

## Full Length Research Paper

# Histological and biochemical evaluations of the liver and kidney of Wistar rats fed with fish meal of *Sarotherodon melanotheron* captured with *Tephrosia vogelii*'s powder

Dougnon Tossou Jacques\*, Kpodekon T. Marc, Houessionon M. Jedirfort and Kiki S. Pascal

Laboratory of Research in Applied Biology (LARBA), Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 BP 2009 Abomey-Calavi, Benin.

Received 23 November, 2015; Accepted 19 January, 2016

To evaluate the toxicity of fish captured with *Tephrosia Vogelii* (TV), an ichthyotoxic plant, the Wistar albino rats were fed with the flour of tilapias *Sarotherodon melanotheron* poisoned with TV leaves powder. This study aimed to evaluate the poisonous effects of TV on various organs of rats, especially the liver and kidney. Three groups of Wistar rats were constituted and fed for 28 days. Histological sections were done on the liver and kidneys. Plasmatic levels of alanine aminotransaminase (ALAT) and aspartate aminotransaminase (ASAT) were measured. The histological sections carried out on their organs presented no lesions. However, the biochemical parameters, ALAT and ASAT showed a slight change. This study shows that the ingestion of fish poisoned with *T. vogelii* does not provoke any digestive lesion in Wistar rats, but the slight changes in biochemical parameters makes it foreseeable to prohibit fishing with *T. vogelii* and to prevent their consumption by humans.

**Key words:** *Tephrosia vogelii*, ichthyotoxic, Wistar rat.

## INTRODUCTION

Fishing provides 44% of halieutics products. There are 90,000 tons of fish used for this purpose in Benin per year (DP, 2011). Fishing contributes to 3% of the National Gross Domestic product (GDP) (Food and Agricultural Organization (FAO), 2008). Moreover, because of increasing demography and fish consumption, which is

9.2 kg per year per capita (ImorouToko, 2011), the demand for halieutics products remains unsatisfied. The use of many prohibited ways for fishing like the powder of *Tephrosia vogelii* in rivers is a secular practice in Africa and American- Southern areas (Kerharo et al., 1974). It is true that this practice allows for the collection of a large

\*Corresponding author. E-mail: dougnonj@yahoo.fr

amounts of fish that are sold in local markets for human consumption. However, the toxicity of *T. vogelii* is not very selective and has been observed in both macrofauna (fish, batrachians and reptiles) and microfauna. *T. vogelii* is very toxic, even with very weak dilution and by simple contact with cold-blooded animals (Elouard et al., 1982). The rotenone, principal component of *T. vogelii*, is carcinogenic, according to Bourgois (1989). Unfortunately in Benin, information about its negative effects on consumers and fishers of fish captured with *T. vogelii* is not evaluated, which is the importance of this study. There are scanty reports in this field. Thus, the aim of our present study was to evaluate: 1) the effect of the powder of *T. vogelii* on the liver and kidney of Wistar rats, 2) the effects of the fish meal poisoned with the powder of *T. vogelii* on the plasmatic level of ALAT of the Wistar rats, and 3) the effects of the fish meal poisoned with the powder of *T. vogelii* on the plasmatic level of ASAT of the Wistar rats.

## MATERIALS AND METHODS

### Animals and experimental model

Thirty (30) male Wistar rats were used in this study. Their weights were between 130 and 150 g. Before assays, they were acclimatized to the conditions of breeding of the Institute of Applied Biomedical Sciences (IABS) at the fairground of Cotonou. They received water and a standard food distributed *ad libitum*. They were divided into three groups of ten (10) rats: group 0 (control): 10 rats fed with a ration without fish meal poisoned during 28 days; group 1: 10 rats fed with a ration of fish meal poisoned at a concentration of 400 powder mg/L of *T. vogelii* during 28 days; and group 2: 10 rats fed with a ration of fish meal poisoned at a concentration of 800 powder mg/L of *T. vogelii* during 28 days.

The temperature of experimental location was maintained at 22°C, the ambient humidity was 60% and a photoperiod of 12 h/24 followed. The fish were captured in the Lac Nokoue; their average weight and length were respectively  $140 \pm 2$  g and  $13 \pm 0.5$  cm.

### Plants

The leaves of *T. vogelii* were collected in the Commune of Bohicon, Benin. The leaves were dried during two weeks at 22°C in the Laboratory of Histology of IABS. They were then reduced to fine powder and conserved in sterile plastic bags. The powder was served to poison fish.

### Laboratory materials

The proportioning of the enzymes was carried out using a spectrophotometer of mark "Thermo Electron"; ALAT and ASAT kits were from "Etablissement Français du Sang Alpes-Méditerranée 149, Bd Baille 13392 Marseille Cedex 5".

### Obtaining the fish meal of *Sarotherodon melanotheron* poisoned with the powder of *Tephrosia vogelii*

*S. melanotheron* fish were fished in Nokoue Lake in the acadjas. They were maintained alive in a container filled with water. Then, fish were poisoned with the powder of *T. vogelii* of four hundreds

(400) and eight hundred (800) milligrams per liter of water. They were then cured and reduced to flour for the food of the Wistar rats.

### Blood sampling and proportioning of ALAT and ASAT

From the caudal vein, 2 ml of blood was taken in vacuum tubes from each rat. Blood sampling was done at days 0, 14 and 28. The proportioning of ALAT and ASAT in sampled bloods was done using commercial kits.

### Autopsy of the rats and removal of their bodies

Following the blood sampling, an autopsy was carried out on 20% of each group. The liver and the kidney of the rats were collected and then fixed in 10% formol for histological examination after coloring with hematoxylin-eosin. Observations were done with a light microscope.

### Statistical analyses

After the treatment, data on the ALAT and the ASAT were analyzed by analysis of variance (ANOVA) (type one) with the software Statistica 6.0 (1998). To know the differences, the test of Newman-Keuls was used with an error risk  $p > 0.05$  (the difference is not significant) and  $p < 0.1\%$  (highly significant difference).

## RESULTS

### Behavioral observation of the rats after intoxication

The poisoned Wistar rats did not present any sign of disease (weakening, diarrhea, or vomiting). No mortality was recorded.

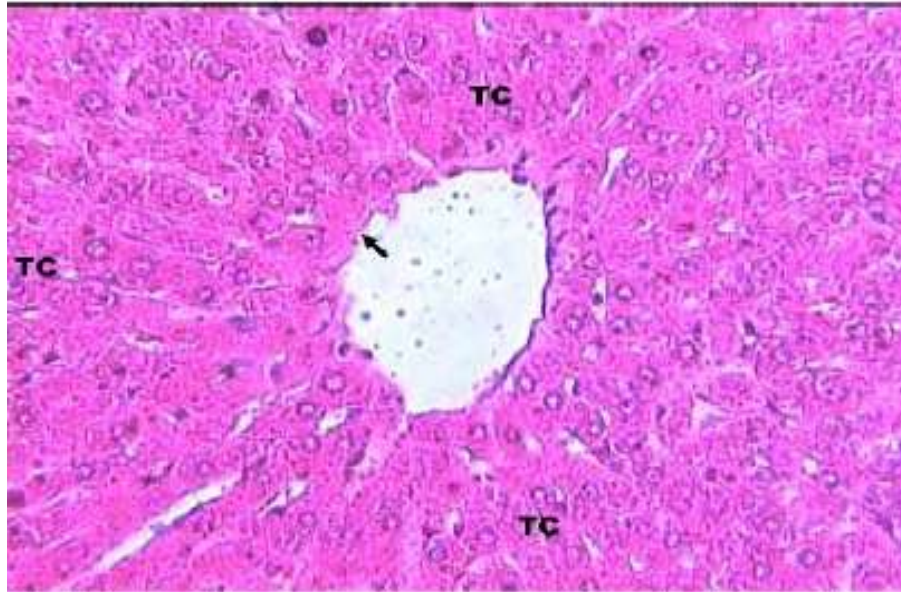
### Histological characteristics of the liver and the kidney of the rat fed with fish poisoned with powder of *T. vogelii*

Among the various organs of the rats fed with the fish meal containing *T. vogelii*, there was slight structural modification on the liver and kidney. The histological sections of the liver and kidney of groups 0, 1 and 2 are presented in Figures 1, 2, 3 and 4.

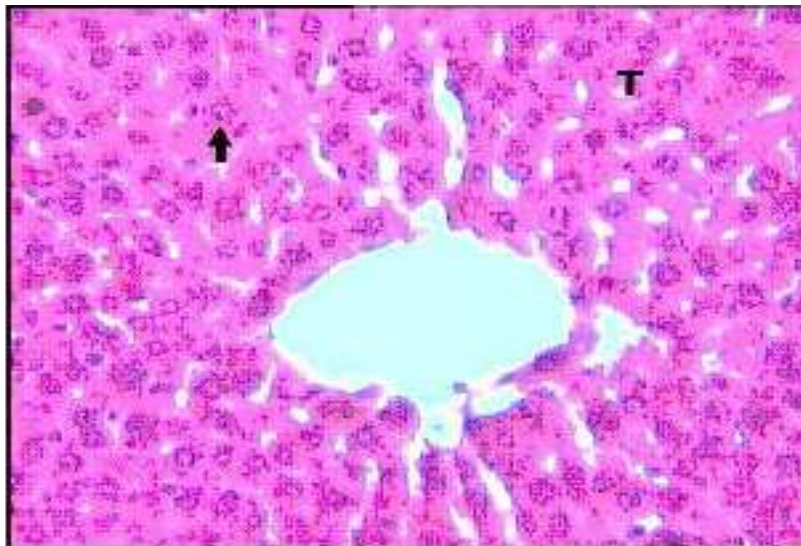
### Biochemical parameters

#### Plasmatic levels of ALAT

The plasmatic levels of the ALAT (UI/L) in the control rats were  $69.3 \pm 0.8$ ,  $70.3 \pm 0.8$  and  $61.6 \pm 4.2$ , respectively at days 0, 14 and 28. Regarding the rats of group 1, the values of the ALAT were  $72.3 \pm 0.5$  at day 0,  $74.4 \pm 0.6$  at day 14, and  $76.2 \pm 0.3$  at day 28. In group 2, the values of the ALAT were  $73.1 \pm 0.1$  at day 0,  $75.2 \pm 0.5$  at day 14, and then  $77.3 \pm 0.2$  at day 28. At day 0, there was no significant difference at 5% threshold between the



**Figure 1.** Normal liver of Wistar rat in group 0:  $\times 20$  (HE). Liver lobule showing a central vein (arrow) around which the liver cells are organized in cell spans (TC) arranged in radial way.

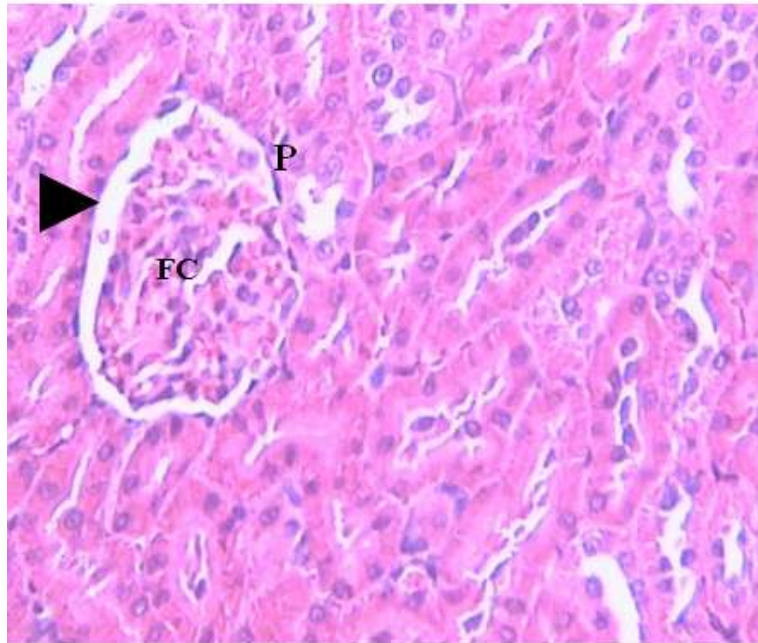


**Figure 2.** Wistar rat liver, groups 1 and 2:  $\times 20$  (HE). Slight necrosis of hepatocyte marked by a homogenization of the structure of cytoplasm that becomes eosinophilic with disappearance of limit cells.

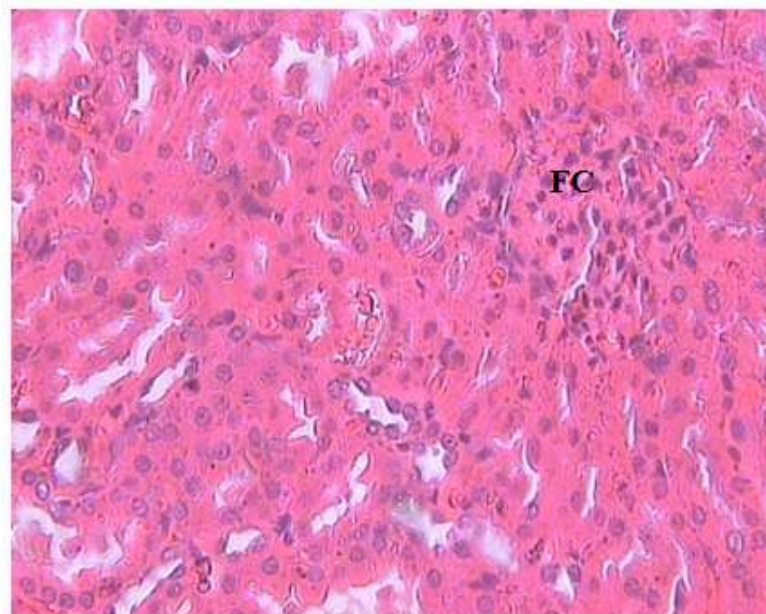
various groups with regard to the plasmatic level of the ALAT. The plasmatic levels of the ALAT did not vary either significantly at days 14 and 28 for group 0; but, in groups 1 and 2, values increased very significantly with the experimental duration. This is more remarkable in the group 2 rats fed with fish poisoned with *T. vogelii* at a rate of 800 mg/L, which have a significant difference at the threshold of 0.1% (Table 1).

#### **Plasmatic levels of ASAT in the Wistar rats**

The plasmatic levels of the ASAT (UI/L) in the control rats were  $41.0 \pm 3.1$ ,  $45.6 \pm 2.3$  and  $47.3 \pm 6.8$ , respectively at days 0, 14 and 28. In group 1, the level of the ASAT was  $47.3 \pm 4.3$  at day 0,  $50.3 \pm 1.2$  at day 14, and  $54.6 \pm 2.1$  at day 28. In group 2, the values of the ASAT were  $47.6 \pm 3.3$  at day 0,  $5.2 \pm 1.1$  at the day 14, and  $53.4 \pm 0.3$  at



**Figure 3.** Normal Wistar rat kidney, group 0:  $\times 20$  (HE). Renal cortex showing a glomerulus with the capillary flocculates (FC), glomerular room (arrowhead) and the vascular pole (P) and the excretory ducts.



**Figure 4.** Wistar rat kidney, groups 1 and 2:  $\times 100$  (HE). Structural changing marked by the thickening of the interstitium with compresses of the flocculus capillary and disappearance of glomerular room (FC).

day 28. At the beginning of the experimentation, there was no significant difference at a threshold of 5% between the various groups with regard to the value of the ASAT. It is the same with the experimental duration

on the plasmatic levels for the ASAT in control group. However, the values of this hepatic enzyme evolved very significantly in groups 1 and 2 as the experimental duration was prolonged (Table 2).

**Table 1.** Plasmatic levels of ALAT (UI/L).

Group	Day		
	0	14	28
Group 0	69.3 ± 0.8 <sup>a</sup>	70.3 ± 0.8 <sup>a</sup>	61.6 ± 4.2 <sup>a</sup>
Group 1	72.3 ± 0.5 <sup>a</sup>	74.4 ± 0.6 <sup>b</sup>	76.2 ± 0.3 <sup>c</sup>
Group 2	73.1 ± 0.1 <sup>a</sup>	75.2 ± 0.5 <sup>b</sup>	77.3 ± 0.2 <sup>c</sup>
Probability	0.75 <sup>NS</sup>	<0.001 <sup>**</sup>	<0.001 <sup>**</sup>

Averages followed by the different letters are highly significant: P <0.001: highly significant; NS= not significant.

**Table 2.** Plasmatic level of ASAT (UI/L).

Group	Day		
	0	14	28
Group 0	41.0 ± 3.1 <sup>a</sup>	45.6 ± 2.3 <sup>a</sup>	47.3 ± 6.8 <sup>a</sup>
Group 1	47.3 ± 4.3 <sup>a</sup>	50.3 ± 1.2 <sup>b</sup>	54.6 ± 2.1 <sup>c</sup>
Group 2	47.6 ± 3.3 <sup>a</sup>	52.1 ± 1.1 <sup>b</sup>	53.4 ± 0.3 <sup>c</sup>
Probability	0.75 <sup>NS</sup>	<0.001 <sup>**</sup>	<0.001 <sup>**</sup>

Averages followed by the different letters are highly significant: P <0.001: highly significant; NS= not significant.

## DISCUSSION

### Effect of the powder of *Tephrosia vogelii* on the liver and kidney of Wistar rats

During our experimentation, no clinical sign of intoxication such as vomiting or hyper-salivation was observed in the Wistar rats. This is justified by the quantity of powder of *T. vogelii* involved which could not have been sufficient to cause the symptoms of a possible intoxication. Indeed, according to Morris and Powell (2000), the absorption of the rotenone, active ingredient of *T. vogelii* was relatively slow and incomplete in the stomach and intestine. Moreover, the liver metabolizes quickly the components of the rotenone (Ling, 2003). In addition, the poisoned fish were eviscerated before being reduced to powder for the food of the rats. This method was adopted in order to imitate the art of fish cooking before their consumption by humans.

The fish meal eaten by Wistar rats was obtained after curing of poisoned fish. According to Ross Robertson and Smith-Vaniz (2008), the rotenone, one of the toxic substances of *T. vogelii*, was thermolabile. The rotenone was then destroyed through the curing of fish used to intoxicate the rats; but, among the various organs of the rats fed with the fish meal poisoned with *T. vogelii*, some lesions were found on the cells and structures of the liver and kidney. Therefore, as little as *T. vogelii* powder in the body can induce several disturbances on the organs. These results are contrary to those of Ross Robertson and Smith-Vaniz (2008) who assert that the poisoned fish kept in their flesh negligible quantity of the toxic

substance which accumulates mostly in the internal organs (internal and gills). These internal organs were not consumed by the rats in the present study.

Indeed, Dzenda et al. (2008) affirmed that the lesions of the liver and kidneys appeared after a chronic intoxication. In the same way, according to Morris and Powell (2000), rats having consumed more than 2.5 mg/kg of rotenone during two years did not develop any pathological signs, which could be due to rotenone. Therefore, from this experimentation, it is noticed that rotenone is not the only substance which could cause lesions on Wistar rats' liver and kidney because the amount of deguelin seems higher compared to rotenone (Kalume et al., 2012). For Dzenda et al. (2008), *T. vogelii* contains, in addition to rotenone, deguelin; while the former is thermolabile, the latter is not destroyed by heat. The biochemical disturbances could be related to the activity of this heat-resisting compound.

### Effects of the fish meal poisoned with the powder of *Tephrosia vogelii* on the plasmatic level of ALAT of the Wistar rats

The normal values of the ALAT in the rats fed with fish not poisoned with *T. vogelii* are in accordance with those defined by Kamdem et al. (1981); they are an average of 74.7 ± 3.8. In addition, the muscular exercise can modify the plasmatic level of the ALAT as remarked by Lecoanet (1981). The levels of the ALAT approximately increased in the rats of groups 1 and 2 fed with fish poisoned with 400 and 800 mg/L powder of *T. vogelii*. These results confirm the remark through histological section because, significantly elevated levels of ALAT suggest the existence of medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. So ALAT is commonly used as a way of screening for liver problems. The increase in its values after the feeding of the rats with fish poisoned with *T. vogelii* indicates a beginning of functional disturbances in these organs. For example, according to Scheurer et al. (2002), in the viral hepatitis, values from 500 to 1500 UI/L were reported in humans; better, values higher than 3000 UI were met in the presence of acute toxic necrosis or serious hypoxia during a hepatic ischemia.

### Effects of the fish meal poisoned with the powder of *Tephrosia vogelii* on the plasmatic level of ASAT of the Wistar rats

The normal values of the ASAT obtained in the rats of group 0 in the present study differ from those reported by Kamdem et al. (1981) which were, respectively 178 ± 20.99 and 200 ± 26 UI/L. These results, on the other hand, harmonize with the rate of 42.9 ± 10.1 UI/L established by Kaneko (1989). The factor 'age' is

responsible for these cases. The consumption of fish meal poisoned by *T. vogelii* increased the rate of the ASAT in the rats of groups 1 and 2. This enzymatic increase, although light, means surely a beginning of functional deteriorations of the liver of these rats. This enzyme is found in many tissues: liver, heart, kidney, muscles, intestines and the ASAT and ALAT ratios (ASAT/ALAT ratio) are commonly measured clinically as biomarkers for liver health. Nevertheless, its activity is more significant in liver, heart and the muscles as remarked by Schuck and Alain (1997). The increase in the rate of ASAT is constant during acute hepatitis (Banting et al., 1975). In the present study, the results of the histological section examination revealed hepatic lesions; however, the significant increase in the biochemical parameters could certify the structural modification on liver. This could be confirmed with structural modifications like necrosis tissue hepatic observed in rats poisoned directly by the powder of *T. vogelii*, according to results of Morris and Powell (2000).

## Conclusion

This study aimed to evaluate toxicity of *T. vogelii*. The livers and kidneys of Wistar rats revealed histological lesions. However, the plasmatic levels of alanine-amino-transaminase and aspartate-amino-transaminase were slightly increased in the rats fed with fish meal of *Sarotherodon melanotheron* captured by *T. vogelii*. Based on the toxicity of *T. vogelii* employed for the fishing, it is urgent and necessary that further studies continue on longer duration, and with higher amounts of intoxication to encircle closely the mode of action of degueline and rotenone which are active substances of *T. vogelii*. Total and fast evisceration of fresh fish and the prohibition of the ichthyotoxic plants as resource of fishing are actually the main ways to protect fish consumers in Benin.

## Conflict of interests

The authors have not declared any conflict of interest

## REFERENCES

- Banting A, Bouchaud C, Lebas H, Marinez P, Rose F(1975). Étude de l'évolution des paramètres sanguins chez le mouton et le lapin à la suite d'une infection artificielle avec *Fasciola hepatica*. Rev. Med. Vet. 126(2):70-249.
- Bourgeois JJ(1989). *Tephrosia vogelii*, une plante ichthyotoxique utilisée pour la pêche aux Comores. Bull. Soc. Roy. Bot. de Belgique 133:81-86.
- Dzenda T, Ayo JO, Adelaiye AB, Auda AO (2008). Ethno medical and veterinary uses of *Tephrosia vogelii* Hook. Austr. J. Med. Herb. 20(2):71.
- Elouard JM, Dejoux C, Troubat JJ (1982). Action de *Tephrosia vogelii* (Leguminosae) employée dans les pêches traditionnelles sur les invertébrés benthiques de la Maraoué (Côte d'Ivoire). Rev. Hydrobiol. Trop. 75(2):177-188.
- ImorouToko I (2011). Techniques de production aquacole, Cours de Master de Pêche et Aquaculture, Ecole Polytechnique d'Abomey-Calavi (Université d'Abomey-Calavi, Bénin).
- Kalume M, Losson B, Angenot L, Tits M, Wauters J-N, Frederich M, Saegerman C (2012). Rotenoid content and *in vitro* acaricidal effect of *Tephrosia vogelii* leaf extract on the tick *Rhipicephalus appendiculatus*. Vet. Parasitol. 190(1):204-209.
- Kamdem L, Magdalou J, Biest G (1981). Effect of aflatoxin B1 on the activity of drug-metabolizing enzymes in rat liver. Toxicol. Appl. Pharmacol. 60(3):570-578.
- Kaneko JJ (1989). Chemical biochemistry of domestic animals, California: Academic Press Inc. 4<sup>th</sup> edition, P. 932.
- Kerharo J, Guichard F, Bouquet A (1974). Les végétaux ichthyotoxiques (poisons de pêche), 1<sup>ère</sup> Partie: Introduction à l'étude des poisons de pêche, Bio. Vég. et mat. Méd. pp. 313-329.
- Lecoanet J (1981). Application des dosages enzymatiques en pathologie du bétail et des animaux de basse-cour. Point Vet. 12(58):39-45.
- Ling N (2003). Rotenone: A review of its toxicity and use for fisheries management, Wellington (New Zealand), Department of Conservation, Science for Conservation, n° 211.
- Morris S, Powell D (2000). Background: Rotenone and Parkinson's disease Centre for safe food, University of Guelph. Retrieved from [www.plant.uoguelph.ca/safefood/chem-haz/rotenone.htm](http://www.plant.uoguelph.ca/safefood/chem-haz/rotenone.htm) the 26 September 2012.
- FAO-Organisation des Nations Unies pour l'Alimentation et l'Agriculture (2008). Vue générale du secteur des pêches national, Profil des pêches et de l'aquaculture par pays. Retrieved from [www.fao.org](http://www.fao.org) the 16 August 2012.
- Ross Robertson D, Smith-Vaniz WF (2008). Roténone: un outil essentiel mais décrié pour l'évaluation de la diversité des poissons marins. BioSci. Mag. 58(2):165-171.
- Scheurer S, Reindl J, Rihs HP (2002). IgE reactivity to profiling in pollen-sensitized subjects with adverse reactions to banana and pineapple. Int. Arch. Allergy Immunol. 128:105-114.
- Schuck S, Alain H (1997). La douleur: Moyens et stratégies thérapeutiques. La revue du praticien. 47:555-569.