analysis, and determination of physical constants, Walter and Cole (1967) isolated and quantitatively determined the cantharidin in *E. pestifera* in North America. It was, also, isolated from *Cyaneolytta sapphirina* Mäklin (Salama et al., 1974) and *Epicauta tomentosa* (Mäklin) (Hammouda and Salama, 1974).

Different meloid species infesting the alfalfa fields in Colorado (USA) had been subjected to determine their cantharidin levels. Significant differences in cantharidin concentrations were found among species *E. pennsylvanica*, spotted blister beetle *Epicauta maculata* (Say) and immaculate blister beetle *Epicauta immaculata* (Say) (Capinera et al., 1985). Cantharidin contents were determined for four *Epicauta* species abundant in northeast Kansas alfalfa fields. The three-striped blister beetle *Epicauta occidentalis* (Werner) and *E. pennsylvanica* typically contained much cantharidin (198-266 µg/beetle), representing nearly 1% of their dry body weight, significantly more toxin than possessed by *E. fabricii* and *E. pestifera*. Two species also exhibited significant intersexual differences in cantharidin content. *E. occidentalis* males had almost four times more cantharidin than females. In contrast, female *E. pennsylvanica* contained approximately six times as much of the substance as males (Blodgett et al., 1991). Cantharidin titre in blister beetles is depended on several factors including, age, sex and mating status (McCormick and Carrel, 1987, Carrel et al., 1993). During the period 1989-1991, an extensive survey of Meloidae in the Chinese provinces, Hebei, Nei Mongol, Xinjiang, Sichuan, Guangxi, Yunnan, was carried out Among 35 meloid species, there were 19 predominant species, such as *E. chinensis*, *Lytta caraganae* Pallas, *Mylabris calida* (Pallas) which were used for determination the quantitative levels of cantharidin. The results also show that the cantharidin levels vary among different species and also among individuals of the same species collected from different localities (Tan et al., 1995).

In addition, some specimens of 9 species from Meloidae were collected on various host-plants from different districts in China. The cantharidin was isolated by from dry bodies of adults and quantitatively analyzed. All specimens contain cantharidin and the content of cantharidin was higher in the male than that in the female of the same species. The cantharidin content in the female collected after the copulation peak was higher than that before copulation peak, this suggested that transfer of cantharidin from male to female occurred during copulation. (Yuling et al., 2001). Considering the titer of cantharidin in Iranian and non-Iranian meloids, no significant difference was found. The male before copulation has a high titer, while it decreased significantly just after copulation when most of the toxin transfers to

the females' sexual organ as a nuptial gift (Nikbakhtzadeh and Tirgari, 2002). Nikbakhtzadeh et al. (2007) precisely measured the titer of the compound in different organs of both sexes in order to study the cantharidin pharmacodynamics in male and female of *M. quadripunctata*. Yang et al. (2001) investigated the effect of mating on the cantharidin content in a field collected sample of the blister beetles *Epicauta mannerhimi*. They determined the changing regularity of cantharidin content in the mated and non-mated beetles and discussed the cause of formation of this regularity. Under laboratory conditions, Wang et al. (2008) analyzed the changes in level of cantharidin at different life stages of the blister beetle *Mylabris cichorii* Linnaeus. Their results showed that larvae accumulated cantharidin as they grow and develop. Adult beetles exhibit a pronounced sexual dimorphism in cantharidin biosynthesis, but total content of cantharidin produced by sex-mixed rearing group was much higher than that by sex-segregated rearing group. The highest cantharidin level presented in abdominal part of adults and lower level in thoracic part. Lowry and Bundy (2011) evaluated the relative toxicity of common blister beetle species in New Mexico in 2010 and determined the cantharidin levels in males and females of six different species. Their results showed that males generally had more cantharidin than females. Females exhibited an increase in cantharidin concentration as dry beetle weight decreased.

The bound cantharidin, free cantharidin and total cantharidin were determined in 12 species of Meloidae in different spaces by gas chromatography in China. The result showed that the total cantharidin contents were all more than 0.35% in Meloidae. The bound cantharidin and free cantharidin contents were partly more than 0.35% in Meloidae. The results suggested that the existing form of the bound cantharidin might be the magnesium cantharidate, calcium cantharidate, potassium cantharidate in Meloidae (Li, 2011). From the methodological view of point, a simple and rapid gas chromatographic analysis was described by Li et al. (2006) for the determination of cantharidin in *M. phalerata* from different places. Also, a simple, convenient and precised method for the determination of cantharidin in *Mylabris* and cantharidin products was presented (Guo et al., 2007). Li et al. (2007) used the gas chromatography to determine the total cantharidin and free cantharidin in eight species of Meloidae. They found that content of total cantharidin was higher than the content of free cantharidin in all meloid species. The gas chromatography was used to compare the change of cantharidin content in *Mylabris* before and after biotransformation. The determined contents were 0.7% and 1.29%, respectively (Xu et al., 2011). A headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) method was developed to determine a type of terpenoid named As cantharidin in the false blister beetles, family Oedemeridae (Mehdinia et al., 2011).

Cantharidin as pesticide and plant growth regulator

Previous authorities focused mainly on the resources investigation, mechanism and function of cantharidin naturally occurring in insects. Also, some institutions and researchers have been engaged in the cantharidin poisoning to animal and human and so many others have working on the use of cantharidin and its analogues in the medical field. Herein, the potentials of cantharidin as a pesticide and a plant growth regulator have been reviewed depending on the available literature.

Cantharidin as pesticide

Cantharidin has been developed as a new type of biopesticides because of its high insecticidal activity. However, the relationship of structure to insecticidal activity of cantharidin has not been ever clarified. The insecticidal activity of cantharidin was assessed on the 6th instar larvae of the Northern armyworm *Leucania separata* (Walker)(Lepidoptera: Noctuidae). Some histopathological effects had been recorded on the blood cells, mid-gut, thoracic ganglia, fat-bodies, Malpighian tubes and testis. The symptoms of cantharidin toxicity were mainly due to nervous injuries (Lu, 1964). Cantharidin exhibited obviously toxic activity on the diamondback moth, *Plutella xylostella* (Linnaeus)(Lepidoptera: Plutellidae)(Zhang et al., 1998). The control ratio of 0.1% cantharidin aqueous solution was 84.0%–98.7% on the cabbage white butterfly, *Pieris rapae* (Linnaeus) (Lepidoptera: Peridae), and 92.2%–99.2% control ratio on the green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae)(Mingjie, 2002). The feasibility of applied techniques on natural watery 0.1% cantharidin was estimated for controlling the damage of the cabbage aphid *Brevicoryne brassicae* Linnaeus, *M. persicae*, and pear aphid *Dysaphis pyri* (Boyer de Fonscolombe) (Homoptera: Aphididae), as well as for *P. xylostella* (Diao et al., 2003). In laboratory tests, 1.5% cantharidin aqueous solution showed significant contact toxicity on the fall armyworm, *Spodoptera frugiperda* (J.E. Smith)(Lepidoptera: Noctuidae)(Wei et al., 2007). A patent of insecticidal cantharidin micro-emulsion was formulated in China. Cantharidin micro-emulsions were assessed on the 3th instar larvae of *P. xylostella*. Cantharidin micro-emulsion had obvious toxic action of contact and stomach digestion. The results of field trials showed that all larval instars could be effectively controlled by a 400 fold dilution of 0.1 % cantharidin micro-emulsion (Li, 2007). Li et al. (2008) treated six pests with cantharidin to assess its insecticidal activity. Their results showed that cantharidin had a contact activity against all tested pests but a stomach activity against some pests. It exhibited no systemic activity or fumigant activity. The same authors found the bamboo-feeding leafhopper *Mukaria pallipes* Li and Chen (Hemiptera: Cicadellidae) as the most sensitive to

cantharidin and the 3rd instar larva was the most insensitive to cantharidin. In a laboratory study on *P. xylostella*, cantharidin exhibited stomach toxicity to 3rd larvae. At a concentration of 2 mg/mL cantharidin, the corrected mortality of the larvae was 100% and LC₅₀ was 515.58 µg/mL (Jinzhong et al., 2011). Cantharidin and its analogue, demethylcantharidin, had obviously insecticidal function on the 3th instar larvae of *P. xylostella*. LD₅₀ of demethylcantharidin and cantharidin were 819.5 µg/g and 8.6875 µg/g, respectively, the former is 94.4 times to the later. Thus, the insecticidal activity of cantharidin was found considerably stronger than its analogue (Yong et al., 2009). Some compounds had been synthesized from cantharidin by replacing the anhydride ring of norcantharidin with an aromatic amine or fatty amine with different electron density, respectively. A bioassay showed that compounds 6(a–m) lacked any larvicidal activity against *P. xylostella*; whereas their ring-opened partners 7(a–m) exhibited a variety of larvicidal activities. It was concluded that the improvement of insecticidal activity required a reasonable combination of both aliphatic amide and aromatic amide moieties (Sun et al., 2013). The lethal and sub-lethal effects of cantharidin were evaluated on *H. armigera* under laboratory conditions. Higher mortality on the larval, pupal and adult stages was recorded in cantharidin-treated beetles in comparison with controls. Reduction in the larval weight and malformation of wings were observed in cantharidin-treated population (Khan et al., 2013a).

Cantharidin is an effective antifeedant and deterrent to insects at certain concentrations (Carrel and Eisner, 1974) although it was not found as repellent to *P. xylostella* (Zhang et al., 1998). Against the same pest, cantharidin micro-emulsion exhibited a deterrent action (Li, 2007). In the laboratory, immersing of both larvae of *P. xylostella* and host-plant leaves into 1.0% cantharidin EC liquid caused a deterrence of the 3th larvae (Feng, 2007). In laboratory tests, 1.5% cantharidin aqueous solution showed significant antifeedant activity on armyworm, *S. frugiperda*. The more concentration of 1.5% cantharidin aqueous solution, the higher antifeeding rate on armyworm except the treatment of 400 mg/L after 24 h (Wei et al., 2007).

Cantharidin synergism and synergists had been reported in the literature. A synergic composition of cantharidin and organic phosphorous insecticide was invented. The invented mixture comprised cantharidin 0.1–10.0%, organic phosphorous insecticide 1–40.0%, and balancing pesticide adjuvant (Yalin et al., 2005). The toxicological effect of cantharidin and five insecticides was assessed, separately and in synergistic mixtures, on *P. xylostella*. The results indicated that cantharidin had different synergistic interaction with these insecticides and the best mixture was cantharidin with chlorfluazuron (Zheng et al., 2007).

In the field of insecticide resistance, cantharidin was assessed. The glutathione S-transferases (GSTs) are one of the major reasons for insecticide resistance.

Therefore, effectiveness of new candidate compounds depends on their ability to inhibit GSTs to prevent metabolic detoxification by insects. Using cantharidin on *H. armigera*, bioassay results of Khan et al. (2013b) showed that the down-regulation of glutathione S-transferase gene (HaGST) at the mRNA transcript ranged from 2.5 to 12.5 folds while biochemical assays showed *in vivo* inhibition of glutathione S-transferases in midgut and *in vitro* inhibition of rHaGST. Binding of cantharidin to HaGST was rationalized by homology and molecular docking simulations using a model glutathione S-transferases (1PN9) as a template structure. Molecular docking simulations also confirmed accurate docking of the cantharidin molecule to the active site of HaGST impeding its catalytic activity.

Functionally, cantharidin inhibits the protein phosphatase 2A (PP2A), an enzyme that operates in the metabolism of glycogen. To widen the understanding of its mode of action, as a biopesticide, some reported works may be fruitful. Obvious pathological changes in the midgut of the orient armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) and *P. xylostella* were observed following treatment with cantharidin. All observations indicated that cantharidin acts on the midgut membrane system (Zhang et al., 2003). Bioactivity of cantharidin was tested, by different laboratory bioassay methods, against eleven pests, such as the housefly *Musca domestica* (Diptera: Muscidae), Euonymus leaf notcher *Pryeria sinica* (Lepidoptera: Zygaenidae), the aphid *M. persicae*, and three mite species: two spotted spider mite *Tetranychus cinnabarinus* (Boisd.), hawthorn spider mite *Tetranychus viennensis* Zacher (Acari: Tetranychidae) and winter grain mite *Penthaleus major* (Dugés) (Acari: Prostigmata). The results showed that the cantharidin has insecticidal effect on different kinds of pests to different degrees, and its main mode of action is contact as well as stomach toxicity (Liu et al., 2010).

On the basis of enzymatic investigation, serine proteinases have been identified in gut extracts of many lepidopteran insects (Houseman et al., 1989) and many of these enzymes are inhibited by proteinase inhibitors. Serine proteinase inhibitors show anti-nutritional effects against several lepidopteran insects (Applebaum, 1985; Macedo et al., 2002). After feeding of the larvae of *P. xylostella* on cantharidin, the dynamics of some digestive enzymes and esterases were determined. The average activities of proteinase, lipase, and acid phosphatase decreased but α-amylase increased. The activities of acetylcholinesterase and carboxylesterase considerably increased. The larvae were lazy in their activity after cantharidin feeding and gradually died (Zhang et al., 2000). The enzymatic activities of cantharidin in the larvae of *M. separate* were assayed. The results showed that cantharidin can affect the metabolic enzymes distinctly, and the inhibition on alkaline phosphatase and polyphenol oxidase (PPO) may be related to its toxic effect (Ma et al., 2010). In a laboratory study on *P. xylostella*, cantharidin at the dosage of 12.5×10^{-3} mg/mL

against 3rd instar larvae enhanced the activities of glutathione-S-transferase and acetylcholinesterase at 4h or 12h post-treatment but prohibited them at 24h post-treatment (Jinzhong et al., 2011).

Molecular biology may help for more understanding the mode of action of cantharidin. In Lepidoptera, morphological changes of the cell livability and cell cycles arrest of insect cell lines Sf9 and Spex-VII were observed after treatment by cantharidin *in vitro* using the transmission electron microscope and flow cytometry. Cantharidin inhibited the growth of spex-VII and Sf9 in a dose-and-time dependent manner, and Sf9 was more sensitive to this compound (Chen et al., 2008). After treatment of 3rd instar larvae of *P. xylostalla* with cantharidin, the higher the dose of cantharidin, severer the DNA damage of lepidopteran cell lines. DNA damage of cantharidin incubated Sf-9 was of obvious dose-effect relation, which would indicate that DNA damage played a role in toxic effect mechanism (Jinzhong et al., 2011). Zhang et al. (2011) obtained results showing that the two cell lines presented apoptosis features after treatment with cantharidin such as chromatin condensation, nucleic fragmentation and apoptotic body formation etc. As the treated concentration of cantharidin increased the ratio of necrotic cells increased.

Cantharidin as plant growth regulator

Cantharidin is a potent plant growth regulator but exhibits a phytotoxic action at certain concentrations and an inhibitory action on the growth at other concentrations. It may be a selective herbicide and a selective mitotic inhibitor (Cutler, 1975). Protein phosphatases catalyze a multitude of reactions crucial to the regulation of nearly every cellular process, such as gene transcription and translation, metabolism, protein-protein interactions, protein activity, and apoptosis (Ayaydin et al., 2000). Protein phosphatases and kinases maintain a sensitive balance between phosphorylated and dephosphorylated forms of proteins playing important roles in signal transduction pathways and regulation of gene expression, cellular proliferation, cell differentiation, apoptosis and other processes in plants. Cantharidin was reported as an inhibitor of types 1 and 2A protein phosphatases for okadaic acid and calyculin A in plants (Smith et al., 1994). For investigating the molecular mechanisms which control the morphogenesis of primary roots of the mouse-ear cress *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) and the organization of cortical microtubules in response to inhibitors of protein phosphatases and kinases, Baskin and Wilson (1997) recorded root swelling and disorganized cortical microtubules after treatment with cantharidin. Gernand et al. (2003) found a high level of serine 28 as well as of serine 10 phosphorylation along the entire mitotic monocentric chromosomes after treatment of mitotic cells with cantharidin. As a potent inhibitor of protein

serine/threonine phosphatases (PPPs), especially PP2A and PP4, 200 μ M (IC(30)) of cantharidin was applied by Basja et al. (2011) on the foliage of 12-day-old *A. thaliana* seedlings and the entire transcriptome profile was determined at 2, 10 and 24 h after treatment. These authors concluded that cantharidin interfered with the activities of PPPs that interact directly or indirectly with receptors or are located near the beginning of signal transduction pathways.

Polar auxin transport, mediated by two distinct plasma membrane-localized auxin influx and efflux carrier proteins/complexes, plays an important role in many plant growth and developmental processes. Protein phosphatases, sensitive to cantharidin and okadaic acid, are involved in regulating AGR1/PIN2-mediated root basipetal auxin transport and gravitropism, as well as auxin response in the root central elongation zone (Shin et al., 2005). The heavy metals had inhibitory action on the modification of plasma membrane H⁺-ATPase (EC 3.6.3.14) activity in cucumber roots. The effect of cantharidin on the inhibitory action of metals on the H⁺-ATPase was determined. The inhibition of the plasma membrane proton pump caused by 100 mM Cd, Cu and Ni was partially diminished in the presence of cantharidin (Janicka-Russak et al., 2008). Cantharidin has an ability to destabilize membranes stronger than that observed with endothall (Dayan and Watson, 2011).

The *in vivo* induction of H₂O₂ production was tested on tobacco cell suspension cultures of the tobacco cultivar (*Nicotiana tabacum* cv. Bright Yellow-2) Linnaeus (Solanaceae). The phosphatase inhibitor cantharidin induced a transient increase in H₂O₂ synthesis (Van Gestelen et al., 1998). Cantharidin induced an increasing synthesis of H₂O₂, also, in Norway spruce *Picea abies* (Linnaeus) (Pinaceae) cells. In addition, the cantharidin-induced release of K⁺ was more pronounced and longer lasting than that caused by other elicitors (Hebe et al., 1999). Cona et al. (2006) examined the specific contribution of polyamine oxidase, a hydrogen peroxide (H₂O₂)-producing enzyme, to the oxidative burst induced in maize *Zea mays* Linnaeus (Poaceae) mesocotyl after treatment with cantharidin. In the maize mesocotyl, N-prenylagmatine and its structural analogue Ro5, as well as diphenyleioidonium, strongly affected the H₂O₂ production as induced by cantharidin.

In mitosis, plant chromosomes are highly phosphorylated in the pericentromeric region only. In order to modulate histone H3 phosphorylation, root meristems of different plant species were treated with cantharidin. Immunostaining revealed a high level of H3 phosphorylation along the whole mitotic chromosome after cantharidin treatment, which resembles the distribution seen exclusively in first meiotic division. Cantharidin affected spindle assembly and chromosome length, but these effects did not seem to be directly linked to changes in H3 phosphorylation (Manzanero et al., 2002). *Oryza grandiglumis* Chitinase IVa(OgChitIVa) cDNA encoding a class IV chitinase was cloned from wild

rice. Southern blot analysis showed that *OgChitIVa* genes are present as two copies in the wild rice genome. The RNA expression of *Og-ChitIVa* was induced by defense/stress signaling chemicals, such as jasmonic acid, salicylic acid, and ethephon or cantharidin and endothall or wounding, and yeast extract (Pak et al., 2009). The mutant seedlings were more sensitive than the wild type to the phosphatase inhibitors okadaic acid and cantharidin. Treatment of wild-type seedlings with the appropriate concentration of cantharidin phenocopied the *trcn1-1* mutation (Tseng and Briggs, 2010).

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) is a derivative of cantharidin and was first synthesized in 1929 (von Bruchhausen and Bersch 1929). In other words, endothall and cantharidin are close chemical analogues. The toxic effects of cantharidin and its analog endothall, were attributable to their high affinity and specificity for a cantharidin-binding protein. Inhibition of PP2A activity may account for the diverse effects and toxicity of cantharidin and its analog, endothall, in mammals and possibly plants (Li and Casida, 1992). Endothall has been used as an aquatic herbicide for several decades ago (Hiltbran 1963, Thayer et al. 2001). In 1960, Pennsalt Chemical Company registered endothall for use as an aquatic herbicide (MacDonald et al., 2002). Endothall is also registered for use as a preharvest defoliant for cotton *Gossypium hirsutum* Linnaeus (Malvales), as a potato vine *Solanum tuberosum* Linnaeus desiccant and harvest aid for alfalfa *Medicago sativa* Linnaeus (Fabaceae) and clover (*Trifolium* spp.) (Anonymous, 2001). Endothall had been reported to cause cell membrane dysfunction (Rikin and Rubin, 1993). Early events of signaling cascade involve changes in protein phosphorylation pattern, which eventually regulate various cellular processes in eukaryotes, including plant defense responses (Conrath et al., 1997). Phosphorylation of proteins is a transient process, can be regulated by using protein phosphatase inhibitors- cantharidin and endothall (Millward et al., 1999). Endothall is known to be a strong inhibitor of plant serine/threonine protein phosphatases controlling many cellular processes (Ayaydin et al., 2000). Treatment of tobacco (*N. tabacum*) cells with cysteine triggers a signal pathway culminating in a large loss of mitochondrial cytochrome (cyt) pathway capacity. The down-regulation of the cyt path requires events outside the mitochondrion and is effectively blocked by cantharidin or endothall, indicating that protein dephosphorylation is one critical process involved (Vanlerberghe et al., 2002). Duke (2010) found that the natural product cantharidin is more phytotoxic than its structural analogue endothall in the laboratory. The phytotoxicity of endothall and cantharidin was directly related to their ability to inhibit plant serine/threonine protein phosphatase activity (Bajsa et al., 2012). As concluded by the latter authors, the molecular target site of endothall in plants is similar to that of cantharidin in animals, namely, serine/threonine

protein phosphatases responsible for regulating an array of biochemical processes. This mode of action is unlike any other commercial herbicide.

Cantharidin effects on lower plants and microflora had been scarcely reported in the literature. Protein serine/threonine phosphatases were found implicating in the regulation of circadian rhythmicity in the marine dinoflagellate *Gonyaulax polyedra*. Cantharidin caused large phase delays but no persistent effect on period, as measured by the bioluminescent glow rhythm (Comolli et al., 1996). The inhibitory action of cantharidin on phytopathogen was examined. The inhibition of cantharidin was assessed on 6 plant pathogenic fungi including *Pellicularia sasakii*, *Pellicularia filamentosa* (Ceratobasidiaceae), *Sclerotinia sclerotiorum* (Sclerotiniaceae), *Glomerella cingulata* (Glomerellaceae). Treatment with 500 mg/L cantharidin to *P. sasakii*, *P. filamentosa*, and *G. cingulata* caused stronger inhibition (Yueli and Guanjun, 2003). An array of chemicals capable of specifically inhibiting/affecting most of the vital systems of the plant cell was employed to evaluate a range of systems vital in promoting non-host resistance in the pea pathogen *Fusarium solani* f. sp. *phaseoli* (Nectriaceae)/ pea endocarp interaction. At low concentrations the treatments with cantharidin, among some other inhibitors of protein phosphatases and kinase inhibitors transiently enhanced the resistance to the pea pathogen (Hartney et al., 2007). The omnivorous plant pathogen *Sclerotinia sclerotiorum* is a necrotrophic, with worldwide distribution. Plant infection depends on the formation of melanin-rich infection cushions, and secretion of hydrolytic enzymes and oxalic acid. Type 2A Ser/Thr phosphatases (PP2As) are involved in the regulation of a variety of cellular process. In the presence of cantharidin, hyphal elongation and sclerotia numbers were impaired whereas sclerotial size increased (Erental et al., 2007). The antifungal activities of cantharidin and demethylcantharidin against the fungus *Valsa mali* (Valsaceae) and other 6 plant pathogens were studied. The results showed that cantharidin affected all tested plant pathogens (Cao et al., 2008). The biodegradation of cantharidin by two species of parasitic fungi of Meloid beetles, *Beauveria bassiana* (Cordycipitaceae) and *Aspergillus* sp., was studied. *B. bassiana* decomposed 90.45% of cantharidin, and *Aspergillus* sp. did not decompose cantharidin (Li et al., 2008).

Cantharidin and environmental safety

Is the cantharidin environmentally safe when be used as natural insecticide? Although cantharidin was reported highly toxic to most mammals, birds and frogs (Carrel and Eisner, 1974; Schmidt, 2002), the watery 0.1% cantharidin was found harmless to crops (Diao et al., 2003). The polyps of the Upside-down jellyfish *Cassiopea andromeda* Forskål (Rhizostomae: Cassiopeidae) produce spindle shaped, freely swimming buds which do

not develop a head (a mouth opening surrounded by tentacles) and a foot (a sticky plate at the opposite end) until settlement to a suited substrate. Cantharidin induced head and foot formation in buds very efficiently in a 30 min treatment (Kehls et al., 1999). Feng (2007) evaluated the safety of cantharidin to non-target organisms in China. It showed no or low toxicity to vegetables and non-target organisms. Also, it had low toxicity to soil microorganisms. The toxicities of 1.0% cantharidin EC to non-target organisms and their safety evaluation were studied, according to "Experimental Guideline for Environmental Safety Evaluation of Chemical Pesticides". The results showed that cantharidin had low toxicities to earthworm and soil microorganisms, high toxicities to bee and silkworm, medium toxicities to tadpole (Feng and Zhang, 2007). In China, also, the study of Cui (2009) indicated that 1.0% cantharidin EC had low toxicity against quails, ladybugs and soil microorganisms, but had a moderate toxicity against fish. The same author concluded that cantharidin can be considered one type of weak motility pesticides, and it was mainly absorbed by soil in the soil-water system in balance and doesn't pollute the environment; Cantharidin degrades fast in soil and water, and it is lower persistent in environmental media.

CONCLUSION

The food utilization and energy metabolism of blister beetles had attracting some research attention in the world. Less attention had been paid to the reproductive physiology and environmental physiology. Unfortunately, the physiological events and biochemical process of blister beetle diapause are scarcely reported in the available literature. On the other hand, cantharidin physiology and biochemistry have gained more interest of researchers all over the world. In general, particular interests to several physiological and biochemical aspects of blister beetles must be created.

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