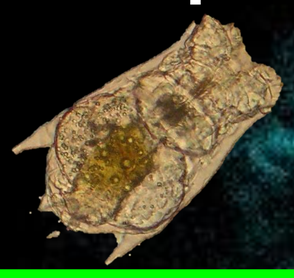
Impact of anti-androgenic substances on gametogenesis of the rotifer, *Brachionus calyciflorus*



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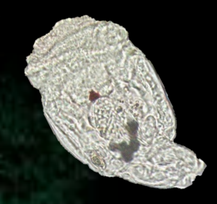

Introduction

Several studies have shown that pollutants with anti-androgenic activity have an impact on the sexual reproduction of the rotifer, *Brachionus calyciflorus* (Joaquim-Justo et al., submitt; Preston et al., 2001). Fenitrohion, an non steroidal anti-androgeninduces a decrease in fertilization rate following exposure ≥0,5mgL^{-1}. Cross mating experiment showed that effects of fenitrothion are due to an impact on males exclusively. In this study, we investigated the impact of fenitrothion on spermatogenesis and observed a decrease in the spermatozoa productionand mobilility with concentrations down to 0,5mgL^{-1}.

Material and methods

Spermatozoa production and study of morphology

Males used for spermatozoa countings were born from control mothers contaminated with 0,5 mgL-1 of fenitrothion and fed with 6x106 cells per ml of Nannochloris atomus. Males ages between 0 and 2 hours were placed between slide and coverslip (18x18mm) in a 10µl drop of water and placed under Leitz Diaplan optic microscope. Within 30 seconds males are crushed and spermatozoa are extruded. Mobile spermatozoa are counted at 400X magnification. For the study of the morphology of spermatozoawe crushed males exposed to 0,5mgL-1 of fenitrothion between 2 coverslips (15x15mm). Then coverslips are fixed in 25% glutharaldhehyde buffered with 0,2M cacodylate, rinsed with 0,1Mcacodylate buffer, passed trough a dehydration series of alcohol , subjected to critical point drying principle and finally metallizing to be observed with ESEM (Philips XL30).

Results and discussion

Number of spermatozoa

The number of spermatozoa in males born from exposed mothers is lower than in control animals at 0,5 mgL^{-1} (p = 0,023) (Table 1).

Table 1 : Number *of spermatozoa extruded from males which mothers had been exposed to fenitrothion and from controls*

	Control	0,05 mg L^{-1}	0,1 mg L^{-1}	0,5 mg L^{-1}	Statistics
EXP 1	38 ± 5			26 ± 3	Mann-Witney p < 0,001
EXP 2	36 ± 8	37 ± 4		31 ± 4	ANOVA 1 p = 0,023
EXP 3	42 ± 3		41 ± 3	39 ± 2	ANOVA 1 p < 0,001

Motility of spermatozoa

Table 2 shows that he number of spermatozoa in males born from exposed mothers is lower than in control animals although results are not statistically different due to the low number of replicates (p = 0,177). However if a t test is applied to a data set of n = 18 for controls and n = 16 for exposed animals by duplicating the data obtained, the decrease observed is then significant (p = 0,047). Likewise, a decrease in the number of mobile spermatozoa and the proportion of mobile spermatozoa is observed although the differences are not statistically significant (p = 0,062 and p = 0,151 respectively) but if the data sets are doubled, then both the number of mobile spermatozoa and the proportion of mobile spermatozoa are statistically different (p = 0,006 and p = 0, 039 respectively). Moreover the factor of decrease in total number of spermatozoa produced in our study is of 1,13 and the factor of decrease in the number of mobile spermatozoa is of 1,46. However, decrease in the production of resting eggs with 0,5 mgL^{-1} of fenitrothion is of 2,52. This indicates that number and mobility are probably not the only factors related to male fertility affected by fenitrothion

Table 2 : M*obility of spermatozoa extruded from males which mothers had been exposed to 0,5 mg/L of fenitrothion and from controls*

	Total number of spermatozoa	Number of mobile spermatozoa	Proportion of mobile spermatozoa
Control	109	49	45%
	102	41	40%
	108	23	21%
	76	14	18%
	106	40	38%
	110	35	32%
	77	51	66%
	80	33	41%
	78	25	32%
n=9	94±16	35±12	37±14%
Fenitrothion	97	26	27%
	61	16	26%
	103	46	45%
	66	21	32%
	79	20	25%
	66	21	32%
	84	18	21%
	106	22	21%
n=8	83±18	24±9	29±8%

Morphology of spermatozoa

No difference was observed in the general aspect and characteristics of the spermatozoa produced (fig 1). Indeed both the head and flagellum present similar morphology and thickness. In another study we showed that the ultrastructure of spermatozoa is also unaffected by fenitrothion.It is possible however that the enzymatic content of spermatozoa or their energetic reserves are altered by fenitrothion, which would decrease their fertilization efficiency.

Fig1 : ESEM observations, Left: control male sperrmatozoid. Right: fenitrothion male sperrmatozoid.

Conclusions

Male fertility in *Brachionus calyciflorus* is negatively affected by fenitrothion. This study shows both the number and motility of spermatozoa produced is decreased with concentration of 0,5mgL^{-1} altough further investigations with higher number of replicates would be indicated. Morphology of spermatozoa seems to be affected. It was also observed that the decrease of resting eggs produced in the presence of 0,5mgL^{-1} is of 2,52 whereas the decrease in number of mobility of sperm produced are respectively 1,13 ans 1,46 which indicates that number and mobility are not the only factor of male fertility affected by fenitrothion.

Acknowledgements

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