

ORIGINAL RESEARCH ARTICLE

Evaluation of LD₅₀ Value of the Phytopesticide Piperidine on the Adult Male Insect *Odontopus varicornis* (Heteroptera: Pyrrhocoridae)

T. Mohan*, Selvisabhanayakam and V. Mathivanan

Department of Zoology, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India.

Received 08 Sep 2011; Revised 12 Nov 2011; Accepted 25 Nov 2011

ABSTRACT

The piperidine structural modification is present in numerous natural alkaloids. These include piperine, which gives black pepper and fire ant venom. The present work has been undertaken to evaluate the sublethal concentration (LD₅₀) value of piperidine on *Odontopus varicornis*. The changes in the mortality of *Odontopus varicornis* was observed at various exposure period for 24, 48, 72 and 96 hours and its sublethal concentration was found to be about 0.15% for 48 hours. When the insects were injected with sublethal concentration 0.15% for 48 hours, led to 50% mortality of the test insects.

Key words: Piperidine, Toxicity, LD₅₀ and *Odontopus varicornis*.

INTRODUCTION

Studies on bioactivity of plant derivatives for pest control continue to increase, but few of them have potential as botanical insecticides. Chemical instability, regulatory barriers and the availability of new cost-effective and relatively safe synthetic products compared to traditional insecticides are hindering the success of botanical products. These insecticides are best suited for organic food production but they have potential to protect food in developing countries^[1]. Insecticides from medicinal plants are an attractive alternative for pest management because they pose low threat to the environment or to human health compared to synthetic insecticides^[2]. Much of the increase in agricultural productivity over the past half century has been the result of controlling these arthropod pests by synthetic chemical pesticides^[3].

Feeding damage and the transmission of plant diseases by insects and mites are some of the major causes of crop loss worldwide. Estimates on a world scale suggest that elimination of insect pests would increase crop production by about a third^[4]. However, control of such pests has become increasingly difficult because of reduced effectiveness of pesticides caused by emergence of pesticidal resistance in arthropod pests. Therefore, an effort is warranted to find alternatives or formulations for improving currently used pesticides. One source of potential new pesticides is natural products produced by

plants. Not only might certain natural products be source of new pesticides, but botanical derivatives may be more environmentally benign than synthetic chemicals. Plants in the Piperaceae are members of traditional pharmacopeia in many Asian and African cultures and have also been used for pest control. *Piper guineense* (Schumacher & Thonn) and *Piper nigrum* (L.) are used as insecticides and molluscicides in several areas of Africa^[5, 6].

Insect pests are a major constraint on crop production, especially in developing countries. Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms^[7]. Many floral volatiles have anti-microbial or anti-viral activity^[8,9,10] and hence act to protect valuable reproductive parts of plants from damage^[11]. However, intensive screening is necessary to select compounds with pesticidal properties, but harmless to the environment and ecosystem. Researches on potential botanical extracts which are safe with little or no residues and naturally derived with minimal technology are urgently needed. There are more than 2400 plant species belonging to 189 plant families which are said to be rich sources of bioactive organic compounds^[12].

Many toxic substances have been synthesized which are lethal to insects at doses which are not toxic to vertebrates^[13]. Acute toxicity is typically associated with the breakdown of tissues and physiological systems at which exceed rates of repair or adaptation and a test made to determine the acute toxicity of a substances is referred to as acute toxicity test^[14]. These acute toxicity tests of different insects were reported for *Gryllotalpa africana*^[15], *Sphaerodema rusticum*^[16], *Laccotrephes ruber*^[17] respectively. The well known generally accepted technique of evaluating the toxicity of pesticide is the determination of sub lethal concentrations (LD₅₀ values), where the toxicity of pesticide to an organisms is assessed by the concentration of the toxic compound in terrestrial environment that kill 50% of the animal exposed for specific period of time. The aim of the study is to provide an examination for the acute toxicity of piperidine, as a chemical compound and some information about the intrinsic toxicity towards the test insect, *Odontopus varicornis*.

MATERIALS AND METHODS

Insect

The insects collected from the fields and gardens were reared in wooden cage, each measuring about 30 × 22 × 28cm at the laboratory temperature of 28 ± 2°C and relative humidity of 80 ± 5%. The insects were fed daily with soaked cotton seeds (*Bombax ceiba*) as well as with seeds of its higher plant, *Stericulia foetida* and *Gossypium* sp. An additional food of the pieces of chow-chow (*Sechium edule*) was also given to these insects.

Piperidine

Density: 0.8629 g/mol.

Molecular wt: 85.15 g/mol

Synthesis Pvt. Ltd.

Toxicity Experiments

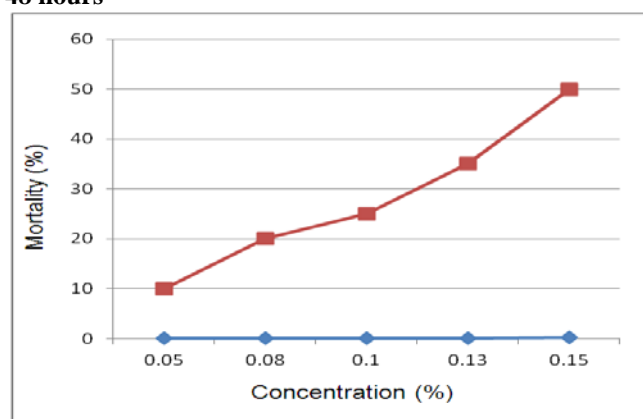
Ten treated insects each allowed into a small insect cage (29 x 25 x 11cm) and observed their mortality from 24 hours thereafter upto 96 hours. Different sublethal concentration, of piperidine which were 0.05, 0.08, 0.1, 0.13 and 0.15%. In five small insect cages, each 10 insects were kept in each. The solutions are prepared over wide range of concentration. The tests were conducted in adult male insect by injecting 0.025 ml to each insect with 2.6 gauge syringe needle. The mortality was recorded every 24 hours for the observation period of 48 hours. The LD₅₀ value was calculated by adopting the method of^[18].

RESULTS AND DISCUSSION

The sublethal concentration (LD₅₀) of piperidine for 24, 48, 72 and 96 hours and its 95 % confidence limits were determined from the data of the toxicity tests to get a basis of reference for analysis and determination of the mode of action for piperidine toxicity on the test insect, *Odontopus varicornis*.

The acute toxicity of the phytopesticide piperidine on *Odontopus varicornis* were assessed and presented in the form of sublethal concentration (LD₅₀) for 48 hours of exposure. The mortality rates of *Odontopus varicornis* in different concentrations of piperidine were studied. The selected concentrations to determine LD₅₀ were ranging from 0.05 to 0.15%. The LD₅₀ value was 0.15% for piperidine (**Table 1**). The toxicity test revealed that the rate of mortality increased with increased concentrations (**Fig 1**).

Fig 1: Percentage Mortality of *Odontopus varicornis* at different concentrations of piperidine, over the period of 48 hours



The toxicity tests were ideally suited for assessing the relative impact of piperidine concentration on *Odontopus varicornis* for 48 hours of exposure. The dried fruits of some Piperaceae are used as flavoring agents in food, but are known to have insecticidal properties^[19,20]. The suitability of these naturally occurring amides as alternatives for the development of new synthetic insecticides has been reviewed^[21]. In spite of this, few amides have been tested for insecticidal activity. Recently, pipernonaline isolated from *P. longum* was shown to have mosquito larvicidal activity^[22]. It has been well recognized that natural plant derivatives could be developed into products suitable for pest control because many of them are selective and often biodegrade into nontoxic products^[23]. In the case of black pepper, it is one of the world's most traded spices and is grown in several equatorial regions of the globe^[24]. Extracts of black pepper contain high proportions of piperine and other active piperamides, which

already have been tested and applied for use in several developing regions [25]. In the present investigation, *Odontopus varicornis* treated with phytopesticide piperidine, the acute toxicity level was expressed in terms of LD₅₀ value. The toxicity graph denotes the median lethal concentration of piperidine at 48 hours of exposure as 0.15%. The trends showed a raise in mortality rate with increasing concentration and exposure period revealing the regular mode of action to continuous absorption of toxicant [26].

Similarly, a declining trend of sub lethal concentration has been reported by many authors for different toxicants in different species of insects, such as the works of *Odontopus varicornis* exposed to dimethoate [27],

Laccotrephes ruber exposed to mercuric chloride [28]. *Odontopus varicornis* exposed to monocrotophos [29], *Gryllotalpa africana* exposed to endosulfan [30], *Sphaerodema rusticum* exposed to heavy metal mercury [31] respectively. The application of LD₅₀ values has gained wide acceptance among toxicologists and is generally the most reliable test assessing potential hazards of aquatic life [32]. LD₅₀ values are differing in species to species for the same toxicants due to the mode of action and responses of the animals [33] and also there can be large inter individual differences in response to a chemical because of subtle genetic differences referred as genetic polymorphisms [34].

Table 1: Percentage Mortality of *Odontopus varicornis* at different concentrations of piperidine, over the period of 48 hours

Exposure Concentration (%)	Number of insects in each concentration	Observed Mortality (%)	Expected Mortality (%)	LD ₅₀ (LD ₉₀)	95% Confidence Limits		Chi Square
					Lower	Upper	
0.05	10	10	10.81				
0.08	10	20	17.46				
0.1	10	25	26.25	0.15289	0.13899	0.17567	0.824
0.13	10	35	36.87	(0.25954)	(0.22350)	(0.32500)	
0.15	10	50	48.16				

ACKNOWLEDGEMENT

The authors are very grateful to the authorities of Annamalai University and to the Head, Department of Zoology for providing all necessary facilities for completion of this work.

REFERENCES

1. Isman M.B. (2006). Botanical insecticides, detergents and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.*, 51(1): 45-66.
2. Moreira M.D., Picanço M.C., Barbosa L.C.A., Guedes R.N.C., Barros E.C and Campos M.R. (2007). Compounds from *Ageratum conyzoides*: Isolation, structural elucidation and insecticidal activity. *Pest Manag. Sci.*, 63(6): 615-621.
3. Duke S.O., Menn J.J and Plimer, J.R. (1993). Challenges of pest control with enhanced toxicological and environmental safety. In: Duke, S.O., Menn, J.J., Plimer, J.R. (Eds.), *Pest Control with Enhanced Environmental Safety*. ACS Symposium Series 524.
4. Van Emden H.F. (1989). *Pest Control*, 2nd Edition. Edward Arnold Publication, London and New York.

5. Su H.C.F and Horvat, R. (1981). Isolation, identification and insecticidal properties of *Piper nigrum* amides. *J. Agri. Food Chem.*, 29: 115-118.
6. Ivbijaro M.F and Bolaji O.O. (1990). Effects of cypermethrin+dimethoate and extracts of *Piper guineense* and *Azadirachta indica* on the pests and yield of cowpea, *Vigna unguiculata*. *J. Agric. Sci.*, 115: 227-231.
7. Sharma A., Kaushal P., Sharma K.C and Kumar R. (2006). Bioefficacy of some plant products against Diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *J. Entomo. Res. Soc.*, 30: 213-217.
8. De Moraes C.M., Mescheer M.C and Tumlinson J.H. (2001). Caterpillar induced nocturnal plant volatiles repel nonspecific females. *Nature*, 410: 577-580.
9. Friedman M., Henika P.R and Mandrell R.E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enteric*. *J. Food Prot.*, 65: 1545-1560.
10. Hammer K.A., Carson C.F and Riley T.V. (2003). Antifungal activity of the

- components of *Melaleuca alternifolia* (tea tree) oil. *J. Appl. Microbiol.*, 95: 853–860.
11. Dudareva N., Pichersky E and Gershenzon J. (2004). Biochemistry of Plant Volatiles. *Plant Physiol.*, 135: 1893–1902.
 12. Rao N.V., Maheswari T.U and Manjula K. (2005). *Review on Botanical Pesticides as Tools of Pest Management*, pp: 1–16. Narosa Publishing House Pvt., Ltd.
 13. Coats J.R. (1982). Insecticide Mode of action Academic Press, New York.
 14. EIFAC. (1983). Revised report on fish toxicity testing Procedures Prepared by EIFAC working Party on toxicity testing Procedures. *Technical Paper*, 24 (1): 1-37.
 15. Sumathi (2002). Studies on the impact of endosulfan on certain selected tissues of the adult male insect *Gryllotalpa africana* (Palisot de Beaurols) (Orthoptera: Gryllotalpidae) in relation to reproduction Ph.D., Thesis, Annamalai University, India.
 16. Rajathi (2004). Studies on the impact of heavy metal mercury in the adult male reproductive Physiology of *Sphaerodema rusticum* (Heteroptera: Belostomatidae) Ph.D., Thesis, Annamalai University, India.
 17. Ramesh Kumar T. (2004). Studies on the impact of heavy metal zinc on certain selected tissues in the adult male *Laccotrephes ruber* (Linn) (Heteroptera; Nepidae) in relation to reproduction. Ph.D., Thesis, Annamalai University, India.
 18. Finney D.J. (1971). Probit analysis, University Press, Cambridge. Pg.No. 333.
 19. Miyakado M., Nakayama M.I., Yoshioka H and Nakatani N. (1979). The piperaceae amides I: structure of pipericide, a new insecticidal amide from *Piper nigrum* L. *Agri. Biol. Chem.*, 43: 1609–1611.
 20. Miyakado M., Nakayama M.I and Ohno N. (1989). Insecticidal unsaturated isobutylamides: from natural products to agrochemical leads. In: Arnason, J.T., Philogene, B.J.R., Morand, P. (Eds.), *Insecticides of Plant Origin*. ACS symposium Series 387. American Chemical Society, New York, pp. 183–187.
 21. Elliot M. (1979). Progress in the design of insecticides. *Chem. Ind.* 759–768.
 22. Lee S.E. (2000). Mosquito larvicidal activity of piperonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. *J. Am. Mosquito Contr. Assoc.* 16: 245–247.
 23. Sukumar K., Perich M.J and Boobar L.R. (1991). Botanical derivatives in mosquito control: a review. *J. Am. Mosq. Control Assoc.*, 7: 210-237.
 24. Simpson B.B and Ogorzaly M.O. (1995). *Economic botany: Plants in our world*. 2nd ed. New York: McGraw-Hill, 742 p.
 25. Arnason J.T., Durst T and Philogène B.J.R. (2002). Prospection d'insecticides phytochimiques de plantes tempérées et tropicales communes ou rares. In: Regnault-Roger C, Philogène B.J.R., Vincent C., editors. *Biopesticides d'origine végétale*. Paris: Editions TEC and DOC. p 37–51.
 26. Jha A and Singh H.N. (1984). Toxicity of seven different insecticides against adult *Tribolium castaneum* (Herbst) Indian. *J. Ent.* 46 (4): 395-397.
 27. Jayakumar C. (1988). Effect of dimethoate (roger) on the accessory reproductive gland, testis, fat body, haemolymph, neurosecretory cell and corpus allatum in *Odontopus varicornis* (Dist) (Hemiptera: Pyrrhocoridae) Ph.D., Thesis, Annamalai University, India.
 28. Pazhanisamy K. (1997). Studies on the effect of heavy metal (mercuric chloride) on histopathological and biochemical change in the selected tissues of the adult male *Laccotrephes rubber* (Linn). (Heteroptera: Nepidae). M.Phil., Thesis, Annamalai University, India.
 29. Ravichandran S. (1996). Toxicological studies on the ovary, fat body and haemolymph of the adult female *Laccotrephes ruber* (Linn). (Heteroptera: Nepidae). Ph.D., Thesis, Annamalai University, India.
 30. Sumathi S., Selvisabhanayakam and Mathivanan V. (2001). Effect of endosulfan on histological changes in the fat body of adult male, *Gryllotalpa Africana* (Orthoptera: Gryllotalpidae). *Indian J. Environ & Ecoplana.*, 5(2) : 261-264.
 31. Selvisabhanayakam., Rajathi V and Mathivanan V. (2002). Toxicity studies on the impact of heavy metal mercury on *Sphaerodema rusticum* (Heteroptera:

- Belostomatidae). *Indian J. Environ. and Ecoplan.*, 6(3): 431-434.
32. Brungs W.A and Mount D.I. (1978). Introduction to a discussion on the use of aquatic toxicity test for evaluation of the effects of toxic substances. In: Estimating the hazards of chemical substances to Aquatic life. ASTMST. P. 657. Chemical Society, New York, pp. 1-13.
33. King S.G. (1992). Some effects of DDT on the guppy and known trout. *Spec. Scient. Res. U. S. F: Sh Wild Sdrr.* 399: 1-22.
34. Curtis D., Klassen John B and Watkins III. (2003). *Essentials of toxicology*, The Mc Graw – Hill Companies, Inc., USA.