International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 3, Issue 3, pp: 440-444, 2018 **http://www.ijzab.com**

Research Article

STUDIES ON THE GC MASS AND HPLC ANALYSIS IN THE DEFENCE SECRETION OF CARABIDAE BEETLE *PHEROPSOPHUS HILARIS*

G. Raja Selvi and T. Ramesh Kumar*

Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India

Article History: Received 25th April 2018; Accepted 9th June 2018; Published 12th June 2018

ABSTRACT

The bombardier beetle *Pheropsophus hilaris* having a pair of defensive organs consisted of many small synthetic lobes, large reservoirs and collecting ducts. The defence secretary lobes of *P. hilaris* secrete aqueous hydrogen peroxide and hydroquinones, which are stored in the large quantity in the collecting reservoirs. The modern research is oriented towards attaining health and economic benefits of mankind. Now- a- days, the farmers are using large number of pesticides and insecticides to control the pest and insect. The poor knowledge among the farmers is the main reason for pollution. In order to reduce pollution by contaminating the amount of pesticides, biological control is also advocated and attempted on different scale. In recent years, in addition to bio control, bio pesticides came into existence for integrated pest management. In view of this, it has been programmed to study the GC MASS and HPLC analysis of the defence secretion of *P. hilaris.*

Keywords: Bio pesticides, Bombardier beetle, Collecting ducts, Hydroquinones, Hydrogen peroxide.

INTRODUCTION

The unique combination of features of the bombardier beetle's defense mechanism strongly exothermic reactions, boiling-hot fluids, and explosive release have been used by creationists and proponents of intelligent design as examples of irreducible complexity that could not have been produced by evolution (Rice *et al*., 2007). However, while the true evolutionary path is still unknown, biologists have shown that the system could in fact have evolved from defenses found in other beetles in incremental steps by natural selection.

Specifically, quinone chemicals are a precursor to sclerotic, a brownish substance produced by beetles and other insects to harden their exoskeleton. Some beetles store excess quinones, including hydroquinone, in small sacs below their skin. Some beetles additionally mix hydrogen peroxide and hydroquinone a common by-product of the metabolism of cells. The chemical reaction produces heat and pressure and some beetles exploit the latter to push out the chemicals in the beetle *Metrius contractus*, which produces a foamy discharge when attacked (Eisner *et al*., 2000).

One of the interesting characteristics of this beetle, and bombardier beetles in general, is that they are capable of spraying boiling hydrogen peroxide and p-benzoquinone out of their abdomen (Beheshti & Mcintosh, 2007). This jet is also accompanied by a characteristics popping sound, and is used as a defense mechanism to fend off predators. In some species the ability to aim this jet is very highly developed; it is capable of targeting individual legs on its body and even being able to shoot predators that have climbed onto their back (Eisner & Aneshansley, 1999). White to the naked eye it appears to be a single burst of fluid and gasses it is actually a series of very rapid pulses (Beheshti & Mcintosh, 2007). It works in a similar manner to pulse jet engines that powered the German $V-_1$ flying bombs in World War II (Gullan and (Cranston *et al*., 2010). Pressure for expulsion is not produced by muscular contraction, but rather from a one way valve that traps the rapidly expanding gasses and funnels them out of the insect abdomen (Gullan & Cranston, 2010).

The mechanism powering this reaction is relativity simple; catalytic decomposition of hydrogen peroxide and

*Corresponding Author: Dr. T. Ramesh Kumar, Associate Professor, Annamalai University, Annamalainagar, Tamilnadu, India. Email: ktrameshau@gmail.com, Mobile: +91 9489361412

oxidation of hydroquinones to p-benzoquinones (Bradford, 1976). These two chemical are stored together in a gland. When necessary, the insect contracts the gland and forces the compounds through a one way value into the reaction chamber (Gullan & Cranston, 2014). This chamber is lined with catalases that catalyze the decomposition of peroxide and peroxidases that catalyze the oxidation of hydroquinones. This reaction is highly exothermic; releasing -202.8 kj/mol (Beheshti & Mcintosh, 2007). The solution rapidly boils and the pressure produced by the gasses forces the one way valve closed and then forces the solution out of the abdominal opening. This solution can be so irritating to the predators that it repels the attack within milliseconds (Eisner *et al*., 2006).

Most noticeable, is the force of the spray, which is ejected during the reaction. The spray is ejected in explosive discharges of about 500 pulses per second, which can surprise and deter large vertebrates (even frogs) and can even send some attackers into seizures. One study records the velocity of the spray to be within a range of 325 to a sliirinmg 1950 cm/s. Additionally, the beetle's spray is astonishingly hot (some are unleashed at 100°C), a feature that seems to be dependent on the biochemistry of the reaction between the hydroquinones, hydrogen peroxides and the catalases and peroxidases that the beetle synthesizes and stores in separate reservoirs. The structure of the defence system of the Bombardier Beetle, as reported in the literature, is complex, consisting of two sets of secretary lobes, collecting canals, collecting reservoirs, one-way valves, sphincter muscles, reaction chambers, exit tubes, and exit nozzles (Schnepf, 1969).

MATERIAL AND METHODS

Collection of Pygidial secretion for chemical analysis

Material for chemical analysis were obtained either of gland reservoirs or as secretion discharged on filter paper. For gland removal, live beetle were placed in a freezer for several minutes and dissected under distilled water. Whole gland reservoirs were placed in dry-ice cooled reaction vials. Collected discharged secretion on filter paper, beetles were held held by one leg with forceps and a small strip of filter paper near the beetle to catch the secretion as it was sprayed. To prevent premature discharge, beetles were temporarily incapacited by cooling them to approximately 10^{o}C and then allowed to warm to room temperature while under observation. Once beetles became active, defensive secretion was collected on a piece of filter paper.

Chemical analysis

Defensive secretion absorbed to filter paper, or excised defensive gland were extracted with dichloromethane (100 µl), and 1 µl of the extract was injected into a GC-MS (HP 5890 gas chromatograph linked to a HP 5970 mass selective detector) by splitless iinjection. Analysis were performed using a 25-m×0.25 mm fused-silica capillary colum coated with DB-5 (5% phenyl methylsilicone) stationary phase (0.25 µm film thickness). The oven temperature was held at 40℃ for 4 min and increased to 260℃ for 10 min (Will, Attygalle, & Herath, 2000).

HPLC analysis

Twenty micolitres of the filtered, derivated amino acid sample was infected into a C-18 reverse phase column and analyzed using sodium acetate buffer with tetrahydrofuran and trithylamine and sodium acetate with methanol as solvent system. The amino acids were identified by comparing their retention time (Rt) with the standard amino acids run at identical (Bradford, 1976).

Procedure - Derivatization of amino acid sample

One ml of OPA reagent was added to each vial containing 200 ml of amino acid samples. The samples were mixed well and kept for 2 minutes for derivatisation. The samples were injected at the rate of 20 µl in the HPLC for amino acid analysis.

GC-MASS Analysis

Extracts were anlaysed by coupled gas chromatography mass spectrometry (GC-MS) on a Hewlet –Packard 5973 mass selective detector coupled with a HP 6890 GC-system. MS-Spectra were recorded in EI mode at 70ev, within a mass range of 40-500 mass units and a scan cycle time of 0.7 sec. A detector temperature of 250℃ to and an injector temperature of 300^{o}C were chosen as a GC condition. A corbowax column (25 m, 0.25 mm) was used. Temperature was programmed from 100℃ to 150℃ at 30℃ (1 bar). A 1-µl sample was injected in splitless mode (Bradford, 1976).

RESULTS AND DISCUSSION

The natural component of the pygidial or defence secretion is benzoquinions and fatty acids secreted by carabid beetles of *P. hilaris* identified by gas chromatography, mass spectrometry and HPLC. Among the more interesting compounds produced by *P. hilaris* are 1,4-quinioines and hydroquinones ejected explosively by members of Brachinini. 1,4-benzoquinone and 2-methyl-1, 4-benzoquinone compound was found to be observed in *P. hilaris* (Figure 1). This beetle secreted these substances at body temperature from the reservoir with no sound. In contrast, Brachinidae beetles have a pair of brownish reaction chamber connected with a reservoir. The sprayed two benzoquinones at about 100ºC making sound.

Two types of fatty acid secreting beetles were found, one secreted formic acid and the other mixed short chain fatty acids. These short chain fatty acids were metabolized from some amino acids, methacrylic acid from valine; angelic acid from isoleucine; senecioic acid from leucine and crotonic acid from lysine, formic acid from serine and glycine was observed by HPLC. The quantities of isoleucine were found to be more of about 29.9 µmoles/ml (Table 1 Figure 2).

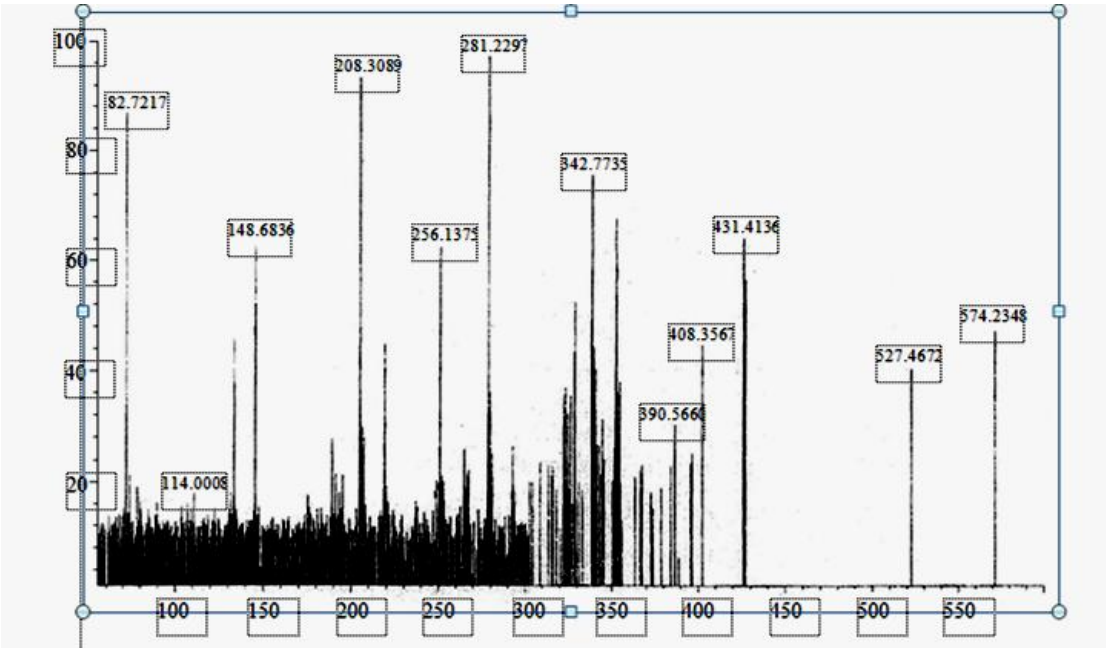

Figure 1. Mass spectra of *Pheropsophus hilaris* defence secretion.

Table 1. Quantity and quality of amino acids in *P. hilaris.*

Amino acid level in µ moles/ml	
Amino acids	Pygidial
Aspartic acid	0.5
Glutamic acid	0.4
Serine	1.5
Histidine	0.6
Glycine	1.2
Threonine	13.4
Alanine	0.8
Arginine	2.2
Tyrosine	0.3
Valine	0.4
Methionine	0.9
Phenylalanine	6.4
Isoleucine	29.9
Leucine	8.2
Lysine	0.6

Pheropsophus hilaris represents the largest exocrine structure in the abdomen. The glands produce a secretion with a strong smell, which the *Pheropsophus hilaris* release when they are disturbed. The unique combination of features of the bombardier beetle's defense mechanism is strongly exothermic reaction, boiling-not gluids, and explosive release. However, while the true evolutionary path is still unknown, biologists have shown that the system could in fact have evolved from defenses found in other beetles in incremental steps by natural selection (Eisner & Aneshansley, 1999). Some beetles additionally store excess foul-smelling quinines including hydroquinone, in small sac below their skin as a natural deterrent against predators. Some beetles additionally mix hydrogen peroxide, a common by product of the metabolism of cells, in which the hydroquinone and some of the catalases that exist in most cell makes, the process more efficient. The chemical reaction produces heat and pressure, and some beetles exploit the latter to push out the chemicals onto the skin; this is the case in the beetle metrius contractus, which produce a foamy discharge when attacked (Eisner *et al*., 2000).

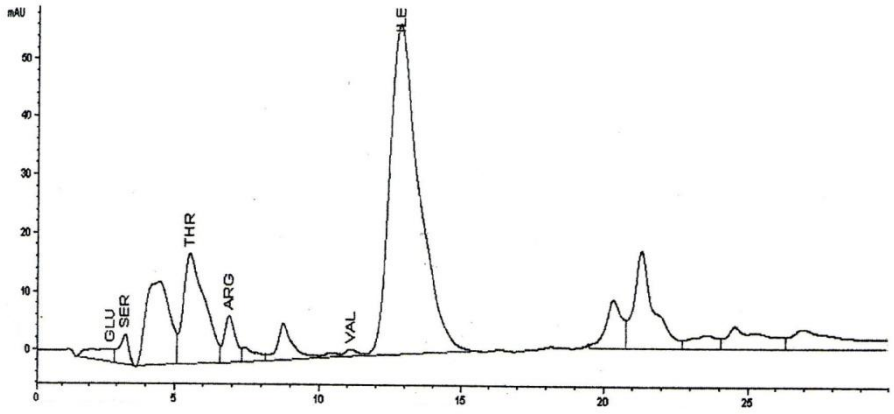

Figure 2. HPLC spectra of amino acids in *P. hilaris*

One of the interesting characteristics of this beetle, and bombardier beetles in general, is that they are capable of spraying boiling hydrogen peroxide and P. hydroquinone out of their abdomen (Beheshti & Mcintosh, 2007). This jet is also accompanied by a characteristing popping sound, and is used as a defense mechanism to fend off predators. In some species the ability to aim this jet is very highly developed; it is capable of targeting individual legs on its body and even being able to shoot predators that have climbed onto their back (Eisner & Aneshansley, 1999).

The secretion from the pygidial glands of dolichoderine ants are generally characterized by a mixture of iridoids and ketones. The iridoids seem to be used for defense, being repulsive to a number of insects where as the ketones; for example, 2-methyl 4-heptatone (V) and 6-methyl-5-hepten-2-one (VI), elicit alarm behavior in conspecific individuals (Holldobler & Wilson, 1990). Besides their occurrence on the pygidial glands of dolichoderine ants, iridoids are also present in plants and some other insects. In plants, they function either as feeding or olfactory attractants and in insects they serve as defensive compounds (Harborne, 2014). One the other hand, the ketones, especially 2-heptanone and 6-methyl-5-hepten-2-one, were found to function as very effective allomones for cockroaches in the genera *Palyzosteria* and *Neostylopiga* wall bank water house, 1970 as well as for beetles in the genus *Dyschirius* (Moore & Brown, 1979).

CONCLUSION

The natural compound of the pygidial secretion is benzoquinious and fattyacids secreted by the caratid beetle of *Pheropsophus hilaris* was identified by gas chromatography, mass spectrometry and HPLC. Among the more interesting compounds produced by *Pheropsophus hilaris* are hydrogen peroxide and hydroquinones 1,4-benzoquinone and 2-methyl-1-4-benzoquinone compound. Two types of fatty acid secreting beetles were found, one secreted formic acid and the other mixed short chain fatty acids.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Annamalai University for the facilities provided to carry out this research work.

REFERENCES

Beheshti, N., & Mcintosh, A. C. (2007). The bombardier beetle and its use of a pressure relief valve system to deliver a periodic pulsed spray. *Bioinspiration and Biomimetics*, *2*(4), 57.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Cranston, P. S., Hardy, N. B., Morse, G. E., Puslednik, L., & Mccluen, S. R. (2010). When molecules and morphology concur: the 'Gondwanan'midges (Diptera: Chironomidae). *Systematic Entomology*, *35*(4), 636-648.

Eisner, T., Aneshansley, D., del Campo, M. L., Eisner, M., Frank, J. H., & Deyrup, M. (2006). Effect of bombardier beetle spray on a wolf spider: repellency and leg autotomy. *Chemoecology*, *16*(4), 185-189.

Eisner, T., & Aneshansley, D. J. (1999). Spray aiming in the bombardier beetle: photographic evidence. *Proceedings of the National Academy of Sciences*, *96*(17), 9705-9709.

Eisner, T., Eisner, M., Aneshansley, D. J., Wu, C.L., & Meinwald, J. (2000). Chemical defense of the mint plant, *Teucrium marum* (Labiatae). *Chemoecology*, *10*(4), 211-216.

Gullan, P., & Cranston, P. (2010). Insect systematics: phylogeny and classification. *The Insects: An Outline of Entomology*. Fourth ed. Wiley-Blackwell, Oxford, 189-222.

Gullan, P. J., & Cranston, P. S. (2014). *The Insects: An*

Outline of Entomology: John Wiley & Sons press.

Harborne, J. B. (2014). *Introduction to Ecological Biochemistry*, Academic press,4th Edition, 1-384

Holldobler, B., & Wilson, E. O. (1990). *The ants*, Belknap Press of Harvard University Press, Cambridge, MA, 1-732.

Moore, B., & Brown, W. (1979). Chemical composition of the defensive secretion in *Dyschirius bonelli* (Coleoptera: Carabidae: Scarittnae) and its taxonomic significance. *Austral Entomology, 18*(2), 123-125.

Rice, S. D., Short, J. W., Carls, M. G., Moles, A., & Spies, R. B. (2007). The Exxon Valdez oil spill. In: Spies, R. B., editor. Long-term Ecological Change in the Northern Gulf of Alaska. Amsterdam Elsevier, 419-520.

Schnepf, E. (1969). *Sekretion und Exkretion bei Pflanzen. Protoplasmatologia, Handbuch der Protoplasma-forschung*, Bd. 8, Physiologie des Protoplasmas, Springer, New York.

Will, K.W., Attygalle, A.B., & Herath, K. (2000). New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework. *Biological Journal of the Linnean Society, 71*(3), 459-481.