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Impact of Oxyfluorfen 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 effectiveness and selectivity of some soil herbicides used in growing oriental tobacco plants of the Plovdiv 7 variety. One of them was the Goal 2E herbicide with active ingredient oxyfluorfen 24%.*

Oxyfluorfen was applied at the dose of 24 g/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 of determining 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 oxyfluorfen 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: *oxyfluorfen, phytotoxicity, tobacco leaf anatomy, stomata number/mm^2, mesophyll.*

1. INTRODUCTION

Non-communicable diseases in plants are most often caused by abiotic factors of the environment, but may also be a consequence of activities of the anthropogenic factor. Human impact on plant life increases in line with the growth of technical progress, but sometimes it may have negative effect due to disruption of the relationship between requirements of plants and the environment, which leads to enhancement of the frequency of development of Non-communicable Diseases. They make plants weaker, create favourable conditions for the penetration of pathogenic organisms into plants, which bring more damage to infested plants. The set of factors has deep reflection into the rhythm of growth and development of plants.

Chemical agents which are used to combat diseases and pests of agricultural crops often cause damage to plants and sometimes even death (Martin, Fletcher, 1972; Muniyappa et al., 1980; Ferrel et al., 1989; Mukharji, 1994; Kamble, 2007 a).

Series of changes occur of their physiological functions which are a consequence of the relevant anatomical changes caused by the activity of the herbicide oxyfluorfen. There are differences in structure and shape of cells, expression of proliferation (budding) in the cambium and phloem in stems, accumulation of large amounts of meristematic cells which destroy stem peel and core of the treated plants (Kamble, 2007 b).

Inside leaves, this herbicide causes drying of cells, budding of cambium in the midrib zone of leaves, degeneration of conductive elements in the vascular bundles, damage of epidermal and mesophyll cells. (Guh, Kuk, 1997; Kamble, 2007 b).

Oxifluorfen reduces the content of chloroplasts in the cells of assimilation parenchyma of leaves (Jung et al., 2008), which is a cause for delay of growth and development of plants which are not resistant to its effects (Choi et al., 1998; Warabi et al., 2001; Ha et al., 2003; Jung et al., 2004; Yang et al., 2006).

The purpose of this study was to determine the impact of oxyfluorfen 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 effectiveness and selectivity of some soil herbicides used in growing oriental tobacco plants of the Plovdiv 7 variety. One of them was the Goal 2E herbicide with active ingredient oxyfluorfen 24 %.

Oxyfluorfen was applied in the dose of 24 g/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 of determining the influence 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). Measurement was performed by measuring screw eyepiece mounted on the microscope. 30 measurements of each indicator were carried out.

3. RESULTS AND DISCUSSION

The leaf of the tobacco plant (Nicotiana tabacum L.) is dorsoventral. The 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. Palisade parenchyma is located immediately below the upper leaf epiderma. 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.

Thickness of the leaf parenchyma (Table 1) in the control plants exceeded the values reported for the treated plants, which applied to both types of assimilation parenchyma. The values of columnar parenchyma (60)74,5±1,7(95) μm, reported in untreated plants significantly exceeded the results obtained for treated plants (47,5)54,1±0,64(60) μm. For aerenchyma those were (97,5)105,8±0,8(112,5) μm in the control plants and (80)89±0,85 (100) μm on the treated plants.

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

Indexes		(min) $\bar{X}$ ±S $\bar{X}$ (max)	S,%	max:min	(min) $\bar{X}$ ±S $\bar{X}$ (max)	S,%	max:min
Variants		non treated			treated		
	palisade parenchyma	(60)74,5±1,7(95)	12,9	1,6	(47,5)54,1±0,64(60)	6,6	1,3
leaf parenchyma (mesophyll), μm	spongy parenchyma	(97,5)105,8±0,8(112,5)	4,2	1,1	(80)89±0,85(100)	5,2	1,25
ad epidermis stomata	number /mm^2	(75)111,39±2,72(133,3)	13.4	1.8	(50)58,6±1,29(75)	12,1	1,5
	length, μm	(25)32,3±0,82(40)	14,1	1,6	(27,5)33±0,48(37,5)	8,1	1,4
	width, μm	(22,5)25,58±0,5(32,5)	10,8	1,4	(22,5)25,25±0,45(35)	9,8	1,5
ab epidermis stomata	number /mm^2	(141,7)159,4±1,9(175)	6,8	1,2	(83,3)113,6±2,45(150)	11,8	1,8
	length, μm	(22,5)29,75±0,72(35)	13,3	1,5	(25)31,91±0,72(42,5)	12,4	1,7
	width, μm	(17,5)24,6±0,57(30)	12,7	1,7	(22,5)27±0,55(32,5)	11,2	1,4

The number of stomata in mm^2 for adaxial (upper) epidermis of the treated plants (50) 58,6±1,29(75) was significantly smaller than that recorded for untreated plants (75)111,39± 2,72(133,3), which was confirmed by the index max**:**min (1,5) and the average values of the control plants 111,39 number/mm^2 and treated plants 58,6 number/mm^2 of stomata. The tendency for reduction of the number of stomata per mm^2, in treated plants was preserved with the abaxial (lower) epidermis (83,3)113,6±2,45(150) number/mm^2 and (141.7)159,4±1,9 (175) number/mm^2 for the control plants. Reduction of the number of stomata per mm^2 of the lower epidermis of treated plants was higher if compared to the upper, which is also confirmed by the index max**:**min (1,8) and was due both to the relatively large number of stomata in the lower epidermis compared to the upper as well as to the negative impact of oxyfluorfen on tobacco plants.

This negative influence was confirmed by the increased values for the length and width of the stomata reported in treated plants, which was associated with atrophy of the closing cells of stomata, and there from followed a disorder of the operation of stomata apparatus. Inefficient operation of stomata of the treated plants was in direct relation to their feeding. Reduced access to CO_2 of the assimilation parenchyma cells leaded to disruption of their growth and development, and was demonstrated by visible signs of phytotoxicity in tobacco plants caused by oxyfluorfen.

4. CONCLUSIONS

Oxifluorfen causes adverse impact to certain anatomical features of the leaves of Oriental tobacco plants of the Plovdiv 7 variety, by reducing the thickness of the assimilation parenchyma of leaves and by causing reduction of the number of stomata per mm^2 of the upper and lower epidermis of leaves as well as causing atrophy of the closing cells of stomata.

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