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Impact of Acetochlor on Some Anatomic Parameters in the Leaves of Oriental Tobacco Plant (*Nicotiana Tabacum L.*)

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Abstract: *In the testing grounds of the Tobacco and Tobacco Products Institute in Plovdiv, near the village of Markovo, in humus-carbonate soil, a field experiment was set up to determine the biological efficiency and selectivity of some soil herbicides used in growing oriental tobacco plants of the Plovdiv 7 variety. One of them was the Harnes herbicide with active ingredient acetochlor 90%.*

Acetochlor was applied at a dose of 150 ml/dka and 250 ml/dka 72 hours prior to tobacco planting. During the vegetation period, some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, leaf and vegetation tip deformation, weak chlorosis, etc.

For the purpose to determine the impact of the herbicide on tobacco leaf anatomy, samples from the midsection of the leaves were taken. The parameters taken into consideration were stomata number/mm^2 and stomata size (μm) from the upper and lower epidermis, and size of the assimilation parenchyma (mesophyll) of the leaf.

It was established that acetochlor caused considerable changes in the tobacco leaf anatomy, which found expression in reduction of stomata number/mm^2, as well as reduction of the thickness of leaf lamina (blade), compared to those in the non-treated control plants.

Keywords: *acetochlor, phytotoxicity, tobacco leaf anatomy, stomata number/mm^2, mesophyll.*

1. INTRODUCTION

Under the conditions of intensive development, agricultural production requires the use of herbicides for weed control in crops. Chemical agents that are applied often cause damage to agricultural crops, which in some cases are reversible in time, but sometimes lead to irreversible changes in the processes of growth and development of plants which could contribute to their perish (Miller et al., 1963; Martin, Fletcher, 1972; Gorske, Hopen, 1978; Muniyappa et al., 1980; Bakale, 1989; Ferrel et al., 1989; Tripathi et al., 1992; Mukharji, 1994; Kamble, 2007 a).

Anatomical changes in tissues and organs of crops, degeneration and malformations, reduction of content of chloroplasts in assimilation parenchyma of leaves, which may cause delays in their development may be added to the irreversible changes caused by herbicides (Guh, Kuk, 1997; Choi et al., 1998; Warabi et al., 2001; Ha et al., 2003; Jung et al., 2004; Yang et al., 2006; Kamble, 2007 b; Jung et al., 2008).

The purpose of this study was to determine the impact of acetochlor on some anatomic parameters in the leaves of the Oriental tobacco plant.

2. MATERIALS AND METHODS

In the testing grounds of the Tobacco and Tobacco Products Institute in Plovdiv, near the village of Markovo, in humus-carbonate soil, a field experiment was set up to determine the biological efficiency and selectivity of some soil herbicides used in growing oriental tobacco plants of the Plovdiv 7 variety. One of them was the Harnes herbicide with active ingredient acetochlor 90 %.

Acetochlor was applied at a dose of 150 ml/dka and 250 ml/dka 72 hours prior to tobacco planting

During the vegetation period, some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, leaf and vegetation tip deformation, weak chlorosis, etc.

For the purpose to determine the impacts of the herbicide on tobacco leaf anatomy, samples from the midsection of the leaves of the damaged plants and from the untreated control plants were taken and

fixed in 70% Ethanol. To examine the anatomic parameters, an Amplival light microscope was used. The parameters taken into consideration were stomata number/mm^2 and stomata size (μm) from the upper and lower epidermis, and size of the assimilation parenchyma (mesophyll) of the leaf – all at combined magnification of 400X (10X ocular and 40X objective). 30 measurements of each parameter were performed.

3. RESULTS AND DISCUSSION

The leaf of the tobacco plant (Nicotiana tabacum L.) is dorsoventral. Stomata are located on both sides of the leaf, characterizing it as amphistomatic. The basic epidermal cells are more or less isodiametric in shape, with undulated, curvy anticlinal walls. The stomatal complex is of the anomocytic type (lacking differentiated subsidiary cells), in which the stomata-surrounding cells are indistinguishable from the other epidermal cells, and the guard cells are bean-shaped.

The mesophyll is heterogeneous, represented by palisade (columnar) and loosely packed (spongy) parenchyma. The palisade parenchyma is located directly underneath the upper epidermis of the leaf. The spongy parenchyma consists of dispersedly situated, isodiametrically shaped parenchyma cells, interspersed with larger or smaller intercellular spaces, which are frequently connected to the stomata on the lower epidermis of the leaf.

Data about dimensions of the assimilation parenchyma (Table 1) on in control plants were significantly higher than those reported for the treated plants. The values for the thickness of the palisade parenchyma (112,5)127,6±1,79(147,5) μm, in untreated plants, were higher than the results obtained for acetochlor treated plants which were (85)95,7±1,31 (112,5) μm at the dose of 150 ml/dka and (65)72,2±0,97(82,5) μm for the dose of 250 ml /dka. Reduction of the thickness of columnar parenchyma was significant in plants, treated by a dose of 250 ml/dka acetochlor, which was confirmed by the average figures - 72,2 μm for the treated and 127,6 μm for the control plants.

Table1: *Impact of acetochlor on some anatomic parameters in the leaves of oriental tobacco, cultivar Plovdiv 7*

Indexes		(min) $\overline{X}$ ±S $\overline{X}$ (max)	S,%	max:min	(min) $\overline{X}$ ±S $\overline{X}$ (max)	S,%	max:min	(min) $\overline{X}$ ±S $\overline{X}$ (max)	S,%	max:min
Variants		non treated			treated - 150 ml/dka			treated – 250 ml/dka		
	palisade parenchyma	(112,5)127,6±1,79(147,5)	7,7	1,3	(85)95,7±1,31(112,5)	7,5	1,3	(65)72,2±0,97(82,5)	7,4	1,3
leaf parenchyma (mesophyll), μm	spongy parenchyma	(95)128,9±1,76(147,5)	7,5	1,5	(117,5)133,9±1,8(152,5)	7,4	1,3	(87,5)98,9±1,14(112,5)	6,3	1,3
ad epidermis stomata	number /mm^2	(83,3)103,6±2,61(125)	13.8	1.5	(58,3)81,1±2,26(108,3)	15,2	1,8	(41,6)57,5±1,61(75)	15,4	1,8
	length, μm	(17,5)27,8±0,75(35)	14,9	2,0	(27,5)32,4±0,54(37,5)	9,1	1,4	(25)30,4±0,43(35)	7,8	1,4
	width, μm	(15)22,8±0,49(27,5)	11,8	1,8	(22,5)26,25±0,42(30)	8,9	1,3	(20)24,3±0,31(27,5)	7,1	1,4
ab epidermis stomata	number /mm^2	(108,3)134,7±2,33(158,3)	9,5	1,5	(141,6)157,5±1,93(175)	6,7	1,2	(141,6)164,4±2,36(200)	7,8	1,4
	length, μm	(20)27,3±0,77(35)	15,5	1,75	(22,5)30,8±0,78(40)	13,8	1,8	(22,5)27,6±0,69(35)	13,8	1,5
	width, μm	(15)22,7±0,67(30)	16,4	2,0	(20)26,8±0,64(35)	13,2	1,7	(20)23,1±0,35(27,5)	8,4	1,4

In spongy cell tissue increase of values was observed which was reported for the cases of plants, treated at the dose of 150 ml/dka (117,5)133,9±1,8(152,5) μm, compared to control plants (95)128,9±1,76 (147,5) μm, but at a dose of 250 ml/dka the results (87,5)98,9± 1,14(112,5) μm were significantly lower than the untreated plants, which was in line with the data for the palisade parenchyma and confirmed the adverse impacts of acetochlor on the thickness of mesophyll of the leaf and that was in close connection to the growth and development of plants.

The number of stomata in mm^2 for adaxial (upper) epidermis with control plants (83,3)103,6±2, 61(125) was bigger compared to the treated plants - (58,3)81,1±2,26(108,3) number/mm^2 stomata at the dose of 150 ml/dka and (41,6)57,5±1,61(75) number/mm^2 for the dose of 250 ml/dka. Reduction of the number of stomata in treated plants was associated with the negative impact of acetochlor on them. In abaxial (lower) epidermis, increase of the number of stomata per mm^2 in treated plants was registered, and the average values were 164,4 number/mm^2 stomata at the dose of 250 ml/dka, 157,5 number/mm^2 of stomata at 150 ml/dka and 134,7 number/mm^2 of stomata reported in untreated plants. Increase of the number of stomata in treated plants was due both to their relatively large number per unit of area for the lower epidermis compared to the upper and to the adverse impacts of acetochlor on them and on the significant deformation of the leaf blade of those. Adverse impact of the herbicide

was confirmed by the increased values of the length and width of the stomata in the upper and lower epidermis in treated plants, which was associated with atrophy of closing cells of the stomata and improper operation of the stomata apparatus.

Reduction of the leaf parenchyma thickness of the leaves of tobacco plants treated with acetochlor, reduction of the number of stomata of adaxial epidermis and their increase by the abaxial as well as the increase of their size, was due to the adverse impact of the herbicide on them, and that was related to their feeding, growth and development. Visible signs of that influence were retarded growth, deformation of leaves and vegetation peak, weak chlorosis.

4. CONCLUSIONS

Acetochlor reduces thickness of the assimilation parenchyma of leaves it causes reduction of the number of stomata per mm^2 of the upper epidermis and their increase in the lower epidermis and causes atrophy of the closing cells of stomata and leaf blades of the Oriental tobacco plants of the Plovdiv 7 variety.

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