

INSECTICIDES BASED ON ROW MATERIAL OF VEGETAL ORIGIN – TOXICITY EFFECT ON MAMMALS

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Abstract: *Melia azedarach* extracts used as insecticide are relatively of recent date procedure, the azadiractinate biological action being discovered in 1993. This study presents some aspects of toxicological action of *Melia azedarach* vegetal extracts on different harmful organisms, such as *Hyphantria cunea* și *Leptinotarsa decemlineata*. In the assesment of the bioinsecticide toxicity, the main step is to establish the reverse or not changings (lack of modification) of skin and eye irritability caused by the bioinsecticide contact. Based on the results obtained by bioinsecticide toxicological tests of *Melia azedarach* extracts we conclude that: (1) all three bioinsecticide of *Melia* extracts show to have low toxicity effect on Wistar white rat, LD₅₀ is higher than 5000 mg.a.i./kg body weight and (2) all three show a very low irritable effect on skin and eye (0 - 1 on Draize et al. scale).

Key words: *Melia azedarach*, vegetal extracts

INTRODUCTION

Melia azedarach extracts used as bioinsecticide products, is a procedure of relatively recent date (JACOBSON, 1989; KOUL, 1990; SCHMUTTERER, 1990; ACHER, 1993), the pattern activity of azadiractine being revealed în 1993 by Mordue and Blackwel.

The bioproducts for agricultural use, made basicaly from vegetale extracts, represent an important orientation in modern agriculture because it presents some great advantages such as: minimising environmental and food pollution, avoiding the apparition of control resistant pests population, and the sustainable use of profitable resource from agriculture system undiscovered untill now. Bioinsecticides may interact both by direct contact and ingestion.

There have been studied eleven *Melia azedarach* extracts obtained from diferent parts of the plant, those that proved a good biological activity against a variety of harmful organisms, have been toxicologically tested in compliance with actual regulations referring to plant protection products registration in Romania.

For the establishment and assessment of bioinsecticide toxicity an important phase is represented by the dermal and ocular irritability, caused by the reversible or irreversible changes which occur following the bioinsecticide application on mammals.

In this study there are presented some toxicological effects of *Melia azedarach* extracts that showed to have good biological activity against some harmful organisms such as: *Hyphantria cunea* and *Leptinotarsa decemlineata*.

MATERIAL AND METHOD

1. Measuring acute oral toxicity (LD₅₀) on white Wistar rats

The following *Melia azedarach* extracts have been tested:

19 E₁ – *Melia azedarach* sawdust extract;

20 E₂ – *Melia azedarach* bark extract;

21 E₃ – *Melia azedarach* leaves extract.

The test animals have been white male Wistar rats weighting 150 –180 g. The animals were partitioned in 5 batches of 10 rats, one batch for each applied doze/rate. As control was added another batch also of 10 rats. The application of test products was made intragastric. The number of animals,

the mode of application and test timing were in compliance with the OECD principles in Guideline no. 401. LD₅₀ was measured by logarithm doze-probit mortality (Bliss, 1938., Finney, 1971).

2. Measuring ocular irritability on rabbits

The test animals were New Zealand, Papillon or White of California rase of domestic rabbits. Both rabbit eyes were treated with sodium fluorosceine in order to aknowledge ocular cornean integrity. In the case of some lesions, the eye was treated by oily vitamin A, untill lesions disparition/healing.

In order to determine ocular irritability, the OECD decision nr.405 propose a doze application of 0,10 ml/eye. In our experiments we used dozes/ rates higher than 0,10 ml/eye, such as 0,15 and 0,20 ml/eye respectively, to increase the results relevance.

The biopesticide application was made by means of a syringe, dropping the test sample in the lacrimal/tear sac, under the down eyelid and keeping both eyelids stuck for a second to avoid the substance waist. The other eye remain as a control. The tested eye will not be rinsed for 24 h from the moment of test substance instillation.

The number of animals used was at least three. The irritation rate is evaluated and recorded at 1, 24, 48, 72, 96, 168 hours and more but not exceeding 21 days from the instillation. The possible eye lesions can be revealed by instilling two drops of sodium fluorosceine in conjunctival sac of treated eye, followed by abundant rinsing with saline solution. The evaluation of ocular lesions (irritability) was made by DRAIZE and coll. (1944) score scale.

3. Measuring dermal/skin irritability on rabbits

For dermal irritability evaluation were used healthy adult rabbits from White of California and New Zealand rase/breed, weighting 2000-2500 g.

Animal preparation: 24 h before the test, the rabbit skin is prepared by scissors cutting and removing the hair using a suspension of sodium sulphure and oxide of zinc in 1 : 2 volume. After aproximatively 1-1,5 minute, the skin surface is clean and must be washed, wiped and greased by a softening cream.

The application of the test substance is made on the saline solution wet skin, the next day. The direct contact between the animal skin and the test substance is made by means of some glass capsules (2–4 ml), fixed in adhesive bands and attached on the animal body. The animals are in containment for 24 h in special gadgets.

The observation period is not limited but must be sufficient for a complete evaluation of the reverse and ireverse effects occured.

Three animals are used for each test product. After the test substance application the observations of the effects are recorded as eritemas and edemas during 24, 48, 72 hours and after the removal of the capsules.

The measuring method for dermal irritation is in compliance owith the OECD decision no. 404, and the recording is made based on DRAIZE and coll. (1944) scale of skin irritation.

RESULTS

- Bioinsecticide oral acute toxicity (LD₅₀)

The tables 1, 2 și 3 present the results of products toxicity based on *Melia azedarach* extracts.

Table 1

Oral acute toxicity LD₅₀ of *Melia azedarach* sawdust extract on white Wistar rat (E₁)

No.	No. of rats	Dose mg a.i./kg body	Log. dose	Mortality %	Probit	LD ₅₀
1	10	1000	3,0	0	-	> 5000
2	10	2000	3,30	0	-	
3	10	3000	3,47	0	-	
4	10	4000	3,60	0	-	
5	10	5000	3,69	0	-	

Table 2Oral acute toxicity LD₅₀ of *Melia azedarach* bark extract on whiteWistar rat (E₂)

No.	No. of rats	Dose mg a.i./kg body	Log. dose	Mortality %	Probit	LD ₅₀
1	10	1000	3,0	0	-	> 5000
2	10	2000	3,30	0	-	
3	10	3000	3,47	0	-	
4	10	4000	3,60	0	-	
5	10	5000	3,69	0	-	

Table 3Oral acute toxicity LD₅₀ of *Melia azedarach* leaves extract on whiteWistar rat (E₃)

No.	No. of rats	Dose mg a.i./kg body	Log. dose	Mortality %	Probit	LD ₅₀
1	10	1000	3,0	0	-	> 5000
2	10	2000	3,30	0	-	
3	10	3000	3,47	0	-	
4	10	4000	3,60	0	-	
5	10	5000	3,69	0	-	

The dates presented in Tables 1, 2 și 3 revealed that *Melia azedarach* extracts showed a low toxicity effect on mammals, the observations which invalidated the scientific literature dates, according to the melia extracts showed a high toxicity effect on the mammals.

- Biopesticides ocular irritability effect

Manufacturing and handling bioinsecticide imply a serious potential hazard for eyes exposure, so it is needed to know the eyes irritability limits.

The results of ocular irritability progress at the exposure of the *Melia azedarach* (E₁, E₂ and E₃) extracts are presented on the tables 1, 2, and 3.

Referring to the ocular irritability showed on the charts, it can be noted that the extract based on *Melia azedarach* showed an insignificant ocular irritable effect. The only eye component slightly irritated at the high dose (0,20 ml) showed to be the eye conjunctive. At this dose of exposure the eye produces an overflow of lacrimal secretion.

The comparison between toxical standard (acetone – a very high irritable effect), and bioinsecticides under observations showed a very low ocular irritable effect. At the end of the testing time (the 4-th day) the eye irritation disappeared, the eye was restored at the normal aspect.

- Biopesticides dermal/skin irritability effect

The development of dermal irritability after the application of bioinsecticide based on *Melia azedarach* extracts are showed in table 4.

Table 4Mammal dermal irritability evolution after *Melia azedarach* sawdust extract application

Product	No. of animals/ Variant	Dose g (ml) a.i./kg body	Specification	Observation time			
				24h	48h	72h	96h
E1	3	4	Edem very, very slighty	1	0	0	0
E2	3	4	Edem very, very slighty	1	0	0	0
E3	3	4	Edem very, very slighty	1	0	0	0
Acetone	3	4	Severe eritem	4	4	3	3
Control	3	-	-	-	-	-	-

The results presented in table 4 about bioinsecticides based on melia extracts showed lack of irritability effect, though at skin contact the products have been adsorbed. Only in the first day was

observed a slightly skin edem, may be caused by the mechanical action of glass capsules with which the insecticide was applied.

CONCLUSIONS

Based on the results obtained by the bioinsecticide toxicological tests of *Melia azedarach* extracts we conclude that:

- All three bioinsecticide based on melia extracts showed a low toxicity effect on white Wistar rat, DL_{50} was higher than 5000 mg. a.i./kg body;
- They showed a low irritability effect for both skin and eyes (according to Draize et al. scale).

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Ocular irritability evolution following different doses of *Melia azedarach* sawdust extracts application (E₁) Table 1

	Observation period / timing test							
	1 st day		2 nd day		3 rd day		4 th day	
Acetone 0,10 ml/eye	cornea =	2	cornea =	2	cornea =	1	cornea =	0
	iris =	0	iris =	0	iris =	0	iris =	0
	conj. =	3	conj. =	2	conj. =	2	conj. =	0
	chem. =	2	chem. =	1	chem. =	1	chem. =	0
	secr. =	3	secr. =	2	secr. =	1	secr. =	0
E ₁ 0,1g a.i./ eye	cornea =	0	cornea =	0	cornea =	0	cornea =	0
	iris =	0	iris =	0	iris =	0	iris =	0
	conj. =	0	conj. =	0	conj. =	0	conj. =	0
	chem. =	0	chem. =	0	chem. =	0	chem. =	0
	secr. =	0	secr. =	0	secr. =	0	secr. =	0
Acetone 0,15 ml/eye	cornea =	4	cornea =	4	cornea =	3	cornea =	0
	iris =	2	iris =	2	iris =	2	iris =	0
	conj. =	3	conj. =	3 ³ 2	conj. =	2 ¹ 2	conj. =	0
	chem. =	3 ⁴ 3	chem. =	2	chem. =	2	chem. =	0
	secr. =	3	secr. =	3	secr. =	2	secr. =	0
E ₁ 0,1 g a.i./ eye	cornea =	0	cornea =	0	cornea =	0	cornea =	0
	iris =	0	iris =	0	iris =	0	iris =	0
	conj. =	0	conj. =	0	conj. =	0	conj. =	0
	chem. =	0	chem. =	0	chem. =	0	chem. =	0
	secr. =	0	secr. =	0	secr. =	0	secr. =	0
Acetone 0,20 ml/eye	cornea =	4	cornea =	4	cornea =	3	cornea =	0
	iris =	2	iris =	2	iris =	2	iris =	0
	conj. =	3	conj. =	3	conj. =	2	conj. =	0
	chem. =	4 ³ 4	chem. =	4 ³ 3	chem. =	3	chem. =	0
	secr. =	4	secr. =	3	secr. =	2	secr. =	0
E ₁ 0,2 g a.i./eye	cornea =	0	cornea =	0	cornea =	0	cornea =	0
	iris =	0	iris =	0	iris =	0	iris =	0
	conj. =	1	conj. =	1	conj. =	0	conj. =	0
	chem. =	0	chem. =	0	chem. =	0	chem. =	0
	secr. =	1	secr. =	1	secr. =	0	secr. =	0

Abbreviations: conj. = conjunctive; chem.= chemosis; secr.= secretion

Ocular irritability evolution following different doses of *Melia azedarach* bark extracts application (E₂) Table 2

	Observation period / timing test							
	1 st day		2 nd day		3 rd day		4 th day	
Acetone 0,10 ml / eye	cornea =	2	cornea =	2	cornea =	1	cornea =	0
	iris =	0	iris =	0	iris =	0	iris =	0
	conj. =	3	conj. =	2	conj. =	2	conj. =	0
	chem. =	2	chem. =	1	chem. =	1	chem. =	0
	secr. =	3	secr. =	2	secr. =	1	secr. =	0

E ₂ 0,1g a.i./ eye	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
Acetone 0,15 ml / eye	cornea = iris = conj. = chem. = secr. =	4 2 3 3 ⁴ 3	cornea = iris = conj. = chem. = secr. =	4 2 3 ³ 2 3	cornea = iris = conj. = chem. = secr. =	3 2 2 ¹ 2 2	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
E ₂ 0,15 g a.i. / eye	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
Acetone 0,20 ml / eye	cornea = iris = conj. = chem. = secr. =	4 2 3 4 ³ 4	cornea = iris = conj. = chem. = secr. =	4 2 3 4 ³ 3	cornea = iris = conj. = chem. = secr. =	3 2 2 3 2	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
E ₂ 0,2g a.i./ eye	cornea = iris = conj. = chem. = secr. =	0 0 1 0 1	cornea = iris = conj. = chem. = secr. =	0 0 1 0 1	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0

Abbreviations: conj. = conjunctive; chem.= chemosis; secr.= secretion

Ocular irritability evolution following different doses of *Melia azedarach* leaves extracts application (E₃)

Table 3

	Observation period / timing test							
	1 st day		2 nd day		3 rd day		4 th day	
Acetone 0,10 ml / eye	cornea = iris = conj. = chem. = secr. =	2 0 3 2 3	cornea = iris = conj. = chem. = secr. =	2 0 2 1 2	cornea = iris = conj. = chem. = secr. =	1 0 2 1 1	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
E ₃ 0,1 g a.i./eye	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
Acetone 0,15 ml / eye	cornea = iris = conj. = chem. = secr. =	4 2 3 3 ⁴ 3	cornea = iris = conj. = chem. = secr. =	4 2 3 ³ 2 3	cornea = iris = conj. = chem. = secr. =	3 2 2 ¹ 2 2	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
E ₃ 0,15 g a.i./eye	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0
Acetone 0,20 ml / eye	cornea = iris = conj. =	4 2 3	cornea = iris = conj. =	4 2 3	cornea = iris = conj. =	3 2 2	cornea = iris = conj. =	0 0 0

	chem. = secr. =	4 ³ 4 4	chem. = secr. =	4 ³ 3 3	chem. = secr. =	3 2	chem. = secr. =	0 0
E ₃ 0,2g a.i./eye	cornea = iris = conj. = chem. = secr. =	0 0 1 0 1	cornea = iris = conj. = chem. = secr. =	0 0 1 0 1	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0	cornea = iris = conj. = chem. = secr. =	0 0 0 0 0

Abbreviations: conj. = conjunctive; chem.= chemosis; secr.= secretion