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Toxicity and Genotoxicity of Pendimethalin in Maize and Onion

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ABSTRACT

Herbicides are applied widely to maize crops. Frequently, these chemicals cause genetic change. Thus, this study was carried out with the aim to modify the Allium test using maize and onion to evaluate the toxicity and genotoxicity of pendimethalin. The effective concentration (EC), mitotic index (MI) and chromosomal aberrations found in treated root meristems of onion (*Allium cepa*) and three cultivars of *Zea mays* (HSSS, Insee2 and Suwan4452) were used as the endpoints of toxic and genotoxic evaluation. The results revealed that pendimethalin affected the toxicity and genotoxicity on three cultivars of maize and on onion. The percentages of recommended dose (7.5 ml/L) of pendimethalin at EC_{30}, EC_{50}, and EC_{70} were 0.05, 0.08 and 0.15% for HSSS, 0.02, 0.04 and 0.08% for Insee2, 0.02, 0.05 and 0.09% for Suwan4452, and 0.07, 0.16 and 0.29% for onion, respectively. Pendimethalin caused a significant increase in both the mitotic index and the frequency of chromosomal aberrations for all plants in the study. The types of chromosomal aberrations detected in root cells were fragments, bridges, laggards, multipolar and micronuclei. Moreover, abnormal metaphase (C-mitosis) and polyploidy, as well as the accumulation of metaphase cells, were also found in treated root cells. The results of this study will provide additional data on the toxicity and genotoxicity of the herbicide and also assist in environmental pollution monitoring.

Keywords: toxicity, genotoxicity, pendimethalin, maize, modified Allium test

INTRODUCTION

Herbicides are potential hazards that may have toxicological and genotoxicological effects on the environment and human health (Ma, 1982). The induction of genetic damage may cause an increased incidence of genetic disease in future generations and contribute to somatic cell diseases including cancer in the present generation (Connell, 1997). Therefore, it is very important to detect compounds that affect genetic material and to avoid human exposure to them. Pendimethalin is a mitotic inhibitor herbicide that can inhibit cell division in root meristems. It also inhibits microtubule synthesis, which is important for the formation of cell walls and spindle fiber. Thus, incomplete cell division and induction of multi-nucleated cells occurs.

Root growth inhibition and adverse effects upon chromosomes provide indications of toxicity and genotoxicity (Rank, 1993). The advantages of the Allium test among other testing

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systems are that it is easy to conduct, has low cost and a good correlation with mammalian test systems (Nielsen, 1994). The positive results from the Allium test should be considered as a warning or an indicator that the tested chemicals may cause a risk to human health and to the environment (Fiskesjö, 1985). On the other hand, maize is grown widely in Thailand and is sprayed directly with pesticides and herbicides in the field. Frequently, these chemicals can induce genetic change. Thus, breeders are locked into a continuous process of selecting cultivars of maize with desirable characters for the next generation of crops.

This study aimed to modify the Allium test using maize and onions to evaluate toxicity levels at EC_{30}, EC_{50} and EC_{70}, which represent the effective concentrations of pendimethalin that inhibit 30, 50 and 70% of root growth, respectively, compared to a control. Moreover, the objective of this study was to investigate the genotoxicity of pendimethalin, which was evaluated by the percentage of aberrant cells identified as having chromosomal aberrations. The results will provide information to: 1) assist with the evaluation of the toxicity and genotoxicity of herbicides on maize; and 2) manage environmental pollution.

MATERIALS AND METHODS

The current study was carried out to determine the toxicity and genotoxicity of the pre-emergence herbicide, pendimethalin, on onions (*Allium cepa* L.) and three cultivars of maize (*Zea mays*; super sweet corn cultivar Hawaiian Sugar Super Sweet from Department of Agriculture (HSSS); super sweet corn cultivar, Insee2; and field corn cultivar, Suwan4452). The toxicity was evaluated in term of toxicity levels and the mitotic index of root growth. Genotoxicity was determined by the percentage of root cells which contained chromosomal aberrations.

The laboratory study for screening the toxicity and genotoxicity of pendimethalin in maize by the modified Allium test was divided into three phases.

1) Preliminary range finding and definitive toxicity test: The toxicity of pendimethalin on the three cultivars of maize and on onion was evaluated by measuring the root bundle length inhibition after 96 h. Concentrations between the highest concentration that inhibited root growth and the lowest concentration that rarely inhibited the root growth were assessed. As a result, definitive concentrations were chosen from the range to establish the full-scale test.

2) Determination toxicity levels: The toxicity levels on the root growth inhibition at EC_{30}, EC_{50}, and EC_{70} were calculated from a plot of root bundle length using the percentage of the control against the concentrations.

3) Determination of mitotic index and chromosomal aberrations: Toxicity and genotoxicity tests were determined as the mitotic index and chromosomal aberration at concentrations of EC_{30}, EC_{50} and EC_{70}. The effect of pendimethalin on the mitotic index in each treated plant was determined in 400 cells per slide and 6 slides per concentration. The genotoxicity of pendimethalin on maize and onion was determined as the percentage of cells which contained chromosomal aberration (aberrant cells). Both the mitotic index and chromosomal aberration were described with descriptive statistics and then a one-way ANOVA was used to compare the means of more than two groups, Scheffe's multiple comparisons procedure was used to compare the means of pair groups.

In addition, all experiments were carried out using tap water as a negative control and 10 mg/L of methyl methanesulfonate herbicide (MMS) as a positive control for comparison with pendimethalin.

RESULTS

Toxicity test

The toxicity of pendimethalin on the

three cultivars of maize (HSSS, Insee2, Suwan4452) and on onion using the modified Allium test were determined for the two parameters of root growth and the mitotic index.

1) Effects on root growth

The polynomial equations that had the maximum R^2 values were selected to estimate the toxicity levels at EC_{30}, EC_{50} and EC_{70} of pendimethalin for the three cultivars of maize and for onion (Table 1).

2) Mitotic index

It was found that pendimethalin at high concentration increased the mitotic index in most cultivars of maize (Figure 1). The mitotic index in the root cells of the treated maize cultivars, Insee2 and HSSS, were highest for EC_{70} followed by EC_{50}, EC_{30}, the control and MMS, respectively. Onion had the highest mitotic index value at EC_{50} (Figure 1).

Genotoxicity test

The percentages of aberrant cells found in the roots of pendimethalin-treated maize and onion at toxicity levels of EC_{30}, EC_{50}, and EC_{70} were significantly ($p>0.05$) different from those of the control. The chromosomal aberrations of

Table 1 Concentration of pendimethalin at EC_{30}, EC_{50} and EC_{70} for three cultivars of maize and for onion, estimated from second-degree polynomial equations.

	Concentration in percentage of recommended dose*		
Plant	EC_{30}	EC_{50}	EC_{70}
HSSS	0.05	0.08	0.15
Insee2	0.02	0.04	0.08
Suwan4452	0.02	0.05	0.09
Onion	0.07	0.16	0.29

* 100 % of recommended dose = 7.5 ml/L

EC = Effective concentration

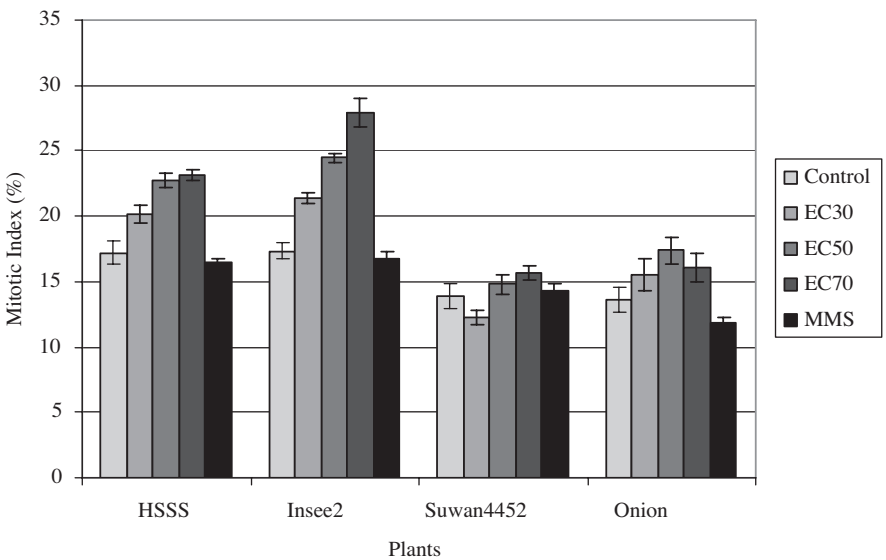

Figure 1 Effect of pendimethalin at three toxicity levels on the mitotic index of treated root cells in maize and onion (Vertical bars shown the standard deviation).

all treated cultivars were highest at EC_{70} followed by EC_{50}, EC_{30}, MMS and the control, respectively. An exception was Suwan4452, where the percentage of aberrant cells was highest at EC_{70} followed by EC_{50}, MMS, EC_{30} and the control, respectively (Figure 2).

Moreover, it was found that pendimethalin induces abnormal metaphase (C-mitosis or double chromosome) (Figure 3A) and polyploidy, as well as the accumulation of metaphase cells. Chromosomal aberrations induced by pendimethalin were found frequently to be in the form of either a: bridge (Figure 3B), fragment (Figure 3C), laggard (Figure 3D), micronucleus (Figure 3E) or multipolar (Figure 3F).

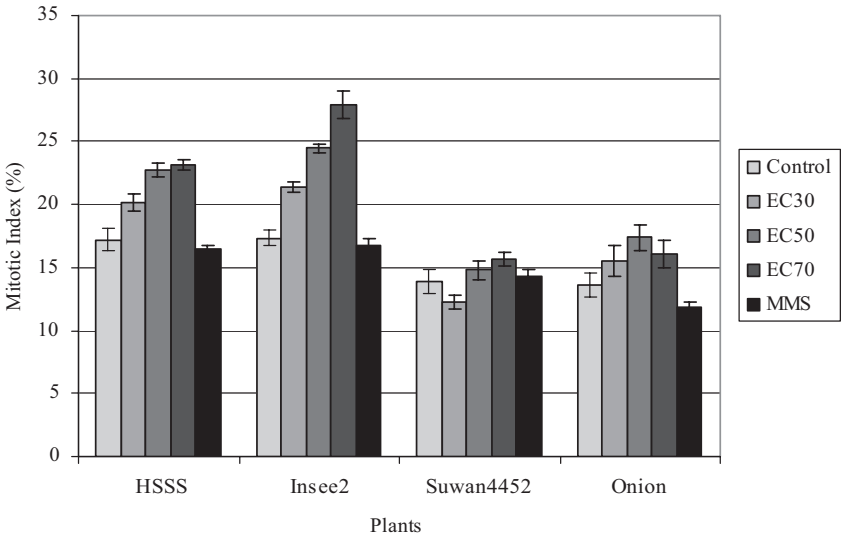

Figure 2 Effect of pendimethalin on chromosomal aberrations detected by percentage of aberrant cells of treated roots in maize and onion (Vertical bars shown the standard deviation).

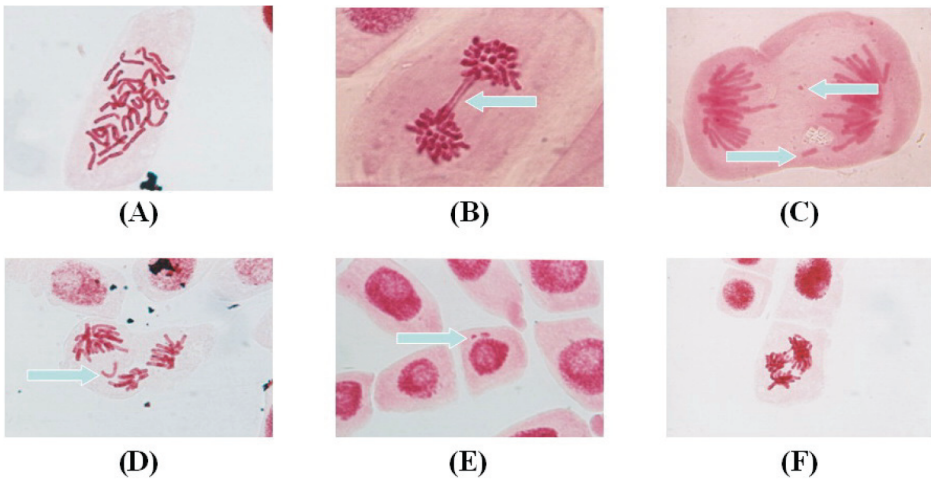

Figure 3 Chromosomal aberrations found in mitotic cells in treated roots of onion: (A) C-mitosis (2n = 32); (B) bridge; (C) fragment; (D) laggard; (E) micronucleus; and (F) multipolar.

DISCUSSION

Growth is one of the best indices for the evaluation of plant responses to environmental stress (Nilsen, 1996). The direct exposure to herbicide results in a rapid accumulation of these elements in the roots. The visible effect of herbicide is stunted root growth. Therefore, the difference in root elongation is a very useful parameter for screening the toxicity of herbicide in plants. The results revealed that pendimethalin affects toxicity and genotoxicity in the three cultivars of maize and in onion. The root growth rate of treated maize and onion after 96 h tended to decline more than that of the control. This occurrence was supported by the inhibiting and poisoning effects of pendimethalin, which were cumulative following exposure time. Genotoxicity can be defined as an alteration of the genome, either by disruption of the DNA itself, or by interference with its proper division and distribution in mitosis. Thus, the risk of pendimethalin affecting genetic material was evaluated by chromosomal aberration in the root meristematic cells of maize and onion. The results indicated that pendimethalin was able to alter mitotic division in root tip cells after 48 h. It caused an increase not only in the mitotic index, but also in the frequency of cells with chromosomal aberration significantly. Moreover, the effect of pendimethalin on mitotic cells was found to be similar to that of colchicine in the type of abnormal metaphase (C-mitosis) and polyploid induction, as well as in the accumulation of metaphase cells. This indicated that pendimethalin could be applied for polyploid induction in a breeding program. Further study should be undertaken to support this result and to optimize the concentration of pendimethalin that induces polyploidy.

In addition, all experiments were carried out using tap water as a negative control and 10 mg/L of MMS as a positive control for use as a comparison with pendimethalin and also to test onion in a comparison with the three cultivars of maize, as the Allium test (including onion and MMS) shows good correlation with mammalian test systems (Nielsen, 1994). The results may be considered as providing a warning or an indicator that the tested chemicals may be a risk to human health and to the environment, based on the results of Fiskesjö (1985).

CONCLUSIONS

The modified Allium test using the three cultivars of maize and using onion was able to be applied in a practical manner for screening the toxicity and genotoxicity of pendimethalin. It was found that pendimethalin decreased the root bundle length and increased the mitotic index, as well as the percentage of aberrant cells in comparison to the control. A greater effect resulted at the higher concentrations of EC_{70}, EC_{50} and EC_{30} respectively. Moreover, the effect of pendimethalin on mitotic cells was found to be exhibited in cells showing abnormal metaphase (C-mitosis) and polyploid induction, as well as the accumulation of metaphase cells.

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