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Acute toxicity and reprotoxicity of aqueous extract of a Moroccan plant (*Tetraclinis articulata)* on freshwater cladoceran *Daphnia magna*.

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Abstract

Previous studies of some biocidal effects on Moroccan's plants have shown that the aqueous extract of Thuya (*Tetraclinis articulata* (Vahl) Mast) has larvicidal activity on different mosquito species in wetlands in Mohammedia. However, the perspective of using this substance as a larvicide should take into account the risk of this plant's extract on aquatic ecosystem species. The aim of the current study is to assess acute and reprotoxicity effects induced by aqueous extract of wood of Thuya (*Tetraclinis articulata* Vahl) on freshwater cladoceran *Daphnia magna*. To do that, *Daphnia magna* was exposed to different concentrations of aqueous extract of sawdust (*Tetraclinis articulata*) for acute and chronic ecotoxicity evaluation. The effective concentrations immobilizing 50% of *Daphnia magna* (EC50) after 24 h and 48 h were 8.171 ± 0.743 mg/L and 6.490 ± 0.541 mg/L, respectively. Reprotoxicity was also assessed after 21 days of exposure to aqueous extract (*Tetraclinis articulata*), the NOEC and LOEC values were 0.49 and 0.83 mg/L, respectively for the number of neonates per female.

1. Introduction

In view of controlling harmful organisms such as insects, mosquitoes etc., several insecticides have been used like Organophosphorus, Pyrethroid and Carbamate as they are a very effective agents against pests. These insecticides are found in air, waters, sediments, plants, animals and their presence can directly affect the health of aquatic and terrestrial organisms.An insecticide is an active substance that has power to kill pests, larvae, eggs and have a large spectrum; it can also have an effect on non-targeted organisms. On the other hand, pests can develop an insecticide resistance [1, 2]. For these reasons and topreserve the environment, significant efforts are thus currently being devoted to search new, highly efficient plant extracts based on plant essential oils or aqueous extract having a biocidal activity [3].These could be suitable for the development of botanical insecticides.

In Morocco, different plants have been the subject of several studies[4, 5, 6] and *Tetraclinis articulata* (*T. articulata*) is one of these plants, especially to combat mosquitoes in wetlands in Mohammedia. The cedar Maghreb *T. articulata (Valh)* Masters is genus of coniferous trees in the Cupressaceae (cypress family)[7]. It is endemic to the South Western Mediterranean, especially in the Maghreb countries. *T. articulata* has an important sociological and economical role; It is also used in traditional and veterinary medicine [8, 9]. Buhagiar *et al.* [10] showed the cytotoxic effects of *T. articulata* essential oil in different human cancer cell lines. Previous studies showed an antimicrobial, antifungal, antioxidant and anti-inflammatory activities of essential oils from leaves, branches and sawdust of *T. articulata* (Vahl) [11, 12, 13]. Several studies showed composition of sawdust, leaves and branches essential oil for *T. articulata.* Barrero *et al.* [14] reported that the main components of essential oil of *T. articulata* are sesquiterpenoid (56.4%), monoterpenoid (20.4%), diterpenoid (23.2%). The major component found in the wood is cedrol (30.7%). Bourkhiss *et al.* [11, 12,13] showed that the yield of essential oils of leaves is 0.22% andthe major components arebornyl acetate (30.74 %),

α-pinene (23.54 %), camphor (17.27 %) and limonene (23.31 %). The yield of essential oils of sawdust is 1.63%, these oils are composed of α-acorenol (20.9%), cedrol (17.9 %), totarol (8.8 %), α-cedrene (8.7 %) and β-acorenol (7.4%). The yield of essential oils of branches is 0.41% and their components are α-pinene (30.22 %), limonene (22.29 %), widdrol (5.41 %), bornyl acetate (4.76 %) [15].

To come up with a new method to combat mosquitoes in wetlands in Mohammedia, Aouinty *et al.* [16] tested the possibility of using an aqueous extract of *T. articulata* to combat four mosquito species. All experiments showed that aqueous extract of *T. articulata* have an important larvicidal activity against mosquitoes. These authors concluded on the interesting possibility of the use aqueous extract as biocide against mosquitoes in wetlands. *T. articulata* was also showed to have a termiticidal activity [17]. All of these toxic effects observed on *T. articulata* extract against different species can have potential damage to ecosystems and suggest the necessity to evaluate their acute and chronic toxicity on key species of aquatic ecosystems. The water flea *Daphnia magna* (*D .magna*) is widely used for acute and chronic toxicity assessment in aquatic ecosystems. Its high sensitivity was largely demonstrated for several mineral and organic pollutants [8. It is easy to culture in laboratory, has high fertility and a parthenogenetic mode of reproduction [18]. In addition, *D. magna* population is representative of Moroccan aquatic ecosystems [19]

2. Experimental

2.1. *Aqueous extract preparation*

The biocide activity of *T. articulata* was explored by aqueous extract of sawdust. 100 mL of distilled water was boiled and 10 g of sawdust of Thuya was transferred to beaker and stirred gently for 30 min. The solution was filtered and the filtrate was recovered (pH of filtrate is 4.5). The pH was adjusted to 7.8 with sodium hydroxide [16].This solution was considered as a stock solution of 100 %.

2.2. *Test organism and culture media*

Experiments were conducted with strains of *D. magna*. Cultures of *D. magna* were maintained at temperature of 20 ± 2°C in ISO (International Organization for Standardization 6341, 1996) medium (containing only 11.76 g/L $CaCl_2$, $2H_2O$; 4.93 g/L $MgSO_4$, $7H_2O$; 2.59 g/L NaHCO3, 0.23 g/L KCL) [20] with a pH of 7.8, a conductivity of 10 µS cm^{-1} and a total hardness of 250 mg $CaCO_3$ L^{-1}. The culture medium was renewed three times weekly and *D. magna* was fed with a mixture of three algal species (5×10^6 *Pseudokirchneriella subcapitata*, 2.5×10^6 *Scenedesmus subspicatus*, 2.5×10^6 *Chlorella vulgaris* /*Daphnia*/day). These algae have been cultured in our laboratory [21, 22].

2.3. *Test with reference substance*

A reference test with potassium dichromate ($K_2Cr_2O_7$) was also performed in our lab to test *D. magna* sensitivity. The test was performed in glass tubes (180 x15 mm) containing 10 mL of test medium. For each concentration, five neonates were exposed to concentration from 0.17 to 2.2 mg/L without feeding during the test for 24 h. For each concentration, we had four replicates. Test tubes were incubated in darkness at 20 ± 2 °C. 24 h-EC50 value should be in the range between 0.9 and 1.5 mg/L as required by ISO 6341, 1996. Results were expressed as effective concentration immobilizing 50% of treated organisms and the 24 h-EC50 was determined by an appropriate statistical method (REGTOX).

2.4. *Acute ecotoxicity*

A preliminary test was conducted to determine the range of concentration for the final test. Acute ecotoxicity test for *D. magna* was performed using immobilization as an endpoint according to ISO 6341, 1996. *D. magna* was exposed to aqueous extract concentrations from 0.5 mg/L to 17 mg/L during 24 h and 48 h. Four replicates of five neonates of *D. magna* (aged < 24 h) were placed in glass test tubes containing 10 mL for each test concentration and control. Tests were conducted in complete darkness at 20 ± 2 °C and the neonates were not fed during the test. The assessment endpoint examined was the immobilization. After 24 h and 48 h of exposure, neonates still moved their antennae but did not swim within 15 second after shaking gently was considered immobile.

2.5. *Chronic ecotoxicity*

The neonates of *D. magna* (aged < 24h) were exposed to nominal concentration from 0.13 to 3.7 mg/L for 21 days. They were exposed individually in 50 mL glass beakers containing 40 mL of test solution, in semi-static

conditions with medium renewal at each 48 h. The experiments were conducted in 10 replicates for control and each concentration and were performed following the OECD guideline procedures (OCDE 211, 2012) [23]. *D. magna* were fed daily with a mixture of three algal species 5×10^6 *Pseudokirchneriella subcapitata, 2*.5×10^6 *Scenedesmus subspicatus, and 2*.5×10^6 *Chlorella vulgaris* /*Daphnia*/day).All test beakers were checked daily for parental mortality and offspring production of neonates were counted and removed when the exposure medium was renewed. The reproductive endpoints assessed were longevity, days of the first brood, number of broods, brood size, total number of neonates produced per female and to determine the NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration).

2.6. *Statistical analyses*

The experimental data was reported as mean values of three measurements ± standard deviation (SD). Toxicity endpoints, such as effective concentration values (EC50) that was used for *D. magna* was estimated using the bootstrap method in the REGTOX Excels macro. To assess the effects of aqueous extract in number of brood, first days brood and brood size, data was first tested for normality and homogeneity of variances using Kolmogorov–Smirnov test and Levene's test, respectively. Then, single factor one-way analysis of variance (ANOVA) to detect significant differences between treated groups and control was used. The NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) was determined for each endpoint. All statistical analyses were performed with Statistica for Windows ($P < 0.05$: STATISTICA version 6 for Windows, Statsoft, Tulsa, OK, USA).

3.Results and discussion

3.1. *Test with reference substance*

At the end of 24 h–testing potassium dichromate ($K_2Cr_2O_7$), we counted immobile *D. magna* and calculate the percentage inhibition for each concentration. The 24 h-EC50 value was 0.955 ± 0.018 mg/L. This value was between 0.9 and 1.5 mg/L confirming the sensitivity of the strain *D. magna*. This result satisfied the validity conditions of ISO standard and validity of biological material used in our laboratory.

3.2 Acute toxicity

The immobilization of *D. magna* was recorded after 24 h and 48 h of exposure to aqueous extract (*T. articulata*). Our results demonstrated significant acute toxicity on*D. magna*. The 24h-EC50and 24h-EC10were 8.177 ± 0.743 mg/L and 3.852 ± 0.782 mg/L, respectively. The 48h-EC50and 48h-EC10 were6.490 ± 0.541 mg/L and 3.308 ± 0.544 mg/L, respectively. These values give an estimate of aqueous extract's acute toxicity and underline that these concentrations cause *D. magna* immobilization and the death of 50% of freshwater microcrustaceans populations. No mortality was observed at control and at both concentrations 0.5 and 1.3 mg/L (Fig 1). Immobilization began at 2.7 mg/L with 5 % and 15% after 24 h and 48 h of exposure, respectively. 35% and 50% of immobility were observed after 24 h at 6.2 and 8 mg/L, respectively. The same percentages were observed after 48 h of exposure at 4.8 and 6.2 mg/L. 80% and 90% of immobility were observed at 10 and 13 mg/L, respectively after 48 h of exposure. At 17 mg/L, 90% and 100% rate of immobility were observed after 24 h and 48 h of exposure, respectively.

As previously mentioned, Aouinty*et al.* [16] tested the effects of aqueous extract on several stages of four mosquito species (*Culexpipiens, Aedescaspius, Culisetalongiareolata, Anopheles maculipennis*) which were collected originally in wetlands in Mohammedia. The 24 h-LC50 values were ranged from 110 to 220 mg/L and from 210 to 530 mg/L, respectively for the 2nd instar and the 4th instar larvae. Our results showed clearly that *D .magna* is more sensitive to aqueous extract of *T. articulate* than wetlands mosquito species. In fact, 24h-EC50 registered for *D. magna* (8.177 mg/L) was 12 times less than 110 mg/L observed for the most sensitive larvae mosquitoes.

No previous study was conducted to evaluate acute toxicity of aqueous extract of *T. articulate* on non-target aquatic ecosystems. However, other extracts of different plants were tested for acute toxicity on *D. magna*. Mousa *et al.* [24] tested the effects of aqueous extract of *Azadirchtaindica* on freshwater community especially *Daphnia sp.* (96h-LC50 = 0.1 g/L). Olaru*et al.* [25] reported thatLC50 values were ranged from 0.3255 to 0.9043 mg/mL for three aqueous extracts (*Euphorbia platyphyllos, Euphorbia stricta* and *Euphorbia cyparissias*). In another study, the 24h and 48h-EC50 were ranged from 82.30 to >1000 mg/L and from 31.25 to >1000 mg/L, respectively for five plant species [26]. Ferreira *et al.* [27] reported that 24h-EC50 was 188.7

µg/mL for aqueous extract of *Moringaoleifera.* All these authors underline the toxicity of aqueous extracts and the high sensitivity of *D. magna.* The 48h-EC50 obtained in this study (6.490 mg/L) inducing 50% of immobility is comparable to 48h-EC50 (0.028 mg/L) for a conventional insecticide such as malathion [28].

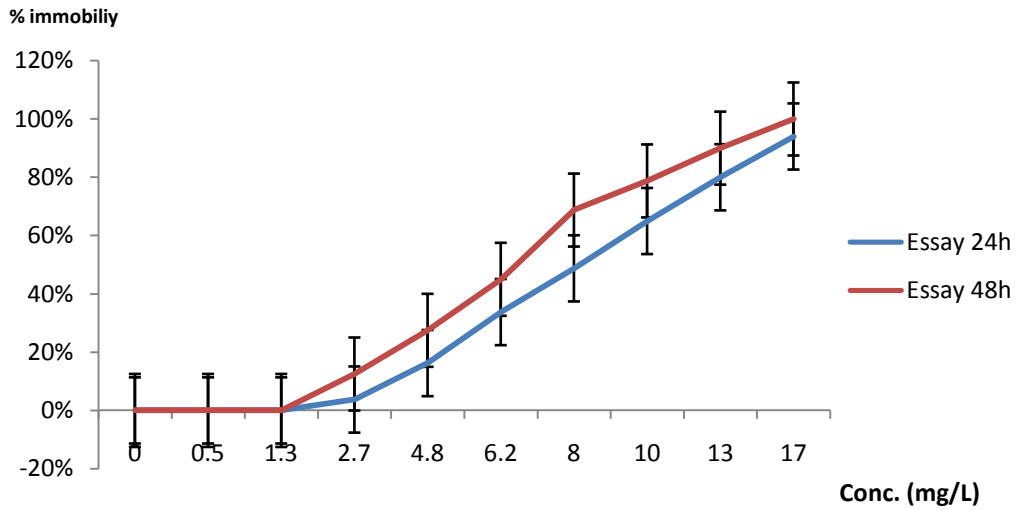

Figure 1: Acute toxicity of aqueous extract of *T. articulata* to *D.magna* after 24 h and 48 h of exposure

3.3. *Chronic toxicity results*

For chronic test, *D. magna* was exposed to a range of concentrations of aqueous extract (0, 0.17, 0.28, 0.49, 0.83, 1.42 and 2.42 mg/L). Effects on survival and different reproductive parameters of *D. magna* were quantified at 21 days of exposure and described in Table 1. We chose to express measurement endpoints in terms of NOEC and LOEC.

Table 1: Longevity, size, molting and reproduction of *Daphnia magna* exposed to concentrations of *T. articulata* aqueous extract in a 21-day life study (values are means ± standard deviation) (*$p < 0.05$)

T. articulata (mg/L)	Longevity (days)	Number of cumulative molts	Day to first brood	Number of neonates per female	Brood size	Number of broods
0	21 ± 0.0	9.9 ± 0.316	7.6 ± 0.69	100.3 ± 7.58	20.47 ± 0.91	4.9 ± 0.31
0.17	21 ± 0.0	9.8 ±0.421	7.8 ± 0.78	93.3 ± 8.55	19.46 ± 1.11	4.8 ± 0.42
0.28	21 ± 0.0	9.5 ± 0.527	8.2 ± 1.03	87.5 ± 11.72	19.45 ± 1.55	4.5 ±0.52
0.49	19.9 ± 1.91	8.1 ± 2.469	8.6 ± 0.51	73.4 ± 21.43	19 ± 1.47	3.9 ± 1.19
0.83	18.8 ± 2.44	7.3 ± 2.21*	8.9 ± 0.87	54.2 ± 16.53*	15.86 ± 1.40*	3.4 ± 0.96*
1.42	17.7 ± 3.16	6.6 ± 2.50*	9.1 ± 0.73*	40 ± 16.27*	12.71 ± 2.71*	3.1 ±1.10*
2.42	16.1 ± 3.41*	6.1 ± 2.33*	9.3 ± 1.15*	32.1 ± 10.20*	11.55 ± 1.27*	2.8 ± 0.91*

The evaluation of toxicity of aqueous extract on 21-days reproduction test showed that both survival and reproduction of *D. magna* decreased with increasing concentration of aqueous extract. The results showed that mortality was 30% (after 19 days) at 0.49 mg/L, 60 % (after 20 days) at 0.83 mg/L, and 80 % (after 19 days) at 2.42 mg/L (Fig 2). The percentage of survival decreased with increased aqueous extract concentrations. This showed that dose-response and time dependent relationship exist. The NOEC and LOEC values were 1.42 and 2.41 mg/L, respectively.

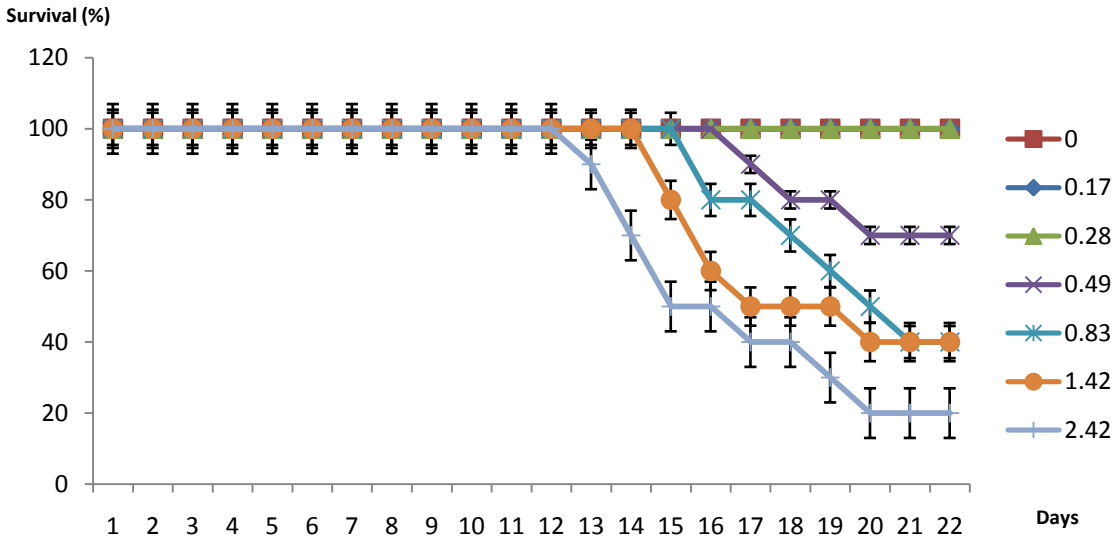

Figure 2: Effects of aqueous extract of *T. articulata* on the survival of *D. magna* for 21 days.

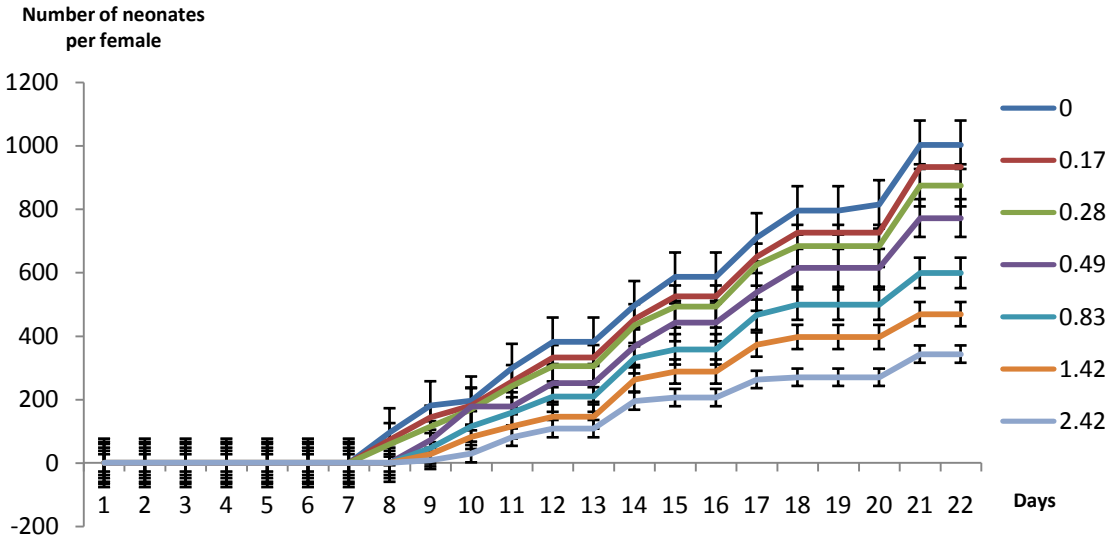

Figure 3: Effects of aqueous extract of *T. articulata* on the number of neonates per female of *D. magna* exposed for 21 days

Reproduction parameters were affected at concentrations lower than survival and an inverse relationship was clearly registered between the number of neonates per female and aqueous extract of *T. articulata* concentrations (Fig 3). After 21 days of exposure, the number of neonates decreased progressively with increased concentration. No significant effect was registered at 0.49 mg/L after 21 days. The number of neonates were 93.3 ± 8.55, 87.5 ± 11.72 and 73.4 ± 21.43 at 0.17, 0.28 and 0.49 mg/L, respectively. At 0.42 mg/L, only 32.1±10.20 neonates were produced, compared to 100.3±7.58 for control. The NOEC and LOEC values were 0.49 mg/L and 0.83 mg/L, respectively for number of neonates. These results clearly showed the high toxic effects of aqueous extract on *D. magna* fecundity during 21 days. In case of the use of *T. articulata* as biocide to combat mosquito species, this generates a major aftereffect on the population's reproduction of *D. magna*.

At 0.49 mg/L, the number of broods was 3.9 compared to 4.9 for control, and this value decreased to 3.4 and 3.1 at 0.83 and 1.42 mg/L, respectively (Fig 4). At 2.42 mg/L, the number of broods was 2.8. Similarly, the brood size was reduced from 20.47 in control to 15.86 at 0.83 mg/L, 12.71 at 1.42 mg/L and 11.55 at 2.42 mg/L (Fig 5). The NOEC value was 0.49 mg/L for both number of broods and brood size. For the parameter "day to first brood", a significant delay was observed at concentration 1.42 mg L^{-1}. The NOEC and LOEC values were 0.83

and 1.42 mg/L, respectively. No significant effect was observed at 0.49 mg/L for number of cumulative molts. The NOEC and LOEC values were 0.49 and 0.83 mg/L, respectively. The longevity was reduced from 21 at control to 18.8, 17.7, and 16.1 at 0.83, 1.42 and 2.42 mg/L, respectively. The NOEC and LOEC values were 1.42 and 2.42 mg/L, respectively for longevity. These results showed that the use of aqueous extract of *T. articulata* can have a major effect on *D. magna* maturity. These findings agree those obtained by other natural compounds [29-31]

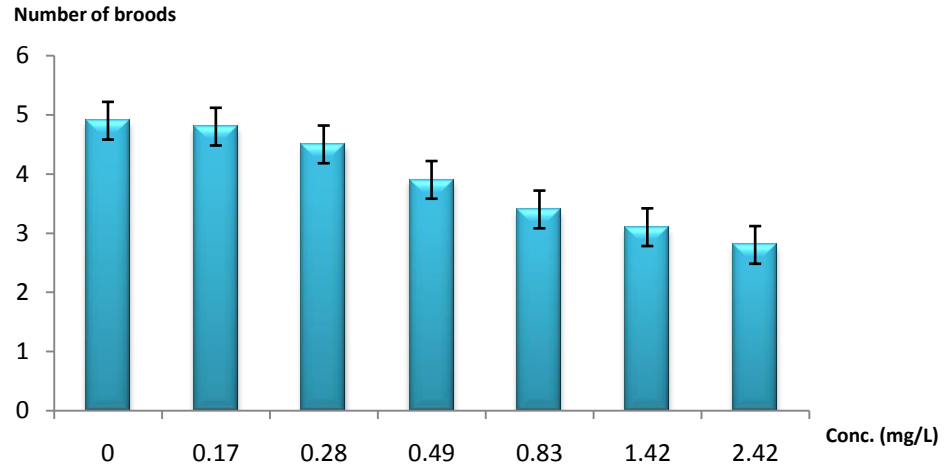

Figure 4: Effects of aqueous extract of *T. articulata* on number of brood exposed for 21 days.

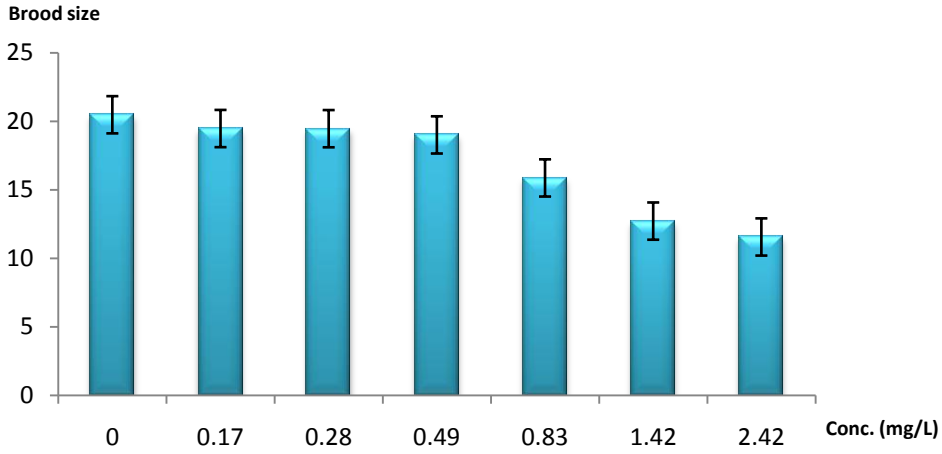

Figure 5: Effects of aqueous extract of *T. articulata* on brood size exposed for 21 days

The present study is the first and the only one conducted on the assessment of *T. articulata* chronic effects on *D. magna*. Mousa et al., [24] showed that aqueous extract of *Azadirachta indica* has also a chronic effect on *Daphnia sp.* Similar results were also obtained by ibuprofen [32], dimethoate and pirimicarb [33]…

Conclusion

Our study is the first one to explore the toxic effects of aqueous extracts of *T. articulata* on aquatic organisms. *D. magna* was selected to serve as a test organism, giving an important role to the food chain of aquatic ecosystems.Our results clearly show significant effects of acute toxicity and reprotoxicity on *D. magna* population. This suggests that the use of the aqueous extracts of *T. articulata* for its biocidal activities in Mohammedia wetlands can generate effects on non-target populations. The balance and dynamics of these ecosystems may be altered. In conclusion, this study draws attention to the need to integrate ecotoxicological approach to study the biological activities of natural substances extracts.

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References

1. Georghiou G.P., Ariaratnam V., Pasternak M.E., Lin C.S., *J. Econ. Entomol.*68 (1975) 461.
2. Sinegre G., Jilien J.L., Gaven B., *Parasitologia* 19 (1977) 79.
3. Zoubiri S.,Baaliouamer A., *J. Saud. Chem. Soc.* 18 (2011) 925.
4. Zidane A., Tits M., Angenot L., Wauters J. N., Frederich M., Dib I., Mekhfi H., Aziz M., Bnouham M., Legssyer A., Ziyyat A., *J. Mater. Environ. Sci.*, 5 (2014) 1368.
5. Sliti S., Ayadi S., Dumarçay S., Khouja M. A., Gérardin P., André E., Perrin D., Abderrabba M., *J. Mater. Environ. Sci.*,7 (2016) 968.
6. Fadel O., Ghazi Z., Mouni L., Benchat N., Ramdani M., Amhamdi H., Wathelet J.P., Asehraou A., Charof R., *J. Mater. Environ. Sci.* 2 (2011) 112.
7. IUCN : http://www.iucnredlist.org/details/30318/0
8. Bellakhadar J., Claisse R., Fleurentin J., Yaunos C., *J. Ethnopharmacol.* 35 (1991) 123.
9. Ziyyat H., Legssyer A., Mekhfi H., Dassoili A., Serhrouchni M., Benjelloun W., *J. Ethnopharmacol.*58 (1997) 45.
10. Buhagiar J.A., Podesta M.T., Wilson A.P., Micallef M.J., Ali S., *Anticancer Res.* 19 (1999) 5435.
11. Bourkhiss M., Hnach M., Bourkhiss B., Ouhssine M., Chaouch A., *Afri. Sci.* 3 (2007) 232.
12. Bourkhiss M., Hnach M., Paolini J., Costa J., Chaouch A., *Bull. Soc. Roy. Sci. de Liège* 78 (2009) 281.
13. Bourkhiss M., Hnach M.,Lakhliffi T.,Bourkhiss B., Farah A., Ouhssine M., Satrani B., *Bull. Soc. Roy. Sci. de liege* 79 (2010) 4.
14. Barrero A.F., Herrador M. M., Arteaga P., Quílez J., Akssira M., Mellouki F., Akkad S., *J. Essent. Oil Res.* 17 (2005) 166.
15. Bourkhiss B., Ouhssine M., Hnach M., Bourkhiss M., Satrani B., Farah A., *Bull. Soc. Pharm. Bordeaux* 146 (2007) 75.
16. Aouinty B., Oufara S., Mellouki F., Mahani S., *Biotechnol. Agron. Soc. Environ*. 10 (2006) 67.
17. El Hanbali F.,Amusant N., Mellouki F., Akssira M., Baudasse C., IRG/WP 07-30418, Jackson Lake Lodge, Wyoming, USA (2007) 20.
18. Koivisto S., *Env. Poll.* 90 (1995) 263.
19. Ramdani M., *Bulletin de l'Institut Scientifique*, Rabat 6 (1982) 105.
20. ISO : Water quality -- Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) -- Acute toxicity test ISO-6341,1996.
21. Manar R., Bessi H., Vasseur P., *Environ. Toxicol.Chem.* 28 (2009) 2150.
22. Toumi E., Boumaiza M., Millet M., Radetski C.M., Camara B.I., Felten V., Ferard J.F., *J. Environmental Science and Health, Part B* 50 (2015) 34.
23. OECD. Guidelines for Testing of Chemicals.*Daphnia magna* Reproduction Test, OECD 211; OECD: Paris, France, 2008.
24. Mousa M. A. A., El-Ashram A. M. M., Hamed M., *8th International Symposium on Tilapia in Aquaculture* (2008) 307.
25. Olaru O.T., Şeremet O.C., Petrescu M., Salagean A., Velescu B.Ş., Nitulescu M.G.,*Romanian J. Biophys*., 24 (2014) 43.
26. Jančula D., Suchomelová J., Gregor J., Smutná M., Maršálek B., Táborská E., *Environmental Toxicology*, 22 (2007) 480.
27. Ferreira P.M.P., Carvalho A. F.U., Farias D. F., Cariolano N. G., Melo V.N. M.M., Queiroz M.G.R., Martins A. M. C. Machado-Neto J. G., *Anais da Academia Brasileira de Ciências*, 81 (2009) 207.
28. Rassoulzadegan M. et Akyurtakli N., *Turk. Zool.*, 26 (2002) 349.
29. Lindsay J., Metcalf J.S., Codd G.A., *Toxicon* 48 (2006) 995–1001
30. Chakri K., Touati L., Alfarhan A.H., Al-Rasheid K.A.S., Samraoui B., *C. R. Biologies* 333 (2010) 836–840
31. M. Bachar, L. Zidane, A. Rochdi, J. Mater. Environ. Sci. 7 (11) (2016) 4175-4204
32. Heckmann LH., Callaghan A, Hooper HL, Connon R, Hutchinson TH, Maund SJ, Sibly RM., *Toxicol Lett.* 172 (2007) 137-45.
33. Andersen TH., Tjørnhøj R, Wollenberger L, Slothuus T, Baun A., *Environ Toxicol Chem.* 25 (2006) 1187-95.

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