Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):1-5

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Acute toxicity of *Nerium oleander* L. essential oil on the honey bee workers *Apis mellifera intermissa* (Buttel-Reepen, 1906)

Abdelkader Harizia

Laboratory Geo - Environment and Development Areas, Faculty of Life Science and Nature, University Mustapha Stambouli of Mascara, Algeria

---

ABSTRACT

A preliminary study of the toxicity of Nerium oleander leaf extract on Apis mellifera intermissa (Buttel-Reepen , 1906) workers bee has been studied under laboratory conditions. Nerium oleander (Apocynacaea) is an ornamental shrub very common in North Africa. Studies concerning contact toxicity towards bees show a 100 % mortality rate with a 25µg/bee dose and 8.94-µg/bee LD_{50} 48 hours after treatment has started. Mortality equals 0 with a 5-µg/bee dose. Controls mortality rate is 3.33%.Tested doses with 10, 15 and 25-µg/bee has the same toxicity 4, 24 and 48 hours after topical application treatment has started. LD_{50} values are 9.26 µg/bee 4 hours (Fig.4) after treatment has started and 8.94 µg/bee at 24and 48 hours. These preliminary LD_{50} results assess that N.oleander extract toxicity is very weak compared to dimethoate (with a 0.16-µg-SA/bee LD_{50}), being the reference toxic insecticide towards bees.

Keywords: Toxicity, bee workers , oleander, mortality, topical application, doses

---

INTRODUCTION

Apis mellifera is endemic to Africa, Europe and parts of western Asia ,ranging from Kirgizia in the east to the most western limits of Europe [1] honey bees are a vital part of our agricultural system. It plays an important role in global food production [2]. They are an indispensable part of the ecological balance in the world for their role in the pollination of many plant species [3]. Beekeeping has suffered enormously from the spread of phytosanitary treatments, thousands of hives have been destroyed by insecticides [4]. It is evident that the effects of honey poisoning will, depending on the active ingredient, sometimes immediate, sometimes delayed.

If there is an immediate effect, the consequences are visible after a few hours and during 2-4 days, sometimes leading to the total extinction of the population [5]. Unfortunately in the last decade, a high mortality of honey bee, *Apis mellifera intermissa*, was frequently reported in countries in Africa [6]. In Algeria, during the last few years the mortality that varies between 11% and 90%, of honey bees was declared by farmers mainly in agricultural regions [3]. Botanicals are a promising source of pest control compounds. Over 2000 species of plants are known to possess some insecticidal activity [7]. These plants have natural allelochemical substances protecting themselves from pests [8]. Experimental toxicity of *N.oleander* has been described in *Culex pipiens* [9], mice [10] and *Schistocerca gregaria*[11]. Essential oils represent a promising track in the fight against pests and research on this subject is numerous. However, most of these studies focus on biological and toxicological effects and not on non-target insects such as bees. *N.olender*'s essential oil causes on desert locust consumption rejection, decreased body weight and an insecticidal effect [12]. The present study was performed on worker honey bees to evaluate the toxic effect of *Nerium oleander's* essential oil. Thus, the main aim of this laboratory experiment was to evaluate the acute toxicity of this plant in local bees. Also to decide on its use in testing subsequent actions against insect pests.

EXPERIMENTAL SECTION

Plant material

N. oleander old leaves were collected during flowering stage in the Mascara region (Mount Beni-Chougrane-Algeria).*N.oleander* (Apocynaceae) spontaneously grows in the Mediterranean region as well as in Asia and Oceania sub-tropical regions. In North Africa *N.oleander* is quite common in steppe area. In Algeria it is also quite common, above all on alluvial deposits and rocky grounds. It grows along North Sahara wadies and reaches Tassili and Hoggar mountains [13]. *N. oleander* is an extremely toxic plant: taken orally it may cause mortal poisoning because of two alkaloids, mainly oleandrin [14]. Mechanisms responsible for oleander cardenolides toxicity are similar to common digitalis glycosides mechanisms inhibiting membrane Na.K ATPase activity and increasing intracellular calcium. Ingesting 0.25 g of dried leaves per kg of body weight is supposed to be mortal in 4 to 24h with no treatment [15]. Nevertheless substances composing oleander are useful in medicine with pharmacological, cardiac, antibacterial and pest control properties [16, 17] .Plant accumulates cardenolide heterosides in all organs. Leaves contain about 1.5% of cardenolides including 0.1% of oleandrine [18]. Fresh or dried leaves are used externally in traditional medicine to heal loss and certain skin disorders [19, 20, 21].

Insect material

Apis mellifera intermissa also called "western honey bee" is a local species living in Maghreb countries. Acute toxicity tests were performed on domestic worker bees collected during summertime with no determined age. They all came from healthy colonies, had no acaricide treatment and were well fed. *A. mellifera intermissa* is an local Algerian race.

Extracting of essential oil

20g of fresh *N.oleander* leaves were subjected to hydrodistillation for 4 at 100°C in a 250ml flask with 2/3 of distilled water.

After hydro-distillation essential oil were dried over anhydrous magnesium sulfate (MgSO4) to remove traces of water and were deposited in a small flask and hermetically sealed to be stored at 4°C[18].For experimentation needs several leaf hydro-distillation was processed under the same conditions. Extracted essential oil was diluted in a determined quantity of acetone. Further on solution was the purpose of biological tests on honeybees.

Acute contact toxicity test on *Apis mellifera intermissa*

Acute toxicity tests on bees were conducted according to OECD guidelines [22]. Bees were anesthetized with low-flow carbon dioxide before depositing 10 individuals per wooden crate (10 x 8,5 x 6cm). Bees were fed throughout the experiment with an aqueous sucrose solution (500g/l) given ad libitum through a hemolysis tube. Bees were kept in darkness in a metal oven at 25 ± 2°C and 50 to 70% relative humidity. Moisture level was controlled with water recipients located in the oven. During test bees were kept in daylight and treated one by one with 1µl *N.oleander* extract locally applied on the backside of thorax using a micropipette. Five doses were tested (25, 20, 15, 10 and 5µg per bee). The range was determined through a preliminary experiment. Three replicates were used for each dose. A fresh aqueous sucrose solution was given to treated bees after every application. Control individuals from 3 wooden crates were treated with 1µl acetone. Mortality was recorded 4, 24 and 48 hours after treatment. Motionless and lying bees were considered as dead. LD_{50} was calculated at 4, 24 and 48 hours .A molecule acute toxicity is measured through oral or contact LD_{50}. It is well known that values are extremely related to temperature, active substances, bee breeds and age of tested individuals [23]. The estimation of toxicity vis-a-vis bee pesticides is carried out mainly through the measurement of the oral LD 50 or contact sublethal effects being mostly obscured , mainly due to a lack of standardized methods and criteria clearly identified [24].

Statistical analysis

XLSTAT-Pro was used to analyze all data. The analysis of variance was performed by ANOVA. Comparisons were conducted with confidence levels 5% of statistical significance. Cochrane test was used to compare mortality of treated individuals.

The determination of LD_{50} and its confidence limit was based on the dose effects analysis logistic regression model using the methodology of logistic regression aims at modeling the probability of success depending on the values of the explanatory variables, which can be numerical or categorical variables.

RESULTS AND DISCUSSION

Acute toxicity and LD_{50} of bees

Figure 1 records after 4, 24 and 48 hours cumulative mortality rate of *A.mellifera* individuals exposed to increasing *N.oleander* extract doses. Mortality reaches a significant 100% rate with a 25-µg/bee dose 48 hours after treatment has started. Mortality equals 0 with a 5-µg/bee dose (Q cal = 262.46, Q theo=22.36, P< 0.0001; alpha =0.05). Controls mortality rate is 3.33%.Tested doses with 10, 15 et 25-µg/bee have the same toxicity 4, 24 and 48 hours after topical application treatment has started ($F_{(1.71)} = 45.79$, $P<0.0001$).

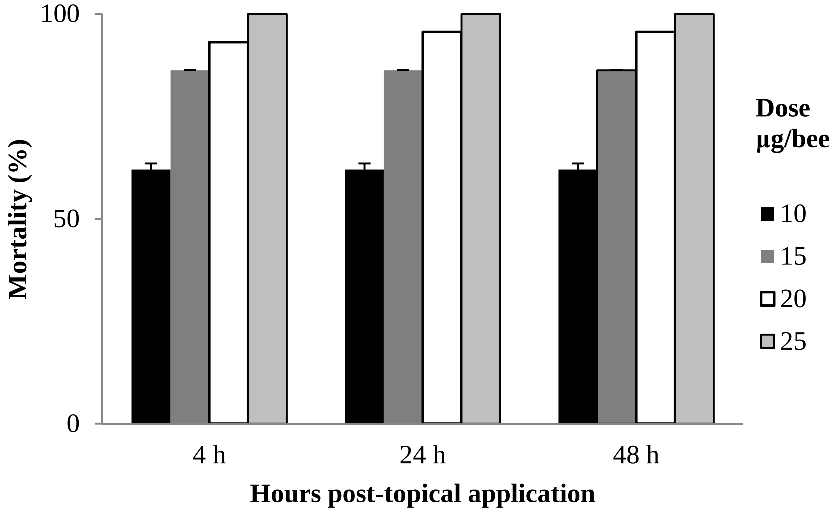

Fig.1. Mortality (%) of bees treated with increasing doses of *N.oleander* essential oil

LD_{50} values are 9.26 µg/bee (confidence limit is 7.69<9.26 <10.85) 4 hours (Fig.2) after treatment has started (Chi^2=50.62, p< 0, 0001) and 8.94 µg/bee (confidence limit is 7.10<8.94 <10.50) at 24and 48 hours (Fig.3) Chi^2=41.04, p< 0, 0001).

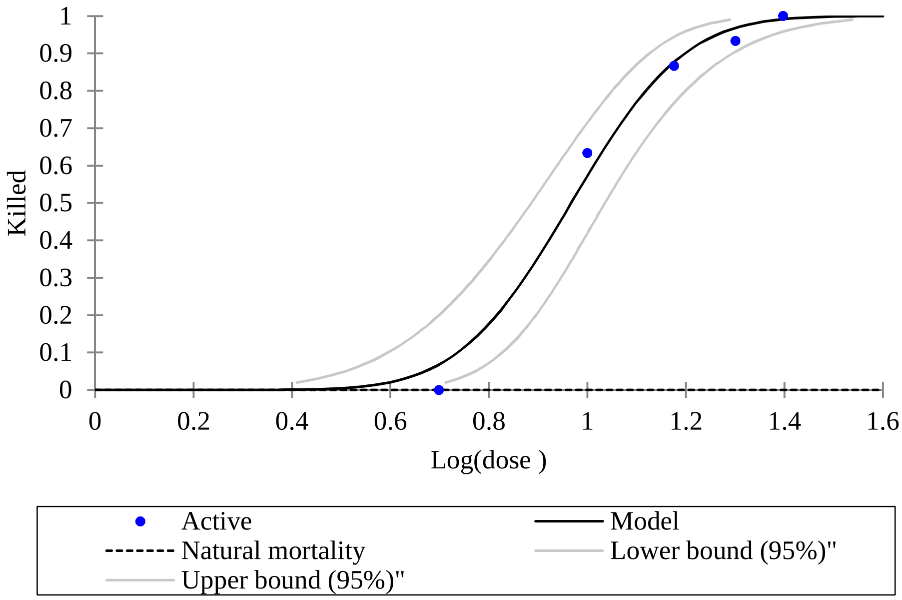

Fig.2. Lethal effect of *N. oleander* essential oil on bees at 4 hours

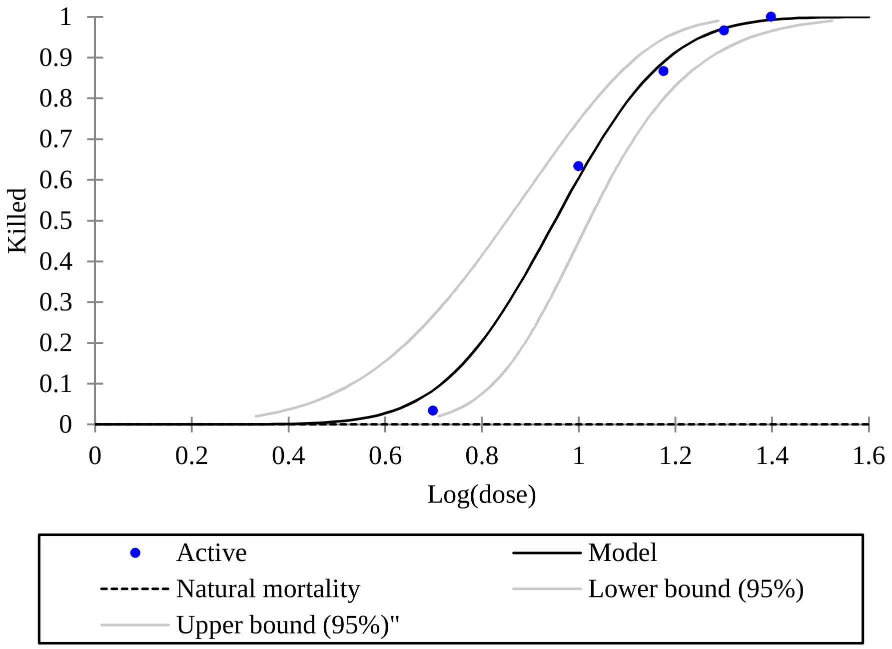

Fig.3. Lethal effect of *N.oleander* essential oil on bees at 48 hours

A 100% mortality of bee individuals is recorded 4 hours after topical application of increasing doses. It is then recorded 24 and 48 hours with a 25-µg/bee dose. Toxicity can thus be evaluated in a laboratory after 24 hours. The weakest mortality rate (3.33%) is recorded with a 5-µg/bee dose 24 and 48 hours after treatment. Mortality rate equals 0 after 4 hours. Nevertheless mortality is not a sufficient factor to evaluate chronic effects of a product [25].
N.oleander leaf extract applied to the bee's thorax goes across the cuticle through waxy canaliculus and is directly dispensed in the organism more particularly in the most lipophilic zones. Toxic molecules generally act on nervous influx conduction disorders [5]. Main *N.oleander* known toxic components are cardiac heterosides [26] .These preliminary results recorded in a laboratory on an individual scale are a first step towards semi-controlled and on-the-field tests. Only after these tests we will be able to assess about *N.oleander* extract innocuousness towards bee populations. These preliminary LD_{50} results assess that *N.oleander* extract toxicity is very weak compared to dimethoate (with a 0.16-µg-SA/bee LD_{50}**)**, being the reference toxic insecticide towards bees. *N.oleander* extract toxicity is also very weak compared to most common synthetic insecticides in pest control such as chlorpiryphos (LD_{50} = 0.059 µg/bee), malathion and deltamethrine (LD_{50} = 0.0015 µg/bee) [27, 28] . Topical application LD_{50} values are not only due to the extract action but also to anesthetic effects. Bees being immobilized with carbon dioxide before topical application can increase extract action. Anesthesia with carbon dioxide has direct effects on the nervous system, *via tracheae*, neuron depolarization. Carbon dioxide gas has indeed effects on acetylcholine and on number of neurotransmitters such as dopamine and octopamine [29].

CONCLUSION

Having LD_{50} as the main criteria to assess *N.oleander* extract toxicity towards bees appears to be insufficient. This is why sublethal effects on bee behavior should be the purpose of further studies also on the components responsible of toxicity. These preliminary results will also allow for further research on biopesticides is based plant extract.

Acknowledgments
I would like to thank team of the Laboratory Geo - Environment and Development Areas for their kind support and also Mr. Abdelkader Louissi for statistical analysis.

REFERENCES

[1] WS Sheppard; MD Meixner. *Apidologie*., **2003**, 34, 367-375.
[2] JP Faucon & M Ribiere. *Bulletin des GTV*., **2003**, 20 , 15-18.
[3] N Adjlane ;SE Doumandji ; N Haddad. *Cahiers Agricultures*., **2012**, 21(4),235-241.
[4] J Louveaux. Pollinisation et productions végétales. INRA-Paris, France, **1984** ; 565-575.
[5] JN Tasei. *Courrier de l'environnement de l'INRA*., **1996**, 29, 9-18.

[6] D Nabti; M Achou and Soltani N. *Advances in Applied Science Research*, **2014**, 5 ,51-55
[7] M Jacobson. Botanical pesticides, past, present and future. In "Insecticides of plant origin ".Amason ,J.T,ed . Proc. Amr. Chem. α Soc. Washington, D C, **1989**; 1-10.
[8] L Idrissi Hassani & M Hermas. *Journal Zoologica baetica.*, **2008**, 19, 71-84.
[9] N Barbouche; B Hajlem; L Georges; & M Ammar**.** *Biotechnol. Agron. Soc. Envir.*, **2001,** 5 ,85-90.
[10] F Maha. *Baghdad Science Journal*. **2011**,8, 366-372.
[11] M Bagari; A Bouhaimi; S Ghaout; &. Chihrane. *Journal Zoologica Baetica.* **2013**, 24, 193-203.
[12] A Harizia and SE Doumandji. *International journal of zoology and reaserch*. **2014**, 4, 19-28
[13] LC Chopra ; BK Abrol ; K LHanda. *Les plantes médicinales des régions arides considérées surtout du point de vue botanique.* Premiére partie, UNESCO, Imprimeries Oberth Paris, France, **1971** ;45-50.
[14] R R Barbosa; J D Fontenele-Neto; B Soto-Blanco. *Research in Veterinary Science*. **2008**, 85 , 279-281.
[15] H Bakkali; M Ababou; T Nassim Sabah; A Moussaoui; A Ennouhi; FZ Fouadi; S Siah; H Ihrai. *Ann Burns Fire Disasters*. **2010** ,23, 128-130.
[16] L Delille. Les plantes médicinales d'Algérie, Berti éditions. Algérie, **2007** ;240.
[17] K A Ibrahim; S Khalagi; D Youssef; I.Khan; M Mesbah. *Enzyme and Microbial Technology*. **2007**, 41, 331-336.
[18] J Bruneton. *Pharmacognosie, Phytochemie- Plantes médicinales-Techniques et documentations*, 3ème édition, Lavoisier,France, **1999** ; 463-730.
[19] F Baba Aissa. Encyclopidie des plantes utiles. Flore d'Algérie et du Maghreb. Ed. Libraire Moderne-Rouïba, Alger. **1999 ;** 80.
[20] M M Bnouha ; H Mekhfi ; A Legssyer ; A Ziyyat. *J. diabetes et Metabolism*. **2002** , 10,33-50.
[21] R O Adom; J. W Gachichi; B Negi; J Tamale; S O Apio. *African health sciences*. **2003**, 3,77-82.
[22] OCDE/OECD. Ligne directrice de l'OCDE pour les essais de produits chimiques : abeille domestique, essai de toxicité aiguë par contact. Éditions OCDE, France,section 2,essai n°214 , **1998.**
[23] MP Chauzat ; JP Faucon ; A.C Martel ; J Lachaize ; N Cougoule & M Aubert. *Phytoma*.**2006** ; 594 ,443-455.
[24] A Decourtye. Etude des effets sublétaux de l'imidaclopride et de l'endosulfan sur l'apprentissage olfactif chez l'abeille domestique Apis mellifera L. DEA de Neurobiologie des Processus de Communication et d'Intégration, Université de Montpellier. **1998.**
[25] Y Aliouane ; A. K EL Hassani ; V Gary ; C Armengaud ; M Lambin ; M Gauthier. *Environm. Toxicol. Chem*. **2009**, 28 , 113-122.
[26] Bruneton, J. *Plantes toxiques : végétaux dangereux pour l'homme et les animaux.* 2ème édition, France, **2001** ; 564.
[27] LE Smart & JH Stevenson. *Bee World* **.1982**, 63, 150-152.
[28] L H Brader ; H Djibo ; F G Faye ; S.Ghaout ; M Lazar ; P N Luzietoso & M A Ould babah,. Evaluation multilatérale de la campagne 2003- 2005 contre le Criquet pèlerin. Ed. FAOUN, Rome, Italie, **2006.**
[29] G Nicolas & D Sillans. *Annu. Rev. Entomol*. **1989**, 34 , 97-116.