Dinitroaniline Herbicide effects on Mitotic Division in Faba Bean: Possible Damage Recovery via Certain Growth Factors` Seed Treatments

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Abstract: A pre-sowing overnight faba bean seeds soaked in tryptophan, methionine amino acids and in a pyrimidine derivative SG93 were cultivated in soil incorporated with the two dinitroaniline herbicides butralin and pedimethalin. The herbicides generally caused the decline of mitotic indices in root tip cells of faba bean and in percentage of divided cells at prophase. The divided cells` percentage at ana-telophase stage of mitosis was significantly inhibited. Maximum inhibitory effect was exerted by the butralin in comparison to control. On the contrary, the exposure to the herbicides resulted in an increase in percentage of divided cells at the metaphase stage .The mitotic abnormalities caused by the herbicides were recorded as stickiness, C-metaphase, anaphase bridges and micronuclei. From the results, the growth factors under test, i.e. tryptophan , methionine and the pyrimidine derivative substance while; showed variable effects; all exerted significant counteraction influence against the herbicide-induced inhibitory damageable symptoms on the mitotic division stages in root tip cells of faba bean.
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1. Introduction:

Studies on the dinitroaniline herbicides revealed that they caused certain damages in mitosis in meristematic cells via interrupting the stability of spindle and microtubules (Upadhya and Looden, 1987; Lloyd et al, 1987). This was attributed to the interfering with tubulin dimer polymerization (Morejohn et al., 1987). Later, the instability of spindle or cortical microtubules under exposure to such herbicides were recorded (Fennell et al, 2006; Baird et al, 2000). In this respect, pendimethalin herbicide caused chromosomal abnormalities in mitotic cell division as micro nuclei and C-mitosis in plant and mammalian cells as well (Dimitrov et al, 2006). Whereas, (Abd El-Aziz and Hassan, 1994) reported a protective action of thiols against butralin - induced chromosome damages in mice. In earlier study, aromatic amino acid tryptophan and adenine were found to restore seedling root growth of lentils (Hassan and Gaweesh, 1989) and tissues` structures of shoot meristematic apical parts of faba bean under exposure to glyphosate herbicide (Hassan et al, 1993). Similar observations were obtained through exogenous application of the pyrimidine derivative substance SG93 (Hassan et al, 2006; Hassan and Gad, 2003).

In the present experiment, we aimed to investigate the damageable action of two dinitroaniline herbicides; herein butralin and pedimethalin; on mitosis and chromosome abnormalities in faba been root tip cells. In addition, to examine the possible counteraction influence of the aromatic amino acid tryptophan and the sulphur containing one, methionine against the induced injurious action of the two herbicides on mitosis in root tip cells of faba bean plant.

2. Material and Methods:

1-**Cultivation and pre-sowing growth factor treatments of faba bean seeds**: Pure line of faba bean (*Vicia faba* L., cv. Giza 643) was obtained from the Agriculture Research Centre, Ministry of Agriculture, Egypt. Uniform seeds were selected and sterilized in 1% sodium hypochlorite solution for 15 minutes (Yenne et al, 1988). Seeds were washed thoroughly with distilled water, soaked overnight (ca. 12 hours) in the following solutions:

1-Distilled water.

2- Growth factors: Methionine amino acid (M), tryptophan (W), and the pyrimidine derivative substance (SG93, Fig. 1), each at 100 and 500 ppm. Treated seeds were then cultivated in pots 30 cm diameter during November at average maximum and minimum temperatures of 25.2 and 13.9 C respectively. Media of cultivation was equal amounts of sieved soil of 2:1 clay and sand v/v. The pots were divided into 14 groups in case of either butralin (But) or pendimethalin (Pen). The soil was incorporated with the two herbicides before cultivation in seven groups. Seeds of faba bean were then sown at 2 cm

depth as 10 seeds per pot. Fertilization with ammonium nitrate, super phosphate (2:1 w/w) per pot and the application procedure of the herbicides calculate *Vis* surface area of the pot. The method is described in detail by (El-Awadi, 2007). Pots were supplied with water according to plant stages requirements. The pots were distributed in a complete randomized block design. Experimental treatments are indicated in the Table 1.

3-Feulgen –Squash technique:

This technique is used in cytology for staining the nucleus at any stage in the cell cycle. The method applied is according to (Darlington and La-Cour, 1976).

Table (1): Experimental treatments

Number	Treatment	Concentration
1	Control (H_2O)	0
2	But or Pen	5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
3	M	100
4	M	500
5	W	100
6	W	500
7	SG93	100
8	SG93	500
9	M + But or Pen	100+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
10	M + But or Pen	500+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
11	W + But or Pen	100+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
12	W + But or Pen	500+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
13	SG93 + But or Pen	100+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$
14	SG93 + But or Pen	500+5.38L ha.$^{-1}$ or 4.76 L ha.$^{-1}$

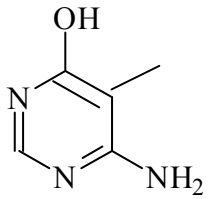

Figure 1: Chemical structure of the pyrimidine derivative (SG93)

3. Results:

Mitotic Division:

Results presented in Table 2 indicated the decline of mitotic indices in root tip cells of faba bean plant as compared to control. Such an influence was true in response to both the herbicides under test, i.e. butralin and pendimethalin. Butralin showed the maximum effect. The sole treatment of the two amino acids methionine and tryptophan at the concentrations 100 and 500 ppm revealed to a significant increase in the mitotic index. The pyrimidine derivative substance SG93 (Fig. 1) followed the same pattern except at its high concentration of 500 ppm. The overnight soaking of the faba bean seeds in the above mentioned growth factors (methionine =M, tryptophan =W, pyrimidine derivative = SG93) in an alternative application regime with pre-sowing of the butralin (B) and pendimethalin (Pen.), showed variable response. Low concentration of methionine (100ppm) resulted in significant increase in the mitotic index under the application of butralin herbicide (5.38L ha.$^{-1}$), whereas significant reduction was observed with high concentration of methionine and SG93 substances. In case with the pendimethalin herbicide (at 4.76 L ha.$^{-1}$) , methionine and tryptophan at the low concentrations of both led to significant increases in the mitotic indices in root cell tips of the faba bean. Other treatments appeared not significant.

Prophase

The percentage of divided cells at prophase had increased due to butralin application in comparison to control (Table 2). On the contrary, the percentage of divided cells at prophase was reduced as a result of pendimethalin application. The pyrimidine derivative (SG93) and methionine both as single treatments significantly elevated the percentage of the prophase divided cells as compared to control.

Except with high concentration of the amino acid tryptophan (W) treatments with each growth factor in interaction with the butralin, herbicide caused significant increase in the percentage of divided cells at prophase.

Pre-soaking overnight treatment of faba bean seeds in high concentrations of tryptophan and the pyrimidine derivative substance (SG93) followed by the application of pendimethalin resulted in a significant increase in the percentage of the divided cells at prophase stage in root tips of the faba bean plants under test.

Metaphase

The application of both the dinitroaniline herbicides butralin and pendimethalin resulted in the increase in percentage of divided cells of the root tips of faba bean at metaphase stage as compared to control. Such an influence was reversed in respect to sole treatments with the growth factors under test with exception with the pyrimidine derivative substance at 100 ppm (Table 2).

In general as shown in Table (2), the interaction treatments between the herbicides and the growth factors resulted in the reduction of percentages of divided cells at metaphase stage of the faba bean root tips.

Ana-Telophase

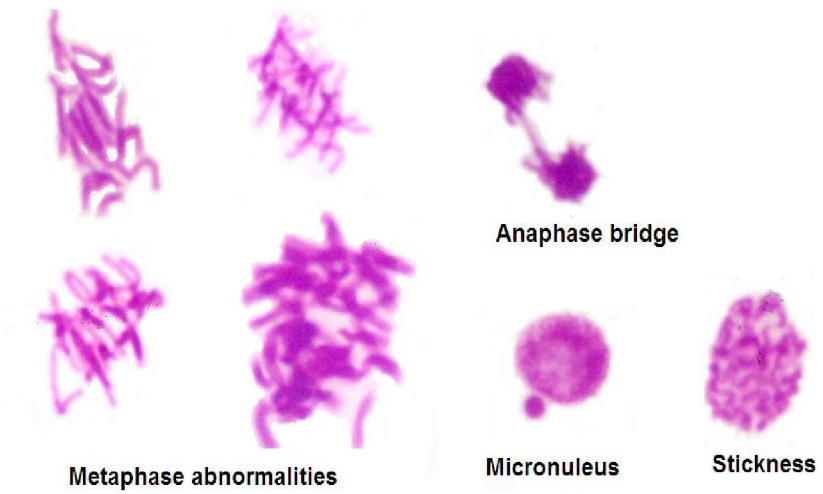

Figure (2): Abnormalities in mitotic division of faba bean (cv, Giza 643) root-tip cells, in response to pre-emergence soil incorporation with the herbicide butralin (at (5.38L ha.$^{-1}$) or pendimethalin (at 4.76 L ha.$^{-1}$).

Table (2): Mitotic indices and percentages of its stages in root tip cells of faba bean (cv. Giza 643), in response to the herbicides butralin (5.38L ha.$^{-1}$) and pendimethalin (4.76 L ha.$^{-1}$), each alone or in combination with seed soaking treatments prior to sowing in methionine, tryptophan and pyrimidine derivative SG93 at 100 and 500ppm . Each value is the mean of 5 randomly choice replicates from 5 different roots. According to one letter symbols of amino acids: M=methionine and W=tryptophan, whereas But=butralin, Pen= pendimethalin.

Treatments		Mitotic index		%of Prophase		% of Metaphase		% of Ana-telophase	
		But (3L Fed.$^{-1}$)	Pen (2LFed.$^{-1}$)	But (3L Fed.$^{-1}$)	Pen (2LFed.$^{-1}$)	But (3L Fed.$^{-1}$)	Pen (2LFed.$^{-1}$)	But (3L Fed.$^{-1}$)	Pen (2LFed.$^{-1}$)
Control (H_2O)		7.60 $\pm_{0.12}$	7.60 $\pm_{0.12}$	42.59 $\pm_{0.51}$	42.59 $\pm_{0.51}$	24.88 $\pm_{0.58}$	24.88 $\pm_{0.58}$	32.53 $\pm_{1.24}$	32.53 $\pm_{1.24}$
Herbicides		3.68** $\pm_{0.03}$	4.47** $\pm_{0.15}$	55.36** $\pm_{0.68}$	36.47* $\pm_{0.34}$	27.23** $\pm_{0.45}$	37.12** $\pm_{0.24}$	17.41** $\pm_{0.63}$	26.41** $\pm_{0.37}$
M	100ppm	8.30* $\pm_{0.26}$	8.30* $\pm_{0.26}$	44.54* $\pm_{0.35}$	44.54* $\pm_{0.35}$	20.38** $\pm_{0.42}$	20.38** $\pm_{0.42}$	35.08** $\pm_{0.39}$	35.08** $\pm_{0.39}$
	500ppm	8.60** $\pm_{0.12}$	8.60** $\pm_{0.12}$	44.88* $\pm_{0.51}$	44.88* $\pm_{0.51}$	21.38** $\pm_{0.39}$	21.38** $\pm_{0.39}$	33.74 $\pm_{1.02}$	33.74 $\pm_{1.02}$
W	100ppm	8.44* $\pm_{0.25}$	8.44** $\pm_{0.25}$	41.86 $\pm_{0.65}$	41.86 $\pm_{0.65}$	23.43* $\pm_{0.47}$	23.43* $\pm_{0.47}$	34.71* $\pm_{0.35}$	34.71** $\pm_{0.35}$
	500ppm	8.52* $\pm_{0.31}$	8.52** $\pm_{0.31}$	42.68 $\pm_{0.70}$	42.68 $\pm_{0.70}$	23.2* $\pm_{0.24}$	23.2** $\pm_{0.24}$	34.12* $\pm_{0.92}$	34.12* $\pm_{0.92}$
SG93	100ppm	8.17* $\pm_{0.14}$	8.17* $\pm_{0.14}$	45.22** $\pm_{0.39}$	45.22* $\pm_{0.39}$	25.13 $\pm_{0.47}$	25.13 $\pm_{0.47}$	29.65** $\pm_{0.44}$	29.65** $\pm_{0.44}$
	500ppm	7.93 $\pm_{0.33}$	7.93 $\pm_{0.33}$	44.83* $\pm_{0.77}$	44.83* $\pm_{0.77}$	23.3* $\pm_{0.39}$	23.30** $\pm_{0.39}$	31.87 $\pm_{0.46}$	31.87 $\pm_{0.46}$
M +Herb.	100ppm	8.64** $\pm_{0.12}$	8.51** $\pm_{0.23}$	44.78* $\pm_{0.50}$	41.31 $\pm_{0.45}$	17.81** $\pm_{0.57}$	22.03** $\pm_{0.45}$	37.41** $\pm_{0.44}$	36.66** $\pm_{0.52}$
	500ppm	6.83* $\pm_{0.17}$	7.83 $\pm_{0.23}$	46.05** $\pm_{0.41}$	41.53 $\pm_{0.48}$	19.05** $\pm_{0.52}$	25.25 $\pm_{0.51}$	34.9** $\pm_{0.38}$	33.22 $\pm_{0.34}$
W +Herb.	100ppm	7.52 $\pm_{0.44}$	8.45** $\pm_{0.27}$	45.21** $\pm_{0.55}$	42.84 $\pm_{0.57}$	18.64** $\pm_{0.75}$	23.8 $\pm_{0.19}$	36.15** $\pm_{0.51}$	33.36 $\pm_{0.53}$
	500ppm	8.15 $\pm_{0.22}$	7.51 $\pm_{0.31}$	42.13 $\pm_{0.43}$	44.14* $\pm_{0.91}$	21.21** $\pm_{0.42}$	23.33* $\pm_{0.50}$	36.66** $\pm_{0.54}$	32.53 $\pm_{0.40}$
SG93 +Herb.	100ppm	7.57 $\pm_{0.42}$	7.76 $\pm_{0.18}$	47.52** $\pm_{1.66}$	41.26 $\pm_{0.52}$	18.91** $\pm_{0.70}$	25.89 $\pm_{0.74}$	33.57 $\pm_{0.63}$	32.85 $\pm_{0.04}$
	500ppm	6.73* $\pm_{0.27}$	7.25 $\pm_{0.22}$	49.05** $\pm_{0.71}$	46.43** $\pm_{0.39}$	16.72** $\pm_{0.29}$	20.54** $\pm_{0.50}$	34.23* $\pm_{0.53}$	33.03 $\pm_{0.18}$
L.S.D	1%	0.87	0.79	2.39	1.92	1.69	1.56	2.25	2.13
	5%	0.75	0.56	1.69	1.36	1.19	1.10	1.59	1.51

* Significant at 5% ; ** Significant at 1%

The divided cells percentage at ana-telophase stage of mitosis was significantly inhibited due to pre-sowing application treatment of both the dinitro-aniline herbicides, i.e. butralin and pendimethalin .Maximum inhibitory effect was exerted by the butralin in comparison to control.

On the other hand, the single overnight faba bean seed soaking in either methionine (M) or tryptophan (W), such a percentage was augmented with an exception regarding the application of the pyrimidine derivative substance (SG93) at both its concentrations used.

The percentage of divided cells of faba bean root tips at the ana-telophase mitotic stage was however, increased in the combination of the alternative treatments with the growth factors preceded the application of the herbicides in comparison to the control (Table 2). Significantly better results were observed with the low and high concentrations of the two amino acids in interaction with the butralin herbicide. Similar results were obtained with pendimithalin in interaction with preceded treatment with methionine at its lower concentration of 100ppm.

The mitotic abnormalities indicating the injurious effect of the dinitroaniline herbicides butralin and pendimethilin are shown in Figure (2). The most predominant damages were chromosome stickness, C-metaphase, anaphase bridges and micronuclei.

4. Discussion:

In earlier studies dinitroaniline herbicides were observed to disrupt mitosis in meristematic cells (Upadhya and Looden, 1987; Stephen et al, 1983) via decreasing the stability of spindle and microtubules (Lloyd et al, 1987). These observations were supported in different investigations (Fennell et al, 2006; Baird et al, 2000 and Breviario and Nick, 2000). In this respect, the dinitroaniline herbicide pendimethalin was found to cause chromosomal abnotmalities in mitotic cell division (Barakat and Hassan, 1997). (Dimitrov et al, 2006) added that such a herbicide induced micro nuclei and C-mitosis in plant and mammalian cells as well. These observations mostly agreed with the present experimental results. The pre-emergence soil incorporation with dintroaniline herbicides butralin and/or pendimethalin caused the swelling of the secondary root tips of faba bean and axial skewing in growth direction of the main root. This coincided with significant decreases in mitotic indices observed in root tip cells of as compared to control in response to exposure to both herbicides under test. The results indicated that dintroaniline herbicide treatments led to chromosome alterations such as C- metaphase, anaphase bridges and micronuclei (Fig. 2). Such symptoms can be referred to mutagenicity (Fernandes et al, 2007) and/or to inappropriate chromosome segregation during mitotic division (Saunders et al, 2000). Fennell et al, (2006) attributed such abnormalities to the disappearance of the cell plate and the microtubule spindle fibres that guide the chromosomes.

The present results are supported by those previously obtained on faba bean under chemical stress of herbicides on faba bean meristematic tissues (Hassan et al, 2006; Hassan et al, 1993) also, reported the protective action of the pyrimidine derivative substance SG93 under salinity stress conditions.

Our present results however, proved that low concentration of methionine led to significant restoration in the mitotic index under the application of butralin herbicide. In case with the pendimethalin herbicide, methionine and tryptophan at the low concentrations of both led to significant increases in the mitotic indices in root cell tips of faba bean as compared to control .This recalled the observations recorded by (Abd El-Aziz and Hassan, 1994) on the protective action of thiols against butralin-induced chromosome damages in mice.

Conclusion:

From present results and literature , the cytological damages correlated to the injurious influence of the dinitroaniline herbicides under test can possibly counteracted via a pre-sowing faba bean seed soaking treatments in certain growth promoting factors . Such factors included the aromatic amino acid tryptophan, a sulphur amino acid methionine and a pyrimidine derivative substance SG93.

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