https://doi.org/10.48047/AFJBS.6.14.2024.720-727

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Larvicidal and ovicidal efficacy of an agricultural pesticide, Thiamethoxam (25%) against *Culex pipiens* mosquito

Khedidja Amira*[1,2] and Nour El-Houda Djeghader[2,3]

[1]Department of Natural Sciences, Higher Normal School of Technological Education of Skikda, Skikda- Algeria.

[2]Laboratory of Physical, Chemistry and Biology of Materials, Skikda- Algeria.

[3] Department of Biology, Badji Mokhtar University, Annaba- Algeria.

***Correspondence:** amira_khedidja@yahoo.com

Volume 6, Issue 14,2024

Received: 10 JULY 2024

Accepted: 31 JULY 2024

Published: 02 AUG 2024

doi:10.48047/AFJBS.6.14.2024.720-727

Abstract

Agricultural pesticides play a significant role in Algeria's agricultural sector, helping to protect crops from pests and diseases, and thus contributing to food security and agricultural productivity.

The objective of this study is to test the insecticidal activity of Oxam (Thiamethoxam 25%), on *Culex pipiens* (Linnaeus, 1758) mosquito. For this, various concentrations (5, 10, 20 and 50 µg/l) were tested on fourth-instar larvae newly molted from the domestic mosquito *Culex pipiens*. Three repetitions for each concentration were carried out with 15 larvae each. Preliminary trials allowed for the selection of this range of concentrations. After estimating LC_{50} and LC_{90}, they were applied to newly laid egg rafts to determine their ovicidal activity.

The results obtained showed that the used pesticide has a larvicidal effect on fourth-instar larvae, as evidenced by the mortality recorded in relation to concentration compared to the control. The lethal concentrations LC_{50} and LC_{90} were estimated at 9.54 and 22.9 µg/l respectively. Additionally, it exhibits ovicidal activity by reducing the hatching rate from 96% in the control group to 93% and 85% for LC_{50} and LC_{90} respectively.

Keywords: Insecticidal, Thiamethoxam, *Culex pipiens*, larvae, LC_{50} and LC_{90}, Hatching rate.

1. Introduction

The use of pesticides, fertilizers, and other phytosanitary products is excessive, often with the recommended doses and usage durations being disregarded due to farmers' lack of awareness. However, these chemical substances are not harmless. They have direct environmental consequences and long-term health impacts due to the infiltration of these nondegradable molecules into the soil, groundwater, and subsequently into ecosystems: affecting plants, animals, and inevitably humans (Aït Hamlet et al., 2019).

For these reasons, and due to the resistance to chemical insecticides, including pyrethroids the primary class used against mosquitoes, there has been renewed interest in Neonicotinoids (Zoh et al., 2021). Neonicotinoids are the fastest-growing class of insecticides due to their purported moderate toxicity to mammals, environment and their effectiveness against insects resistant to other classes of pesticides (Hataba et al., 2014; da Silva Mesquita et al., 2020). The term "neonicotinoids" refers to a class of insecticides comprising several substances used in agriculture and by individuals. The seven active substances in this family: imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin, and dinotefuran, are or have been available on the global market for phytosanitary or biocidal use (Qiong et al., 2012). They act agonistically on insect nicotinic acetylcholine receptors, causing nervous stimulation at low concentrations, but leading to receptor blockage, paralysis, and death at higher concentrations (Kundoo et al., 2018).
The purpose of this study is to evaluate the larvicidal and ovicidal effect of an agricultural pesticide Oxam, Thiamethoxam (25%) on the fourth instar larvae and eggs of the domestic mosquito *Culex pipiens* (Linnaeus, 1758).

2. Materials and methods

Mosquitoes rearing

In the laboratory setting, *Culex pipiens*, the most abundant mosquito species in Algeria, was bred under controlled conditions. They were housed in breeding containers filled with dechlorinated water and maintained at a temperature range of 25°C to 26°C, with a natural photoperiod of 12 hours of light. Their diet consisted of a daily mixture of biscuit and yeast, with proportions of 75% and 25% respectively (Amira et al., 2013).

The pesticide

Oxam, typically refers to Oxamyl; is an agricultural pesticide, its active ingredient is Thiamethoxam (25%), it is a neonicotinoid compound with broad-spectrum insecticidal properties (Mitchell et al., 2017). It is used in the Northeast region of Algeria against biting and sucking insects that affect cereals, fruit trees, and vegetable crops. It is applied in cultivation at doses ranging from 800 to 4000 mg/L of insecticide (commercial formulation), corresponding to 200 to 1000 mg/L of its active ingredient thiamethoxam (Aït Hamlet et al., 2019). It works by inhibiting the activity of acetylcholinesterase, an enzyme essential for proper nerve function in insects and nematodes. By disrupting the nervous system of these pests, oxam effectively kills them or prevents them from causing further damage to crops.

Toxicological essay

Larvicidal activity

For experimentation, Oxam (Thiamethoxam, 25%), was used in various concentrations (5, 10, 20 and 50 µg/l). Subsequently, 15 newly molted fourth-instar larvae of *Culex pipiens* were introduced into testing plastic cups containing 500 ml of water with cited concentration, with three replicates for each one. After a 24-hour exposure period, the water was replaced, and food was replenished. Mortality rates were recorded daily until the end of the instar (Amira et al., 2013). The mortality percentage was corrested in cases where mortality was observed in the control group. Subsequently, the toxicity data were analyzed to determine the lethal concentrations (Amira et al., 2018).

Ovicidal activity

Newly laid egg rafts of *Cx. pipiens* were taken from the cages immediately after being laid and placed into jars containing 500 ml of tap water to reduce desiccation. They were then treated with two concentrations LC_{50} = 9.54 and LC_{90} =22.9 µg/l. Each concentration contains 3 egg rafts, Number of laid eggs was estimated by counting the number of eggs using a binocular microscope. Number of laid eggs was estimated by counting the number of first instar larvae after eclosion. The Hatching Rate (H) was calculated using the following formula:

H (%) = (Number of hatched eggs/ Total number of eggs) × 100 (Bouaziz et al., 2017).

Statistical analysis

For statistical analysis, the One-way ANOVA test was used to assess the significance between control and treated groups. The statistical analyses were performed using MINITAB software.

3. Results and discussion

The toxicological study focused on the treatment of newly molted fourth instar larvae of *Culex pipiens* with various concentrations (5, 10, 20, and 50 µg/l) of the pesticide Oxam. The results obtained from the treatment are expressed as the mean (m±SE) of mortality percentage. They revealed an increase in observed mortality in all treated groups compared to the control from 33.33% with the lowest concentration ($p \leq 0.05$) to 100% with the highest one ($p \leq 0.001$), and the same observation was made for the corrected mortality (Table 1).

Table 1: The observed and corrected mortality (%) of fourth larval stage of *Cx. pipiens* exposed to different concentrations of Oxam (Thiamethoxam, 25%), *n=45*.

Concentrations	Observed mortality (%)	Corrected mortality (%)
Control	8.88 ± 2.96	/
C1= 5 µg/l	33.33 ± 4.44 *	26.55 ± 8.42
C2= 10 µg/l	48.88 ± 5.92 **	43.59 ± 8.54
C3= 20 µg/l	73.33 ± 4.44 ***	72.7 ± 7.93
C4= 50 µg/l	100 ± 0.00 ***	100 ± 0.00

** = p≤0.05; ** = p≤0.01; *** = p≤0.001.*

Subsequently, a transformation of the corrected mortality means into probits and the tested concentrations into decimal logarithms was performed. The regression line equation, shown in Figure 1, was determined, with a coefficient of determination ($R^2 = 0.87$) indicating a very strong correlation between the probits and the decimal logarithms.

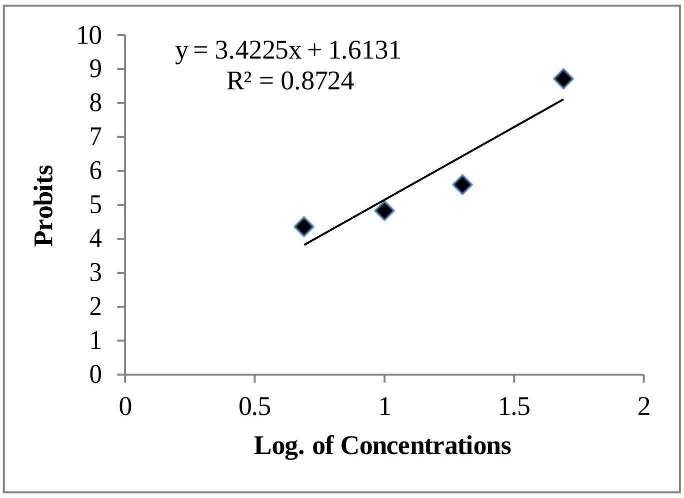

Figure 1: Regression equation of Oxam (Thiamethoxam, 25%) against fourth instar larvae of *Cx. pipiens.*

The lethal concentrations, LC_{50} and LC_{90}, were estimated from the regression equation and line. The LC_{50} is 9.54 µg/l with a confidence interval of 8.67 to 10.49 µg/l, and the LC_{90} is 22.9 µg/l with a confidence interval of 20.81 to 25.19 µg/l (Table 2).

Table 2: Lethal Concentrations: LC_{50} and LC_{90} (µg/l) with Fiducial Limits (FL) of Oxam (Thiamethoxam, 25%) against fourth instar larvae of *Cx. pipiens.*

Treated stage	R^2	Slope	LC_{50} (FL) µg/l	LC_{90} (FL) µg/l
L_4	87.24	1.97	9.54 (8.67 - 10.49)	22.9 (20.81 - 25.19)

The results show that Oxam (Thiamethoxam, 25%) significantly reduced the egg hatching percentage ($p \leq 0.05$) after treatment with the LC_{50}, with a reduction about 3% and ($p \leq 0.01$) after treatment with the LC_{90}, with a reduction of more than 10% compared with the control group (Table 3).

Table 3: Ovicidal activity of Oxam (Thiamethoxam, 25%) against *Cx. pipiens*, n=3 egg rafts.

Concentrations	Number of laid eggs	Hatching Rate (H) (%)
Control	206	96. 01 ± 0.94
LC_{50}= 9.54 µg/l	218	93.14 ± 0.76 *
LC_{90}= 22.9 µg/l	204	85.37 ± 2,82 **

** = p≤0.05; ** = p≤0.01*

The widespread use of neonicotinoids has raised concerns about the development of resistance in mosquito populations. Continuous exposure to sub-lethal doses can select for resistant individuals, making future control efforts more challenging. Monitoring for resistance and implementing integrated pest management strategies are crucial to mitigate this risk.

Several studies have evaluated the effectiveness of neonicotinoids against mosquito larvae, with varying results. Dinotefuran against susceptible and resistant strains of *An. gambiae, Ae. aegypti*, and *Cx. Quinquefasciatus* (Corbel et al., 2004) and Imidacloprid has shown an insecticidal activity against *Aedes aegypti* (Tomé et al., 2014), Clothianidin against *Anopheles arabiensis* (Dagg et al., 2019).

Recent researchs have shown that three neonicotinoids which have significant larvicidal activity against Anopheles species with high mortality rates at relatively low concentrations; LC_{50} values after 24 hours for acetamiprid, clothianidin and thiamethoxam are 13.6, 18.6 and 9.66 µg/l respectively (Ashu et al., 2023). Acetamiprid, imidacloprid and clothianidin have shown a larvicidal activity against *Anopheles gambiae* (Ambadiang et al., 2023). Not only mosquitoes, neonicotinoids affect other non target invertebrates like honey bee *Apis mellifera*, *Mysidopsis bahia*, *Gammarus roeseli*, mayflies *Epeorus spp*. and *Baetis spp*, water flea, *Ceriodaphnia dubia*, amphipod *Gammarus pulex* and others (Chahbar et al., 2011; Pisa et al., 2015 and Chahbar-Adidou et al., 2018).

Moreover, the treatment of newly laid *Cx. pipiens* eggs significantly reduced the hatching percentage. These results can be explained by the effect of the pesticide used on the desiccation of the eggshells, as the larvae develop normally (without treatment) inside them but do not hatch.

Field trials have also demonstrated the potential of neonicotinoids to reduce mosquito populations. However, environmental factors such as water quality, presence of organic matter, and sunlight can affect the stability and efficacy of these insecticides. In real-world settings, higher doses may be required to achieve the same level of control observed in laboratory conditions.

Conclusion

Neonicotinoids show promise as effective larvicides against *Culex pipiens*, offering a potent tool for mosquito control. Sustainable use of these insecticides requires integrated pest management approaches, including rotation with other larvicides, habitat management, and ongoing resistance monitoring. Balancing efficacy and environmental safety is key to the successful implementation of neonicotinoids in mosquito control programs.

References

Aït Hamlet, S., Djekoun, M., Bensoltane, S. and Berrebbah, H. (2019). Effets du Thiamethoxame et de la Tefluthrine sur les biomarqueurs physiologiques de l'escargot terrestre *Helix aspersa*. *Revue Agrobiologia*. 9(2), 1491-1498.

Ambadiang, M., Fouet, C., Ashu, F.A., Penlap-Beng, V. and Kamdem, C. (2023). Chronic exposure of mosquito larvae to pesticide residues endangers a new generation of agrochemicals repurposed for malaria prevention. doi: https://doi.org/10.1101/2023.04.18.537423

Amira, K., Boudjelida, H. and Farine, J.P. (2013). Effect of an insect growth regulator (halofenozide) on the cuticular hydrocarbons of *Culex pipiens* larvae. *African Entomology*. 21(2), 343–348.

Amira, K., Touahria, C., Djeghader, N.E.H. and Boudjelida, H. 2018. Laboratory study of the larvicidal efficacy of a local plant *Hertia cheirifolia* against the most abundant mosquito species, in Algeria. *Journal of Entomology and Zoology Studies*. 6(1), 258-262. *https://www.entomoljournal.com*

Ashu, F.A., Fouet, C., Ambadiang, M., Penlap-Beng, V. and Kamdem, C. (2023). Enhancing the efficacy of neonicotinoids against mosquitoes and overcoming resistance issues. doi: https://doi.org/10.1101/2023.04.18.537427

Assatse, T., Tchouakui, M., Mugenzi, L., Menze, B., Nguiffo-Nguete, D. et al., (2023). Anopheles funestus Populations across Africa Are Broadly Susceptible to Neonicotinoids but with Signals of Possible Cross-Resistance from the GSTe2 Gene. *Tropical Medicine and Infectious Disease*. 8(244), 1-13.

Bouaziz, A., Amira, K., Djeghader, N.E.H., Aissaoui, L. and Boudjelida, H. (2017). Impact of an insect growth regulator on the development and reproduction potency of mosquito. *Journal of Entomology and Zoology Studies*. 5(3), 1662-1667.

Chahbar, N., Belzunces, L.P. and Doumandji, S. (2011). Effet d'un insecticide utilise en protection des végétaux: Thiamethoxam sur l'abeille saharienne *Apis mellifera sahariensis. Algerian journal of arid environment*. 1(2), 11-21.

Chahbar-Adidou, N., Hamadi, K., Acheuk, F. and Doumandji, S. (2018). Toxicité subchronique du Thiaméthoxame insecticide utilisé en protection des végétaux sur deux espèces locales d'*Apis mellifera* L. *Agriculture Journal*. http://revue -agro.univ-setif.dz/

Corbel, V., Duchon, S., Zaim, M. and Hougard, J.M. (2004). Dinotefuran: A Potential Neonicotinoid Insecticide Against Resistant Mosquitoes. *Journal of Medical Entomol*ogy. 41(4), 712-717.

da Silva Mesquita, R., Kyrylchuk, A., Grafova, I., Kliukovskyi, D., Bezdudnyy, A. et al. (2020). Synthesis, molecular docking studies, and larvicidal activity evaluation of new fluorinated neonicotinoids against *Anopheles darlingi* larvae. *PLoS ONE*. 15(2), e0227811. https://doi.org/10.1371/journal.pone.0227811

Dagg, K., Irish, S., Wiegand, R.E., Shililu, J., Yewhalaw, D. and Messenger, L.A. (2019). Evaluation of toxicity of clothianidin (neonicotinoid) and chlorfenapyr (pyrrole) insecticides and cross-resistance to other public health insecticides in Anopheles arabiensis from Ethiopia. *Malaria journal*. *18*, 1-11.

Hataba, A.A., Keshta, A.T., Mead, H. and El-Shafey, N. (2014). Hematological, Biochemical and Histological alterations induced by oral administration of Thiamethoxam and Acetamiprid in male rats. *BiochemistryLetters*. 10(1), 113-125.

Kundoo, A.A., Dar, S.A., Mushtaq, M., Bashir, Z., Dar, M.S. et al. (2018). Role of neonicotinoids in insect pest management: A review. *Journal of Entomology and Zoology Studies*. *6*(1), 333-9.

Linné, C. 1758 - Systema naturae per regna fria naturae, Edition 10, Holmia, 1, 82.

Mitchell, E.A.D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser G. and Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Published in Science*. 358(6359), 109-111.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J.M., Downs, C.A. et al. (2015). Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental science and pollution research*. *22,* 68-102.

Tomé, H.W., Pascini, T.V., Dângelo, R.A.C., Guedes, R.N.C. and Martins, G.F. (2014). Survival and swimming behavior of insecticide-exposed larvae and pupae of the yellow fever mosquito *Aedes aegypti*. *Parasites & Vectors*. 7(195), 1-9.

Qiong, R.A.O., Xu, Y.H., Chen, L.U.O., Zhang, H.Y., Jones, C.M. et al. (2012). Characterisation of neonicotinoid and pymetrozine resistance in strains of Bemisiatabaci (Hemiptera: Aleyrodidae) from China. *Journal of Integrative Agriculture*. 11(2), 321-326.

Zoh, M.G., Gaude, T., Prud'Homme, S., Riaz, M.A., David, J.P. et al. (2021). Molecular bases of P450-mediated resistance to the neonicotinoid insecti- cide imidacloprid in the mosquito *Ae. aegypti*. *Aquatic Toxicology*. 236, pp.105860.