Pesticide Biochemistry and Physiology 99 (2011) 61–64

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Determination of genotoxicity of Fenaminosulf by *Allium* and Comet tests

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ARTICLE INFO

Article history:
Received 29 July 2010
Accepted 25 October 2010
Available online 12 November 2010

Keywords:
DNA damage
Dexon
Mitotic index
Comet assay

ABSTRACT

Genotoxic effects of Fenaminosulf, fungicide and micro-biocide, were examined by using mitotic index (MI), mitotic phase, and Comet assay on the root meristem cells of *Allium cepa*. In the *Allium* root growth inhibition test, EC_{50} value was firstly determined as 25 ppm and, 12.5 ($0.5 \times EC_{50}$), 25 (EC_{50}) and 50 ($2 \times EC_{50}$) ppm concentrations of Fenaminosulf were introduced to onion tuber roots. Distilled water was used as a negative control. All obtained data were subjected to statistical analyses by using SPSS 15.0 for Windows software. For comparison purposes, Duncan multiple range tests by using one-way analysis of variance (ANOVA) were employed and $p < 0.05$ was accepted as significant value. While MI (except in 24 h at 12.5 and 50 ppm) and prophase index increased, metaphase, anaphase and telophase indexes decreased in all concentrations compared to control at each exposure time. A significant increase in DNA damage was also observed at the concentration of 25 ppm in 24 h, 25 and 50 ppm in 96 h by Comet assay.

1. Introduction

Pesticides are used widely to improve crop yields in agriculture. These chemicals or their derivatives accumulate in the organisms and cause risk of mutagenicity, carcinogenicity, or teratogenicity. A great number of studies have been carried out for years all over the world due to the recognition of the importance of their mentioned effects.

Fenaminosulf is also known as; p-dimethylaminobenzenediazo sodium sulfonate, sodium 4-dimethylaminobenzenediazosulfonate, DAS, Dexon, diazoben, and Bayer 22555. It is an aromatic diazo compound, a fungicide and a micro-biocide for control for beans, beets, corn, cotton, cucumbers, peas, sorghum, spinach, and sugar beets. It is also known as the active ingredient in commercial fungicides for use on avocados, ornamentals, sugarcane, lawns, and turf [1–3].

Higher plants not only constitute an important material in the study of cytogenetic and mutagenic effect of chemicals such as *Vicia faba* [4], *Tradescantia paludosa* [5], *Pisum sativum* [6], *Hordeum vulgare* [7], *Crepis capillaris* [8], and *Allium cepa*, but also have been considered more sensitive and easier tools to perform the bioassays when compared to animals. Among them, *Allium* test is one of the best-established test systems used in order to determine the toxicity in the laboratories [9–15]. Not only can onions be stored and handled but also its macroscopic and microscopic parameters are observed easily. Moreover, this system is well correlated with the data obtained from eukaryotic and prokaryotic systems [16–18].

The Comet assay (single cell gel electrophoresis, SCGE) is a technically simple, highly sensitive, fast and economic test which detects *in vitro* and *in vivo* genotoxicity in any cell types examined. It has been introduced to detect even the small changes in the single and double stranded DNA structure, such as repair activities, its packing mode and its entirety besides requiring just few cells for its execution [19–24,14].

The aim of this study was to evaluate the genotoxic effects of Fenaminosulf on mitotic index, mitotic phases and damage on DNA by employing *Allium* and Comet assays.

2. Materials and methods

2.1. Materials

2.1.1. Organisms

A. cepa ($2n = 16$) onion bulbs, 25–30 mm diameter, without any treatment, were purchased from a local supermarket.

2.1.2. Chemicals

Fenaminosulf (Cas no. 140-56-7) was purchased from Fluka. Some chemical properties of the Fenaminosulf are given in Table 1. The other chemicals used in the study were obtained from Sigma–Aldrich.

2.2. Methods

2.2.1. EC_{50} determination

Prior to initiating the *Allium* test, outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordia. In order to determine effective concentrations (EC_{50}), a series of six bulbs were placed in distilled water for 24 h and afterwards the

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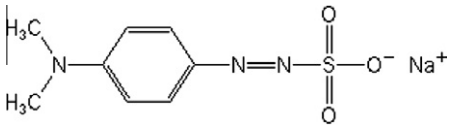
doi:10.1016/j.pestbp.2010.10.006

Table 1
Some chemical properties of the Fenaminosulf.

Chemical name	Synonyms	Chemical structure	Molecular weight	Usage as
Fenaminosulf	Dexon p-dimethylaminodiazobenzenesulphonate		251.24 g/mol	Fungicide micro-biocide

best growing five bulbs, exposed for 4 days to different aqueous concentrations of Fenaminosulf solutions (100, 75, 50, 25, 10 and 5 ppm, respectively) at room temperature (~21 °C ± 4 °C), were used. The test concentrations were renewed at every 24 h during the experiments. On the 5th day, root lengths (lengths of 10 roots from each bulb) were measured from both Fenaminosulf exposed bulbs and control group. EC_{50} value was considered as the concentration which retards the growth of root 50% when compared to the control.

2.2.2. Mitotic index analysis

In the determination of application doses, $2 \times EC_{50}$ (50 ppm), EC_{50} (25 ppm) and $0.5 \times EC_{50}$ (12.5 ppm) and control group were used at 24 and 96 h. Fixation and staining of the root tip cells were carried out as reported earlier [14,15]. The mitotic index (MI) and the frequencies of chromosomal aberrations (CA) were carried out according to Saxena et al. [12]. For each test group, five slides (1 root tip/slide) were prepared by squashing root tips with in solution of ethanol (99%) and glacial acetic acid (3:1). Slides were randomly coded and scored blindly. For MI, the different stages of mitosis were counted in a total of 5000–6000 cells (1000 cells/slide) per concentration, and expressed as a percentage.

2.2.3. Application of the Comet assay (single cell gel electrophoresis)

Twenty milligrams of root tips (from 20 to 30 seedlings) was placed in a petri dish kept on ice and spread with 1 mL of ice-cold Tris–$MgCl_2$ buffer (0.2 M Tris, pH 7.5; 4 mM $MgCl_2$–$6H_2O$; 0.5% w/v Triton X-100). The roots were immediately chopped with a fresh razor blade and isolated root nuclei collected in the buffer. The preparation of microscope slides with isolated nuclei was as previously described [14]. Each microscope slide was pre-coated with a layer of 1% normal melting point agarose and thoroughly dried at room temperature. Next, 100 μL of 0.8% low melting point agarose at 37 °C was mixed with 20 μL of the nuclear suspension and dropped on top of the first layer. The slides were allowed to solidify for 2 min on an ice-cooled tray and were then immersed in ice-cold lysing solution (1 M NaCl; 30 mM NaOH, 0.5% w/v SDS, pH 12.3) for 1 h. Subsequent to lysing, the slides were placed in a horizontal gel electrophoresis chamber and the DNA was allowed to unwind for 1 h in the electrophoretic buffer, containing 30 mM NaOH and 1.5 mM EDTA at pH 12.3 [25]. Electrophoresis was then conducted for 20 min at 25 V (1 V cm^{-1}) in the chamber cooled on ice. Following electrophoresis, the slides were rinsed three times with water, dried for 1 h at room temperature and stained with 50 μL ethidium bromide (20 μg ml^{-1}) for 5 min, and covered with a cover slip. For each slide, 100 randomly chosen nuclei were analyzed using a fluorescence microscope (Olympus, Japan). Three slides were evaluated per treatment and each treatment was repeated at least twice. Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either 0, 1, 2, 3, or 4 so that the total scores of slide could be between 0 and 400 arbitrary units (AU $microgel^{-1}$) [26].

2.3. Statistical analysis

The data of root length, MI, mitotic phases and comet scores, expressed as percentages, and the levels of significance in different treatment groups were analyzed. Duncan multiple range tests were performed by using one-way analysis of variance (ANOVA) on SPSS 15.0 version for Windows software.

3. Results

In *Allium* root growth test results are shown in Table 2. The effective concentration (EC_{50}) was found approximately as 25 ppm. It can also be easily seen that the effect of Fenaminosulf in *Allium* root growth was dose-dependent.

Table 3 summarizes the effect of Fenaminosulf on MI and mitotic phases in the root meristematic cells of *A. cepa* treated for 24 and 96 h. At all concentrations used in the incubations of root increased MI compared to control at each exposure time except in 24 h at 12.5 and 50 ppm. The highest values were obtained from 96 h examination of 50 ppm, and the lowest one in 24 h applications of 12.5 ppm concentrations of Fenaminosulf. The variation of MI showed statistically significant results ($p < 0.05$) only in 24 h at 12.5 ppm and 96 h at 25 and 50 ppm of Fenaminosulf.

All concentrations of Fenaminosulf used in the experiment caused changes in the percentage of particular phases' distribution in comparison to the control (Table 3). The percentage values of particular mitotic phases of control in the 24 h experiment were 84.48 ± 0.81% for prophase, 5.01 ± 1.01% for metaphase, 2.41 ± 0.15% for anaphase and 8.09 ± 0.34% for telophase. For 96 h experiment, the values were 86.75 ± 2.16%, 0.85 ± 0.35%, 2.57 ± 1.05%, 9.82 ± 3.56%, respectively. The characteristic effect caused by tested preparations was an increase of prophase index and simultaneous decrease of metaphase, anaphase and telophase index when compared to control. There were no metaphase cells in 96 h at 50 ppm and anaphase cells in 96 h at 25 and 50 ppm applications of Fenaminosulf.

Results obtained from Comet assay are summarized in Table 4. As it can be seen, exposure of Fenaminosulf increased the DNA damage at all concentrations applied except in 96 h at 12.5 ppm. Comet assay results showed that DNA damage was significantly high in 24 h at 50 ppm, in 96 h 25 and 50 ppm compared to negative control. There was no difference between negative control and 12.5 ppm Fenaminosulf in 96 h treatment.

4. Discussion

Allium test is one of the best-established test systems to detect environmental genotoxics and mutagens [9–13,15]. Dose-

Table 2
Results of the *Allium* root growth inhibition test.

Doses (ppm)	Average length (cm) ± SD[A]	Growth (%)
Control	2.62 ± 0.27^{a}	100
5	1.58 ± 0.18^{b}	60.31
10	1.50 ± 0.13^{b}	57.25
25	1.26 ± 0.21^{c}	48.09
50	1.11 ± 0.17^{d}	42.37
75	1.07 ± 0.26^{d}	40.84
100	0.96 ± 0.12^{e}	36.64

[A] Means with the same letter do not differ statistically at the level of 0.05, and SD (Standard deviation).