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Biotoxicity and determination of leaf polyphenols of *Nerium oleander* and *Eucalyptus camaldulensis* on *Gambusia affinis* in Aïn Chkef river of Morocco.

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Abstract

We try to understand some of the mechanisms involved in the phenomenon of the natural contamination of aquatic receptors followed by the main stages of the degradation of leaves Oleander (*Nerium oleonder*) and those of *Eucalyptus camaldulensis* in water.

The content of polyphenols in leaves is one of the main causes of this toxicity, but other substances may also intervene in particular: alkaloids, the saponins, the terpenoids and other water-soluble organic substances has direct effect (toxicity) and indirect (deoxygenation) that should be extracted and to determine the effect. The deoxygenation of water by eucalyptus leaves and Oleander showed that the leaves of Oleander desoxygenent much the receiver medium than the Eucalyptus leave. However the rate of polyphenols released by the leaves of Oleander is significantly lower than that observed in Eucalyptus leaves.

Keywords: Polyphenols, toxicity, leaves *aquatic*, *Nerium oleonder*, *Eucalyptus camaldulensis*.

1. Introduction

When the leaves reach in water they are divided into two fractions:

- A soluble fraction (sugars, pigments, polyphenols, flavonoids, terpenes) entering the stock of organic matter dissolved from the system.

- And a fraction of coarse residues.

The polyphenols rate released by the sheet of water depends on their condition (dried or fresh) the studied species of leaf and the incubation temperature. Distribution and content may vary depending on tissues and organs as well as the seasons Bate-smith (1965), Bate-smith (1962), Barlocher (1990).

Some of these phenolic compounds especially the tannins are considered inhibitors that can slow the rate of decomposition of leaf gas Bunn (1988), Barlocher (1990). They act primarily by inhibiting the action of bacteria and fungi intervened in the process of decomposition of the leaves in water and to a lesser extent that of invertebrates Feeny (1976). Some litters may well persist for more than three years in the water

because of the high concentration of tannins in the leaves.

In a general way the polyphenols particularly tannins that persist even in their leaves after abscission Feeny (1976), shall protect producers against attack of fungi and herbivores. Their oxidation products have inhibitory effects on some enzymatic systems. By comparative studies on the leaves of Alder and Poplar have shown for leaves of alder in addition to their strong cuticle complex nitrogen-polyphenols can inhibit the enzymatic activity of colonizing microorganisms that sword and prevents the use of its nitrogen by bacteria and fungi. By cons in poplar nitrogen although in small quantity may be more available for microorganisms and invertebrates.

Our work thus opens research perspectives toward complementary studies and larger scale in order to know the trophic role (energetics value) and toxic effects “natural” the main vegetable species bordering our waterways these being strongly influenced by the nature of riparian vegetation whose contributions to the aquatic environment mainly in the form of leaves deep can change the biochemical and biological qualities of water It would be interesting and even useful to establish a typology of vegetable species based on these two main parameters; Trophic and toxicity values.

Nerium oleander

Eucalyptus camaldulensis

Gambusia affinis

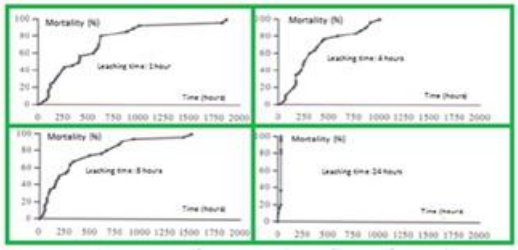

Figure 2, Percentage charge of mortality of *Gambisa* in function of time in the presence of leachates Laurier (*in* ESSAFI and BOURAADA, 2015)

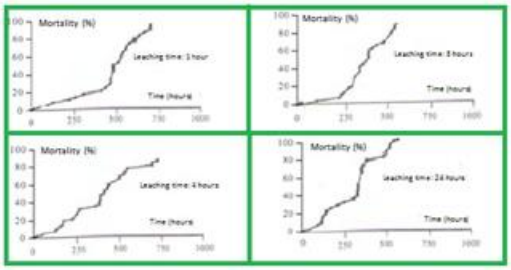

Figure 2, Percentage charge of mortality of *Gambisa* in function of time in the presence of leachates *Eucalyptus*. (*in* ESSAFI and BOURAADA, 2015)

ESSAFI and BOURAADA (2015) confirmed that the comparative analysis of the toxic effect of both vegetable species studied shows that the leaves of Oleonder are very toxic compared to those of Eucalyptus. Leaching time proves a limiting factor in the mortality of *Gambisia*. The toxicity of Eucalyptus leaves and Oleonder could be due to their content of polyphenols and other toxic water soluble substance. As a measure that augments leaching duration toxicity also increases which resulted in a decreasein the TL (50). Thus it was found that there is a significant relationship between the toxicity of the leaves and

their phenolic content. Incubation of Gambisia in these Lessivas causes mortality to 100% in 31h probably because of their oxygen scavenger because the dried leaves of Oleonder desoxygenent much the receiver medium than those of *Eucalyptus*. The mortality rate of Gambisia is growing as and as leaching time increases. The Lessivas 24 hours of Oleonder leaves appear very toxic. They are 15 times more toxic than those obtained after one hour. Whereas those *Eucalyptus* values TL 50 decreases slightly.

In establishing such a typology it would be possible to master the reforestation and deforestation of the banks of rivers by selecting tree species including the expected benefits outweigh the disadvantages such a measure may contribute to the protection of the environment by reducing the intake of toxic elements (polyphenols and other water-soluble organic substances: alkaloids, saponins, terpenoids) from the leaves of some species.

Studying the phenomenon of decomposition of particulate organic matter with all processes physical chemical and biological and their positive and negative effects on aquatic receptors is not easy it is a complex subject that can be addressed under different angles

2. Materials and Methods

2.1- Description of the study site

The study was carried out in the river AinChkef (Figure 1). It a small stream whose source has AinChkef roughly 7 km from the city of Fes the bottom of a circus cliffs (coordinates Lambert: X = 534.75 y = 374). At the level of the source, the water is etale in a depression 4.5 m in length and 3 to 4m wide before pouring the rest of the way. Part of the water from the source is used to power the AinChkef complex.

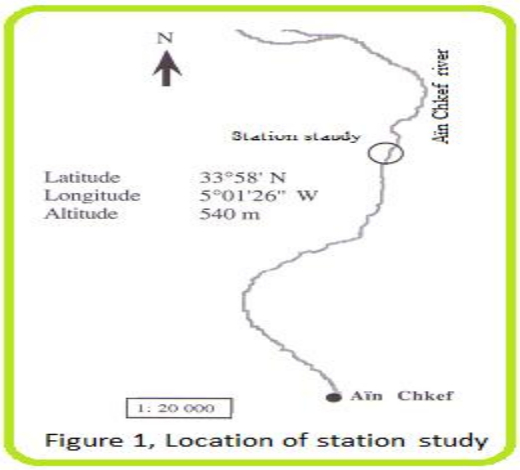

Figure 1, Location of station study

Aquatic vegetation is constituted of filamentous green algae. Riparian vegetation is mainly composed of *Equisandum fluviatilis*,*Scirpus lacustris*, *Salix* sp and *Typha latifolia*. The slopes of the valley are mostly covered with *Eucalyptus camaldulensis*. Le bed of the river is covered mostly set and mud with some stones seats. The aquatic fauna is dominated by *Gambusia affinis* mainly (Little fish that plays the important role in the fight against Anopheles, consuming a large amount of larvae) and *Melanopsis prosobranch* gastropod considered as main dilaceration hardwood inputs to freshwater. The bags of leaves (left batch of three bag for each point of levy) were deposited all along the banks and camouflaged under the cover of riparian vegetation that lead in water and in the tangle of roots to avoid any vandalism.

2.2- Splitting polyphenols

In this part we are interested to the study of total polyphenols fraction tannins in order to determine their role in the phenomenon of deoxygenation (tannins being recognized as enzyme inhibitors.)

The tannins and the lignins contain hydroxyl aromatic moieties that reduce phosphomolybdic acid giving a blue color which can be measured by spectrophotometry Rodier (1996). The reagent employed being the reagent folinciocalteu. The blue coloration produced after 10 mm measures 600 nm. The content of polyphenols is determined by reference to a range stallion made under the same conditions with phenol.

2.3- Methanoic extraction

- 4 g of powder of each of the two kinds of sheets are placed in 20 ml of methanol and the mixture is stirred for 5 min;
- The mixture is then centrifuged at 1500 revolutions/min left at rest and then filtered;
- The pellet is repeated a second time in 10 ml of methanol;
- Then separating the base of the supernatant corresponding to the phenolic moiety;
- The crude extracts are evaporated has its (reduced pressure) by a rotary evaporator at a temperature of 65° C;
- The residue is recovered in still suits water;
- Then diethyl ether was added (2V);
- Two phases are obtained; an ethereal phase and an aqueous phase which is filtered under a separatory funnel and recovering only the aqueous phase;
- The aqueous phase is evaporated to dryness;
- Are finally added methanol and the solution is obtained containing polyphenols.

2.4- Determination of polyphenols

- A test sample suitable for the solution to be determined (10 ml);
- After adding 0.4 ml of the folin reagent, 5 minutes later 20 ml of an aqueous solution of NaCO 3 (20%) are added;
- The blue color produced after 10 min is measured at 600 nm.
- A suitable test sample to the solution assay (10 ml);
- After addition of 0.4 ml of Folin 5 min later 20 ml of an aqueous solution of NaCO3 (20%) the blue color is measured after 10 min at 600 nm.

3. Results and Discussion

Table I: The polyphenols concentration on *Eucalyptus* leaves and Oleander

	Optical Density (OD)	concentration of polyphénols
Eucalyptus	4	60.78 mg/1
Laurier rose	1.715	26.16 mg/1

A comparative study of the polyphenols concentration on *Eucalyptus* leaves and Oleander (Table I) showed that the rate of polyphenols from dried leaves cede of Eucalyptus is significantly higher than that ceded by the leaves of Oleander.

The study of the deoxygenation of water by Eucalyptus leaves and Oleander showed that the leaves of Oleander, desoxygenent much the receiver medium than the Eucalyptus leaves. However the rate of polyphenols released by the leaves of Oleander is significantly lower than that observed in the leaves of *Eucalyptus*. The concentration of polyphenols

The studies conducted on the leaves of pine and fir Tremolieres (1983) shows that these two species have the same phenol content and tannins but present oxygen scavenger opposing effects. The results obtained on the fir and pine reinforce ours and show that even if needles Tree possess twice as polyphenols than the Pin they are six times less toxic Haddy (1993). Found similar results for the dead leaves of Oleander and Willow. Willow leaves are much richer in polyphenols that the leaves of Oleander which particularly show more toxic despite their low polyphenols. Also the presence or absences of polyphenols in the leaves do not explain the totality toxic effects observes. The polyphenols though toxic are not the only extractable responsible for observed mortality among mosquitofish and *Melanopsis* (Essafi, 2000).

The natural contamination process of water triggered by falling leaves therefore seems likely toxic complex. The effect could be indirect (biochemical and microbiological deoxygenation of water in the presence of the polyphenol-polyphenol oxidase system) and / or direct either due to extractable substances (polyphenols alkaloids saponsosides ... etc.) or biochemical transformation products oxidative polyphenols or otherwise to the fermentation by-products without excluding possibly the dystrophiants melanin effects.

4. Conclusion

This phenomenon of degradation of leaves and apparently showed as many authors have us very beneficial for microbial populations fungal and aquatic invertebrates must not hide their actions sometimes very toxic on environments aquatic receptors especially during the early stages of decomposition of the leaves in water.

The deoxygenation of water by eucalyptus leaves and Oleander showed that the leaves of Oleander desoxygenent much the receiver medium, than the Eucalyptus leaves. However the rate of polyphenols released by the leaves of Oleander is significantly lower than that observed in Eucalyptus leaves.

The polyphenol content of the leaves is one of the main causes of this toxicity but other substances may also intervene in particular alkaloids the saponins the terpenoids and other water-soluble organic substances has direct effect (toxicity) and indirect (deoxygenation) that should be extracted and to determine the effect. At the toxicity of the leaves it still has to understand the processes involved in the phenomenon of natural water contamination by leachates leaves a study the kinetics of polyphenoxydase and compounds polyphenolic to follow and quantify the emergence of forms quinones upon oxidation of polyphenols and finally to isolate quantify and study the agents responsible for the toxicity of the main vegetable species retained.

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