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Cantharidin: A chemical precursor for the development of novel bioinsecticides

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Bioinsecticides based on natural toxins offer an alternative means of pest management with the potential to counter insecticide resistance and reduce our heavy reliance on conventional insecticides. Cantharidin is a natural toxin generally produced by the beetles belonging to the family, Meloidae. It has been a drug of choice in both folk and traditional medicine, especially for the topical treatment of viral skin infections such as warts and molluscum. Historically, it has also been used as an aphrodisiac. Besides, cantharidin is also being used as an insecticide in the form of an emulsifiable concentrate (EC) for the control of lepidopteran pests. Although cantharidin has been proved highly effective against a variety of insect pests, its chemical synthesis and potential toxicity to non-target organisms have been a serious concern. A great deal of research is being carried out to synthesize its bioactive analogues with high bioactivity and improved safety profile to non-target organisms or the environment. Many promising analogues of cantharidin have already been synthesized and their effectiveness to several pest species has been reported. Due to the unique mode of action, these analogues will help to reduce the development of insecticide resistance and may be more cost-effective than cantharidin-based insecticides.

Keywords: Cantharidin, insecticide, emulsifiable concentrate, structural relationship activity, protein serine/threonine phosphatase

INTRODUCTION

Cantharidin (*exo*-1,2-*cis*-dimethyl-3,6-epoxy-hexahydrophthalic anhydride) is a widely distributed compound in the insects belonging to order Coleoptera and family Meloidae, some species of Tenebrionidae, Cerambycidae, and Fulgoridae [1-3]. Some species of the Meloidae, commonly known as blister beetles, secrete a chemical blistering compound, cantharidin as a defensive mean. The beetles belonging to this family are considered cosmopolitan, however, their presence in New Zealand, Antarctic regions, tropical and subtropical savannas has not been reported [3].

Several species belonging to the insect family Meloidae produce a poisonous compound with comparable toxicity to strychnine and cyanide, used by the insect as a defensive tool against predators. Its toxicity has been observed in several organs such as the digestive tract, and kidneys in mammals. However, despite its poisonous effects on several organs, historically cantharidin has been used as a medicine for centuries [4].

In 1810, Robiquet first obtained the crude crystals of cantharidin from *Lytta vesicatoria* in Spain [5]. Later on in 1877, a chemist named Piccard determined the molecular formula of cantharidin $C_{10}H_{12}O_4$ [6] and Gadamer in 1914 identified the molecular structure of cantharidin (Fig. 1) [7]. The structure and chemical properties have been well documented in the past [8, 9].

Cantharidin is a white crystalline compound having a molecular weight of 196.2 g/mol, melting point of 215-216 °C and boiling point of 326.9±35.0 °C at 760 mmHg. Its solubility in organic solvents such as chloroform, acetone, dichloromethane, ethyl acetate is better compared to that in ether [10, 11].

The biosynthesis of cantharidin has been investigated by several scientists, however, its exact biosynthetic pathway has not been entirely understood [12]. Biosynthesis of cantharidin in *Mylabris calida* was investigated through the protein expression at early and advanced stages [13]. The process of cantharidin biosynthesis was also studied [14] while investigating the cantharidin biosynthesis and mevalonate pathway relationship.

Compounds based on natural sources are still important and may be used as precursors for the synthetical development of new bioactive molecules [15]. Compounds of natural origin and their products will play a pivotal role in the development of new compounds [15, 16].

Biopesticides based on cantharidin as an active ingredient have been developed and marketed especially in China for the control of lepidopteran pests in general. The high toxicity of cantharidin has already been documented against various insect pests. Different emulsifiable concentrate (EC) formulations have been developed and successfully used as an insecticide against the different orders of insects.

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The use of cantharidin for large-scale agricultural use has raised environmental concern for its potential non-target effects. Chemical synthesis of cantharidin and its extraction from blister beetle is a tedious job. At present intensive research is being done to synthesize bioactive analogues of cantharidin with an objective of low-cost production and reduced non-target effects. The present review on cantharidin was carried out on its biosynthesis, chemical synthesis, insecticidal use, non-target effects, and structure-activity relationship. Due to the development of insecticide resistance and the pursuit of new chemical molecules, it is very timely to review the body of literature available on various aspects of cantharidin, especially insecticidal use, and to set it in future contexts.

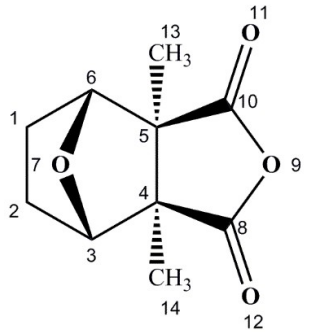


Figure 1. Structural formula of cantharidin and different atomic sites

SYNTHESIS OF CANTHARIDIN

Biosynthesis of cantharidin in insects

Cantharidin is a poisonous defensive compound, widely found in Meloidae beetles [3]. The production of cantharidin is gender-biased and only male insects can secrete it, whereas female insects cannot synthesize cantharidin [17], however, they acquire it by mating with the male. Cantharidin is found in both males and females after mating and its synthesis is continued after mating [18]. The highest quantity of cantharidin is found in the male gonads suggesting that this organ may be involved in the biosynthesis of cantharidin. The idea has been rejected that accessory glands are involved in the biosynthesis of cantharidin [19]. The cantharidin content in salivary glands and digestive tract was higher in the larvae, whereas in adults the level of cantharidin is higher in the hemolymph which is discharged from the leg joints [20].

The previous research shows that cantharidin can be synthesized at various developmental stages, except eggs where cantharidin was detected only on the surface [21]. The content of cantharidin in larvae of *Mylabris cicharii* increased with the development of larvae [22]. Change of cantharidin contents in a different age of *Epicauta chinesis* was assessed by gas chromatography and it was found that there were two peaks of cantharidin synthesis, which were at larval pseudopodae and adult eclosion after 6-8 days [23]. The content of cantharidin in males was increased and the content of cantharidin in females kept in isolation for 60-90 days gradually decreased to a very low level. The above studies indicated that cantharidin could be synthesized before eclosion, and cantharidin could not be produced by a female, it is rather transferred to the female at copulation.

The biosynthesis of cantharidin *in vivo* is a complex process and it is very difficult to determine its exact pathway. However, its biosynthesis through mevalonate pathway is widely accepted [24, 25]. The isotope-labeled acetate and mevalonate in insect feeding experiments suggested the formation of cantharidin by either linkage of two isoprene units in tail-to-tail or head-to-tail configuration. Subsequently, 10 carbon molecules of cantharidin were derived from mevalonate or farnesol precursor by a series of ^{3}H and ^{14}C-labeled farnesol in incorporation experiments [26]. The stable isotope labeling technique was used to determine the transformation of farnesol in the biosynthesis of cantharidin in the male blister beetle *Epicauta pestifera* [19]. In insects, metabolic transformation responsible for the biosynthesis of juvenile hormone oxidizes farnesol to methyl farnesoate in blister beetles suggesting both synthesis of JH and cantharidin sharing the same pathway [27]. Injecting the juvenile hormone synthesis inhibitor, 6-fluoromevalonate (FMVA), can cause a significant reduction in cantharidin production [28]. Recently, a mevalonate pathway gene, 3-hydroxymethyl coenzyme A reductase (HMGR) from the blister beetle *Epicauta mannerheimi* (Maklin) was cloned by RACE technology [29]. The phylogenetic investigation disclosed that EmHMGR has the closest association with HMGRs in chrysomelids. Three genes were identified from *Epicauta chinensis* (methyl farnesoate epoxidase (*EcMFE*), juvenile hormone acid O-methyltransferase (*EcJHAMT*) and juvenile hormone epoxide hydrolase (*EcJHEH*) [30]. It was further demonstrated that interference of *EcMFE* and *EcJHEH* significantly inhibited the biosynthesis of cantharidin in male *E. chinensis* after mating, but the interference of *EcJHAMT did not* influence the biosynthesis of cantharidin.

Chemical synthesis of cantharidin

Earlier, cantharidin was being extracted from the bodies of Meloidae beetles, however, the provision of raw materials and the extraction process was

cumbersome and low yielding. The chemical synthesis program has become a good alternate solution for the production of cantharidin.

Bruchhausen has tried furan and dimethylmaleic anhydride as raw material, by Diels-Alder reaction for the synthesis of cantharidin [31]. However, the synthesis of cantharidin under natural conditions is prone to dehydrogenation and leads to a spontaneous retro Diels-Alder reaction.

In the 1950s, Gilbert Stork synthesized cantharidin in a multistep chemical reaction. In the final step, he purified liquid diene through chromatography and used ethyl acetate to ozonize it at -60°C [32]. The crude cantharidin with a melting point of 209-212°C was obtained by the decomposition of the ozonide with hydrogen peroxide. The crude cantharidin was recrystallized from acetone with a melting point of 212-213°C. The synthetic cantharidin was identified by comparing its X-ray powder diffraction pattern and infrared spectrum with those of natural cantharidin.

Subsequently, another chemist, Gnther Otto Schenck, improved this method. He used 1,4-butadiene and 3,4-dimethyl maleic anhydride as raw material, through the classic 7-step reaction to obtain the final product cantharidin [33]. Although some of the key steps of this method are still very demanding as regards reaction conditions, for a long time this method has been the main method of cantharidin synthesis.

In 1980, William G. Dauben proposed a two-step synthesis of cantharidin solution [34]. The reaction of furan and 2,5-dihydrothiophene-3,4-dicarboxylic anhydride in the presence of methylene chloride at 15 kbar at room temperature for 6 h gave a cycloadducts mixture of isomers. The Raney nickel desulfurization of one of the isomers gave cantharidin as identified by IR, NMR spectroscopy and melting point. Although this method is simple, the extremely high pressure required for the reaction limits its use for a large amount of synthetic cantharidin. So far, low-cost synthesis of cantharidin at a commercial scale was not successful.

CANTHARIDIN AS AN INSECTICIDE

Historical perspective of cantharidin use as an insecticide

Cantharidin has toxic effects on *Phyllopertha horticola*, *Malacosoma neustria,* and *Pyrrhocoris apterus* before the emergence of chemical insecticides [35]. The strong antifeedant activity of cantharidin was reported against insects [17].

Several canthariphilous insects were confirmed to have lower cantharidin contents, namely, ceratopogonids, *Atrichopogon oedemerarum* and *A. trifasciatus* trapped in the field [36]. Three canthariphilous insects were reported to have been attracted towards cantharidin bait in different parts of Africa. From the beetle family Anthicidae, two *Aulacoderus,* seven *Formicomus,* two *Mecynotarsus,* 11 *Notoxus,* three *Tomoderus,* and one *Cyclodinus, Omonadus, Pseudoleptaleus, Sapintus,* and *Tenuicomus* species were noted. The chrysomelid species *Barombiella vicina* and *Barombiella* sp. (Coleoptera: Chrysomelidae) were trapped at cantharidin besides *Pallenothriocera rufimembrls* (Coleoptera: Cleridae) [37].

The toxicity of cantharidin as an insecticide was determined in the laboratory, as well as by field tests for effective control of pests. Cantharidin was found highly effective in a laboratory bioassay against *Plutella xylostella* [38]. There are further reports of strong contact, stomach poisoning and antifeedant activity of cantharidin against larvae of *Plutella xylostella*.

In a previous research, 1.5% cantharidin aqueous solution had strong antifeedant activity, contact activity to armyworm, *Spodoptera frugiperda* [39]. The contact LD_{50} of the 4th instar larvae was 0.45 mg/ kg, the antifeedant EC_{50} value was 2.56 mg/ L. The effects of cantharidin on 6 different pests, *Mukaria pallipes*, *Bambusiphaga furca*, *Agrotis ipislon*, *Nilaparvata lugens, Sogatella furcifera* and *Plutella xylostella* were evaluated [40]. Cantharidin showed contact, stomach activity, but no systemic and fumigation activity was observed. Effects of 1.0 % cantharidin EC were tested using different bioassay methods on *Musca domestica*, *Stiophilus zeamais*, *Pryeria sinica*, *Lipaphis erysimi*, *Macrosiphoniella sanborni*, *Myzus persicae*, *Macrosiphum roswomm*, *Hyaloptera amygdali*, *Tetranychus cinnabarinus*, *Phethaleus major*, *Tetranychus viennensis* and *Myzus persicae*, and different degrees of toxicity against these pests were found [41]. Cantharidin also showed a significant synergistic effect. It has been reported that cantharidin mixed with different groups of insecticides such as abamectin, endosulfan, chlorfluazuron, bisultap, and methomyl showed different levels of synergism, and the best mix was found to be cantharidin with chlorfluazuron [42]. Recently, the researchers found that a sublethal dose of cantharidin can cause abnormalities in population parameters such as intrinsic rate of increase (r), finite rate of increase (rm), net reproductive rate (R_0) and mean generation time (T) index of *Helicoverpa armigera* [43]. The fertility and fecundity were also significantly affected. Besides, its effects on morphological abnormalities were also reported. The sublethal dose of cantharidin

caused similar effects in the armyworm, *Mythimna separata* under laboratory conditions [44].

Field efficacy tests showed that 0.1 % aqueous cantharidin solution was effective against certain sucking pests such as *Brevicoryne brassicae*, *Pieris rapae*, *Myzus persicae,* and *Schizaphis piricola* and chewing pests such as *Plutella xylostella* under field conditions [45]. The control of 0.01 % of cantharidin aqueous solution against the green peach aphid, *Myzus persicae,* and oriental aphid, *Schizaphis piricola* reached 90.2 % and 88 %, respectively, in the field control trials [46]. Toxicity and sublethal effects of cantharidin were documented against housefly, *Musca domestica*. Both low and high concentrations of cantharidin either caused negative effects on population parameters or caused mortality [47]. In more recent studies a microemulsion of norcantharidin was tested against *P. xylostella* in a laboratory bioassay and acute LC_{50} at 12.477 mg/L was determined [48].

Safety of cantharidin against non-target organisms

Although cantharidin has good toxicity to many insect pests, it is important to evaluate whether cantharidin has a negative impact on non-target organisms and the environment, which is an important prerequisite for the development of new pesticides. The toxicity of 1.0 % cantharidin EC to five different organisms such as bees, silkworms, tadpoles, earthworms, and soil microorganisms was determined [11]. The results showed that 1.0 % cantharidin EC showed low toxicity to earthworms and soil microbes, high toxicity to bees and silkworms, and moderate toxicity to tadpoles. Toxicity of pure cantharidin and 1.0 % cantharidin EC to some non-target organisms according to the "Experimental Guideline for Environmental Safety Evaluation of Chemical Pesticides" was also computed [49]. It was found that cantharidin and 1.0 % cantharidin EC showed low toxicity against quail, ladybugs and soil microorganisms, whereas moderate toxicity to fish.

Cantharidin and norcantharidin induced adverse effects on soil invertase and phosphatase activity and fungal gene structure, but the effect was transient in nature. The adverse effects of these biopesticides vanished within two weeks after application in soil. The degradation of cantharidin and norcantharidin in the soil can be completed within a few days in soil [50].

Insecticidal mode of action of cantharidin and its derivatives

Histological observation can directly detect the changes of cells and tissues after cantharidin poisoning, and provide a pathologic basis for clarification of insecticidal mechanism. For the first time in 1964, poisoning symptoms and other cytological changes after the treatment with cantharidin were observed in various tissues of late instar *Mythimna separata* [51]. The poisoning process of the armyworm is divided into three stages: paralysis, coma, and death. After the poisoning, the number of blood cells in the body was decreased; the mesenteric epithelium was separated from the basement membrane; the parietal cells of the Malpighian tubules were disintegrated and the lumen was filled with pus; the nerve cells were blurred and the nerve fibers were assembled and dissolved. Moreover, symptoms such as cell nucleus swelling, partial disintegration; male germ cell division held at the spermatocyte stage were also observed. It is speculated that cantharidin first acts on the nervous system, breaking the ring neurons, hindering nerve conduction, resulting in muscle movement, showing paralysis symptoms, and other tissue lesions as secondary symptoms. Mesenteric tissues of *M. separata* and *Plutella xylostella* were investigated by optical microscopy and transmission electron microscopy after the cantharidin poisoning [52]. There were obvious histopathological changes, such as cell microvilli shedding, mitochondrial dissolution, rupture, ribosome shedding, swelling of nuclei and so on, and it was speculated that there might be cantharidin-specific binding sites in the midgut cell membrane. Effects of cantharidin on cell proliferation were also reported [53]. Although cantharidin was found to inhibit the growth of both spex-VII and Sf9 in a dose-dependent manner, Sf9 showed more sensitivity towards cantharidin. Moreover, both cells showed apoptotic features such as chromatin condensation, nucleic fragmentation, intact cell membrane and formation of the apoptotic body. Cantharidin poisoning has also been reported to have an impact on the level of enzyme activity in insects. In a study, it was found that cantharidin had no significant effect on the activities of phosphatase and larval digestive enzymes of *Plutella xylostella* such as protease, lipase, and α-amylase but the activities of acetylcholinesterase and carboxylesterase significantly increased [54].

Changes of alkaline phosphatase, acid phosphatase, carboxylesterase, glutathione S-transferase and cytochrome P450 enzyme system in 5^{th} instar larvae of *Mythimna separata,* Walker at different times after feeding with cantharidin were investigated [55]. The activities of PPO and alkaline phosphatase decreased with time, the acid phosphatase activity increased at a later stage, the activity of glutathione S-transferase at first increased

and later on decreased; cytochrome P450 enzyme activity was inhibited at first, afterward activated. It was suggested that the toxicity of cantharidin to *Mythimna separata* may be related to its inhibitory activity on alkaline phosphatase and polyphenol oxidase. The activity of carboxylesterase increased with the increase in treatment time.

Although some scholars have done a lot of research on the insecticidal mechanism of cantharidin, at present, the specific mechanism of cantharidin is still unclear, and the main role of cantharidin in insects has not been reported. The main target of cantharidin in mammals is PP2A. In addition, it has also strong inhibitory activity on the protein serine/threonine phosphatase (PSP) family such as PP1, PP4, PP5, PP6, PP7, which catalyze the dephosphorylation of substrate proteins, participate in almost all physiological processes [56]. Once the enzymatic activity of PSPs is inhibited or lost, it can cause the disorder of normal cell activities and even lead to cell apoptosis. The current studies on the effects of cantharidin on PSPs are largely concentrated in mammals, plants, whereas, its role in the regulation of insects' PSPs has not been widely reported until 2014. The PSPs family is considered to be one of the most conserved proteins in eukaryotes. In a recent study, cantharidin, okadaic acid, and endothall were tested for their inhibitory effects on protein phosphatase 5 (PP5) in *Helicoverpa armigera*, *Mythmna separata,* and *Plutella xylostella*. Strong inhibitory effects of cantharidin were noticed on HaPP5, MsPP5 and PxPP5 compared to okadaic acid and endothall [57]. Apart from its inhibitory effects on PPs family cantharidin was found to have strong inhibitory effects on heat shock protein (HSP) at the transcriptional level. In the experiment, it was found that cantharidin in *P. xylostella* down-regulates sHSP19.23, sHSP 19.5, sHSP 20.06, sHSP 20.09, sHSP 20.1, sHSP 21.9, sHSP 23.4, sHSP 27.5 and sHSP 28.9 (Fig. 2).

Cantharidin and its analogue cantharidin-24 were used in combination with cry2ab on *Mythmna separata* and its effects on growth, hydrolytic and detoxifying enzymes were investigated. The mixture of cantharidin and its analogue with cry2ab had adverse effects on larval weight, In addition, alkaline phosphatase and acid phosphatase were inhibited, whereas glutathione S-transferase was unregulated in sublethal concentration. It was further suggested that the combination of cantharidin and its analogue has a potential in pest management [58].

The rationale behind the use of cantharidin and its analogues as an insecticide

Due to the high dependence on chemical insecticides in pest control, serious 3R (resistance, resurgence, residue) problems, especially the rapid development of insecticide resistance, have been caused.

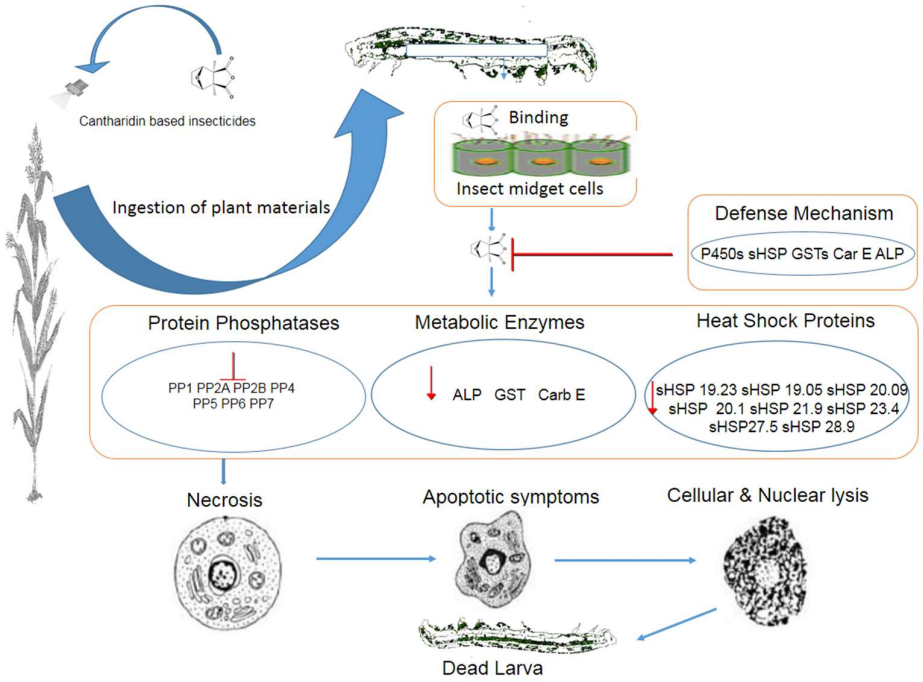


Figure 2. Putative model for the toxicity of cantharidin in insects

However, the development of insect resistance to biopesticides is relatively slow [59]. Biopesticides generally having better selectivity, less pressure on the environment, non-target biological safety, are becoming the focus of new pesticide research and development. Cantharidin is a kind of defensive toxin produced by the insects of Meloidae. A lot of studies have shown that it has good toxicity to many kinds of insects, and cantharidin can also be used in combination with other traditional chemical insecticides showing a significant synergistic effect. Cantharidin is of potential use in pest control and other agricultural applications.

Structural relationship of cantharidin's derivatives to insecticidal activity

Investigations into the structural relationship of cantharidin and its analogues with insecticidal and PPs inhibitory activity has been already documented. The methyl at either atomic site 4-C or 5-C does not significantly affect the activity of PP2B [60], however, it is considered beneficial for the PP1 and PP2A inhibition. Substitution at 3-C or 6-C causes decrease in inhibition capabilities to all PPs. Substitution at both positions will abolish the activity towards PPs. The oxygen bridge is essential for its activity. Anhydride oxygen at site 9 is considered good for its activity towards PP2A but S is considered better (Table 1) [61].

In a recent investigation complete loss of bioactivity was observed when anhydride oxygen of norcantharidin was replaced with nitrogen. The replacement of a cyclic anhydride oxygen atom with N–H and N–alkyl or aryl caused a total loss of larvicidal activity of the compound. Aliphatic amide moiety substituents containing $–CH_3$, $–CH(CH_3)_2$ and $–CH_2(CH_2)_2CH_3$ were used to see the effect of structure-activity relationship. The results showed that the compound containing $–CH_3$ showed significantly higher mortality on *Plutella xylostella* larvae compared to other moieties when used in a concentration of 500 µg mL^{-1}. Subsequently, electron-contributing $–OCH_3$ and electron-drawing $–CF_3$, $–OCF_3$, F and $–CO_2H$ substituents were substituted with aniline ring of the compound to see the effect of electron movement on activity. It was observed that the position of the substituents on the aniline ring has a direct impact on larval mortality. Moreover, substituents with electron-drawing ability demonstrated high larval kill against *Plutella xylostella* compared to the substituents with electron-contributing substituents (Table 2) [62].

Table 1. Structural parts of cantharidin molecule for bioactivity

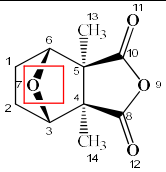
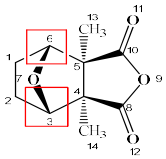
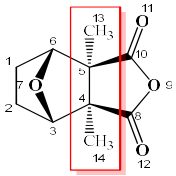
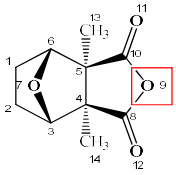
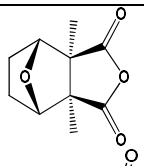
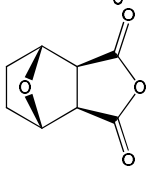
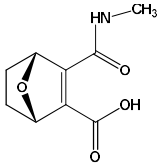
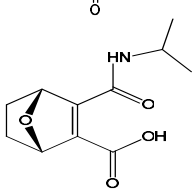
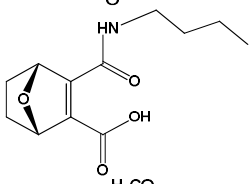
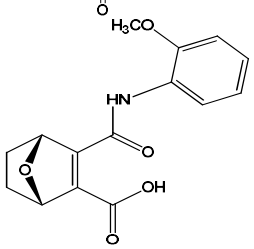
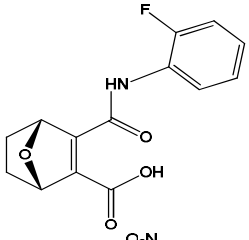
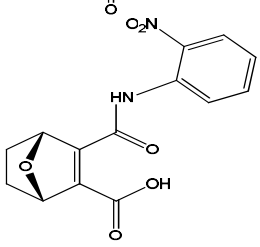
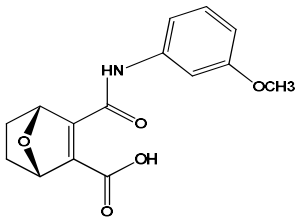
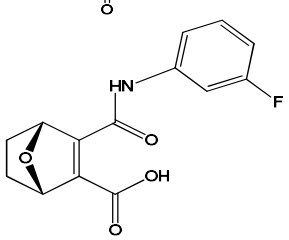
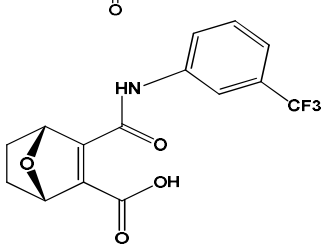
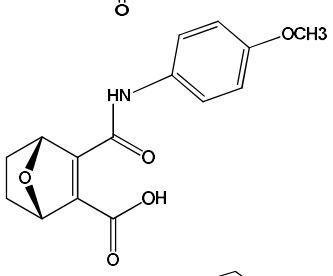
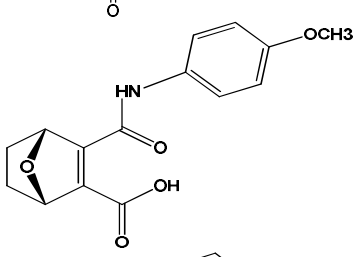
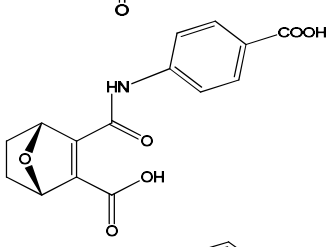
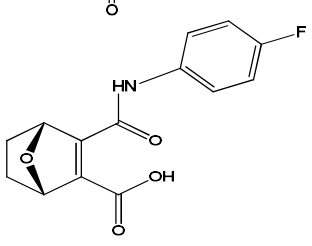
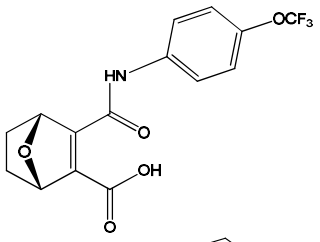
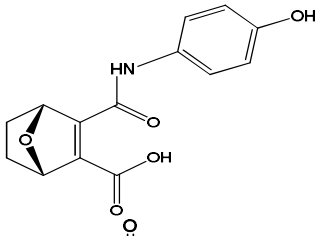
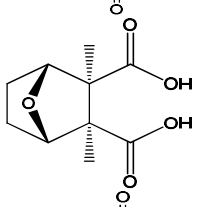
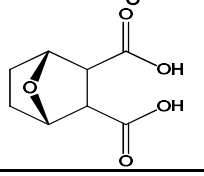
Atomic site	Structural part	Function
7		Bridging of O is indispensable for activity
3, 6		Substitution at either site may result in decreased inhibitory activity for all PPs, whereas substitution at both ends results in total loss of inhibitory activity.
13, 14		Sites 5 and 4: CH_3 at these sites are favorable for inhibitory activity to PP1 and PP2A.
9		Oxygen is considered good, whereas S is considered better.

Table 2. Cantharidin, its analogues and derivatives for insecticidal action.

S. No.	Structure	Insect	Mortality	References
1		*Helicoverpa armigera*; *Plutella xylostella*; *Mythimna separata*	+++++	Khan *et al*., 2014 [43] Fan *et al*., 2017 [58] Sun *et al*., 2013 [62]
2		*Plutella xylostella*; *Mythimna separata*	+++++	Shao *et al.,* 2018 [48] Fan *et al*., 2017 [58] Sun *et al*., 2013 [62]
3		*Plutella xylostella*	+++	Sun *et al*., 2013 [62]
4		*Plutella xylostella*	+	Sun *et al*., 2013 [62]
5		*Plutella xylostella*	+	Sun *et al*., 2013 [62]
6		*Plutella xylostella*	++	Sun *et al*., 2013 [62]
7		*Plutella xylostella*	+++	Sun *et al*., 2013 [62]
8		*Plutella xylostella*	+++++	Sun *et al*., 2013 [62]

9		*Plutella xylostella*	++	Sun *et al*., 2013 [62]
10		*Plutella xylostella*	+++	Sun *et al*., 2013 [62]
11		*Plutella xylostella*	++	Sun *et al*., 2013 [62]
12		*Plutella xylostella*	+	Sun *et al*., 2013 [62]
13		*Plutella xylostella*	+	Sun *et al*., 2013 [62]
14		*Mythimna separata*; *Plutella xylostella*	+++++	Fan *et al*., 2017 [58] Sun *et al*., 2013 [62]
15		*Plutella xylostella*	++	Sun *et al*., 2013 [62]

16		*Plutella xylostella*	+	Sun *et al*., 2013 [62]
17		*Mythimna separata*; *Plutella xylostella*	+++	Fan *et al*., 2017 [58] Sun *et al*., 2013 [62]
18		*Plutella xylostella*	+++++	Sun *et al*., 2013 [62]
19		*Plutella xylostella*	+++++	Sun *et al*., 2013 [62]

A plus sign (+) in the table above indicates level of mortality. (+) = 20% and (+++++) = 100%.

CONCLUSION

Pest control of either agriculturally or medically important pests generally relies on the application of insecticides. The indiscriminate and extensive use of insecticides is becoming ineffective owing to the resistance developed by insects against a broad range of insecticides. The introduction of cantharidin or its analogues with a novel mode of action for pest management will help to overcome the insecticide resistance problem.

The use of cantharidin as an insecticide or as a synergist for the control of Lepidoptera pests has been an established fact. However, the widespread use of cantharidin may raise environmental concerns. Though its safety has been established for some non-target organisms, still it is toxic for other organisms. This problem can be addressed by restricted application of cantharidin as a synergist.

As the extraction or chemical synthesis of cantharidin is a tedious process, it is, therefore, necessary to synthesize analogues and derivatives which may be effective on one hand and easy to produce chemically on the other hand. Norcantharidin may be a better candidate for the synthesis of effective analogues. The unique toxicological and insecticidal properties of these compounds will create an upsurge in research activities in the pesticide industry.

REFERENCES

1. G. S. Wang, *J. Ethnopharmacol.,* **26**, 147 (1989).
2. D. Mebs, W. Pogoda, M. Schneider, G. Kauert, *Toxicon.,* **53**, 466 (2009).
3. K. S. Ghoneim, *Int. Res. J. Biochem. Bioinforma.,* **3**, 75 (2013).
4. C. E. P. Galvis, L. Y. V. Mendez, V. V. Kouznetsov, *Chem. Biol. Drug Des.,* **82**, 477 (2013).
5. R. B. Woodward, R. N. Loftfield, *J. Am. Chem. Soc.,* **63**, 3167 (1941).
6. J. Piccard, *Ber. Dtsch. Chem. Ges.,* **10**, 1506 (1877).
7. V. J. Gadamer, *Arch. Pharm.,* **252**, 609 (1914).
8. Y. Y. Dang, C. Zhu, *J. Chinese Materia Medica,* **34**, 2088 (2009).
9. Y. J. Dang, C. H. Hu, L. N. An, C. Y. Zhu, *Methods Find. Exp. Clin. Pharmacol.,* **32**, 157 (2010).
10. T. S. Huang. Encyclopedia of chemicals & drugs, The University Book Publishing Company, Taipei, China, 1982, p. 117.
11. J. X. Feng, Y. L. Zhang, *Acta Agriculturae Boreali-Occidentalis Sin.,* **16**, 275 (2007).
12. J. Coloe, D. S. Morrell, *Pediatr. Dermatol.,* **26**, 405 (2009).
13. H. Jia, Y. Liu, W. Yan, J. Jia, *Development,* **136**, 307 (2009).
14. E. J. Davidowitz, I. Chatterjee, J. G. Moe, *Curr. Top. Biotechnol.,* **4**, 47 (2008).
15. K. Bonness, I. V. Aragon, B. Rutland, S. Ofori-Acquah, N. M. Dean, R. E. Honkanen, *Mol. Cancer Ther.,* **5**, 2727 (2006).

16. H. Brustel, G. Kakiopoulos, *Biocosme Mésogéen (Nice),* **26**, 65 (2009).
17. J. E. Carrel, T. Eisner, *Science,* **183**, 755 (1974).
18. J. R. Sierra, W. D. Woggon, H. Schmid, *Experientia,* **32**, 142 (1976).
19. J. P. McCormick, J. E. Carrel, Cantharidin biosynthesis and function in meloid beetles, Pheromone Biochemistry, Academic Press, Orlando, Florida, 1987, p. 307.
20. J. E. Carrel, M. H. McCairel, A. J. Slagle, J. P. Doom, J. Brill, J. P. McCormick, *Experientia,* **49**, 171 (1993).
21. J. E. Carrel, W. Thompson, M. McLaughlin, *Am. Zool.,* **13**, 1258 (1973).
22. H. R. Wang, Z. K. Wang, J. Chen, Y. W. Yang, Y. Q. Cao, Y. X. Xia, Y. P. Yin, *Acta. Entomol. Sin.,* **51**, 264 (2008).
23. L. Qiurong, Study on the artificial rearing technology of blister beetle and cantharidin biosynthesis in vivo [Master Thesis], Shaanxi, Northwest Agriculture and Forestry University, 2008.
24. C. Schlatter, E. E. Waldner, H. Schmid, *Experientia,* **24**, 994 (1968).
25. H. Guenther, E. Ramstad, H. G. Floss, *J. Pharm. Sci.,* **58**, 1274 (1969).
26. M. G. Peter, H. R. Waespe, W. D. Woggon, H. Schmid, *Helv. Chim. Acta,* **60**, 1262 (1977).
27. S. J. Kramer, J. H. Law, *Acc. Chem. Res.,* **13**, 297 (1980).
28. J. E. Carrel, J. P. Doom, J. P. McCormick, *J. Chem. Ecol.,* **12,** 741 (1986).
29. J. Ming, T. Huo, S. M. Lu, Y. L. Zhang, *Acta Entomol. Sin.,* **55**, 860 (2012).
30. J. Ming, S. Lü, Y. L. Zhang, *Sci. Rep.,* **7**, 2308 (2017). DOI:10.1038/s41598-017-02393-w
31. F. von Bruchhausen, *Ber. Dtsch. Pharm. Ges.,* **266**, 697 (1928).
32. G. Stork, E. E. van Temelen, L. J. Friedman, A. W. Burgstahler, *J. Am. Chem. Soc.,* **73**, 4501 (1951).
33. G. O. Schenck, R. Wirtz, *Naturwissenschaften*, **40**, 581 (1953).
34. W. G. Dauben, H. O. Krabbenhoft, *J. Am. Chem. Soc.,* **98**, 1992 (1976).
35. V. K. Gornitz, *Arb. Physiol. Angew. Entomol.*, **4**, 116 (1937).
36. M. Frenzel, K. Dettner, *J. Chem. Ecol.,* **20**,1795 (1994).
37. C. Hemp, K. Dettner, *Entomol. Gen.,* **22**, 97 (1997).
38. Z. Y. Zhang, F. Yuan, X. Zhang, *Acta Phytophyl. Sin.,* **25**, 166 (1998).
39. L. X. Wei, Q. L. Liang, H. M. Shen, S. Y. Yang, *Agrochemicals,* **46**, 272 (2007).
40. X. F. Li, X. S. Chen, X. H. Hou, *Guizhou Agric. Sci.,* **36**, 65 (2008).
41. R. R. Liu, Y. Ma, Z. Q. Ma, Y. L. Zhang, *J. Northwest Agric. For. Univ.,* **38**, 181 (2010).
42. S. Zhang, Y. L. Zhangp. A. Fenxia, *Acta Phytophyl., Sin.,* **34**, 83 (2007).
43. R. A. Khan, M. Rashid, D. Wang, Y. L. Zhang, *Pest Manage. Sci.,* **70**, 39 (2014).
44. M. Rashid, R. A. Khan, Y. Zhang, *J. Econ. Entomol.,* **106**, 2177 (2013).
45. S. Diao, J. Wang, M. Gu, H. Jiang, S. Zhou, *Agrochem.,* **42**, 36 (2003).
46. G. Mingjie, *China Fruits,* **5**, 35 (2007).
47. H. Yasoob, H. A. A. Khan, Y. Zhang, *J. Econ. Entomol.,* **110**, 2539 (2017).
48. H. Shao, N. Xi, Y. L. Zhang, *Sci. Rep.,* **8,** 10565 (2018). https://doi.org/10.1038/s41598-018-28626-0
49. F. L. Cui, X. Li, Z. Q. Ma, Y. L. Zhang, *J. Environ. Entomol.,* **31**, 143 (2009).
50. H. Shao, Y. Zhang, *Sci. Rep.,* **7**, 5521 (2017). https://doi.org/10.1038/s41598-017-05923-8.
51. B. L. Lu, *Acta Entomol. Sin.,* **13**, 824 (1964).
52. Y. L. Hang, Y. Zhou, Z. Y. Zhang, *Acta Entomol. Sin.,* **46**, 272 (2003).
53. L. P. Chen, B. D. Yang, Z. Y. Zhang, *J. Beijing Uni. Agric.,* **23**, 17 (2008).
54. Z. Y. Zhang, F. Yuan, X. Zhang, *Acta Phytophyl. Sin.,* **7**, 355 (2000).
55. Y. Ma, R. R. Liu, Z. Q. Ma, Y. L. Zhang, *Acta Entomol. Sin.*, **53**, 870 (2010).
56. Y. M. Li, J. E. Casida, *Proc. Natl. Acad. Sci.,* **89**, 11867 (1992).
57. X. Chen, S. Lu, Y. Zhang, *Plos One,* **9**, e97437, (2014). https://doi.org/10.1371/journal.pone.0097437.
58. J. B. Fan, S. Van de Velde, W. B. Sun, H. Yu, Y. L. Zhang, D. Wang, *J. Appl. Entomol.,* **141**, 363 (2017).
59. Y. Li, H. Sun, H. Yasoob, Z. Tian, Y. Li, R. Li, S. Zheng, J. Liu ,Y. Zhang, *Pestic. Biochem. Phys.,* 172 (2021).
60. M. Sodeoka, Y. Baba, S. Kobayashi, N. Hirukawa, *Bioorg. Med. Chem. Lett.,* **7**, 1833 (1997).
61. W. N. Zeng, Y. Lu., *Chinese J. Org. Chem.,* **26,** 579 (2006).
62. W. Sun, Z. Liu, Y. Zhang, *Int. J. Mol. Sci.,* **14**, 1 (2013).