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Original article

Effects of Imazamox Applications on Stem Anatomy in Sunflower (*Helianthus annuus* L.) Cultivars

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Abstract

The aim of this study was to examine the effects of different doses of imazamox, an imidazolinone (IMI) group herbicide commonly used in sunflower agriculture, on the stem anatomical structure of sunflower cultivars. In this study, four different sunflower cultivars were used as SN:8 which is unresisting to IMI and resistant groups to IMI which are SN:9, SN:10 and SN:14. Seedlings coming to 4-6 leaf stage were applied with 3 different doses of herbicides (1 dose (3.125 ml/l), 2 doses (6.25 ml/l) and 3 doses (9.375 ml/l). When the cross sections of stem samples taken 7 days after the herbicide application were examined under light microscope, decreases in epiderma cell sizes due to dose increase, increases and decreases in collenchymavlayer thicknesses and cell lines, decreases in cortex parenchyma layer thicknesses, sclerenchymal layer thicknesses and significant increases and decreases were observed between the groups and doses in trachea cells. According to these data, it can be concluded that both the effects of herbicide dose changes and anatomical changes in stem sections can be used to determine resistant cultivars.

Keywords: Helianthus annuus L., stem anatomy, herbicide, imazamox.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops cultivated in many countries of the world. The oil of sunflower is a high quality vegetable oil and is used in the meals and as margarine in human nutrition (Kaya and Atakişi, 2003). Nowadays, the increase in the need for edible oil due to the population increase causes the oil production to remain inadequate. Therefore, an increase in sunflower production is required (Kaya, 2004).

The increase in production in agriculture is possible either by increasing the existing cultivation areas or by increasing the yield per unit area. For the increase in production, increasing the yield per unit area has become more important due to the limited increase in planting areas. Yield is the result of a common interaction between genotype and environment. Environmental conditions are factors such as climate, soil structure and cultivation techniques. In order to get high efficiency, yield potential, diseases, stamina strength and laying resistance, germination power, self-fertilization ability, seed size, vegetation period, oil content should be considered while choosing the sunflower cultivars (Cosge and Ulukan, 2005).

Today, hybrid sunflower seeds are preferred by producers in Turkey and all around the world because of its high yield and quality potential, homogeneity, maturation and ease of cultural practices. The use of hybrid seeds is one of the leading factors in the increase in sunflower yield (Semerci and Ozer, 2011).

Another factor directly affecting productivity in sunflower production is weeds. Weeds compete with sunflower for light, water and nutrients, resulting in loss of yield and quality. This problem was solved by the use of IMI group herbicide-resistant sunflower hybrids and IMI group herbicides produced by Clearfield® technology (Tan et al., 2005; Pfenning et al., 2008; Kaya et al., 2013).

IMI resistant sunflower hybrids and IMI group herbicides in weed control are advantageous since they are widespread, economic, easy to apply and showing quick results. However, some undesirable anatomical and physiological damages may occur in sunflower plants produced as a result of overdose and wrong application of herbicides. This situation leads to a decrease in production efficiency (Anastasov, 2010; Balabanova et al. 2016). In this study, the effect of different dose applications of imazamox that is a systemic effective post-emergence herbicide belonging to IMI group which is widely used in weed control in sunflower agriculture on the anatomical structure of sunflower cultivars, was examined.

Material and Methods

Supply of Materials

The sunflower (*Helianthus annuus* L.) seeds used in the study were obtained from Trakya Agricultural Research Institute. Four different types of sunflowers were used. These cultivars are; SN:8 which is unresisting to IMI and resistant groups to IMI which are SN:9, SN:10 and SN:14.

Formation of Experimental Groups

Experimental groups were formed by applying 1 dose (3.125 ml/l), 2 doses (6.25 ml/l), 3 doses (9.375 ml/l) of herbicides as control to the sunflower cultivars (Agricultural use: 125 ml/da). The seeds were planted in the botanical garden and left to grow. Seedlings coming to 4-6 leaf stage were applied herbicides. In this application, it was adapted to the study based on the dose used in agriculture. 7 days after the herbicide application, stem samples were taken and fixed for anatomical examination and stored in 70% alcohol.

Anatomical Examinations

Stem sections from sunflower varieties were covered with glycerin-gelatin (50% glycerin - 50% gelatin) environment prepared at 50 ^{o}C using safranin dye and turned into permanent preparate. These preparates were examined under Olympus CX21 light microscope and photographed with photomicroscope camera.

Results and Discussıon

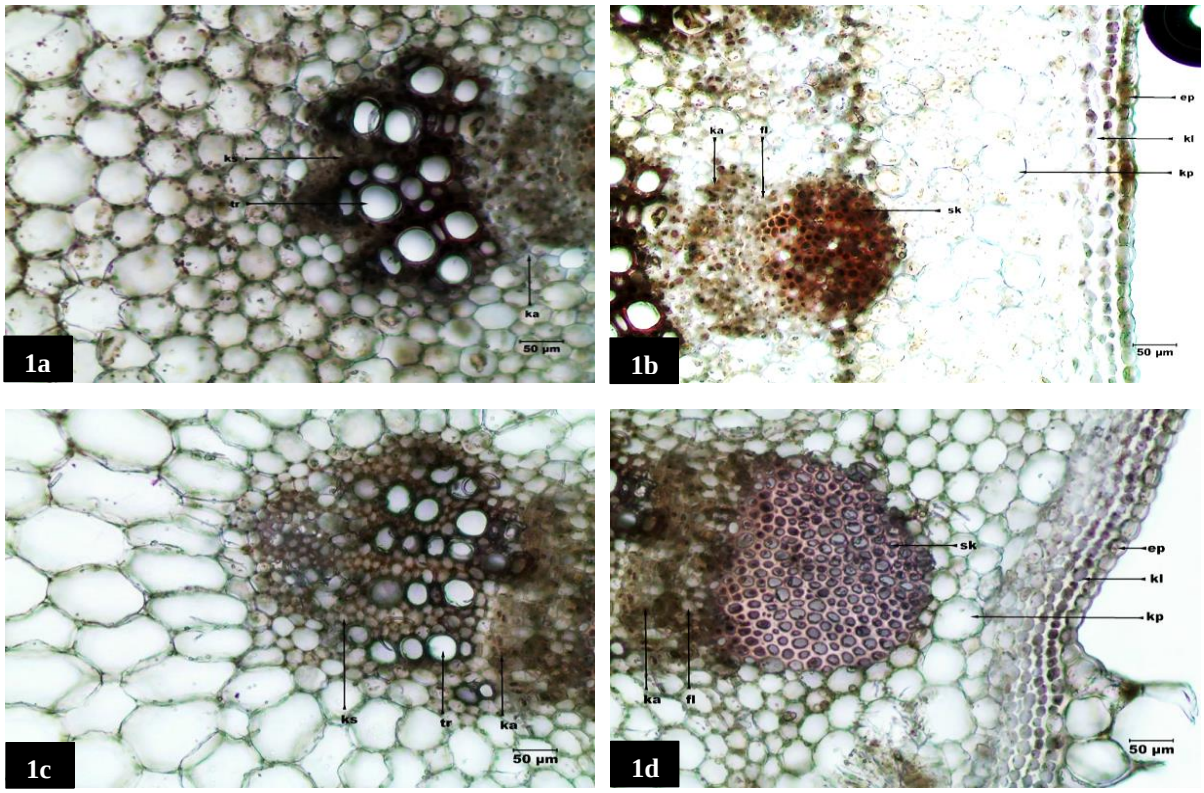
Modern agricultural techniques and inputs are used to increase yield and quality in agricultural production. One of the most important agricultural inputs is pesticides. Pesticides increase crop quantity and quality by protecting plants against disease, pests and weeds. It is widely used in agricultural areas due to its easy applicability, short-term effect and economy (Tiryaki et al., 2010; Torun, 2017).

The widespread use of pesticides in agriculture has brought many problems. Overdose and misapplication lead to environmental pollution, threaten the health of human beings and other living organisms, cause endurance in organisms and residues in food. In addition, overdose of pesticides causes phytotoxicity on plants and causes various adversities in the anatomical structure of the plant (Ozturk et al., 2006; Kamble, 2007; Kamble, 2013; Öztürk Çalı, 2013; Ashraf and Murtaza, 2016; Anastasov and Kalinova, 2017).

In this study, the differences between light cross microscope studies of stem cross sections of sunflower cultivars on which different dosage applications of imazamox herbicide were applied that is unresisting to IMI group herbicide (SN: 8) and resistant to IMI group herbicide (SN: 9, SN10 and SN: 14) were tried to be determined.

Stem Anatomy Results of SN:8 Cultivar

In the control group of SN:8, the cross section of the stem was examined and it was found that the epiderma layer consisted of single row cells with an average width of 17 µm and a length of 26 µm. The collenchyma layer has 2 rows and the thickness of the layer is about 40 µm. The cortex parenchyma is approximately 230 µm thick. The average sclerenchyma layer is 160 µm. The tracheas were measured as 45 µm and the tracheitis as 20 µm. In the experimental group where 1 dose of herbicide was applied, the epiderma layer consisted of cells with average size of 17 µm and length of 26 µm. The collenchyma layer is 4-5 rows. The thickness of the collenchyma layer was measured as 75 µm on average. The thickness of the cortex parenchyma layer varies between 60-220 µm. The sclerenchyma layer is approximately 245µm thick. The tracheas were measured as 34 µm and tracheitis as 15 µm. In the group where 2 doses of herbicides were applied, the cells forming the epidermis had an average width of 19 µm and a length of 30 µm. The collenchyma layer has 4-5 rows and the average thickness of the layer is 85 µm. The cortex parenchyma layer thickness varies between 54-190 µm. The sclerenchyma layer is 189 µm. Tracheas are 40 µm and tracheids are 16 µm. In the experimental group where 3 doses of herbicides were applied, the epiderma layer consisted of cells with an average size of 17µm and length of 26 µm. The collenchyma layer is formed of 4-5 rows and the layer thickness is 86 µm. The cortex parenchyma layer thickness varies between 43-186 µm. The thickness of the sclerenchyma layer is 240 µm. The tracheas are 36 µm and tracheids 20 µm (Fig. 1).



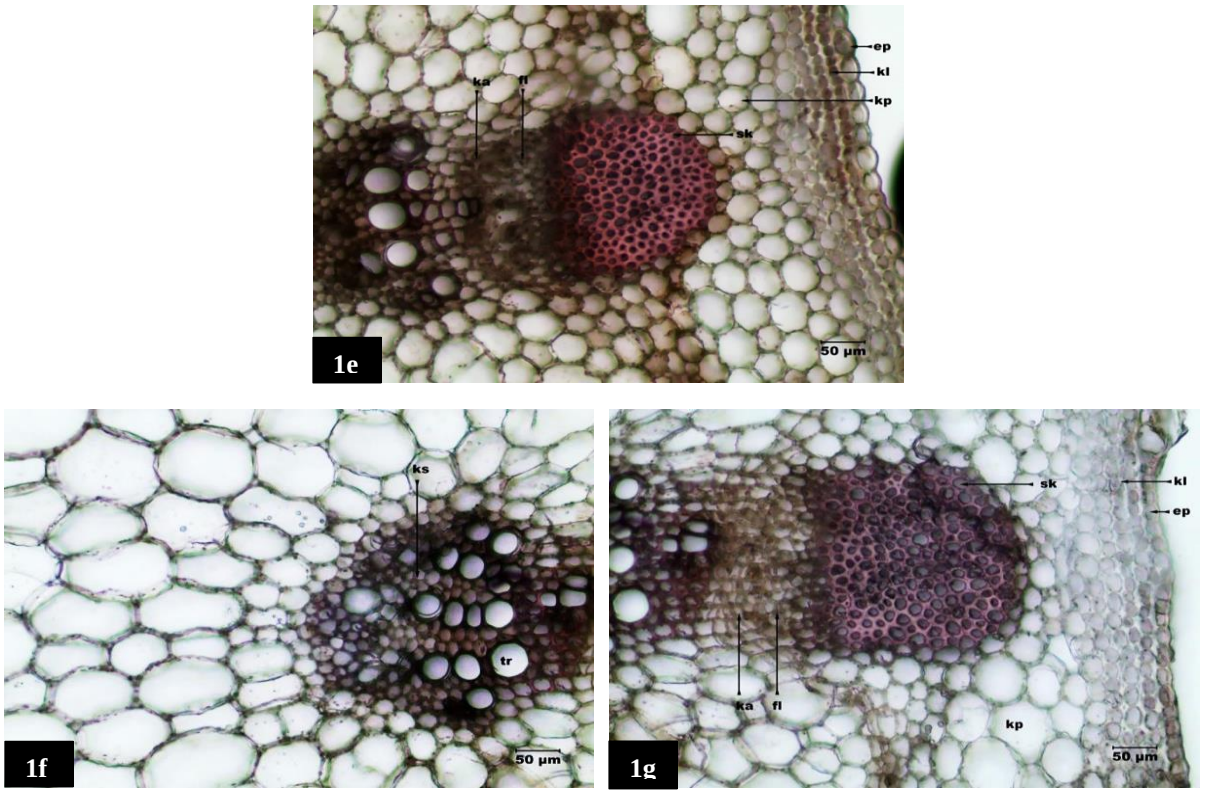


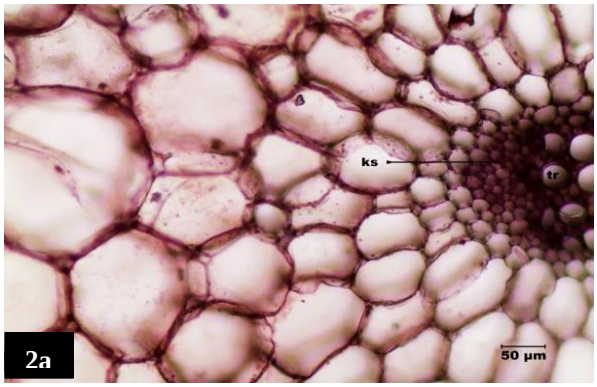
Figure 1. Stem cross sections of SN:8 cultivar. a, b- control, c, d-1 dose, e- 2 doses, f, g- 3 doses. ep: epidermis, kl: collenchyma, kp: cortex parenchyma, ka: cambium, fl: phloem, sk: sclerenchyma ka: cambium, ks: xylem, tr: trachea.

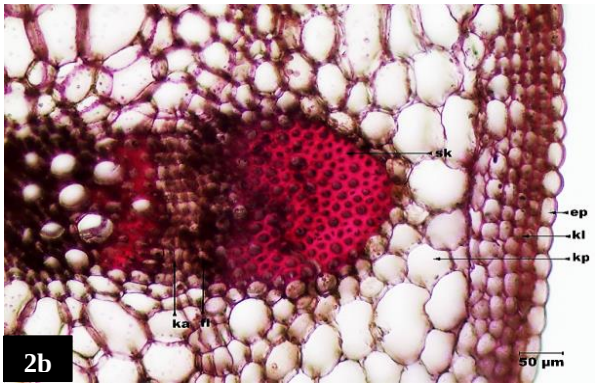
Stem Anatomy Results of SN: 9 Cultivar

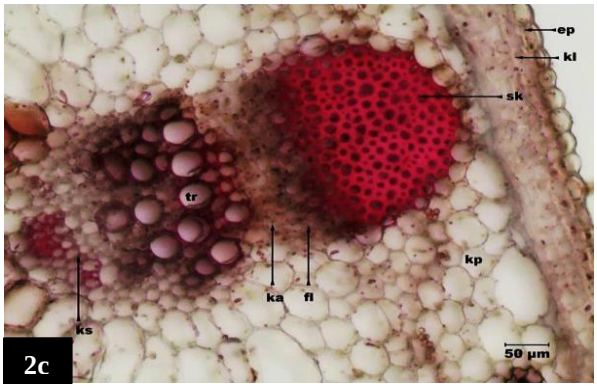
When the stem cross sections were examined in the control group of SN: 9, the average width of epiderma cells was measured as 18 µm and the length was 32 µm. The collenchyma layer has 4-5 rows and the thickness of the layer is 80 µm. The cortex parenchyma layer thickness varies between 66-220 µm. The sclerenchyma layer is 188 µm thick. The tracheas are 36 µm and the tracheids are 21µm. In the group where 1 dose of herbicide is applied, the average width of epiderma cells is 20 µm and the length is 30 µm. Collenchyma layer has 4 rows and the thickness of the layer is 68 µm. The cortex parenchyma is in the range of 85-145 µm. The sclerankyma layer is 190 µm thick. The tracheas were measured as 37 µm and the tracheitis as 18 µm. In the experimental group where 2 doses of herbicides were applied, the average width of epiderma cells was 14 µm and the length was 16 µm. The collenchyma layer is in 5-6 rows. The average thickness of the collenchyma layer is 82 µm. The thickness of the cortex parenchyma varies between 128-324 µm. The sclerenchyma layer was measured at 145 µm. Tracheas are 30 µm and tracheitis are 17 µm.In the 3 dose herbicide applied group, average width of epiderma cells was 15µm and length was 26 µm. The collenchyma layer has 4 rows, with an average thickness of 75 µm. The thickness of the cortex parenchyma varies between 112-143 µm. The sclerenchyma layer is 147 µm. Tracheas were measured as 23 µm and tracheitis as 12 µm (Fig. 2).

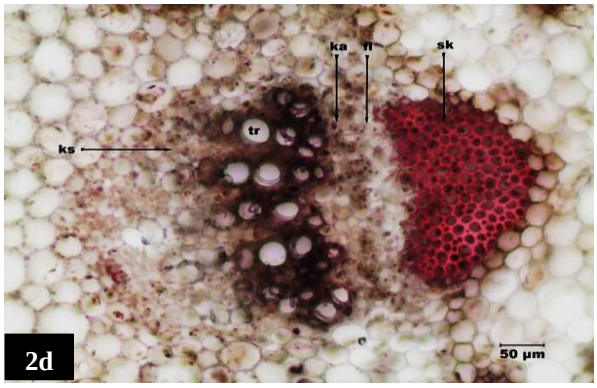
Stem Anatomy Results of SN: 10 Cultivar

The average width of epiderma cells was measured as 17 µm and length 26 µm in the stem cross sections of the control group plants that belong to SN:10 cultivar. The collenchyma layer is 3-4 rows. The thickness of the collenchyma layer was measured as 50-90 µm. The layer thickness of the cortex parenchyma is 100 µm. The sclerenchyma layer is 176 µm thick. Tracheas are 35 µm and tracheitis are 16 µm. In the experimental group where 1 dose of herbicide was applied, the average width of epiderma cells was measured as 18µm and the length was 29 µm. The collenchyma layer has 2-3 rows and the average thickness of the layer is 65 µm. The thickness of the cortex parenchyma layer varies between 67 and 115 µm. The sclerenchyma layer is 107 µm thick. The tracheas are 45 µm and tracheids are 21 µm.









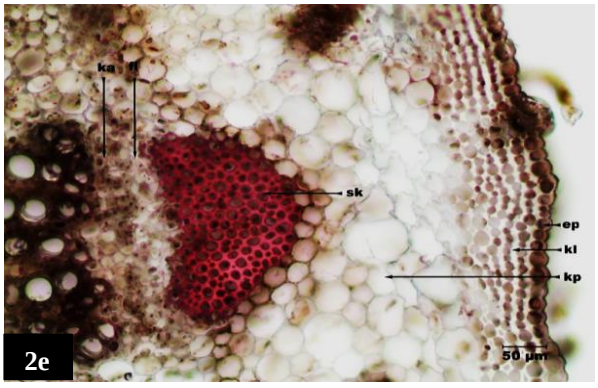


Figure 2. Stem cross sections of SN:9 cultivar. a, b- control, c- 1 dose, d, e- 2 doses, f- 3 doses. ep: epidermis, kl: collenchyma, kp: cortex parenchyma, ka: cambium, fl: phloem, sk: sclerenchyma, ka: cambium, ks: xylem, tr: trachea.

In the experimental group where 2 doses of herbicides were applied, the average width of the epiderma cells was 12 µm and the length was 18 µm. Collenchyma layer consists of 2-3 rows. The thickness of the collenchyma layer is 40-62 µm. The cortex parenchyma is approximately 80 µm thick. The sclerenchyma layer was measured as 180 µm. The tracheas are 42 µm and the tracheids are 26 µm. When the stem cross-sections of plants treated with 3 doses of herbicide were examined, the average width of the epiderma cells was 17 µm and the length was 24 µm. The cell walls and borders of the collenchyma cells are not clear. The thickness of the collenchyma layer is 55-82 µm. The thickness of the cortex parenchyma varies between 38-102 µm. The thickness of the sclerenchyma layer was measured as 172 µm. Tracheas are 45 µm and tracheids are 18 µm (Fig. 3).

Stem Anatomy Results of SN: 14 Cultivar

When the stem cross sections of control plants belonging to SN: 14 were examined, average width of epiderma cells was measured as 21µm and length was 27 µm. Kollenkima layer consists of 3-4 rows. The thickness of the collenchyma layer is 48-67 µm. The thickness of the cortex parenchyma layer varies between 45-171 µm. The sclerenchyma layer is 130 µm. The tracheas were measured as 25 µm and the tracheitis as 12 µm. In the experimental group of SN:14 cultivar in which 1 dose of herbicide was applied, average width of epiderma cells was measured as 22µm and length was 27 µm. The collenchyma layer is 3-4 rows and the layer thickness is 55-80 µm. The thickness of the cortex parenchyma varies between 125-235 µm. The sclerenchyma layer is 122 µm thick. The tracheas are 39 µm and the tracheids are 23 µm. In the stem cross sections of the experimental group plants with 2 doses of herbicide application, average width of epiderma cells was measured as 15µm and length was 26µm. The thickness of the collenchyma layer is 50-90 µm and the cell lines are not clear. The thickness of the cortex parenchyma varies between 125-235 µm. The sclerenchyma layer is 107 µm thick. The tracheas are 37 µm and the tracheids are 19 µm. In the experimental group where 3 doses of herbicides were applied, the width of epiderma cells was measured as 16 µm and length was 21 µm. The collenchyma

layer is 4-5 rows and 50-90 µm thick. The thickness of the cortex parenchyma varies between 150-180 µm. The sclerenchyma layer is 145 µm. Tracheas are 33 µm and tracheids are 14 µm (Fig. 4).

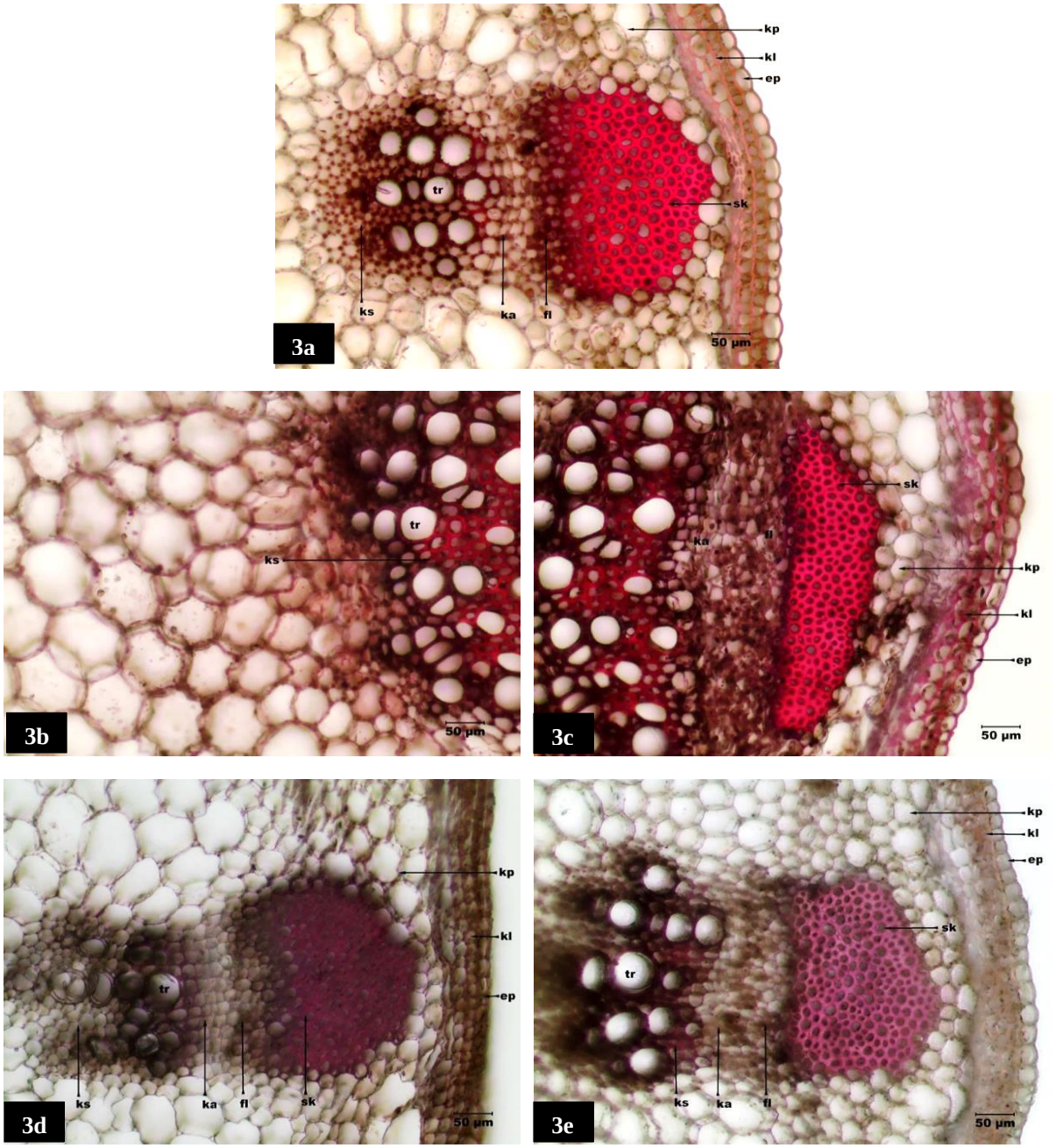


Figure 3. Stem cross sections of SN:10 cultivar. a-control, b, c-1 dose, d-2 doses, e-3 doses. ep: epidermis, kl: collenchyma, kp: cortex parenchyma, ka: cambium, fl: phloem, sk: sclerenchyma, ka: cambium, ks: xylem, tr: trachea.

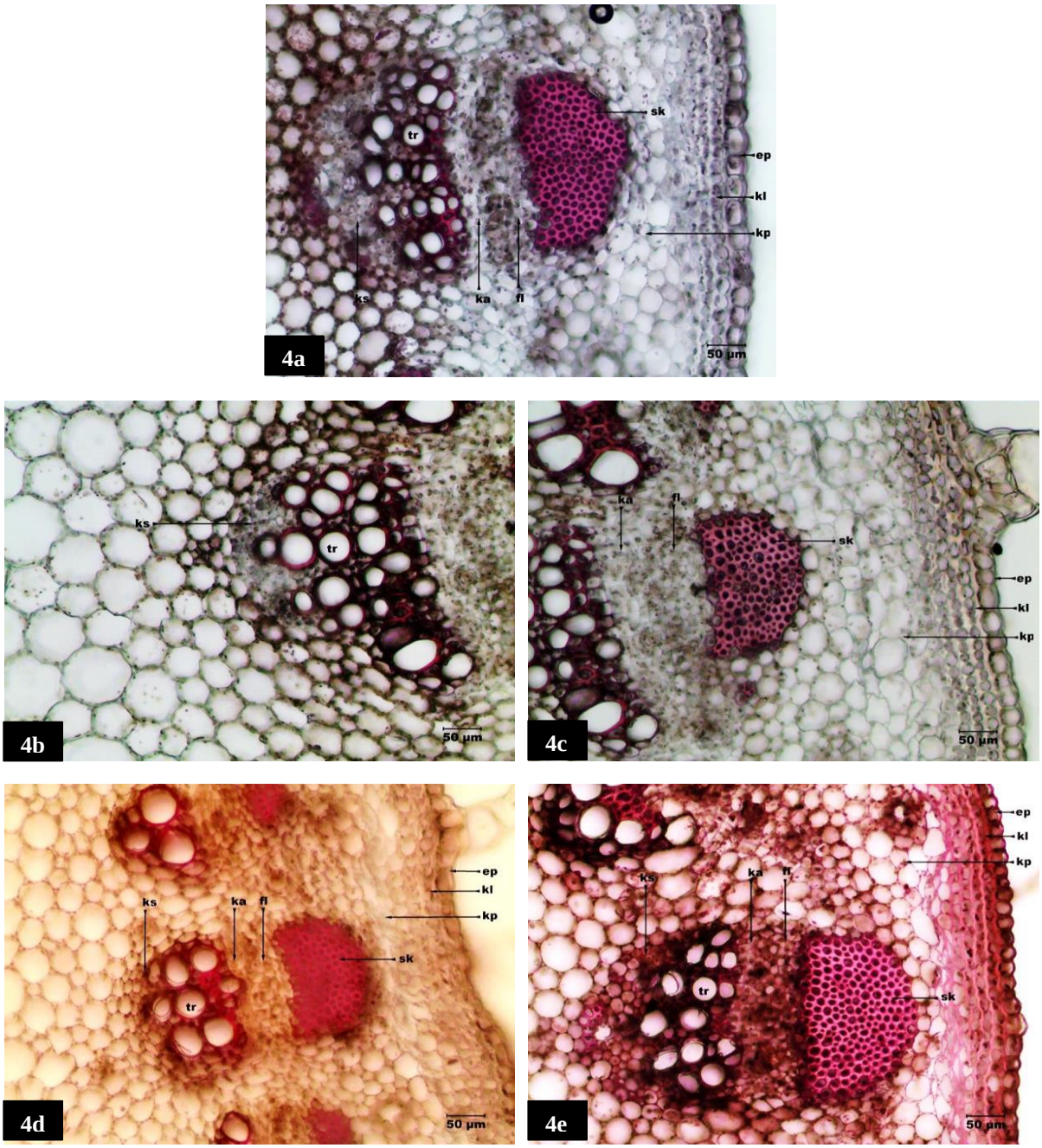


Figure 4. Stem cross sections of SN:14 cultivar. a-control, b, c-1 dose, d-2 doses, e-3 doses. ep: epidermis, kl: collenchyma, kp: cortex parenchyma, ka: cambium, fl: phloem, sk: sclerenchyma, ka: cambium, ks: xylem, en: trachea.

When the results of stem anatomy of SN: 8, SN: 9, SN: 10 and SN: 14 were evaluated, decreases in epiderma cell sizes, increases and decreases in collenchyma layer thickness and cell lines, decreases in cortex parenchyma layer thicknesses, significant increases and decreases in sclerenchyma layer thicknesses and trachea cells between groups and doses were observed due to increase of dosage. According to other studies, it is stated that herbicides, insecticides and fungicidal pesticides used in plant production cause anatomical changes in plants (Tort et al., 2004; Aksoy et al., 2013; Kaya and Ozturk Calı, 2015). Our study has parallels with these studies. According to these data, it was concluded that the anatomical changes caused by the effects of herbicide dose changes can be used to determine resistant cultivars.

Conclusion

As a result of the study, it was observed that increasing dose applications of imazamox herbicide used against weed control in sunflower agriculture caused various adversities in sunflower stem anatomical structure. Increases and decreases in cell layer sizes in stem cross sections of herbicide treated groups were determined when compared to control group. These changes in cell layer values prominently show up especially in groups in which 3 doses of imazamox were applied. Especially in SN: 8 and SN: 9 sunflower cultivars, 3 dose herbicide application, a decrease in trachea and tracheid sizes and an increase in SN: 10 and SN: 14 groups were detected. It is thought that these changes in the trachea and tracheid which are the transmission tissue elements, will adversely affect the transmission of water and nutrients, that is one of the metabolic activities of the plant. In the light of these findings, it was concluded that during plant growth and production, the increase in the use of herbicides by the producers would not cause problems up to 2 doses and problems may occur in the use of 3 doses or more.

Additional Declaration

Research and publication ethics principles were comply with in this study. Authors contributed equally to the study.

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