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Short communication

Genotoxicity evaluation of the insecticide endosulfan in the wetland macrophyte *Bidens laevis* L.

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Endosulfan causes a concentration-dependent increase of chromosome aberrations in the macrophyte Bidens laevis.

Abstract

The frequency of micronuclei (MN) and chromosome aberrations in anaphase–telophase (CAAT) was determined in root tips of the wetland macrophyte *Bidens laevis* exposed to environmentally relevant concentrations of endosulfan (0.01, 0.02, 0.5 and 5 μg/L) for 48 h. MN frequency varied from 0 in negative controls and plants exposed to 0.01 μg/L endosulfan to 0–3 in plants exposed to 5 μg/L. Moreover, a significant concentration-dependent increase of CAAT was observed. The higher proportion of laggards and vagrand chromosomes observed at 5 μg/L would indicate that endosulfan interacts with the spindle interrupting normal chromosome migration. Endosulfan resulted genotoxic to *B. laevis*, a species of potential value for bioassays and *in situ* monitoring of environmental contamination by pesticides.

Keywords: Endosulfan; Wetland macrophyte; Micronuclei; Chromosome aberrations

1. Introduction

Endosulfan is one of the few organochlorine insecticides still in use around the world for protecting cereal, vegetable and fruit crops from a variety of insects (Jergentz et al., 2004). According to the EPA, USA, endosulfan concentrations above 0.22 μg/L (acute) and 0.05 μg/L (chronic) have adverse impact on the health of aquatic organisms (Mersie et al., 2003). During and immediately after the spraying season, endosulfan concentrations in rivers near and downstream of Australian cotton-growing areas are broadly in the range of 0.02–0.2 μg/L (Raupach et al., 2001). However, runoff in Southern USA contain concentrations even higher than 100 μg/L (Mersie et al., 2003).

Endosulfan is genotoxic in bacteria (Chaudhuri et al., 1999), fishes (Neuparth et al., 2006) and mammalian germinal cells (Pandey et al., 1990; Lu et al., 2000), but its genotoxicity in plants has not yet been tested.

Terrestrial indicator plants are used in most bioassays (Rank, 2003). In the *in situ* assays, transferred indicator plants are used to detect genotoxins in the environment. However, they cannot reveal the actual genotoxic impact on populations chronically exposed in the field and can give false-positive results (Latuzka et al., 2003). These problems can be overcome if standard indicator species permanently growing at the monitoring site are used. In this work, the species *Bidens laevis* L. (Asteraceae), a perennial macrophyte distributed from USA, Mexico and Colombia to Chile, Uruguay and Argentina, is

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used to test its applicability as biomonitoring plant for genotoxic effects. It grows commonly in wetlands and fresh marshes (Cabrera et al., 2000). The main objective of this study was to evaluate possible genotoxic effects of the insecticide endosulfan in this species.

2. Materials and methods

2.1. Material handling and analysis

Seeds of *B. laevis* were collected in La Brava pond (37°53′ South, 57°59′ West), Argentine in May 2005. They were sterilized in a 50% solution of commercial bleach during 5 min followed by several rinses in distilled water and placed in Petri dishes with humid filter paper for germination. Seedlings were transferred to pots containing a mixture of soil: sphagnum moss (3:1 w/w), and grown in a greenhouse until exposure.

2.2. Genotoxicity test

2.2.1. Experimental solutions

Stock solutions of the test substances (6,7,8,9,10,10-hexachlor −1, 5,5α,6,9,9α-hexahydro-6,9-methane −2,4,3-benzo(*e*)dioxatiepin-3-oxide) (Riedel–de Haen) were prepared in dimethyl sulfoxide (DMSO). Exposure solutions were prepared according to commercial formulas (α-isomer 70% and β-isomer 30%). Pure endosulfan (α-endosulfan, β-endosulfan) was used because some formulations contain epichlorohydrin as a stabilizer, a known genotoxic chemical (Public Health Service, 2002).

2.2.2. Treatments

Seven treatments were used: two negative controls (a) Co−1, that consisted of Hoagland solution, and (b) Co−2, in which DMSO was added to the Hoagland solution at 0.004% (similar to the concentration of DMSO used in endosulfan exposures); one positive control (Co+) mutagen, 10 mg/L of methyl methanesulfonate (MMS) and four concentrations of endosulfan: 0.01, 0.02, 0.5 and 5 μg/L. Each treatment was carried out using six independent two-months old plants. Each plant was exposed to a volume of 330 ml for 48 h under a photoperiod of 12 h light/12 h darkness and a temperature of 22 °C.

After exposure, the plants were transferred to Hoagland solution during 24 h for recovery.

2.2.3. Sample preparation for microscopic observation

Root tips (1 cm length) were fixed in ethanol:glacial acetic acid (3:1, v/v) during 24 h and maintained in 70% alcohol in a refrigerator until analysis. Root tips were macerated in 1 N HCl at 60 °C during 10 min and stained with Feulgen reagent for 2 h in darkness, squashed in a 1–2% carmine 45% acetic acid solution and observed in an optic microscope.

For chromosome counts, root tips were pretreated with 8-hydroxyquinoline (0.25 g/L) for 4 h, fixed, hydrolyzed, stained, squashed and observed in a similar manner than the exposed samples.

2.2.4. Calculations and statistics

For each treatment, 1000 interphasic cells and 200 cells in anaphase–telophase were observed, respectively, to detect MN and CAAT. Data were expressed in terms of total number of MN per 1000 interphasic cells and median of chromosome aberrations per 200 cells in anaphase–telophase, respectively. The aberration type was expressed as the number of a specific aberration per 200 cells in anaphase–telophase. The two categories were (1) those indicating interaction with the spindle (including vagrant and laggard chromosomes and chromosome in three groups at early telophase) and (2) those indicating clastogenicity (including bridges, fragments and ring chromosomes). The mitotic index (MI) was calculated as the relation between the number of cells undergoing any stage of mitosis per 1000 cells.

Table 1
Total number of micronuclei per 1000 interphasic cells in *Bidens laevis* exposed to endosulfan

Plant No.	Controls			Endosulfan (μg/l)			
	Co−1	Co−2	Co+	0.01	0.02	0.5	5
1	0	0	1	0	0	0	2
2	0	0	0	0	2	1	3
3	0	0	2	0	1	1	1
4	0	0	2	0	0	1	0
5	0	0	1	0	1	2	0
6	0	0	1	0	1	1	0

Co−1: Hoagland solution, Co−2: Hoagland solution + DMSO, Co+: MMS.

Differences between samples were tested by analysis of variance (Kruskal–Wallis nonparametric tests) and between treatments (*a posteriori*) by the Dunn test (Zar, 1999).

3. Results and discussion

3.1. Genotoxic effect of endosulfan in B. laevis

In negative controls and plants exposed to the lower concentration, MN were not detected. In positive controls and plants exposed to the higher concentrations, the response was positive (Table 1). The percentage of CAAT varied from 1 to 3.5% and from 2 to 9.5% in Co−2 and Co+, respectively (Fig. 1). The frequency of CAAT, higher at 5 μg/L in comparison with Co−2 ($p < 0.05$), and the significant increase of CAAT at concentrations of 0.01–5 μg/L revealed a concentration-dependent genotoxic effect, similar to that found in the fish *Sparus aurata* L. (Neuparth et al., 2006) and mammalian germ cells (Pandey et al., 1990). Endosulfan concentrations as low as 5 μg/L resulted genotoxic to *B. laevis* while concentrations of 0.01 and 0.02 μg/L (Fig. 1), which corresponds to the maximum permitted quantities (MPQ) for protection of aquatic life in superficial freshwater in Australia–New Zealand (Raupach et al., 2001) and Argentina (Rovedatti et al.,

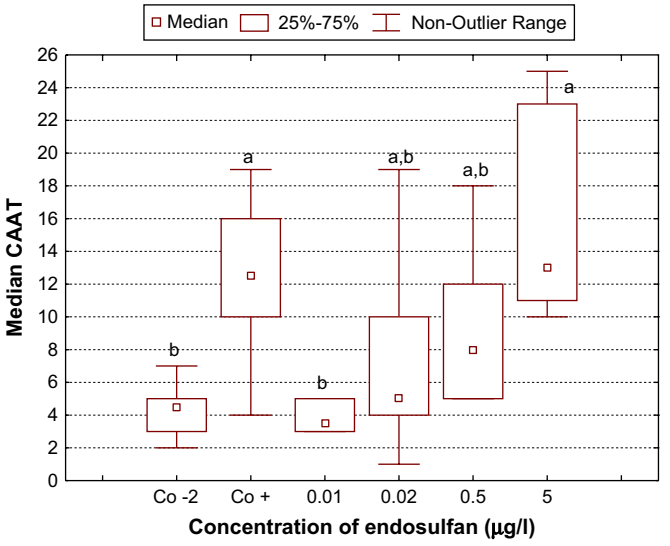

Fig. 1. Median chromosome aberrations in anaphase–telophase in *Bidens laevis* exposed to endosulfan. Different letters indicate significant differences among treatments. $n = 6$.

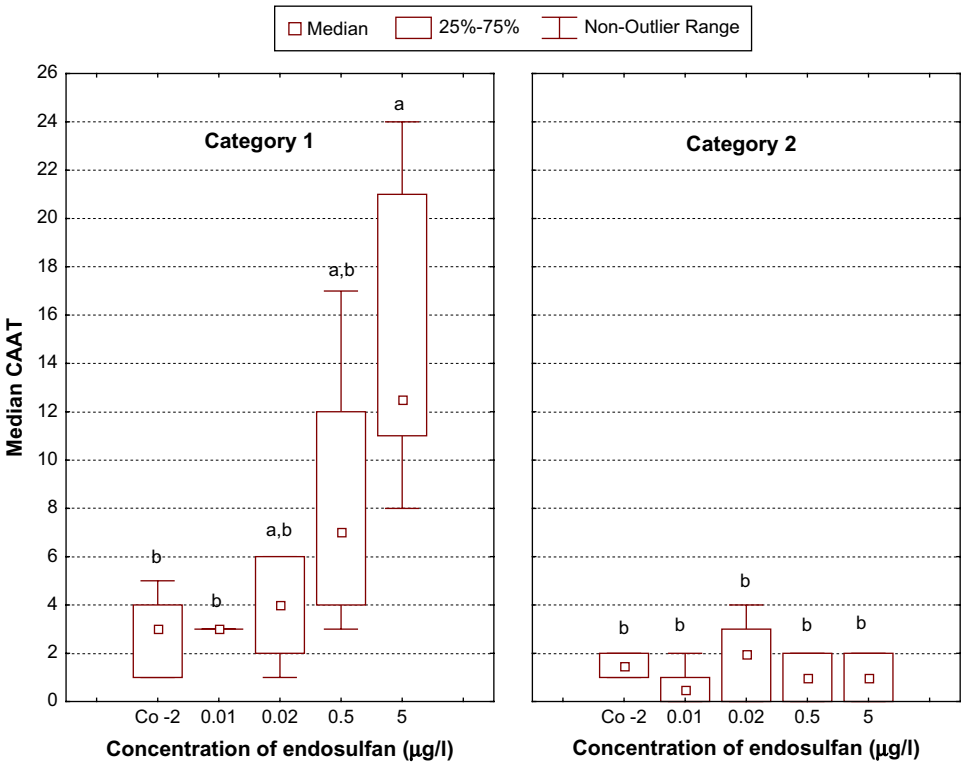

Fig. 2. Median chromosome aberrations per category in 200 anaphase–telophase from *Bidens laevis* exposed to endosulfan. Different letters indicate significant differences among treatments. $n = 6$. Category 1: interaction with the spindle (laggard and vagrand chromosomes, three chromosome groups at early telophase), Category 2: clastogenicity (bridges, fragments and ring chromosomes).

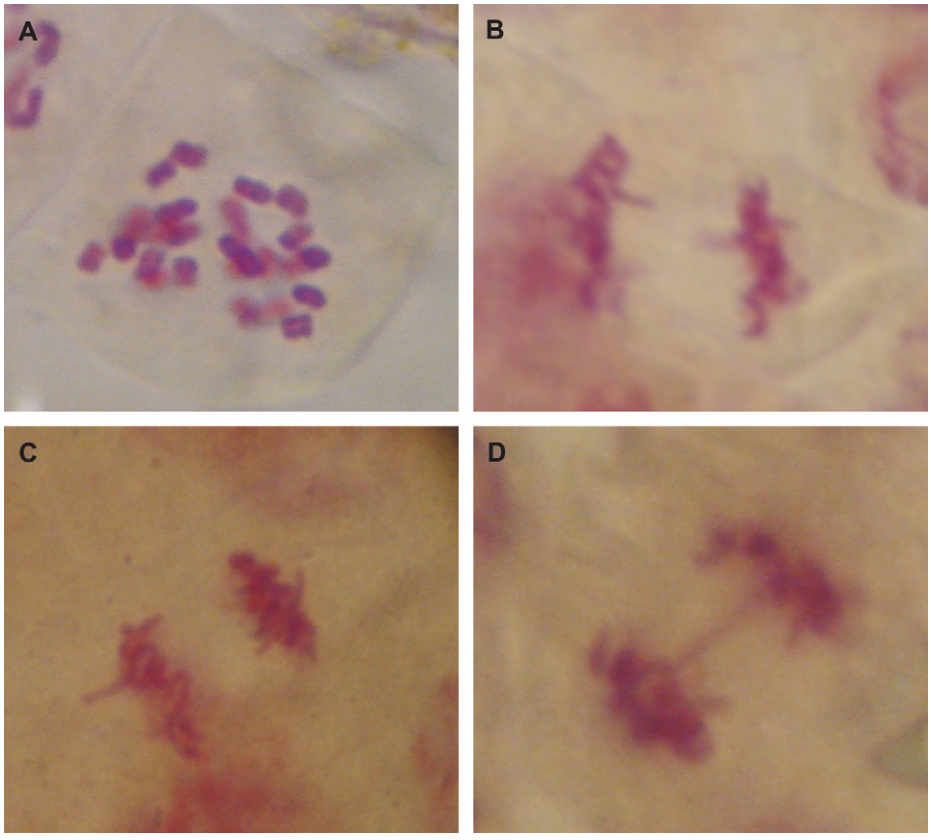

Fig. 3. Mitosis in *Bidens laevis*: normal metaphase with $2n = 24$ (A) and main chromosome aberrations in anaphase–telophase, laggard pair (B), vagrand (C) and bridge (D). 1000×.

2001) respectively, did not generate genotoxic effects. At 5 μg/L endosulfan, significant differences were found for category 1 in comparison to the Co−2 ($p < 0.05$). None of the tested concentrations presented differences for category 2 in comparison to the Co−2 ($p > 0.05$) (Fig. 2). Concentrations of 5 μg/L generated genotoxicity by interaction with the mitotic spindle, as judged by the predominance of laggard and vagrand chromosomes (Fig. 3). An immediate effect in the partial or entire inactivation of the spindle mechanism has been previously seen in plants exposed to other cyclodiene pesticides such as heptachlor, aldrin and dieldrin (Jain and Sarbhoy, 1987).

3.2. Potential of *B. laevis* for genotoxicity assays

B. laevis has large and clearly visible chromosomes, and a somatic chromosome number of $2n = 24$ (Fig. 3). Seeds had a high percentage of germination (>70%) and mortality of seedlings and young plants was almost null. The mitotic index varied from 4 to 12% in all treatments (data not shown), allowing the scoring of enough number of cells in the desired stages per slide preparation. Although *B. laevis* has not been used previously for genotoxicity testing of other pollutants, in this work we show that it is sensitive to 10 mg/L MMS, the concentration used in *Allium* CAAT tests (Rank, 2003) and to environmentally relevant concentrations of endosulfan. Moreover, its response was similar to that of *Allium* exposed to ethyl methanesulphonate (EMS) (Rank and Nielsen, 1997).

Grant and Owens (2001) proposed the transplanting of pea seedlings of *Vicia faba* in sites of potential pollution. To establish if *B. laevis* can be used as a sensitive and reliable species for genetic assays more research focused on the analysis of its response to other known mutagens is necessary. Notwithstanding, it has the potential value for bioassaying other environment xenobiotics as an extension to terrestrial species. If *B. laevis* results sensitive, it can possibly provide data more environmentally relevant for freshwater ecosystems since it belongs to the aquatic environment.

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