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Phytotoxic effects of herbicide Attribut and surfactant BioPower on the root, stem, and leaf anatomy of *Triticum aestivum* 'Pehlivan'

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Abstract: This study was performed in order to determine toxic effects of different doses of Attribut (propoxycarbazone-sodium) used in wheat fields and BioPower (sodium alkyl ether sulphate) surfactant added to Attribut on *Triticum aestivum* L. 'Pehlivan'. The doses used in the study were 0.42 mM Attribut, 0.82 mM Attribut, and 1.68 mM Attribut with or without 0.25% BioPower. Laboratory studies showed that the effective concentration value corresponded to the farmer dose, 0.42 mM Attribut. Applications were done by seed treatment. The effects of each of the doses applied to wheat plants were monitored in terms of anatomical parameters. Attribut doses of 0.82 mM and above had toxic effects on wheat plants, and these effects increased with the use of BioPower. As a response to the stress, the diameter in the general structure of the roots narrowed, the cuticle thickened, cleavages in parenchyma cells developed, and cell walls in the sclerenchyma cells and endodermis thickened.

Key words: Anatomy, phytotoxic effect, propoxycarbazone-sodium, sodium alkyl ether sulphate, wheat

1. Introduction

Pesticides are one class of compounds that, despite their benefits, may produce a wide range of toxic side effects that pose a potential hazard to the environment. As reliance on pesticides increases, human health problems related to pesticides become more intense. There is continuing and extensive research into the effects of herbicide use on the environment, including changes in native plant communities (Nemes-Kosa & Cserhati, 1995; Spawn et al., 1997) and their impact on human health, including cancer, reproductive effects, genetic diseases, and aging (Carbonell et al., 1995; Bain & LeBlanc, 1996; Ribas et al., 1997). In recent years the toxic effects of boron, niacin, and cadmium have become very popular subjects in the area of toxicity in plant materials (Demiray & Eşiz Dereboylu, 2013; Tran & Popova, 2013).

Attribut (propoxycarbazone-sodium) is an herbicide developed for postemergence grass control in cereals. It is triazolone herbicide, and it works by inhibiting acetolactate synthase (ALS). Sulfonylurea herbicides kill weeds by inhibiting the enzyme ALS, which is essential for their growth. They work on a broad range of grasses and broadleaf weeds. The ALS isoenzymes are responsible for the biosynthesis of not only L-isoleucine, but also all 3 branched-chain amino acids (L-valine, L-leucine, and L-isoleucine), thus making Attribut a protein synthesis inhibitor (Babczinski, 2002). Attribut is used for rye, triticale, and winter wheat. It is also effective on grass weeds like black grass, brome, wind grass, quack grass (couch), and wild oats. Surfactants are used to improve the effectiveness of foliar-applied herbicides, other pesticides, and defoliants by reducing the surface tension of aqueous systems. However, there is evidence that surfactants are capable of exerting inhibitory as well as stimulatory effects upon plant growth and behaviour (Parr & Norman, 1965). In this study, BioPower (sodium alkyl ether sulphate), an anionic surfactant, was used to increase the effects of Attribut (propoxycarbazone-sodium) on wheat leaf surfaces. Some of the phytotoxic and inhibitory effects of different surfactants in plant systems are as follows. Vatsol OT repressed growth of sorghum leaves (Foy, 1961) and Tween-20, Tween-80, and Surfax 505 increased the growth of leaves and roots in barley (Parr & Norman, 1964). Responses produced by plants against external stress factors include wall thickening, increase in hair, and alterations in cleavages (cell division) (Yüce et al., 1998). Many studies have evidenced that wall thickening occurs as a resistance mechanism under stress conditions caused by interaction with chemicals at higher doses (Aktaç et al., 2007). The walls of all plant cells contain a matrix composed of noncellulose polysaccharides and cellulose microfibrils embedded into the matrix. Furthermore, phenolic

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compounds containing structural proteins, glycoprotein, and lignin are added into the structure of the wall at the matrix. In particular, the polysaccharide compounds in the matrix create a difference in the cell walls. In monocots, the lignified secondary wall includes heteroxylans containing glucuronoarabinoxylans and glucomannans in small amounts, while dicot plants include heteroxylans containing 4-O-methyl-glucuronoxylans in high amounts and glucomannans in small amounts. Harris and Smith (2006) studied the structure of polysaccharides in detail relating to the plant cell wall. Pectin was observed in the primary wall in dicot plants, while arabinoxylans and (1*3),(1*4)-b-glucans were seen in cereals included in the family Poaceae from monocots. According to Dixon and Lamb (1990), hypersensitive response, the most sensitive response to stress and a plant defence mechanism, involves biochemical processes such as ethylene and phytoalexin synthesis in the plant; wall thickening due to callose, lignin, and relevant compound deposits; and deposition of hydroxyproline-rich glycoprotein on the cell wall. In plant defence against pathogens, there are 3 groups of phenolic compounds: lignin and relevant polyphenolics, phenylpropanoids (phenolics and phytoalexins), and salicylic acid (Kuc, 1995; Wu et al., 1997; Wendehenne et al., 1998). Considering the studies that include the effects of pesticides on plant anatomic nature, it is apparent that such studies are few in number. In the conducted studies, it was seen that pesticides cause certain alterations in plant anatomic nature that lead to structural differences.

The aim of this study was to investigate the phytotoxic effects of the anionic surfactant BioPower and Attribut herbicide on the morphology and anatomy of *Triticum aestivum* L. 'Pehlivan' under laboratory and field conditions.

2. Materials and methods

Germinated seeds, roots, seedlings, and leaves of wheat (*Triticum aestivum* L. 'Pehlivan') taken from the Trakya Agricultural Research Institute were used as the study material. Attribut (propoxycarbazone-sodium) was used as the herbicide and BioPower (sodium alkyl ether sulphate) as the surfactant. One hundred wheat germs from each group were treated with stock aqueous solutions of 4 different propoxycarbazone sodium doses (0.21 mM, 0.42 mM, 0.84 mM, and 1.68 mM) prepared with modified Hoagland medium for each test to determine the half-maximal effective concentration (EC_{50}) values in laboratory studies (Ouzounidou et al., 1997). The average EC value was close to the value of 0.42 mM Attribut, which ranges between 0.21 mM and 0.84 mM, and this value corresponds to the farmer dose (FD) or 2A. In the Table, symbols 1A to 4A are given for the treatments without BioPower and symbols 1B to 4B are given for treatments with BioPower.

The tests were performed as a seed treatment under laboratory and field conditions. Forty seeds were planted in different pots (20 cm in diameter) and filled with soil of the same characteristics (pH: 5.5–6.0, NO_3-N: 40–50 ppm, P: 15–20 ppm, K: 70–100 ppm, Ca: 40–60 ppm, and Mg: 20–25 ppm) in both applications. The control group was placed in Hoagland medium. Doses of 0.21 mM, 0.42 mM, 0.84 mM, and 1.68 mM Attribut and the same doses including 0.25% BioPower surfactant were applied to treated seeds for 15 h before planting in pots. Test cultivations were conducted in fields owned by the Trakya Agricultural Research Institute in 2 subsequent years at 2 different locations. Doses of 0.21 mM, 0.42 mM, 0.84 mM, and 1.68 mM Attribut and the same doses including 0.25% BioPower surfactant were applied to 50 g of wheat seeds, before they were planted, for 15 h to observe the effects of BioPower.

One parcel was allocated for the nontreated control group. Roots, stems, and leaves were placed into 70% alcohol for anatomical examinations. Cross-sections of roots, stems, and leaves from wheat grown under field conditions were dyed with a mixture of 1% safranin-Alcian blue 8GX at a ratio of 4:6 (Davis & Barnett, 1997). The sections were placed in a drop of dye and left for 5 min. An Olympus photomicroscope was used to photograph the sections.

3. Results

Cross-sections of roots, stems, and leaves originating from seeds treated before cultivation with the doses given in the Table were examined. Safranin dyes the lignin in cell walls to red and Alcian blue dyes cellulosed walls to blue, thus enabling identification of parenchyma and sclerenchyma cells. Therefore, red zones in photos correspond to areas dyed by safranin; blue-green, dark, or light blue zones correspond to areas dyed by Alcian blue 8GX dye. Alcian blue dyes both sulphated and carboxylated mucopolysaccharides and sulphated and carboxylated

Table. Doses used in the study.

Control	
1A - FD/2 (0.21 mM Attribut)	1B - FDS/2 (0.21 mM Attribut + 0.25% BioPower)
2A - FD (0.42 mM Attribut)	2B - FDS (0.42 mM Attribut + 0.25% BioPower)
3A - FD × 2 (0.84 mM Attribut)	3B - FDS × 2 (0.84 mM Attribut + 0.25% BioPower)
4A - FD × 4 (1.68 mM Attribut)	4B - FDS × 4 (1.68 mM Attribut + 0.25% BioPower)

sialomucins (glycoprotein). Examining the cross-sections of roots of the control group from outside to inside, the cortex site composed of the cuticle and a single layer of epidermis was the outermost layer, followed by a single layer of parenchyma, 3–4 layers of sclerenchyma, 4–5 layers of parenchyma, and the central cylindrical site (stele). A single layer of endodermis was located at the outermost part of the central cylinder, followed by 13–14 xylem arcs with phloem cells among them and 6–7 tracheas toward the centre. Sclerenchyma takes place in the internal part. The comparison of 2A and 2B with the control group showed that root cross-sections were similar to each other. It was observed comparing 3A and 3B with the control group that the root cross-sections and central cylinder diameters become narrower. The trachea number was 4–5 in 3B, while it was 6 in the control group (Figure 1). The

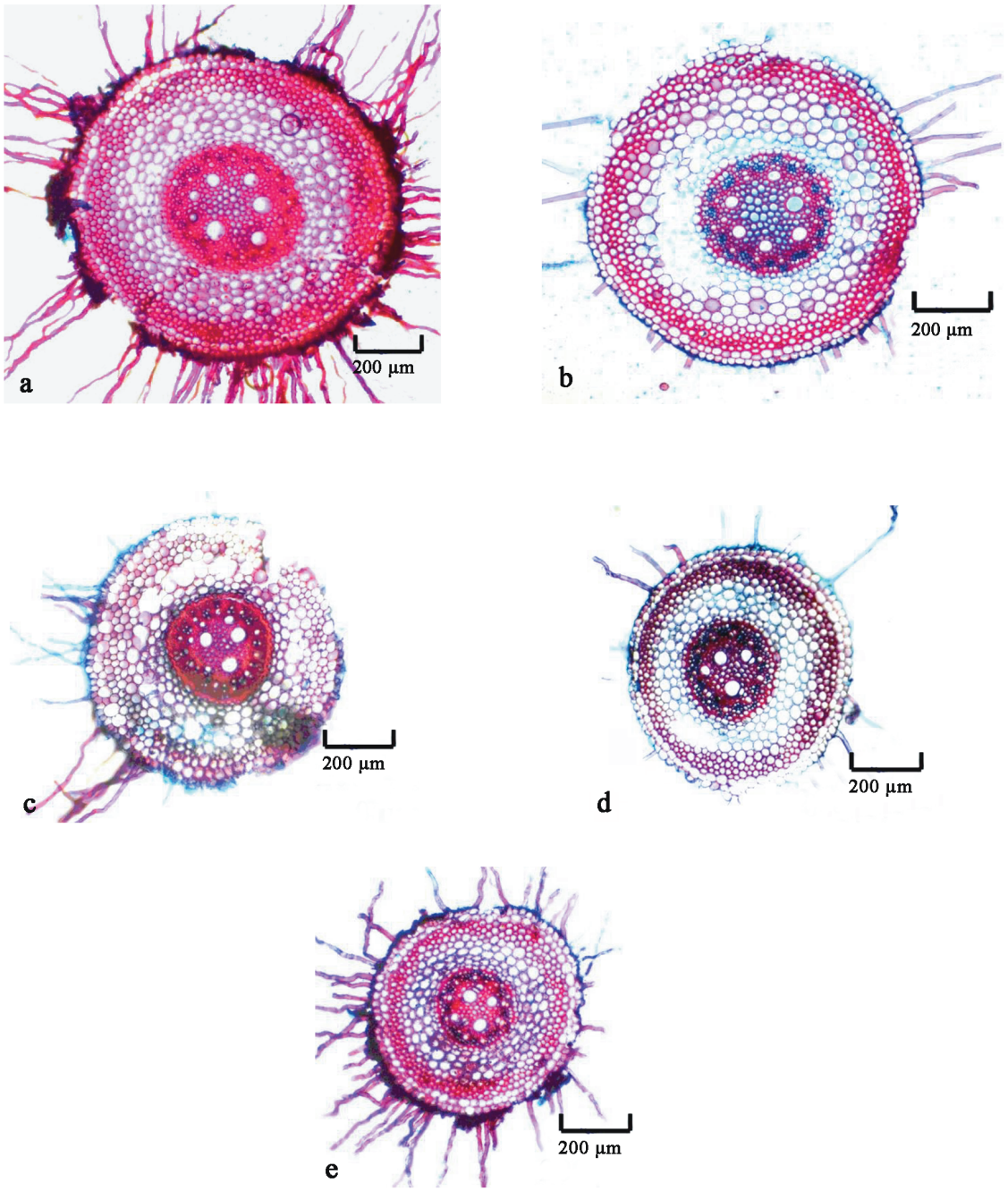

Figure 1. Root cross-sections stained with Alcian blue and safranin dye after seed treatment. a- control, b- 3A (0.84 mM Attribut), c- 3B (0.84 mM Attribut + 0.25% BioPower), d- 4A (1.68 mM Attribut), e- 4B (1.68 mM Attribut + 0.25% BioPower). Scale bar = 200 µM.

cortex examinations revealed that sclerenchyma cell wall thicknesses under the epidermis and the lignin amount were reduced in 3B (Figures 2A and 2B). Disruption was observed in cortex parenchyma cells in 3A and 3B (Figures 2C and 2D). Cell walls of the endodermis were thicker in 3B due to the increase in lignin amount, compared with 3A (Figures 2E and 2F). Toxic effects occurred in 4A and 4B and mostly appeared as a narrowing diameter and a decreased number of xylem arcs as compared with the other doses. Fourteen xylem arcs and 6 tracheas were seen in the central cylinder in the control, while these numbers in 4A and 4B were reduced to 9 and 4, respectively (Figure 1). According to the Alcian blue dying, the cuticle layer in 4B was thicker than in 4A. In both of these treatments, according to the safranin dying, sclerenchyma cell wall thicknesses under the epidermis were lower, and lignin

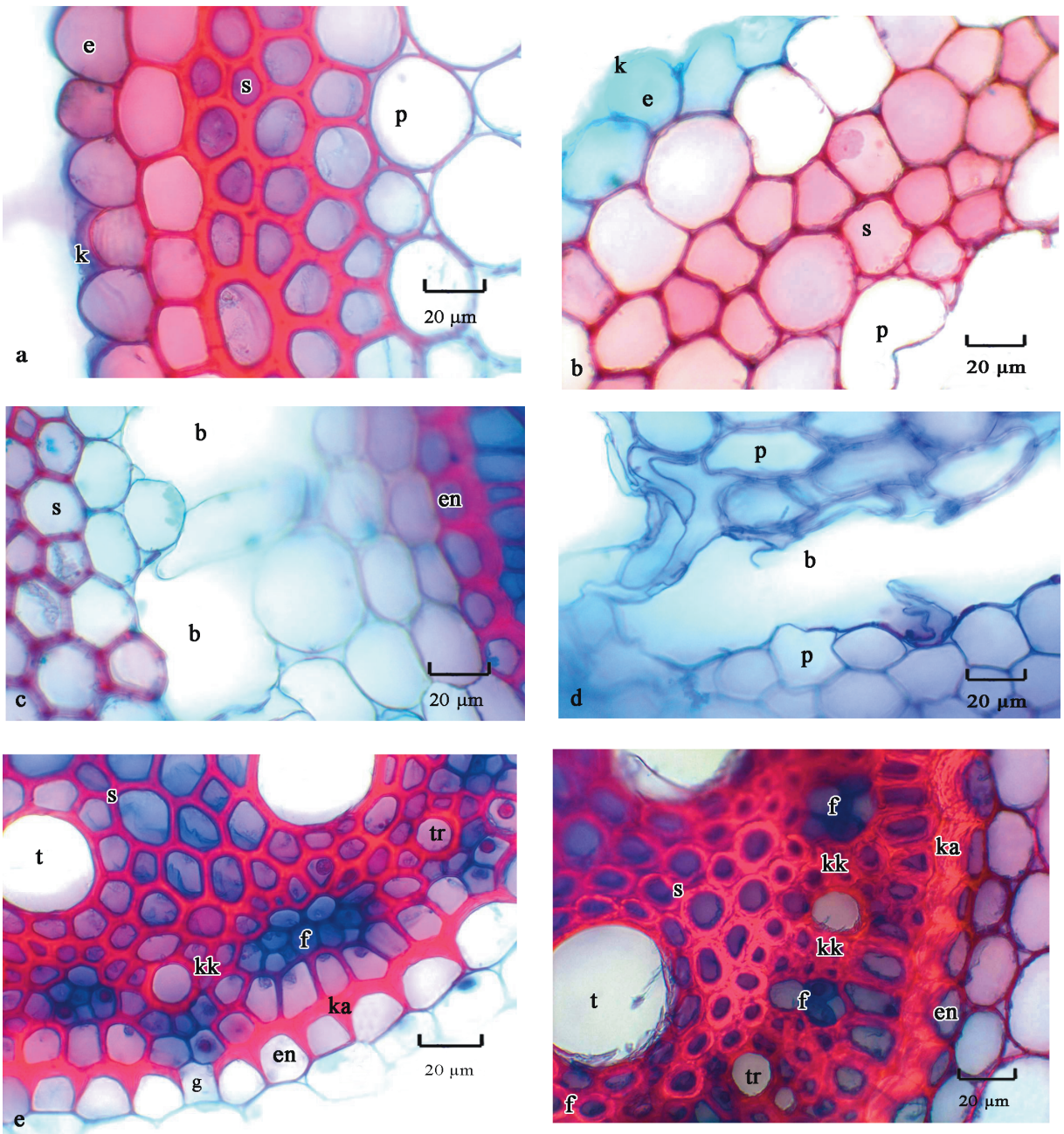

Figure 2. Root cross-sections stained with Alcian blue and safranin dye after seed treatment: a, c, e- 3A (0.84 mM Attribut); b, d, f- 3B (0.84 mM Attribut + 0.25% BioPower). k: cuticula, e: epidermis, s: sclerenchyma, p: parenchyma, b: emptiness, ka: thickness of cell wall in endodermis, en: endodermis, kk: xylem arc, f: phloem, g: cell for water transport, t: trachea, tr: tracheid. Scale bar = 20 µm.

content in these walls was reduced compared with the control. Examining stem sections of control material from the outside towards the inside, the cuticle at the outermost layer was followed by a single layer of epidermis, 4–5 layers of parenchyma, 4–7 layers of sclerenchyma, and quite a high number of cortex parenchyma cells. Vascular bundles showed an untidy layout, small at the circle and growing up toward the centre. Stem sections were similar to each other in 2A and 2B when compared with the control group. In stem cross-sections of 3A and 3B, the lignin amount was very low in cell walls in the area, while there were 4–7 layers of sclerenchyma in control. Because safranin normally dyes the walls, including lignin, to red, it was concluded that the lignin content decreased because dyeing was not observed in the walls of 3A and 3B (Figure 3). In 3A, 3B, 4A, and 4B, lignin was not contained by cell walls under the epidermis, while in the control group there were sclerenchyma cell walls containing lignin under the epidermis.

In 3B and in the control, the area housing sclerenchyma cells turned slightly pink under safranin staining. Because the morphology of the stem structure was already significantly altered in 4A and 4B, sclerenchyma cells under the epidermis were not observed. In the case of the control, 2A, and 2B, the cell walls of the sclerenchyma surrounding the xylem and phloem turned red under safranin because they contained lignin, while in 3A and 3B they turned blue under Alcian blue because they did not contain lignin (Figure 3). Conducting tissues containing xylem and phloem were similar to each other in stem cross-sections in the control, 2A, and 2B. In 4A and 4B, growth of the xylem and phloem was reduced. In 4B cuticle thickness increased compared with 4A. In cross-sections of leaves belonging to the control, 8–9 layered chlorenchyma cells were observed. Main vessels were surrounded by 7 layers of sclerenchyma cells and, on the other side, 4–5 layers of sclerenchyma cells and 2–3 layers of parenchyma cells. The leaf cross-sections were similar to each other in the control, 2A, and 2B. In 3A and 3B, leaf width became narrower compared with the control. This narrowing became visible because the chlorenchyma layer was composed of 8–9 layers in the control, while it was composed of 5–6 layers in 3A and 3B. An increase in sclerenchyma cells surrounding the main vessel was observed in 3B compared with 2A. When Attribut 4B and 4A applications with and without BioPower surfactant additive were compared with the control group, leaf width narrowed, main vessels became smaller, and the number of sclerenchyma and parenchyma cells surrounding the main vessels decreased depending on the increase in dose, compared with the control.

4. Discussion

There are studies on the anatomic toxicity of pesticides conducted by Çalı (2007), Öztürk and Tort (2004), and Öztürk et al. (2006). Cireli and Önür (1983) reported that leaf epidermis cell areas in *Vicia faba* L. treated with Stomp 330 E herbicide decreased, and length of leaflets was reduced. In the present study, cell contents in chlorenchyma decreased in leaves in 4A and 4B. Disruptions were also seen in leaf cross-sections collected from both of the groups to which Akrobat and Sadofan were applied (Tort et al., 2004). Öztürk et al. (2006) reported that the assimilator palisade parenchyma layer covered a smaller area in stem cross-sections taken from tomato plants treated with Metalaxyl at different doses compared with the control, and distortions occurred in the cells building this layer depending on the increase in dose. Similar to the aforementioned studies, according to the examination of the root cross-sections, disruption was observed in parenchyma cells in 3A and 3B in our study (Figure 3). Tort et al. (2004) applied Akrobat and Sandofan fungicides to tomato plants at the dose recommended on their labels and reported that assimilator palisade parenchyma and xylem cells decreased. In our study, as in previous studies, the number of vascular bundles in the roots decreased in 4A and 4B compared with the control (Figure 1). Furthermore, it was observed that in 3A and 3B the structure of vascular bundles significantly changed in the stem compared with the control (Figure 3). In 4A and 4B, anatomic nature in the stem emerged in the form of overlapping leaves. It was seen in the examination of sclerenchyma cell walls under the epidermis in the cortex that the walls became thinner, and lignin content in walls decreased at the same doses, compared with the control (Figures 2A and 2B). In 3B leaf width became thicker in leaf cross-sections. Hossain et al. (2006) observed that pectin and hemicelluloses in roots increase under Al stress; this stress causes hemicellulosic ruptures, and this increase in hemicelluloses triggers the increase in glucose, xylose, and arabinose in neutral sugars. According to these data, Al stress in the cell wall can modify the mechanical properties of polysaccharides in the cell wall by promoting arabinoxylan, beta-glucan, and ferulic acid synthesis. In a study conducted on *Pinus radiata* D.Don, Schmitt et al. (2006) observed that an increase occurred in lignin depending on stress in tracheids, and the galactan covered a larger area in the S_2 region of the cell wall covers. Xue et al. (2008) found in their study that apoplastic peroxidase synthesis increases, H_2O_2 forms, and lignin synthesis increases depending on the signal systems of the nitric oxide and methyl jasmonic acid brought under Al stress. In recent years, Lemcoff et al. (2002) suggested that an increase in cell-wall elasticity, which is largely established by thick and stiff cell walls in drought roots of *Eucalyptus*

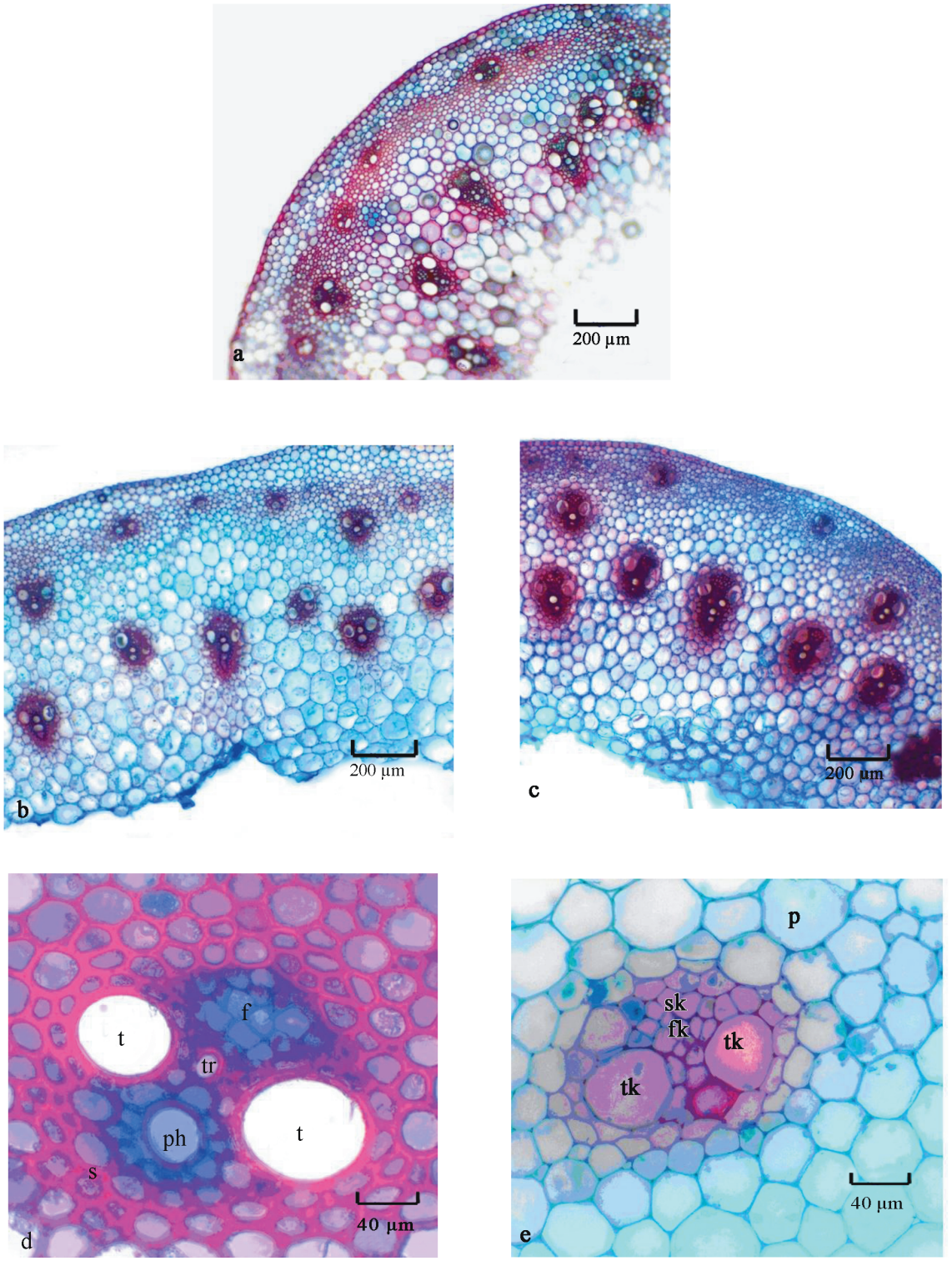

Figure 3. Stem cross-sections stained with Alcian blue and safranin dye after seed treatment. a- control, b- 3A (0.84 mM Attribut), c- 3B (0.84 mM Attribut + 0.25% BioPower). Scale bar = 200 µm. d- control with vascular bundles, e- 3A (0.84 mM Attribut) with vascular bundles. p: parenchyma, s: sclerenchyma, sk: the place where sclerenchyma will be in control, t: trachea, tk: the place where trachea will be in control, f: phloem, fk: the place where phloem will be in control. Scale bar = 40 µm.

camaldulensis Dehnh., is involved in plant drought resistance by maintaining water uptake. Jones et al. (2006) reported that callose production in the roots of *Zea mays* L. increases in response to Al stress. Vollenweider et al. (2006) determined in their study that the cell walls thickened in response to Cd. Aktaç et al. (2007) observed thickening on the cell walls of *Allium cepa* L. root treated by Raxil pesticide as a response to the stress caused by pesticides at higher concentrations.

Results similar to those of previous studies were obtained in our study. According to the examinations of cross-sections of wheat roots, stems, and leaves, an increase in lignin was observed at the endodermis and sclerenchyma walls in the central cylinder in the root in 3B compared with the control (Figures 2E and 2F). A thickening in the cuticle was observed in the root in 4B, compared with the control. In the leaves of 3A and 3B, an increase was observed in sclerenchyma cells compared with the control.

Our study emphasises once again that the awareness of farmers should be raised regarding damage from chemicals, especially those that are deposited in the soil. The collection of pesticides in the soil may occur over time because these chemicals are commonly used to prevent diseases emerging in the field. Farmers should be trained to apply these chemicals properly. In this study, FD × 2 (3A), FDS × 2 (3B), FD × 4 (4A), and FDS × 4 (4B) applications negatively affected wheat morphological and anatomical nature compared with the control, taking into account Attribut deposition in the soil. Evaluating the results from another point of view, Attribut applications including BioPower surfactant additive increased the effects of Attribut on wheat. Thus, we can add a surfactant to an herbicide to increase the herbicide's effect and use the herbicide in smaller amounts. As a result, our staple crop, wheat, will be less contaminated by herbicides, and human health will be preserved. We believe that molecular and cytogenetic studies should be conducted in the future to examine cell structure in detail and learn how Attribut herbicide applications affect cell metabolism in plants.

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