International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 16-09-2025, Accepted: 15-10-2025, Published: 31-10-2025
Volume 10, Issue 10, 2025, Page No. 136-139

Determination of toxicity (LD_{50}) value of phytopesticide *Azadirachta indica* on the adult male insect *Odontopus varicornis* (*Heteroptera: Pyrrhocoridae*)

Navamani A[1,] Rajathi V[2*]
[1] Research Scholar, PG and Research Department of Zoology, Government Arts College, B. Mutlur, Tamil Nadu, India
[2] Associate Professor, PG and Research Department of Zoology, Government Arts College, B. Mutlur, Tamil Nadu, India

Abstract
Azadirachta indica often known as neem, structural modifications both naturally and through chemical process. specifically, azadirachtin and other bioactive compounds extracted from *Azadirachta indica* represent a valuable source of phytopesticides. These plant-based pesticides function as antifeedants, disrupting insect growth and reproduction, and repelling pests. Among these, phytopesticide play a crucial role as agents for insect control. Plant-based products have long been utilized for the control of domestic pests. The global search for natural, eco-friendly insecticidal substances continues to gain momentum. In this context, the present study was undertaken to determine the median lethal dose (LD_{50}) of *Azadirachta indica* against the test insect *Odontopus Varicornis*. Changes in the mortality of *Odontopus Varicornis* were observed at different exposure periods of 12, 24, 48, 72, and 96 hours. The median lethal dose was determined to be approximately 0.05 ppm at 48 hours, which resulted in 50% mortality of the test insects.

Keywords: *Azadirachta indica*, phytopesticide, toxicity, LD_{50}, *Odontopus varicournis*

Introduction

There are many plants that may be used to make botanical insecticides, and most of the time, using botanic preparations is much safer than using many of the conventional pesticides that are now on the market (Isman, Murray B 2017) [16]. Pesticides are products used to protect plants (Wojciechowska, Marta *et al.,* 2016) [32]. The findings from the toxicity tests were used to calculate confidence limits, which served as a foundation for analysing and determining the mode of action for piperidine toxicity in the test insect (Mohan, T. *et al.,* 2011) [20]. Botanical insecticides are natural substances having insecticidal characteristics that have been used in crop protection for as long as agriculture has existed (Grdisa, Martina, Kristina Grsic 2013) [13]. In this study, 59 substances were tested for acute oral toxicity in silkworms (Usui, Kimihito, *et al.,* 2016) [31]. Chemical control alone should not be the main focus; instead, integrated pest management (IPM) strategies should be developed to control D. koenigii by combining all available control approaches in a way that works for that particular pest (Saeed, Shafqat *et al.,* 2016) [28]. We provide a technique for determining the Acute Insecticide Toxicity Loading (AITL) on US agricultural fields and their environs, as well as an analysis of the evolution of AITL between 1992 and 2014 (Di Bartolomeis Michael, *et al.,* 2019) [9].

The herb *Azadirachta indica* has several uses, and using it m edicinally will benefit society (Srivastava, Santosh Kumar, *et al.,* 2020) [30]. Controlling insects in crops is one of the most important global challenges (*Ahmed*, Nazeer, *et al.,* 2021) [1]. Botanical pesticides are currently being utilised as an alternative to manmade chemical pesticides to manage insect pests in agriculture. (Dalavayi Haritha, *et al.,* 2021) [7]. Synthetic pesticides have accumulated in the environment, causing adverse effects on human health and ecosystems. (Bilal, Tuybia *et al.,* 2022) [5]. This paper reviews various studies on the use of non-toxic natural food preservatives (Funmilayo, Festus Okunlade A, *et al.,* 2023) [12]. Knowledge intensive management systems are necessar y for the efficient application of biopesticides (Kiri Idris Z. *et al.,* 2024) [18]. The purpose of this study is to evaluate the acute toxicity and analyze the biological activity of lancing leaf extracts (Ikhwan Afifah *et al.,* 2025) [15].

The most widely accepted method for evaluating phytopesticide toxicity is the determination of doses, expressed as the median lethal dose (LD_{50}). This measure assesses by identifying the dose of the compound in the environment that causes 50% mortality in the test organisms over a specified period. The aim of this study is to evaluate the toxicity of *Azadirachta indica* as a phytopesticide and to provide insights into its intrinsic toxicity against the test insect, *Odontopus Varicornis*.

Materials and Methods

Insect collection and Rearing technique

Adult male insects of *Odontopus Varicornis*, collected from the government arts college campus botanical gardens, were brought to the zoology laboratory and reared in six plastic tubs, having a dimension 30x22x28 cm, at a laboratory temperature of 28°±1°C and relative humidity of 80±5%. *Odontopus Varicornis* feeds mainly on the seeds of Malvaceae (mallow) plants the insects were fed daily with soaked cotton seeds (Bombax ceiba) as well as seeds of its host plant, Stericulia foetida and Gossypium sp. The insects were thrived well on these foods. The insect cages were cleaned properly, every alternatlive days, by removing the excreta and other waste materials. The egg laid by them were transferred to another cage and thus, a continuous culture was maintained.

Azadirachta indica

Azadirachta indica was purchased from SBL world class homoeopathy Campany, formula: $C35H44O16$, Density: 0.933 g/ml, Molecular weight: 720.7 g/mol, for manufacturing in india by SBL Pvt. Ltd.

Toxicity Experiments and Determination of lethal dose (LD_{50})

10 insects were kept in each plastic tubs. The solutions are prepared over wide range of dose. The tests were conducted in adult male insect by injecting toxicity to each insect with micro syringe needle. Different median lethal dose, of *Azadirachta indica* which were 0.01, 0.02, 0.03, 0.04, and 0.05 ppm. Observed their mortality from 12 hours thereafter up to 96 hours. The alterations in the mortality of were detected at various exposure times. The LD_{50} value was calculated adopting the method of Finney 1971.

Result

SPSS 25 statistical software, was used to analyse the experiment's results for the mortality percentage. Table 1 and Figure 1 show the current mortality value of *Odontopus Varicornis* insect after injection with varying doses of *Azadirachta indica* for varying durations. The data from the toxicity tests were used to calculate the median lethal dose (LD_{50}) of *Azadirachta indica* for 12, 24, 48, 72, and 96 hours, along with its 95% confidence limits. This served as a foundation for analysis and the identification of the mode of action for *Azadirachta indica* toxicity on the test insect, *Odontopus Varicornis*. The median lethal dose (LD_{50}) for 48 hours of exposure was used to measure the phytopesticide *Azadirachta indica*'s to *Odontopus Varicornis*. Mortality rates examined at varying *Azadirachta indica* doses. The doses selected to determine ranged from 0.01, 0.02, 0.03, 0.04, to 0.05 ppm. The LD_{50} value for *Azadirachta indica* was found to be 0.054. The toxicity test revealed that mortality rates increased progressively with increasing doses, indicating a clear dose-dependent effect. 50% of the test insects died after receiving an injection of a median lethal dose of 0.05 ppm for 48 hours.

Table 1: LD_{50} value and 95% confidence limit of *Azadirachta indica* as well as the variation between upper and lower confidence limits for the significant adult male insect *Odontopus Varicornis*

Exposure time (in Hours)	Number of insects	Number of insects died in exposure dose (ppm)					LD 50	95 % confidence limits	
		0.01	0.02	0.03	0.04	0.05		Upper	Lower
12	10	0	1	2	3	4	0.069	2.399	0.041
24	10	1	2	3	4	6	0.044	0.086	0.034
48	10	1	2	3	4	5	0.054	174.0	0.033
72	10	4	6	7	8	9	0.014	0.022	0.002
96	10	6	7	8	9	10	0.009	0.016	0.000

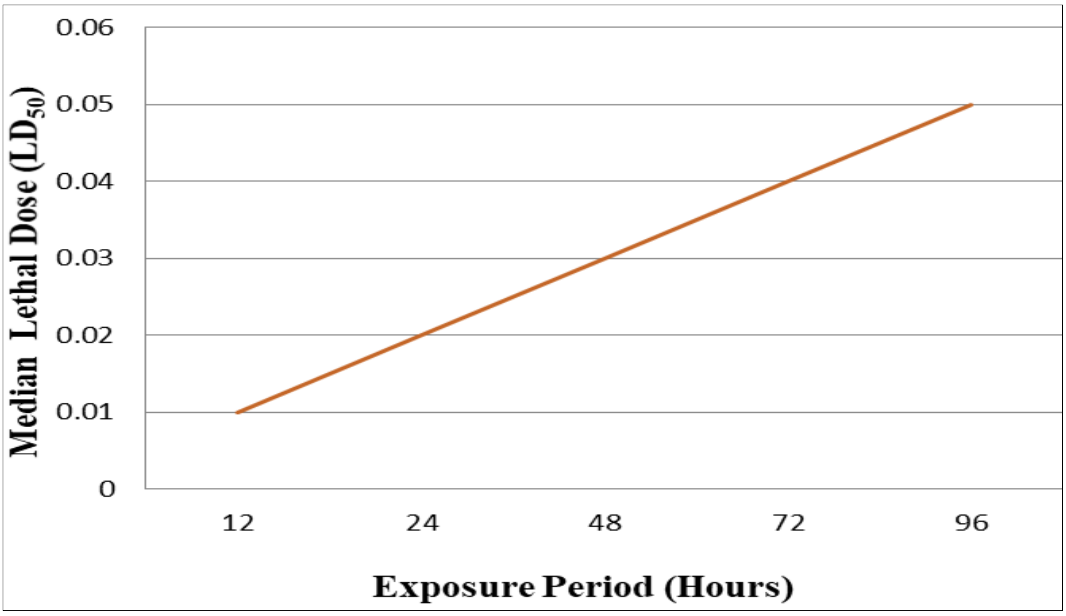

Fig1: Toxicity curve of insect, *Odontopus Varicornis* Exposed to Phytopesticide *Azadirachta indica*

Discussion

We expect that futsure research will be conducted for each pesticide group in an effort to obtain more precise and comparable mean and standard deviation results. (Bakr, Reda FA, *et al*., 2010) [2]. The alterations in the acute and subacute studies did not point to any harmful consequences of these substances (Bhutia Yangchen Doma *et al*., 2010) [4]. No essential oil ingredient totally stopped ootheca hatching, indicating that several field treatments may be necessary to stop reinfestation (Phillips, Alicia K., *et al*., 2010) [24]. According to LD_{50}, exposures to thorax and wing applications were acutely lethal to adults and caterpillars (Hoang Tham C. *et al*., 2011) [14]. The way that piperidine affects the body is indicated by the toxicity levels. It shows if the effect of the activity is cumulative, irregular, or regular. The acute toxicity level in the current study of *Odontopus Varicornis* treated with piperidine was reported in terms of LD_{50} (Selvisabhanayagam *et al*., 2013) [29]. Given the toxicological nature of the surroundings, these behavioural alterations may be regarded as signs of stress (Rani G., and A. K. Kumaraguru 2014) [27]. The food intake, exposure levels, and caterpillar reaction were used to calculate the LD_{50} (Hoang, Tham C. and Gary M. Rand 2015) [14]. To save native species, it is critical to develop management strategies that take this increased susceptibility into consideration (da Costa, Leticia Mariano *et al.,* 2015) [6]. These findings demonstrated that tadpole species were impacted differently based on the pesticide's chemical characteristics and dose response (Junges, C. M., *et al.,* 2017) [17] As anticipated, deltamethrin was the most dangerous active ingredient for 2-d-old female M. rotundata when comparing the LD_{50} values of the various active ingredients; its confidence intervals did not overlap with those of the other two insecticides (Piccolomini Alyssa M., *et al.,* 2018) [25]. Our screening analysis shows an increase in pesticide toxicity loading over the last 26 years, despite some simplifying assumptions. This could endanger the health of pollinators like honey bees and could also lead to a decline in beneficial insect populations, as well as insectivorous birds and other insect consumers (DiBartolomeis, Michael, *et al.,* 2019) [9]. It can be shown that the animals do not die at concentrations of 1.43 mg, while the deadly dose is 27.50 mg (De Jesus, Rosa *et al.,* 2021) [8]. Since LD_{50} does not accurately reflect a substance's toxicity, using it to categorise substances may not be very useful (Pillai, Sadasivan Kalathil, *et al.,* 2021) [26]. Dichloromethane and hexane crude extracts were effective against final instar nymphs and adult P. americana, indicating their potential to disrupt the life cycle and serve as insect control agents (Phayakkaphon Anon *et al.,* 2021) [23]. The highly active ligand is created against all tested bacterial species, according to the activity data (Kyhoiesh Hussein Ali K., *et al.,* 2021) [19]. Following the aforementioned treatments, the levels of MRCC I and ATP dropped, and there were clear dose-effect and time-effect correlations (Zhang can-Xin *et al.,* 2022) [33]. Similar to the Consensus approach, the top five compounds with the lowest LD_{50} values and the greatest potential for harm (Noga Maciej A. Michalska *et al.,* 2024) [22]. To close data gaps before experimental study and get ready for the impending usage of nerve agents, in silico techniques for predicting different parameters are essential (Noga Maciej *et al.,* 2023) [21]. Most research on how pesticides affect beneficial insects concentrates on pollinators like honey bees (Bartling Merle-Theresa *et al.,* 2024) [3]. It was advised to take a lower dose since there was a substantial difference in blood glucose levels between groups but no significant difference between the clinical schemistry groups of liver and kidney function (Farag, Ahmed A. Gh *et al.,* 2025) [10].

Conclusions

According to the findings of this study, *Odontopus Varicornis* insects are somewhat harmed by phytopesticide containing *Azadirachta indica*. Exposure to modest amounts of *Azadirachta indica* causes significant behavioural changes by acting as a repellant and inhibiting insect development this fact needs to be considered.

References

1. Ahmed N, Shahnaz A, Khan AM, Siddiqui TZ, Rahman A, Bano S, *et al*. Botanical insecticides are a non-toxic alternative to conventional pesticides in the control of insects and pests. Global Decline of Insects, 2021.
2. Bakr RFA, Hegazi EM, El-Sheikh ME, Moursy LE, El-Kholy MY. A mathematical model for estimating the LC50 or LD50 among an insect life cycle. Egyptian Academic Journal of Biological Sciences an Entomology,2010:3(2):75–81.
3. Bartling MT, Buschhaus C, Brühl CA. Current insights into sublethal effects of pesticides on insects. International Journal of Molecular Sciences,2024:25(11):6007.
4. Bhutia YD, Sharma R, Suresh R, Srivastava C. Acute and sub-acute toxicity of an insect pheromone, N-heneicosane and combination with insect growth regulator, diflubenzuron, for establishing no observed adverse effect level, NOAEL. Indian Journal of Experimental Biology,2010:48(7):744–751.
5. Bilal T, Qamar U, Ahmad S, Imran A, Naz A, Khan SA, *et al*. Botanicals their use as antimicrobial antifungal and anti-insecticides. Pharma Innovation,2022:11:1521–1528.
6. da Costa LM, dos Santos SF, Alves TA, de Souza BA, Lima MAV, Malaspina O, *et al*. Determination of acute lethal doses LD50 and LC50 of imidacloprid for the native bee Melipona scutellaris Latreille 1811 (Hymenoptera: Apidae). Sociobiology,2015:62(4):578–582.
7. Dalavayi Haritha M, Bala S, Choudhury D. Eco-friendly plant based on botanical pesticides. Plant Archives,2021:21(1):2197–2204.
8. De Jesus R, Iglesias J, Paredes Y, Castillo J, Poma E, Andrade H, *et al*. Determination of medium lethal dose LD50 and acute toxicity of formulation Cytoreg, an ionic mixture of strong and weak acids. Latin American Journal of Development,2021:3(3):1121–1126.
9. DiBartolomeis M, Kegley SE, Mineau P, Radford R, Klein K. An assessment of acute insecticide toxicity loading, AITL. of chemical pesticides used on agricultural land in the United States. PLOS ONE,2019:14(8):0220029.
10. Farag AAG, Ahmed LA, Hegazy AM, Hassan NM, Khalil WF. Acute oral toxicity of two products from a microbial pest control agent, *Beauveria bassiana*, on physiological status aspects of male albino rats. Egyptian Journal of Hospital Medicine,2022:86(1):943–951.
11. Finney DJ. Probit analysis. University Press Cambridge, 1971, 333.
12. Funmilayo FOA, Adegoke AA, Oyekanmi AS, Fadeyibi OE. The study of plant products as botanical pesticides for pest management to reduce post harvest losses, a review, 2023.
13. Grdisa M, Grsic K. Botanical insecticides in plant protection. Agriculturae Conspectus Scientificus,2013:78(2):85–93.
14. Hoang TC, Rand GM. Acute toxicity and risk assessment of permethrin naled and dichlorvos to larval butterflies via ingestion of contaminated foliage. Chemosphere,2015:120:714–721.
15. Ikhwan A, Latifah S, Mukhlish M, Wulandari NA, Salsabila SR, Hanif M, *et al*. Acute toxicity test of lancing leaf extract *Solanum mauritianum Scop*. as well as determination of LD50 values and histopathology in male mice, Wistar rat. Jurnal Penelitian Pendidikan IPA,2025:11(6):23–33.
16. Isman MB. Bridging the gap moving botanical insecticides from the laboratory to the farm. Industrial Crops and Products,2017:110:10–14.
17. Junges CM, Althaus A, Crestani M, de Oliveira SL, Baldisserotto B, Hartz SM, *et al*. Acute toxicity and etho-toxicity of three insecticides used for mosquito

control on amphibian tadpoles. Water Air and Soil Pollution,2017:228(4):143.
18. Kiri IZ, Muhammad S, Jibril HM, Sulaiman MK, Usman MM, Umar A, *et al*. The use of botanicals as pesticides: history development and emerging challenges. Dutse Journal of Pure and Applied Sciences,2024:10(1a):191–207.
19. Kyhoiesh HAK, Al-Sammarrae AH, Al-Khazaali MH, Al-Juboori FY, Al-Taai KA, Al-Fekaiki DF, *et al*. Synthesis spectral characterization lethal dose LD50 and acute toxicity studies of 1 4-Bis imidazolylazo benzene, BIAB. Heliyon, 2021, 7(9).
20. Mohan T, Selvisabhanayakam, Mathivanan V. Evaluation of LD50 value of the phytopesticide piperidine on the adult male insect *Odontopus Varicornis, Heteroptera, Pyrrhocoridae*, 2011, 1705–1709.
21. Noga M, Michalska A, Jurowski K. Application of toxicology in silico methods for prediction of acute toxicity, LD50, for Novichoks. Archives of Toxicology,2023:97(6):1691–1700.
22. Noga M, Michalska A, Jurowski K. The prediction of acute toxicity, LD50. for organophosphorus-based chemical warfare agents (V-series) using toxicology in silico methods. Archives of Toxicology,2024:98(1):267–275.
23. Phayakkaphon A, Sawatdee S, Ritthong P, Ngernsoungnern P, Ngernsoungnern A, Chareonviriyaphap T, *et al*. Oral toxicity of various Stemona collinsiae crude extracts against nymph and adult stages of American cockroach *Periplaneta Americana, Dictyoptera*, *Blattodea*. Heliyon, 2021, 7(9).
24. Phillips AK, Appel AG, Sims SR. Topical toxicity of essential oils to the German cockroach, *Dictyoptera*, *Blattellidae*. Journal of Economic Entomology,2010:103(2):448–459.
25. Piccolomini AM, Ascher J, Marshall J, Pitts-Singer TL, Vaughan M, Danforth BN, *et al*. Acute toxicity of permethrin deltamethrin and etofenprox to the alfalfa leafcutting bee. Journal of Economic Entomology,2018:111(3):1001–1005.
26. Pillai SK, Subramanian V, Menon PG, Nair AS, Subair TM, John M, *et al*. John William Trevan's concept of median lethal dose, LD50/LC50, more misused than used. Journal of Pre-Clinical and Clinical Research, 2021, 15(3).
27. Rani G, Kumaraguru AK. Behavioural responses and acute toxicity of *Clarias batrachus* to synthetic pyrethroid insecticide λ-cyhalothrin. SAARC Journal of Agriculture,2014:15:99–109.
28. Saeed S, Naqqash MN, Jaleel W. Toxicological studies on some important chemicals against *Dysdercus koenigii* Fabr, *Hemiptera, Pyrrhocoridae.* Pakistan Journal of Zoology, 2016, 48(5).
29. Selvisabhanayakam, Mohan T, Mathivanan V. Sub lethal effect of LD50 value of the phytopesticide piperidine on the adult male insect *Odontopus Varicornis, Heteroptera, Pyrrhocoridae*. International Journal of Current Zoological Research,2013:1:17–21.
30. Srivastava SK, Singh A, Suman S, Yadav B, Maurya S. Phytochemicals of *Azadirachta indica* source of active medicinal constituent used for cure of various diseases a review. Journal of Scientific Research,2020:64(1):385–390.
31. Usui K, Ichikawa R, Okuhara H, Namiki T, Sugimura Y, Kadota Y, *et al*. Acute oral toxicity test of chemical compounds in silkworms. Drug Discoveries and Therapeutics,2016:10(1):57–61.
32. Wojciechowska M, Stepnowski P, Gołębiowski M. The use of insecticides to control insect pests. Invertebrate Survival Journal,2016:13(1):210–220.
33. Zhang CX, Yang HJ, Wang C, Tian Y, Liu ZD, Liu F, *et al*. Sublethal effects of tolfenpyrad on the development reproduction and predatory ability of Chrysoperla sinica. Ecotoxicology and Environmental Safety,2022:236:113482.