

ORIGINAL ARTICLE

## Effect of plant hormones on the cambial activity of *Cerasus vulgaris* Miller under stress conditions with Zn

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### Abstract

In the present study, the effects of 10, 20, 30 ppm hormone mixtures (indole-3-acetic acid + gibberellic acid + kinetin) with 0.1, 0.3, 0.5 and 1 ppm zinc (Zn) concentrations alone and their mixtures on the cambial activity of sour cherry (*Cerasus vulgaris* Miller) cuttings were investigated. Morphological and anatomical developments of the plants were observed. The leaves of the plants treated with zinc were found to be greener than the control. Plants treated with zinc faded earlier than the control. The cambial zone thickness, the cambial zone cell line, the radial and tangential lengths of the cambial zone cells decreased with increasing concentrations of zinc and increased with increasing concentrations of hormones. The radial and tangential wall widths of the cambial zone cells increased with increasing zinc concentrations and decreased with increasing hormone concentrations. As a result, in the 0.1, 0.3, 0.5 and 1 ppm Zn concentrations, the cambial zone thickness decreased by 10, 28, 50 and 65%, respectively, compared to the control. Thirty ppm hormone mixture – H.M. (indole-3-acetic acid + gibberellic acid + kinetin) increased the cambial zone thickness by 65, 15, 5% in 0.1, 0.3 and 0.5 Zn, respectively, compared to the control. It was found that plant hormones importantly improved the harmful effects of zinc on the cambial activity of the plant cuttings.

**Keywords:** *Cerasus vulgaris*, gibberellic acid, indole-3-acetic acid, kinetin, sour cherry, zinc

## Introduction

Zinc (Zn) is a trace element required for plant growth and development. Zinc is involved in structural and functional integrity of the cell membrane, protein metabolism, gene expression and photosynthetic carbon metabolism (Broadley *et al.* 2011). Zinc is especially required for the growth of young tissues. Zinc is deficient in 50% of the world's agricultural soils and is recognized as the world's most critical micronutrient deficiency in crops. Deficiency causes stunting and the formation of small leaves. In humans, Zn deficiency contributes to 800,000 child deaths annually (Williams 2015). Zinc also enables the plant to withstand conditions such as drought, salinity, cold and pathogenic infections that cause oxidative stresses (Cakmak 2000).

Zinc also enhances auxin synthesis in plants at specific concentrations (Li *et al.* 2013).

When zinc is in the root environment of plants at appropriate rates, it improves the growth of the plant. Several members of the Zn-regulated transporters and iron (Fe) regulated transporter-like protein (ZIP) gen have been characterized and shown to be involved in metal uptake and transport. OsZIP1, OsZIP3, OsZIP4, OsZIP5 and OsZIPH are reported to encode rice plasma membrane Zn transporters and are induced by Zn deficiency (Bashir *et al.* 2012). However zinc can have toxic effects on the plant, when this ratio increases because of the abrasive effect on the zinc-containing materials of mining and smelting processes, agricultural

fertilizers and sewage water (Aydemir and Ince 1988). This toxicity limit is 83, 170, 660 mg · kg<sup>-1</sup> in leaves, hulls and roots of the tea plant, respectively (Venkatesan *et al.* 2006).

Toxicity of zinc leads to a decrease in yield, root growth and leaf expansion in plants and spreads a reddish brown phenolic compound over the plant (Haktanir and Arcak 1998). Excessive zinc causes spiral leaf formation in the 4th week, leaf fold in the 6th week, and split shell in the 8th week in peanut plants (Davis and Parker 1993). Zinc toxicity leads to increased hydrophobicity of the cell membrane at the cellular level, prolongation of cell division, reduction of protein, RNA and fatty acid content, DNA denaturation and even death of the cell (Powell *et al.* 1986a; Powell *et al.* 1986b; Binder *et al.* 2001; Chang *et al.* 2005).

Kinetin enhances protein and RNA synthesis and encourages cell division (Kende and Zeevaart 1997). The regulator containing 35 mg · dm<sup>-3</sup> synthetic auxin, 35 mg · dm<sup>-3</sup> cytokinin and 1 mg · dm<sup>-3</sup> titanium has increased stomatal width and the length of the stomatal pore in the stomata of the upper epidermis of the leaf blade. The application positively affected the length and mass of shoots, the number of shoots, and also the mass of inflorescence and roots (Sosnowski 2018). Moreover, a mixture of auxin and cytokine triggered the highest nitrate reductase activity in alfalfa roots and raised the ratio of total chlorophyll content to carotenoids. Synthetic auxin caused the decrease of the levels of most parameters compared to the control (Sosnowski *et al.* 2019). Indole acetic acid (IAA) and/or kinetin treatments have positive effects on growth criteria (plant height, leaf numbers, fresh and dry weights per plant), photosynthetic pigments (chlorophyll a, b and carotenoids), total carbohydrate, polysaccharide, free amino acid, proline and total phenolic contents in the two cultivars (Osborne 1962; Sadak *et al.* 2013). Indole acetic acid does not activate cambium during dormancy, but it increases cell division in active cambium (Funada *et al.* 2002). Indole acetic acid is known to play a key role in cell division and elongation too (Rehman *et al.* 2012). The treatment of IAA and gibberellic acid (GA) enhances growth parameters and the accumulation of flavonoids and other phenolic compounds in buckwheat sprouts (Park *et al.* 2017). Gibberellic acid increases prolongation of the trunk by increasing division of the cell (Sachs *et al.* 1959). Gibberellic acid also increases the development of the trunk in plants with badge shape too (Kende and Zeevaart 1997).

As can be seen from the above information, zinc in high proportions decreases the growth and development of plants while indole acetic acid, gibberellic acid and kinetin increase it. Recovery of structure damage and low yields in plants, which is caused by heavy metals such as zinc, is becoming important. The rapidly growing world population is around 7.5 billion today

and it is getting harder to meet its nutritional needs. For this, it is necessary to find way of evaluating the available arable areas optimally and rehabilitating the toxic compounds in these areas.

When we look at it from this point of view, studies that determine which hormones decrease the toxicity of heavy metals (Fatima and Chaudry 2004) such as zinc, which reduces the growth and development of the plant (Deef 2008), are gaining importance. The aim of this study was to detect the effects of indole-3-acetic acid, gibberellic acid and kinetin mixtures on cambial activity in sour cherry (*Cerasus vulgaris* Miller) cuttings exposed to zinc stress. When we consider the increasing world population and pollution, it is clear that such studies are important.

## Materials and Methods

Sour cherry (*C. vulgaris* Miller) cuttings were used in our study. Sour cherry branches were obtained from the campus area of Yuzuncu Yil University. The results were evaluated according to variance analysis and Duncan test.

### Preparation of stock solutions

Stock solutions used in this study were prepared in the laboratory. The Zn applied to the plant was ZnCl<sub>2</sub> (Martha 1983). The atomic weight of ZnCl<sub>2</sub> is 136.28 g · mol<sup>-1</sup>. The atomic weight of Zn is 65.3 g · mol<sup>-1</sup>. When 208.7 mg ZnCl<sub>2</sub> is taken, there are 100 mg Zn in the solution. We added water to 208.7 mg ZnCl<sub>2</sub>, until the total volume was 4 liters. Four liters 25 ppm stock solution was prepared.

Forty ml of 25 ppm Zn stock solution was taken. It contained 1 mg Zn. This was taken and 960 ml pure water was added, and 1 liter 1 ppm Zn stock solution was prepared.

When 20 ml of the 25 ppm Zn stock solution was taken, there was 0.5 mg Zn in the solution. When 980 ml pure water was added to this solution, it made 1 liter 0.5 ppm Zn solution. When 12 ml of 25 ppm stock Zn solution was taken, it contained 0.3 mg Zn. This was taken and 988 ml pure water was added. Consequently 1 liter 0.3 ppm Zn solution was prepared.

When 4 ml of 25 ppm solution was taken, it contained 0.1 mg Zn. When this was taken and 996 ml purified water was added, it made 1 liter Zn solution.

One hundred mg IAA was taken and dissolved with 95% ethyl alcohol to obtain a total volume of 1.5 liters. Bidistilled water was added. One hundred mg of GA was taken and dissolved in 95% ethyl alcohol. To obtain a total volume of 1.5 liters, it was completed with pure water. One hundred mg of kinetin

was taken and dissolved with a few drops of HCl. To obtain a total volume of 1.5 liters, it was completed with pure water.

One and a half liters IAA, gibberellic acid and kine-tin were mixed in a container. A half liter of bidistilled water was added to this container. Total volume was 5 liters. Thus, 5 liters 60 ppm stock hormone mixture solution was prepared (Martha 1983).

A hormone mixture (H.M.) (166.6 ml of 60 ppm) solution was taken. There were 10 mg H.M. in this. This was completed with bidistilled water, until there was a total volume of 1 liter. Thus, 1 liter 10 ppm H.M. solution was prepared. From 60 ppm solution 333.3 ml were taken. There were 20 mg H.M. in this. This was completed with bidistilled water, until the total volume was 1 liter. As a result, 1 liter 20 ppm H.M. solution was prepared. Of 60 ppm H.M., 500 ml solution was taken. There were 30 mg H.M. in this. This was completed with bidistilled water, until the total volume was 1 liter. Thus, 1 liter 30 ppm H.M. solution was prepared. From 25 ppm zinc solution 40 ml and 166.6 ml from 60 ppm hormone mixture solution were taken and this was completed with bidistilled water, until a total of 1 liter and 1 liter 1 ppm Zn + 10 ppm H.M. solution consequently was prepared.

Forty ml of 25 ppm zinc solution and 333.3 ml of 60 ppm hormone mixture solution were mixed with bidistilled water, until total volume was 1 liter. Lastly 1 liter 1 ppm Zn + 20 ppm H.M. solution was prepared. Forty ml of 25 ppm zinc solution and 500 ml of 60 ppm hormone mixture solution were mixed with bidistilled water to arrive at 1 liter. As a result, 1 liter 1 ppm Zn + 30 ppm H.M. solution was prepared. Twenty ml of 25 ppm zinc solution and 166.6 ml of 60 ppm hormone mixture solution were mixed with bidistilled water to give 1 liter total volume. Thus, 1 liter of 0.5 ppm Zn + 10 ppm H.M. solution was prepared. Twenty ml from 25 ppm zinc solution and 333.3 ml from 60 ppm H.M. solution were taken and bidistilled water was added to this, until the total volume was 1 liter. One liter of 0.5 ppm Zn + 20 ppm H.M. solution, therefore was prepared. Twenty ml from the 25 ppm zinc solution and 500 ml from 60 ppm H.M. were taken and bidistilled water was added to prepare 1 liter total volume. Lastly, 1 liter of 0.5 ppm Zn + 30 ppm H.M. solution was prepared. Twelve ml of 25 ppm zinc solution and 166.6 ml of 60 ppm H.M. solution were mixed with bidistilled water to give 1 liter of total volume. As a result, 1 liter 0.3 ppm Zn + 10 ppm H.M. solution was prepared. Twelve ml of 25 ppm zinc solution and 333.3 ml of 60 ppm H.M. solution were mixed with bidistilled water to give 1 liter total volume. Thus, 1 liter 0.3 ppm Zn + 20 ppm H.M. solution was prepared. Twelve ml of the 25 ppm zinc solution was taken and mixed with 500 ml of 60 ppm H.M., and was completed with bidistilled water to prepare 1 liter total volume.

One liter 0.3 ppm Zn + 30 ppm of H.M. solution, was prepared. Four ml of 25 ppm zinc solution and 166.6 ml of 60 ppm H.M. solution were mixed with pure water to give a 1 liter total volume. As a result, 1 liter 0.1 ppm Zn + 10 ppm H.M. solution was prepared. Four ml 25 ppm zinc solution and 333.3 ml 60 ppm H.M. solution were mixed with pure water, then pure water was added until total volume was 1 liter. Thus, 1 liter 0.1 ppm Zn + 20 ppm H.M. solution was prepared. Four ml of 25 ppm zinc solution and 500 ml of 60 ppm H.M. solution were mixed with pure water to give 1 liter total volume and consequently 1 liter 0.1 ppm Zn + 30 ppm H.M. solution was prepared.

### Grouping plants

There were 20 groups and each group contained five sour cherry cuttings.

- 1 – Control: bidistilled water
- 2 – 10 ppm H.M.
- 3 – 20 ppm H.M.
- 4 – 30 ppm H.M.
- 5 – 0.1 ppm Zn
- 6 – 0.1 ppm Zn + 10 ppm H.M.
- 7 – 0.1 ppm Zn + 20 ppm H.M.
- 8 – 0.1 ppm Zn + 30 ppm H.M.
- 9 – 0.3 ppm Zn
- 10 – 0.3 ppm Zn + 10 ppm H.M.
- 11 – 0.3 ppm Zn + 20 ppm H.M.
- 12 – 0.3 ppm Zn + 30 ppm H.M.
- 13 – 0.5 ppm Zn
- 14 – 0.5 ppm Zn + 10 ppm H.M.
- 15 – 0.5 ppm Zn + 20 ppm H.M.
- 16 – 0.5 ppm Zn + 30 ppm H.M.
- 17 – 1 ppm Zn
- 18 – 1 ppm Zn + 10 ppm H.M.
- 19 – 1 ppm Zn + 20 ppm H.M.
- 20 – 1 ppm Zn + 30 ppm H.M.

### Plant growing method

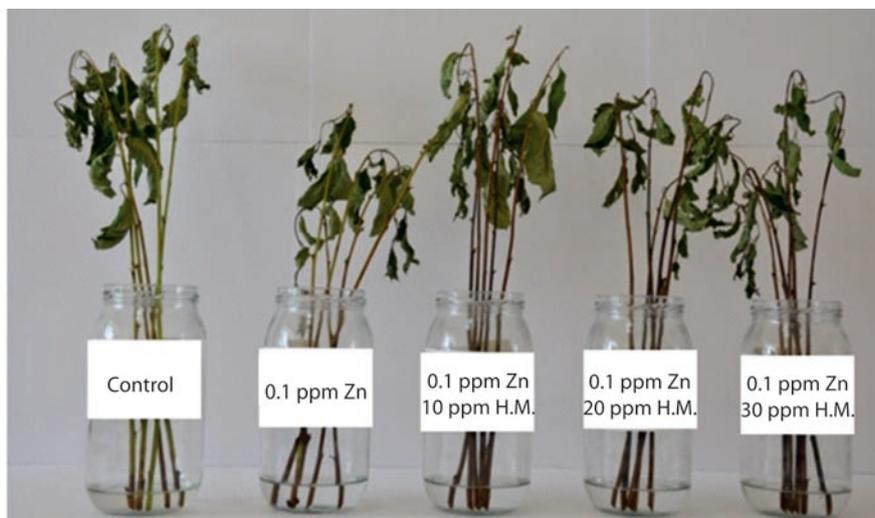
The cuttings of the plants were taken and placed in glass jars (Figs. 1–5). Stock solution was given once a week. Once a week, branch tips were cut 1 mm at a 45-degree angle to ensure easy transport. The temperature was 25°C and the humidity was 60.3%. Fluorescent lamps with a luminosity of 2,600 were used for 14 h in a day. The other times were dark. The progress of the plants was regularly monitored and recorded every week. The experiment lasted for 4 weeks.

### Sampling and analysis operations

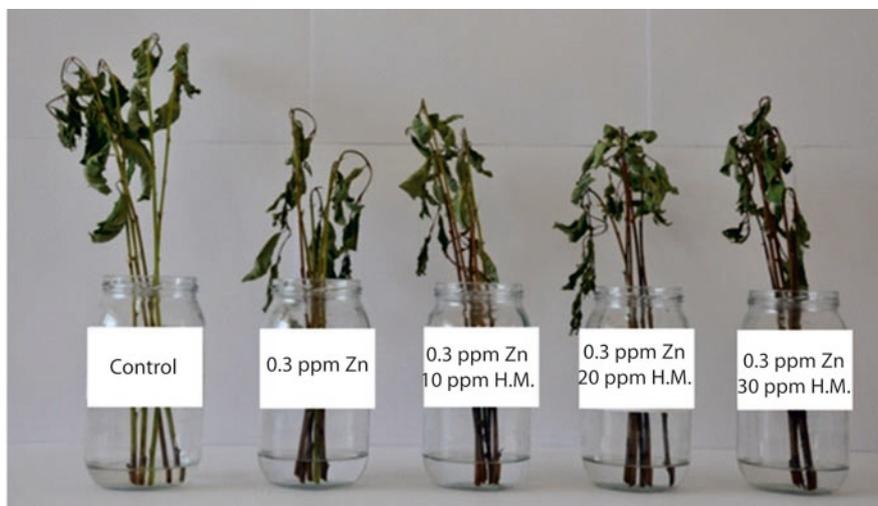
After 4 weeks for the anatomical examinations, sour cherry cuttings were put into 70% ethyl alcohol. Care



**Fig. 1.** General appearance of sour cherries (*Cerasus vulgaris* Miller) at different hormone concentrations. H.M. = hormone mixture



**Fig. 2.** General appearance of sour cherries (*Cerasus vulgaris* Miller) at different hormone concentrations and 0.1 ppm Zn. H.M. = hormone mixture



**Fig. 3.** General appearance of sour cherries (*Cerasus vulgaris* Miller) at different hormone concentrations and 0.3 ppm Zn. H.M. = hormone mixture



**Fig. 4.** General appearance of sour cherries (*Cerasus vulgaris* Miller) at different hormone concentrations and 0.5 ppm Zn. H.M. = hormone mixture



**Fig. 5.** General appearance of sour cherries (*Cerasus vulgaris* Miller) at different hormone concentrations and 1 ppm Zn. H.M. = hormone mixture

was taken to keep the working environment clean. Cross-sections were taken from the 1st internode (Xu *et al.* 2006).

Cross-sections were closed with glycerin medium. The cambial zone thickness of the sections, the cambial zone cell line, the radial width of the cambial zone cells, the tangential width of the cambial zone cells, the radial and tangential wall thicknesses of the cambial zone cells were photographed. A photograph of the thoma hemocytometer was also taken of the same features. We compared thoma hemocytometer and cross-section photos on the Adobe Photoshop CS4 and we recorded cambial zone thickness, radial width of cambial zone cells, tangential width of cambial zone cells, radial wall thickness of cambial zone cells and the tangential wall thickness of the cambial zone cells. The

results were evaluated according to variance analysis and Duncan test.

## Results

### Morphological observations

It was observed that the upper ends of the plants were bent 3 days after the start of the experiment. During the early days of the experiment, plant cuttings absorbed greater amounts of solution per unit time. Towards the end of the experiment, daily consumption declined steadily and the leaves started drying, but the body remained alive until the end of the experiment. The leaves turned slightly yellow (Figs. 1–5).

## Anatomical observations

### Cambial zone cell line

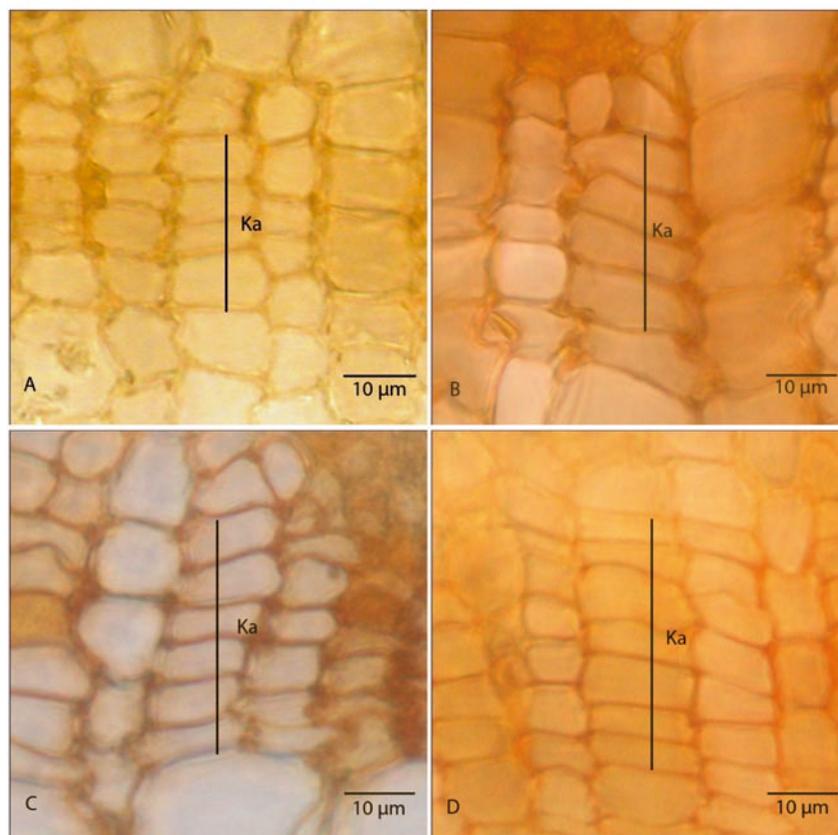
The cambial zone cell line in the 10, 20, 30 ppm H.M. series increased by 9, 28 and 49%, respectively, compared with the control (Fig. 6). In 0.1, 0.3, 0.5 and 1 ppm Zn concentration series, the cambial zone cell line decreased by 6, 23, 43 and 59%, respectively, compared with the control (Fig. 7). The cambial zone cell line in the 0.1 ppm Zn + 10 ppm H.M., 0.1 ppm Zn + 20 ppm H.M. and 0.1 ppm Zn + 30 ppm H.M. series increased by 1, 16 and 37%, respectively, compared to the control (Fig. 8). The cambial zone cell line in the 0.3 ppm Zn + 10 ppm H.M. and 0.3 ppm Zn + 20 ppm H.M. series decreased by 16% and 1%, respectively, but it increased by 4% in the 0.3 ppm Zn + 30 ppm H.M. compared to the control (Fig. 9).

The cambial zone cell line in the 0.5 ppm Zn + 10 ppm H.M. and 0.5 ppm Zn + 20 ppm H.M. series decreased by 23% and 4% compared to the control. It was seen that the cambial zone cell line in the 0.5 ppm Zn + 30 ppm H.M. was equal to the control's (Fig. 10). The cambial zone cell line decreased in the 1 ppm Zn + 10 ppm H.M., 1 ppm Zn + 20 ppm H.M. and 1 ppm Zn + 30 ppm H.M. series by 32, 14 and 7%, respectively, compared to the control (Table 1, Figs. 11–13).

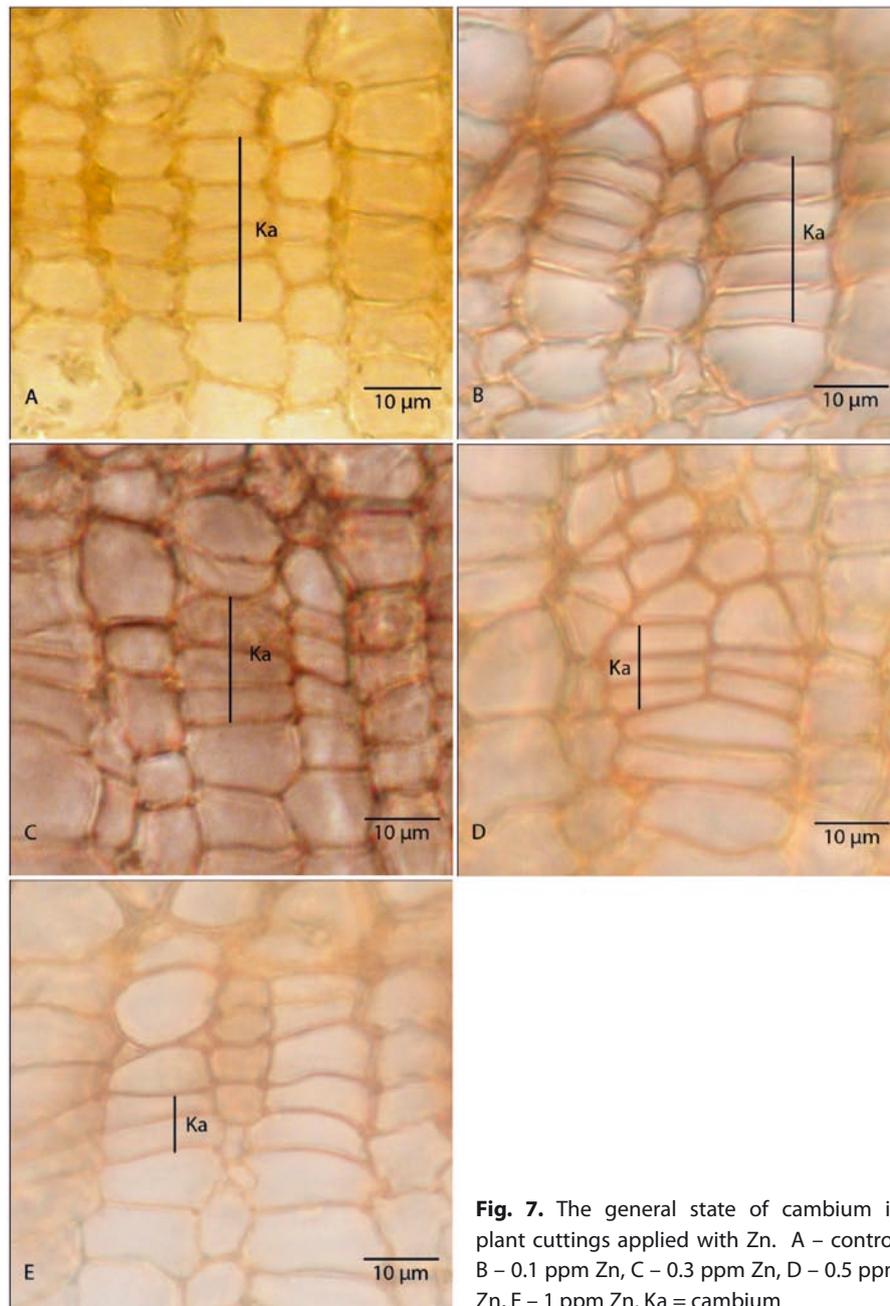
### Cambial zone thickness

The cambial zone thickness increased by 9, 34 and 81% in the 10, 20, 30 ppm H.M. series, respectively, compared to the control (Fig. 6). In the 0.1, 0.3, 0.5 and 1 ppm Zn concentrations, the cambial zone thickness decreased by 10, 28, 50 and 65%, respectively, compared to the control (Fig. 7). The cambial zone thickness in the 0.1 ppm Zn + 10 ppm H.M., 0.1 ppm Zn + 20 ppm H.M. and 0.1 ppm Zn + 30 ppm H.M. were increased by 1, 28 and 65%, respectively, compared to the control (Fig. 8).

The cambial zone thickness decreased by 17% in the 0.3 ppm Zn + 10 ppm H.M., but increased by 6% and 15% in the 0.3 ppm Zn + 20 ppm H.M. and 0.3 ppm Zn + 30 ppm H.M., respectively, compared to the control (Fig. 9 and 14). The cambial zone thickness decreased by 26% and 8% in the 0.5 ppm Zn + 10 ppm H.M. and 0.5 ppm Zn + 20 ppm H.M., respectively, compared to the control (Figs. 10 and 14). The cambial zone thickness went up by 5% in the 0.5 ppm Zn + 30 ppm H.M., but decreased by 36, 17 and 7% in the 1 ppm Zn + 10 ppm H.M., 1 ppm Zn + 20 ppm H.M. and 1 ppm Zn + 30 ppm H.M., respectively, compared to the control (Table 2, Figs. 13–15).



**Fig. 6.** The general state of cambium in plant cuttings applied with hormone mixture (H.M.). A – control, B – 10 ppm H.M., C – 20 ppm H.M., D – 30 ppm H.M. Ka = cambium



**Fig. 7.** The general state of cambium in plant cuttings applied with Zn. A – control, B – 0.1 ppm Zn, C – 0.3 ppm Zn, D – 0.5 ppm Zn, E – 1 ppm Zn. Ka = cambium

### Radial width of cambial zone cells

The radial width of the cambial zone cells was increased by 2, 11 and 23% in the 10, 20 and 30 ppm H.M. (Fig. 6), respectively, compared to the control. In the 0.1, 0.3, 0.5 and 1 ppm Zn series, the radial width of the cambial zone cells decreased by 5, 6, 12 and 16%, respectively, compared to the control (Fig. 7).

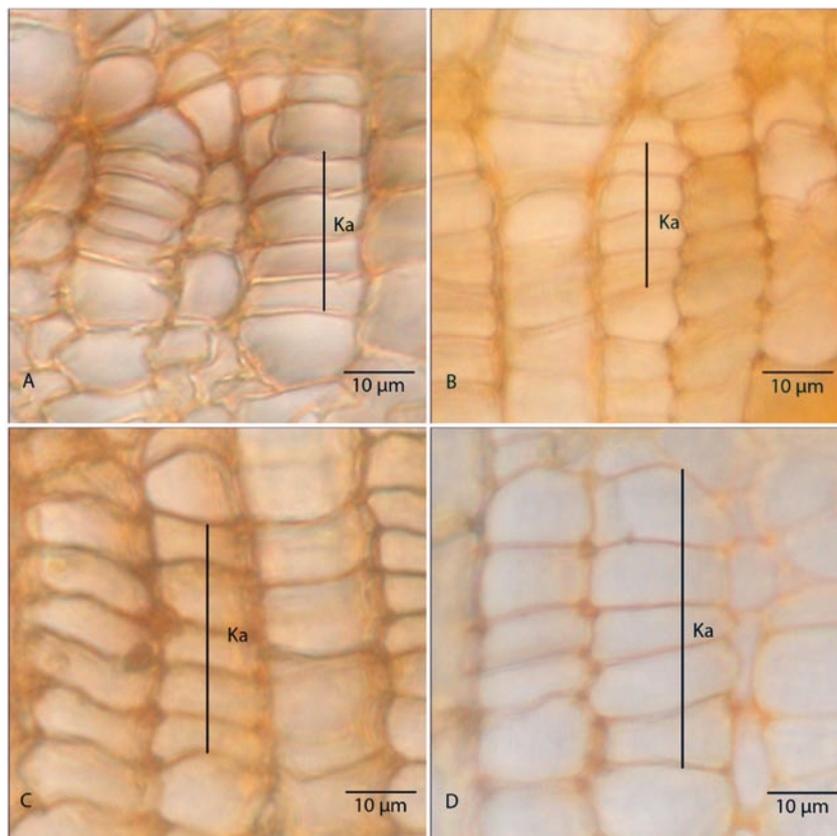
The radial width of the cambial zone cells decreased by 3% in the 0.1 ppm Zn + 10 ppm H.M., while it increased by 6% and 20% in the 0.1 ppm Zn + 20 ppm H.M. and 0.1 ppm Zn + 30 ppm H.M., respectively, compared to the control (Fig. 8).

The radial width of the cambial zone cells decreased by 17% in the 0.3 ppm Zn + 10 ppm H.M., although it

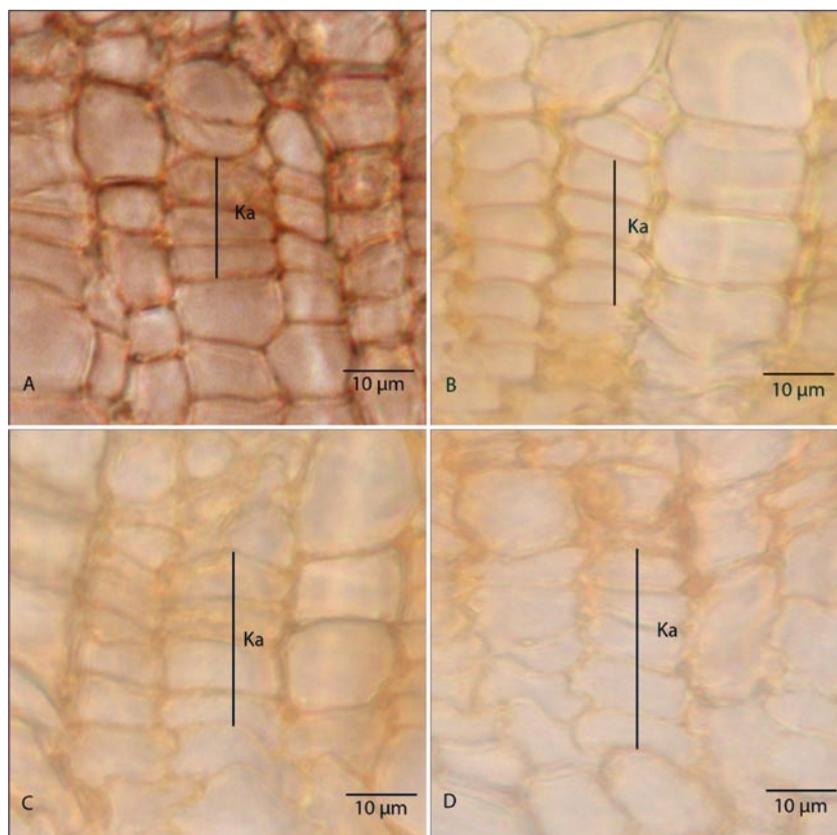
increased by 2 and 11% in the 0.3 ppm Zn + 20 ppm H.M. and 0.3 ppm Zn + 30 ppm H.M., respectively, compared to the control (Fig. 9).

The radial width of the cambial zone cells decreased by 4% and 1% in the 0.5 ppm Zn + 10 ppm H.M. and 0.5 ppm Zn + 20 ppm H.M., respectively, but it increased by 5% in the 0.5 ppm Zn + 30 ppm H.M., compared to the control (Fig. 10).

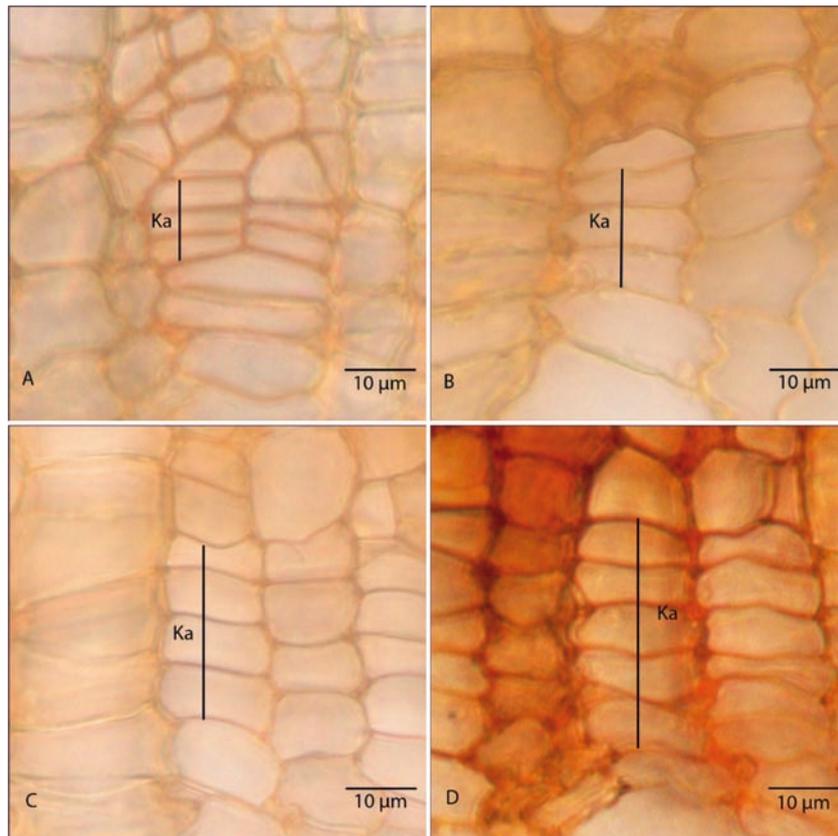
The radial width of the cambial zone cells decreased by 15% and 14% in the 1 ppm Zn + 10 ppm H.M. and 1 ppm Zn + 20 ppm H.M., respectively, compared to the control while there was no difference between 1 ppm Zn + 30 ppm H.M. and the control (Table 3, Figs. 13, 16, 17).



**Fig. 8.** The general state of cambium in plant cuttings applied with 0.1 ppm Zn and hormone mixture (H.M.). A – 0.1 ppm Zn, B – 0.1 ppm Zn + 10 ppm H.M., C – 0.1 ppm Zn + 20 ppm H.M., D – 0.1 ppm Zn + 30 ppm H.M. Ka = cambium



**Fig. 9.** The general state of cambium in plant cuttings applied with 0.3 ppm Zn and hormone mixture (H.M.). A – 0.3 ppm Zn, B – 0.3 ppm Zn + 10 ppm H.M., C – 0.3 ppm Zn + 20 ppm H.M., D – 0.3 ppm Zn + 30 ppm H.M. Ka = cambium



**Fig. 10.** The general state of cambium in plant cuttings applied with 0.5 ppm Zn and hormone mixture (H.M.). A – 0.5 ppm Zn, B – 0.5 ppm Zn + 10 ppm H.M., C – 0.5 ppm Zn + 20 ppm H.M., D – 0.5 ppm Zn + 30 ppm H.M. Ka = cambium

**Table 1.** Cambial zone cell line in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

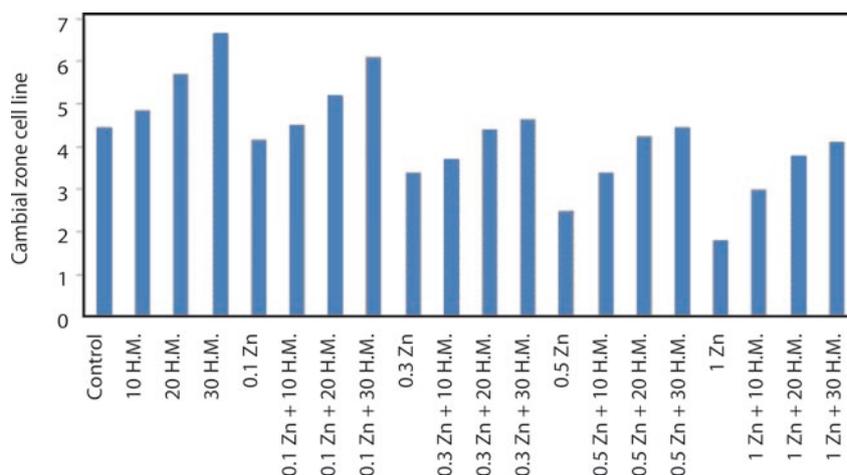
Groups	Cambial zone cell line
Control	4.45 ± 0.51 def
10 ppm H.M.	4.85 ± 0.37 cd
20 ppm H.M.	5.70 ± 1.13 b
30 ppm H.M.	6.65 ± 0.59 a
0.1 ppm Zn	4.15 ± 0.81 fg
0.1 ppm Zn + 10 ppm H.M.	4.50 ± 0.51 def
0.1 ppm Zn + 20 ppm H.M.	5.20 ± 0.83 c
0.1 ppm Zn + 30 ppm H.M.	6.10 ± 0.91 b
0.3 ppm Zn	3.40 ± 0.94 ij
0.3 ppm Zn + 10 ppm H.M.	3.70 ± 0.47 hi
0.3 ppm Zn + 20 ppm H.M.	4.40 ± 0.68 def
0.3 ppm Zn + 30 ppm H.M.	4.65 ± 0.49 de
0.5 ppm Zn	2.50 ± 0.51 k
0.5 ppm Zn + 10 ppm H.M.	3.40 ± 0.50 ij
0.5 ppm Zn + 20 ppm H.M.	4.25 ± 0.44 ef
0.5 ppm Zn + 30 ppm H.M.	4.45 ± 0.51 def
1 ppm Zn	1.80 ± 0.62 l
1 ppm Zn + 10 ppm H.M.	3.00 ± 0.73 j
1 ppm Zn + 20 ppm H.M.	3.80 ± 0.77 ghi
1 ppm Zn + 30 ppm H.M.	4.10 ± 0.31 fgh

a, b, c, d, e, f, g, h, i, j, k, l – the difference between groups that do not contain the same letters is significant ( $p < 0.05$ ), H.M. = hormone mixture

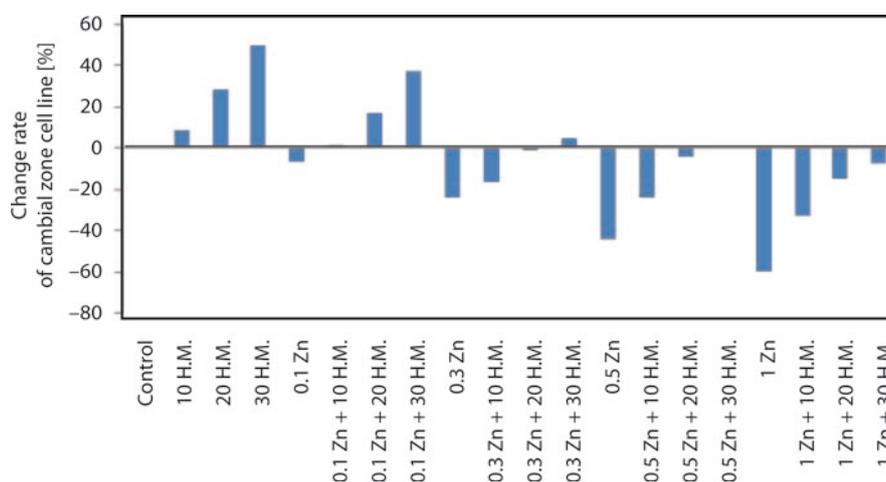
**Table 2.** Cambial zone thickness in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

Groups	Cambial zone thickness [µm]
Control	23.55 ± 4.85 efg
10 ppm H.M.	25.76 ± 3.70 e
20 ppm H.M.	31.60 ± 4.47 c
30 ppm H.M.	42.83 ± 6.17 a
0.1 ppm Zn	21.01 ± 5.48 hi
0.1 ppm Zn + 10 ppm H.M.	23.98 ± 2.96 efg
0.1 ppm Zn + 20 ppm H.M.	30.28 ± 6.69 cd
0.1 ppm Zn + 30 ppm H.M.	38.95 ± 10.08 b
0.3 ppm Zn	16.76 ± 4.43 jk
0.3 ppm Zn + 10 ppm H.M.	19.49 ± 4.06 ij
0.3 ppm Zn + 20 ppm H.M.	25.08 ± 5.77 ef
0.3 ppm Zn + 30 ppm H.M.	27.12 ± 3.50 de
0.5 ppm Zn	11.75 ± 3.80 l
0.5 ppm Zn + 10 ppm H.M.	17.21 ± 2.77 jk
0.5 ppm Zn + 20 ppm H.M.	21.47 ± 7.35 ghi
0.5 ppm Zn + 30 ppm H.M.	24.88 ± 4.13 efg
1 ppm Zn	8.01 ± 3.02 m
1 ppm Zn + 10 ppm H.M.	14.90 ± 4.90 kl
1 ppm Zn + 20 ppm H.M.	19.38 ± 4.86 ij
1 ppm Zn + 30 ppm H.M.	21.71 ± 2.64 fghi

a, b, c, d, e, f, g, h, i, j, k, l – the difference between groups that do not contain the same letter is significant ( $p < 0.05$ ), H.M. = hormone mixture



**Fig. 11.** Cambial zone cell line in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 12.** Change rate of cambial zone cell line in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc

### Tangential width of cambial zone cells

Tangential width of cambial zone cells was increased by 1, 2 and 3% in the 10, 20 and 30 ppm H.M. (Fig. 6), while it was decreased by 7, 12, 16 and 22% in the 0.1, 0.3, 0.5 and 1 ppm Zn concentrations (Fig. 7). There was no statistically significant difference between the tangential extent of the cambial zone cells of 0.1 ppm Zn + 10 ppm H.M. and the control. Tangential width of cambial zone cells in the 0.1 ppm Zn + 20 ppm H.M. and 0.1 ppm Zn + 30 ppm H.M. increased by 8% and 13%, respectively, compared to the control (Fig. 8).

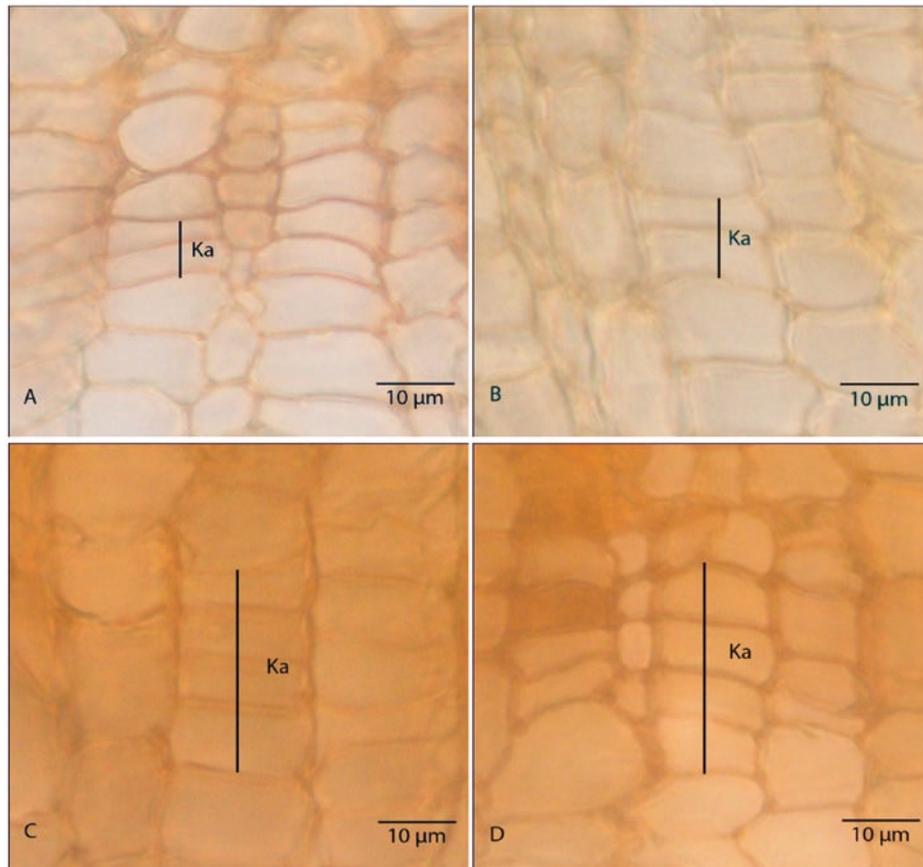
Tangential width of cambial zone cells in 0.3 ppm Zn + 10 ppm H.M. decreased by 3%, although it increased by 2% and 9% in the 0.3 ppm Zn + 20 ppm H.M. and 0.3 ppm Zn + 30 ppm H.M., respectively, compared to the control (Fig. 9).

Tangential width of cambial zone cells in the 0.5 ppm Zn + 10 ppm H.M. and 0.5 ppm Zn + 20 ppm H.M. decreased by 3% and 1%, respectively, but it increased by 1% in 0.5 ppm Zn + 30 ppm H.M., compared to the control (Fig. 10).

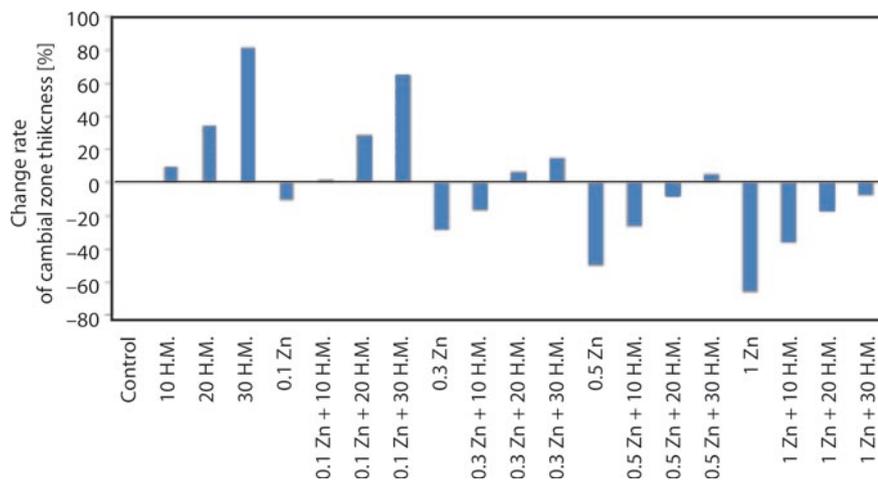
Tangential width of cambial zone cells in the 1 ppm Zn + 10 ppm H.M., 1 ppm Zn + 20 ppm H.M. and 1 ppm Zn + 30 ppm H.M. decreased by 9, 5 and 4%, respectively, compared to the control (Table 4, Figs. 13 and 19).

### Radial wall thickness of cambial zone cells

Radial wall thickness of cambial zone cells in the 10, 20 and 30 ppm H.M. decreased by 9, 17 and 19%, respectively (Fig. 6), while it increased by 6, 11, 16 and 23% in the 0.1, 0.3, 0.5 ve 1 ppm Zn, respectively, compared to the control (Fig. 7).



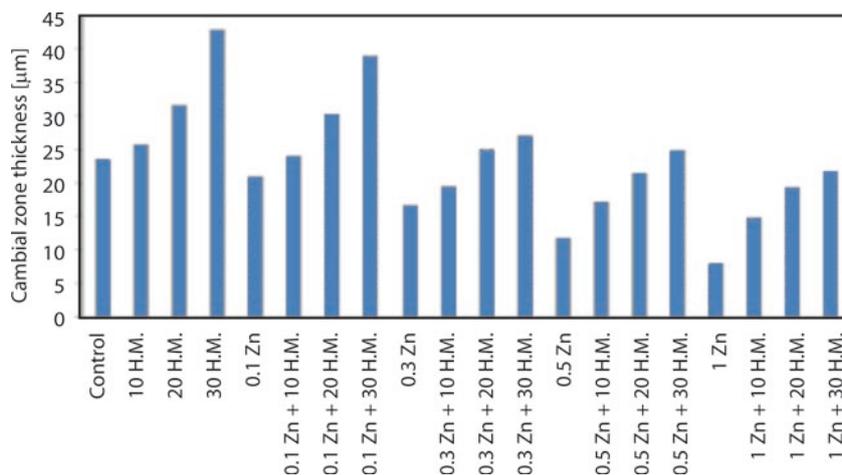
**Fig. 13.** The general state of cambium in plant cuttings applied with 1 ppm Zn and hormone mixture (H.M.). A – 1 ppm Zn, B – 1 ppm Zn + 10 ppm H.M., C – 1 ppm Zn + 20 ppm H.M., D – 1 ppm Zn + 30 ppm H.M. Ka = cambium



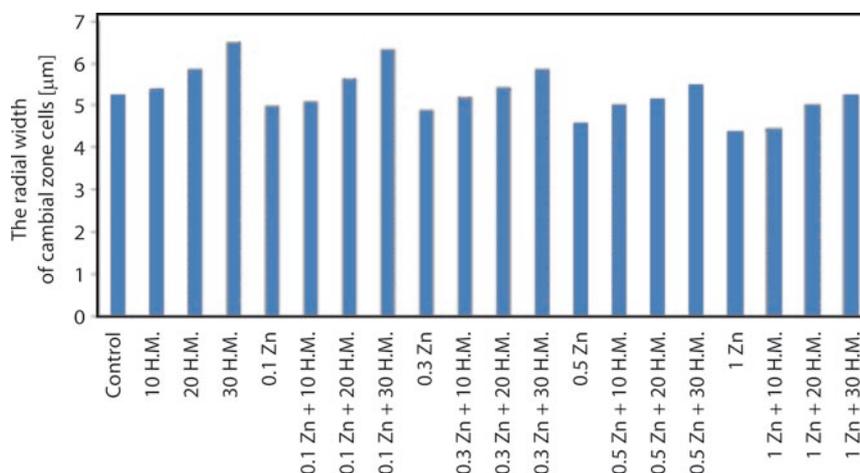
**Fig. 14.** Change rate of cambial zone thickness in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc

Radial wall thickness of cambial zone cells in 0.1 ppm Zn + 10 ppm H.M. increased by 1%, although it decreased by 2% and 6% in the 0.1 ppm Zn + 20 ppm H.M. and 0.1 ppm Zn + 30 ppm H.M., respectively, compared to the control (Fig. 8).

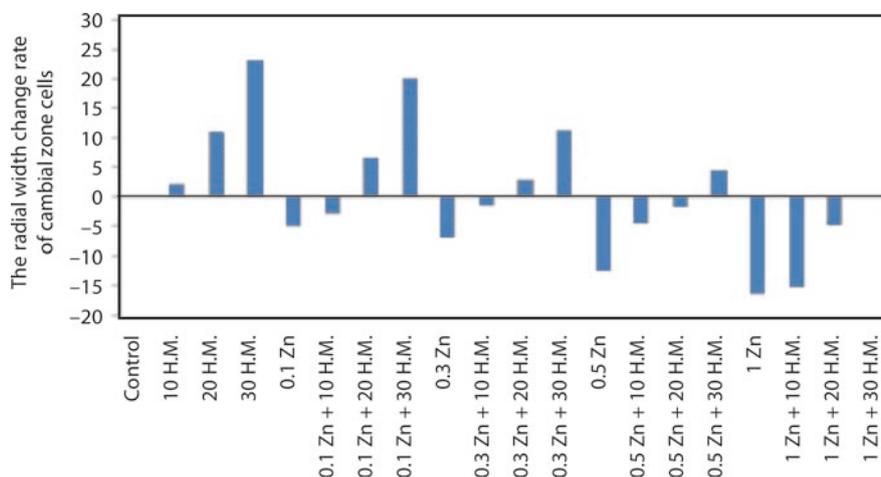
Radial wall thickness of cambial zone cells in 0.3 ppm Zn + 10 ppm H.M. increased by 4%, but it decreased by 4% in 0.3 ppm Zn + 30 ppm H.M., respectively, compared to the control. There was no statistically significant difference with regard to radial wall



**Fig. 15.** Cambial zone thickness in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 16.** The radial width of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 17.** The radial width change rate of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc

**Table 3.** The radial width of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

Groups	The radial width of cambial zone cells [ $\mu\text{m}$ ]
Control	5.27 $\pm$ 1.26 bcd
10 ppm H.M.	5.39 $\pm$ 0.68 bc
20 ppm H.M.	5.86 $\pm$ 0.97 ab
30 ppm H.M.	6.50 $\pm$ 1.29 a
0.1 ppm Zn	5.01 $\pm$ 0.86 cde
0.1 ppm Zn + 10 ppm H.M.	5.11 $\pm$ 0.53 cde
0.1 ppm Zn + 20 ppm H.M.	5.62 $\pm$ 0.68 bc
0.1 ppm Zn + 30 ppm H.M.	6.33 $\pm$ 1.20 a
0.3 ppm Zn	4.91 $\pm$ 0.90 cdef
0.3 ppm Zn + 10 ppm H.M.	5.20 $\pm$ 0.77 bcd
0.3 ppm Zn + 20 ppm H.M.	5.42 $\pm$ 1.09 bc
0.3 ppm Zn + 30 ppm H.M.	5.86 $\pm$ 0.81 ab
0.5 ppm Zn	4.61 $\pm$ 0.87 def
0.5 ppm Zn + 10 ppm H.M.	5.03 $\pm$ 0.83 cde
0.5 ppm Zn + 20 ppm H.M.	5.18 $\pm$ 1.65 bcd
0.5 ppm Zn + 30 ppm H.M.	5.51 $\pm$ 0.78 bc
1 ppm Zn	4.40 $\pm$ 0.67 f
1 ppm Zn + 10 ppm H.M.	4.46 $\pm$ 1.29 ef
1 ppm Zn + 20 ppm H.M.	5.02 $\pm$ 0.45 cde
1 ppm Zn + 30 ppm H.M.	5.26 $\pm$ 0.50 bcd

a, b, c, d, e, f – the difference between groups that do not contain the same letter is significant ( $p < 0.05$ ), H.M. = hormone mixture

**Table 4.** Tangential width of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

Groups	Tangential width of cambial zone cells [ $\mu\text{m}$ ]
Control	15.99 $\pm$ 2.09 efg
10 ppm H.M.	17.15 $\pm$ 1.18 d
20 ppm H.M.	17.95 $\pm$ 0.92 bc
30 ppm H.M.	19.47 $\pm$ 1.25 a
0.1 ppm Zn	14.82 $\pm$ 0.91 ij
0.1 ppm Zn + 10 ppm H.M.	16.10 $\pm$ 0.93 ef
0.1 ppm Zn + 20 ppm H.M.	17.32 $\pm$ 1.04 cd
0.1 ppm Zn + 30 ppm H.M.	18.14 $\pm$ 0.86 b
0.3 ppm Zn	13.99 $\pm$ 0.80 kl
0.3 ppm Zn + 10 ppm H.M.	15.48 $\pm$ 0.88 fghi
0.3 ppm Zn + 20 ppm H.M.	16.41 $\pm$ 1.05 e
0.3 ppm Zn + 30 ppm H.M.	17.46 $\pm$ 0.92 bcd
0.5 ppm Zn	13.40 $\pm$ 0.48 i
0.5 ppm Zn + 10 ppm H.M.	15.49 $\pm$ 0.86 fghi
0.5 ppm Zn + 20 ppm H.M.	15.78 $\pm$ 0.70 efgh
0.5 ppm Zn + 30 ppm H.M.	16.19 $\pm$ 0.83 ef
1 ppm Zn	12.44 $\pm$ 1.21 m
1 ppm Zn + 10 ppm H.M.	14.51 $\pm$ 1.08 jk
1 ppm Zn + 20 ppm H.M.	15.07 $\pm$ 1.22 hij
1 ppm Zn + 30 ppm H.M.	15.29 $\pm$ 0.99 ghi

a, b, c, d, e, f, g, h, i, j, k, l – the difference between groups that do not contain the same letter is significant ( $p < 0.05$ ), H.M. = hormone mixture

thickness of cambial zone cells between the 0.3 ppm Zn + 20 ppm H.M. and the control (Fig. 9).

Radial wall thickness of cambial zone cells in the 0.5 ppm Zn + 10 ppm H.M., 0.5 ppm Zn + 20 ppm H.M. and 0.5 ppm Zn + 30 ppm H.M. increased by 8, 4 and 1%, respectively, compared to the control (Fig. 10).

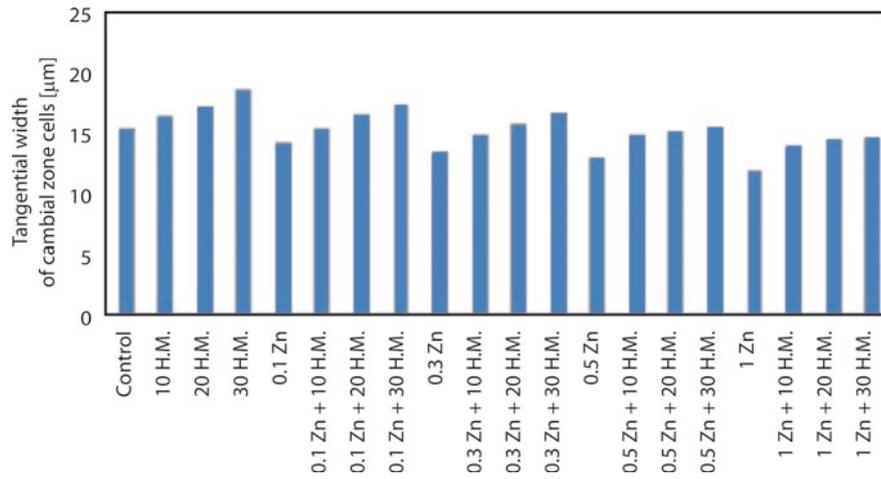
Radial wall thickness of cambial zone cells in the 1 ppm Zn + 10 ppm H.M., 1 ppm Zn + 20 ppm H.M. and 1 ppm Zn + 30 ppm H.M. increased by 11, 7 and 5%, respectively, compared to the control (Table 5, Figs. 13, 20, 21).

### The tangential wall thickness of the cambial zone cells

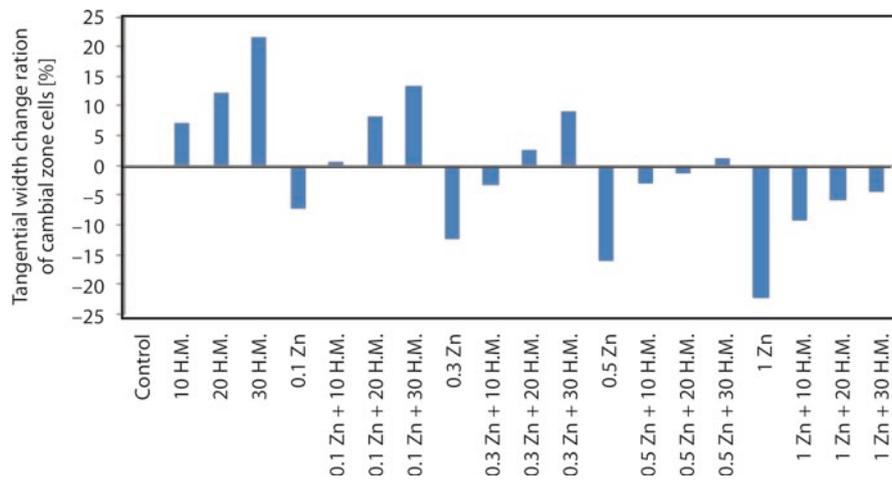
The tangential wall thickness of the cambial zone cells was decreased by 5, 18 and 30% in 10, 20, 30 ppm H.M. series, respectively, compared to the control group (Fig. 6). The tangential wall thickness of cambial zone cells in the 0.1, 0.3, 0.5 and 1 ppm Zn concentration series increased by 4, 7, 9 and 20%, respectively, compared to the control (Fig. 7). The tangential wall thickness of the cambial zone cells in the plants applied 0.1 ppm Zn + 10 ppm H.M. increased by 3%, compared to

the control. There was no statistically significant difference with regard to the tangential wall thickness of the cambial zone cells between the plants applied 0.1 ppm Zn + 20 ppm H.M. and the control. The tangential wall thickness of the cambial zone cells of the series applied 0.1 ppm Zn + 30 ppm H.M. was reduced by 3%, compared to the control series (Fig. 8).

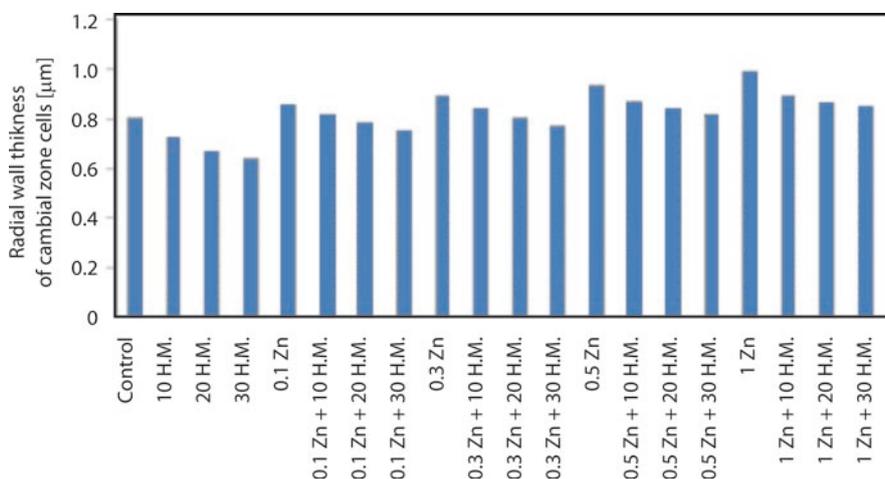
The tangential wall thickness of cambial zone cells in the 0.3 ppm Zn + 10 ppm H.M. and 0.3 ppm Zn + 20 ppm H.M. series increased by 5% and 2%, respectively, compared to the control. The differences between the control and the plants applied 0.3 ppm Zn + 30 ppm H.M. with regard to the tangential wall thickness of cambial zone cells were not statistically significant (Fig. 9). The tangential wall thickness of cambial zone cells in the plants applied 0.5 ppm Zn + 10 ppm H.M., 0.5 ppm Zn + 20 ppm H.M. and 0.5 ppm Zn + 30 ppm H.M. increased by 6, 5 and 3%, respectively, compared to the control (Fig. 10). The tangential wall thickness of cambial zone cells in the plants applied 1 ppm Zn + 10 ppm H.M., 1 ppm Zn + 20 ppm H.M. and 1 ppm Zn + 30 ppm H.M. series were increased by 10, 9, and 6%, respectively, compared to the control (Table 6, Figs. 22, 23).



**Fig. 18.** Tangential width of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 19.** Tangential width change ration of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 20.** Radial wall thickness of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc

**Table 5.** Radial wall thickness of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

Groups	Radial wall thickness of cambial zone cells [ $\mu\text{m}$ ]
Control	080 $\pm$ 0.05 fg
10 ppm H.M.	0.72 $\pm$ 0.05 i
20 ppm H.M.	0.66 $\pm$ 0.04 j
30 ppm H.M.	0.64 $\pm$ 0.04 j
0.1 ppm Zn	0.85 $\pm$ 0.05 cdef
0.1 ppm Zn + 10 ppm H.M.	0.81 $\pm$ 0.05 efg
0.1 ppm Zn + 20 ppm H.M.	0.78 $\pm$ 0.05 gh
0.1 ppm Zn + 30 ppm H.M.	0.75 $\pm$ 0.08 hi
0.3 ppm Zn	0.89 $\pm$ 0.05 bc
0.3 ppm Zn + 10 ppm H.M.	0.84 $\pm$ 0.09 def
0.3 ppm Zn + 20 ppm H.M.	0.80 $\pm$ 0.06 fg
0.3 ppm Zn + 30 ppm H.M.	0.77 $\pm$ 0.07 ghi
0.5 ppm Zn	0.93 $\pm$ 0.05 b
0.5 ppm Zn + 10 ppm H.M.	0.87 $\pm$ 0.07 cd
0.5 ppm Zn + 20 ppm H.M.	0.84 $\pm$ 0.07 def
0.5 ppm Zn + 30 ppm H.M.	0.81 $\pm$ 0.09 efg
1 ppm Zn	0.99 $\pm$ 0.08 a
1 ppm Zn + 10 ppm H.M.	0.89 $\pm$ 0.05 bc
1 ppm Zn + 20 ppm H.M.	0.86 $\pm$ 0.07 cde
1 ppm Zn + 30 ppm H.M.	0.85 $\pm$ 0.08 cdef

a, b, c, d, e, f, g, h, i, j – the difference between groups that do not contain the same letter is significant ( $p < 0.05$ ), H.M. = hormone mixture

**Table 6.** Tangential wall thickness of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings

Groups	Tangential wall thickness of cambrian zone cells [ $\mu\text{m}$ ]
Control	0.82 $\pm$ 0.06 cdef
10 ppm H.M.	0.78 $\pm$ 0.11 f
20 ppm H.M.	0.67 $\pm$ 0.19 g
30 ppm H.M.	0.57 $\pm$ 0.11 h
0.1 ppm Zn	0.86 $\pm$ 0.08 bcde
0.1 ppm Zn + 10 ppm H.M.	0.85 $\pm$ 0.09 bcdef
0.1 ppm Zn + 20 ppm H.M.	0.83 $\pm$ 0.06 cdef
0.1 ppm Zn + 30 ppm H.M.	0.80 $\pm$ 0.06 ef
0.3 ppm Zn	0.89 $\pm$ 0.08 bcd
0.3 ppm Zn + 10 ppm H.M.	0.87 $\pm$ 0.13 bcde
0.3 ppm Zn + 20 ppm H.M.	0.85 $\pm$ 0.11 bcde
0.3 ppm Zn + 30 ppm H.M.	0.82 $\pm$ 0.10 def
0.5 ppm Zn	0.91 $\pm$ 0.09 b
0.5 ppm Zn + 10 ppm H.M.	0.88 $\pm$ 0.10 bcd
0.5 ppm Zn + 20 ppm H.M.	0.87 $\pm$ 0.08 bcde
0.5 ppm Zn + 30 ppm H.M.	0.85 $\pm$ 0.08 bcdef
1 ppm Zn	1.00 $\pm$ 0.10 a
1 ppm Zn + 10 ppm H.M.	0.91 $\pm$ 0.08 b
1 ppm Zn + 20 ppm H.M.	0.90 $\pm$ 0.08 bc
1 ppm Zn + 30 ppm H.M.	0.88 $\pm$ 0.10 bcd

a, b, c, d, e, f, g, h – the difference between groups that do not contain the same letters is significant ( $p < 0.05$ ), H.M. = hormone mixture

## Discussion

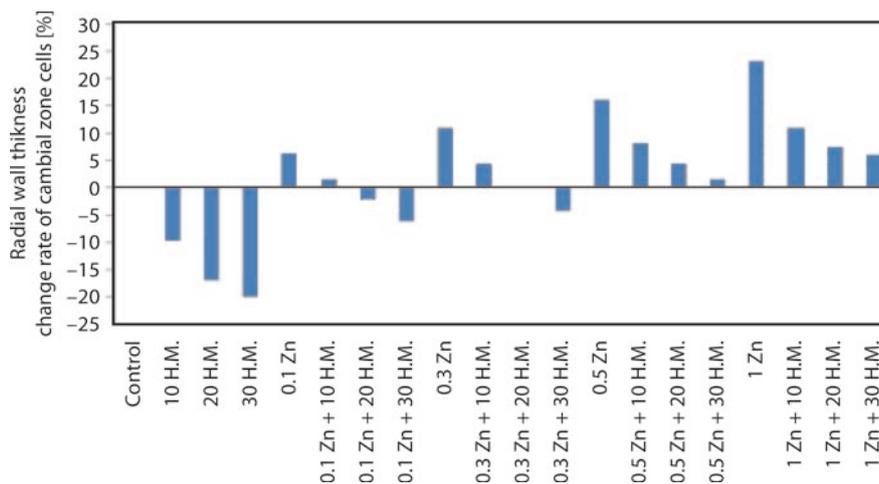
In the current study, it was seen that the leaves of plants subjected to the zinc series were defoliated more than the plants in the control series. Deef (2008) observed that root length, plant weight and the number of leaves in the tomato fidelity, which was zinc-treated at toxic levels, decreased significantly. His study is parallel to our study about the number of leaves.

The plant leaves, where zinc was applied, dried more prematurely than the leaves of the plants in the control series. The intake of water in the plants, in which zinc was applied, decreased more towards the end of the experiment. Broadley *et al.* (2012) reported that the water content of zinc treated plant tissues decreased. In the study in which zinc was applied, the early drying of the leaves relative to the control may have been due to decreased water in the tissue, which is caused by zinc as stated above.

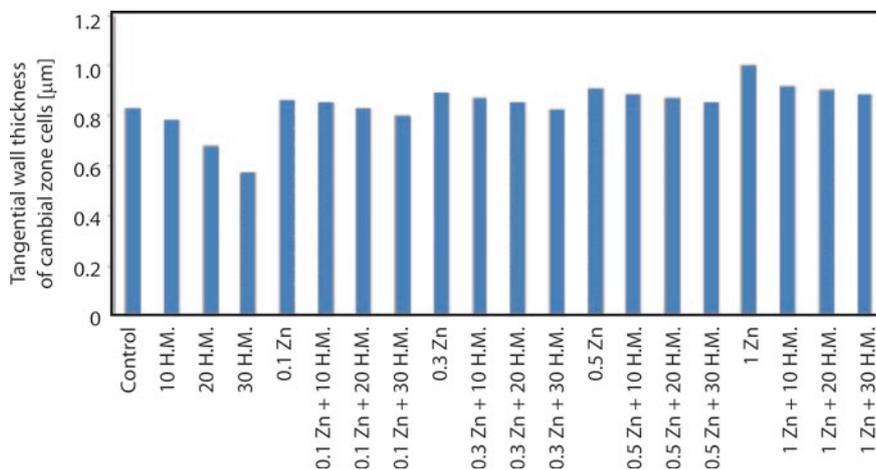
In the current study, it was observed that the leaves of the plants with applied zinc were darker green than the control. Jain *et al.* (2010) reported that excessive zinc decreases the amount of copper and iron and

increases the amount of manganese, thereby increasing the amount of chlorophyll a, chlorophyll b and carotenoid in the plant. In addition, researchers also observed that the number of roots and their length in experimental plants decreased and at the same time the leaves were dark green. When we compared our experiment with the above experiment in terms of the effect of excessive zinc on the color of the plant leaves, the results were similar.

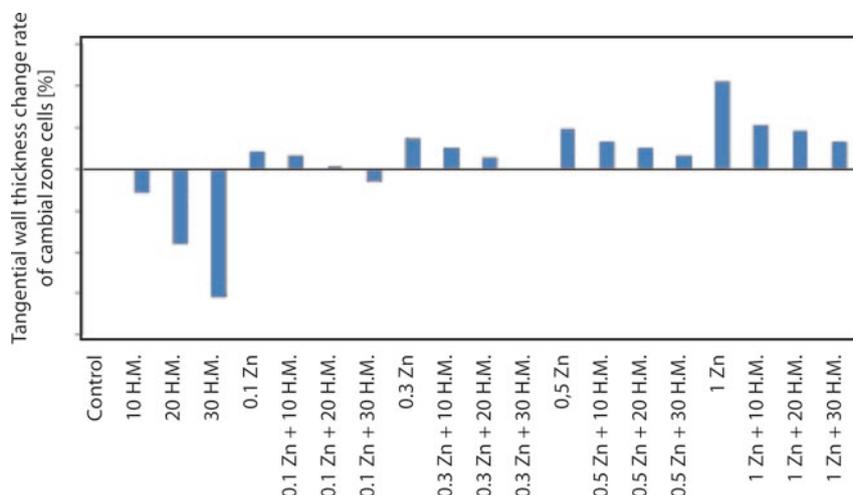
When the zinc-sensitive *Festuca rubra* L. were exposed to 0.1 and 0.2  $\mu\text{g} \cdot \text{cm}^{-3}$  zinc, the nucleus volume of the cells in the G2 phase, average nucleus volume, RNA content and protein content decreased by 43–65%, 13–25%, 9–44% and 6–17%, respectively. When *F. rubra* plants were exposed to 0.1 and 0.2  $\mu\text{g} \cdot \text{cm}^{-3}$  zinc, the cell division time increased by 40% and 132% (Powell *et al.* 1986b). Davies *et al.* (1991) reported that the protein content of mitotic cells decreased by 25% after 12 h and by 50% after 96 h when zinc-sensitive individuals of *F. rubra* were exposed to 120  $\mu\text{m}$  zinc. Borboa and Torre (1996) subjected onion (*Allium cepa*) to very high amounts of zinc and cadmium. G2 phase increased 1.7-fold in zinc-treated cells and increased 2.7-fold in cadmium-treated cells. The



**Fig. 21.** Radial wall thickness change rate of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 22.** Tangential wall thickness of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc



**Fig. 23.** Tangential wall thickness change rate of cambial zone cells in the cross-sections taken from the body of *Cerasus vulgaris* Miller cuttings. H.M. = hormone mixture, Zn = Zinc

researchers stated that the cellular injuries caused by these heavy metals were repaired before anaphase and that the cell division cycle increased 1.5 times in treatment with zinc and doubled in treatment with cadmium. Chang *et al.* (2005) noted that rice (*Oryza sativa* L.) plant cells died in 5–10 mM zinc applications. Researchers have indicated that the cause of cell death is from an increase of free oxygen species and protein phosphatases due to excessive zinc. In our study the cambial zone cell line of the plants in the series treated with 0.1, 0.3, 0.5, and 1 ppm Zn decreased by 6, 23, 43 and 59% and the cambial zone thickness decreased by 10, 28, 50, 65%, respectively. The reason for these decreases may be a decrease in protein and RNA content in the cells or in the number of divided cells due to the prolongation of cell division time when compared with the control, as recorded in the above research results.

Broadley *et al.* (2011) reported that the water content of zinc treated plant tissues decreased. In our study, the radial extent of cambial zone cells was reduced by 5, 6, 12, 16%, The tangential extent of cambial zone cells decreased by 7, 12, 16, 22% in plants subjected to 0.1, 0.3, 0.5 and 1 ppm Zn when compared to the plants in the control series, respectively. The reduction of the radial and tangential widths in the cells may be due to the reduction of water content in the cell caused by the zinc.

The walls of dividing cells are thinner than the cells that are not dividing. In our work, the radial wall thickness of the cambial zone cells increased by 6, 11, 16 and 23%, and the tangential wall thickness of the cambial zone cells increased by 4, 7, 9 and 20% in the plants applied 0.1, 0.3, 0.5, and 1 ppm Zn, compared with the control. This may be due to the reduction in cell divisions.

Chaudhry and Khan (2000) found that GA<sub>3</sub>, applied to *Cicer arietinum* L., increased the body length significantly. Indole acetic acid and kinetin increased cambium activation and differentiation of metaxylem members. Applied IAA + GA<sub>3</sub> and GA<sub>3</sub> + kinetin mixtures significantly increased trunk diameter, trunk length and cambium diameter. Researchers have applied IAA + GA<sub>3</sub> + kinetin mixtures to this plant, but have not observed any significant effect on plant diameter, length and cambium. In a another study, application of GA<sub>3</sub> and kinetin significantly increased chlorophyll content, growth and yield attributes in both genotypes under water stress conditions. However, IAA application did not improve the grain yield in rice genotypes under water stress. The positive impact of GA<sub>3</sub> and kinetin spray was imitated in the form of enhanced solute accumulation; enhanced growth and greater grain assimilate deposition (Khan *et al.* 2015). At a concentration of 0.5 mg · l<sup>-1</sup>, both IAA and GA

(shoot length, root length, and fresh weight) (Park *et al.* 2017). Suzuki (1981) stated that the hypocotyl diameter of *Raphinus satinus* L. is increased by the application of kinetin. This effect of kinetin was more enhanced by 1-naphthaleneacetic acid (NAA) administration. NAA and GA<sub>3</sub> alone did not increase the hypocotyl diameter. The investigator also stated that cytokines acted as regulators in the development of all radishes. In another study, the plant growth regulators GA<sub>3</sub> (50, 100, 150, 200 ppm) and NAA (15, 25, 35, 45 ppm) were applied as foliar application 15 and 45 days after sowing. Looking at the results, it was noticed that the morphological and yield characters showed significant increments of plant height (41 cm) with the foliar spray of 200 ppm GA<sub>3</sub> as well as the number of leaves (36.6/plant), number of branches (8.2/plant), flowers at 50 DAS (8.2/plant), number of pods (14.7/plant), number of seeds (8.5/pod), pod length (8.8 cm), and 100 seed weight (24.8 g). Physiological characteristics were significantly increased: chlorophyll a (1.914 mg · g<sup>-1</sup>) and b (1.983 mg · g<sup>-1</sup>), total chlorophyll (3.894 mg · g<sup>-1</sup> fresh weight) and carotenoid (3.293 mg · g<sup>-1</sup> fresh weight) and the quality character like protein (245 mg · g<sup>-1</sup>) and carbohydrate (643 mg · g<sup>-1</sup>) compared to the control (Sadak *et al.* 2013; Singh *et al.* 2015). Ugglia *et al.* (1998) reported that there is a strong relationship between the IAA's concentration change in the radial direction and the rate of cambial growth and that the IAA gave position information to the plants. Yang *et al.* (1996) reported that IAA increased cell expansion and that GA<sub>3</sub> increased both cell count and cell prolongation. Robards *et al.* (1969) reported that the formation and differentiation of xylem cells was increased by the IAA + GA<sub>3</sub> + FAB (6-furfuryl aminopurine) mixture. Tileklioğlu and Algan (1992) investigated the effect of 5, 10, 15 mg · l<sup>-1</sup> IAA + GA<sub>3</sub> + kinetin on the cambial activity of *Coleus* sp. Researchers have observed cell divisions in the cambium at these concentrations. The dividing cells in this study were added to the xylem and the wall thickness of the xylem elements that was homogeneous. Wang *et al.* (1997) noted that the gibberellins applied to the upper end of the *Pinus sylvestris* L. increased both the elongation and the formation of the floem in the plant. In addition to these observations, researchers noted that IAA + gibberellins also increased elongation, floem formation and xylem formation in the plant.

In the current study, 10, 20, 30 ppm hormone mixtures (IAA + GA<sub>3</sub> + kinetin) were applied to cherry (*C. vulgaris*), and the cambial zone cell line was increased by 9, 28 and 49%, compared to the control, respectively. The difference between 10 ppm H.M. and control was not statistically significant. When we compared 10 and 20 ppm. H.M. with the control and each other, the difference between them was statisti-

cally significant. The cambial zone thickness of 10, 20, 30 ppm H.M. series increased by 9, 34 and 81% compared to the control, respectively. The difference between 10 ppm H.M. and the control with regard to cambial zone thickness was not statistically significant. When we compared 20 and 30 ppm H.M. series with each other and the control, the difference between them was statistically significant. Radial width of cambial zone cells increased by 2, 11 and 23% at the 10, 20, 30 ppm H.M. series, compared to the control, respectively. The difference between 10 ppm, 20 ppm and the control was not statistically significant. The difference between 30 ppm H.M. and the control was statistically significant. The tangential width of the cambial zone cells at 10, 20, 30 ppm H.M. increased by 1, 2 and 3%, compared to the control, respectively. The difference between 10, 20, 30 ppm H.M. series and the control was statistically significant. Even though the results of our studies with the above studies are mostly similar, there were some different points. It is natural, because of the differences in the doses, methods, hormone mixtures and species used in the studies.

Cosgrove (2001) noted that thinning of the cell wall was seen in the expanding cells stimulated by the auxin. Zakrzewski (1983) reported that IAA, GA<sub>3</sub>, kinetin, and sucrose both acted on cambial activity and vascular bundles and that the effect changed, depending on the concentration. The investigator stated that the enhancing effect of IAA and GA<sub>3</sub> on cambial activity could be increased or decreased by kinetin, depending on the season. Chen *et al.* (2010) reported that in the *Populustomentosa* plant, the inactive cambium shows a multilayer structure, has smaller pores and denser microfibrils than the active cambium. In these studies, it was noted that IAA, GA<sub>3</sub>, and kinetin may increase cambial activity, which may decrease microfibrillation in active cambium too. Cosgrove (1997) also noted that the walls of developing cells were different from normal cells in many ways. Generally they were thinner, more easily damaged by mechanical effects, had a different polymer structure and did not have more cross-covalent bonds. In our study, it was seen that the cambial zone cell line and the cambial zone thickness increased in the same way with increasing hormone mixtures in the plants applied 10, 20 and 30 ppm H.M. This showed that cambium activity in the plant increased more in proportion to the increased hormone concentrations. In the current study, the radial wall thickness of the cambial zone cells was reduced by 9, 17 and 19% in the plants treated 10, 20 and 30 ppm H.M., compared to the control. The difference between each one of these series and the control was statistically significant. The difference between the radial wall thicknesses of the plant applied 20 and 30 ppm H.M. was not statistically significant. Tangential wall thickness decreased by 5, 18 and 30%, respectively, in the

plants applied 10, 20 and 30 ppm H.M., compared to the control. The difference between 10 ppm H.M. and the control was not statistically significant with regard to tangential wall thickness. When we compared 20 and 30 ppm H.M. with each other and the control, the difference between them was statistically significant. In our study, the reduction of both radial wall thickness and tangential wall thickness of cambial zonal cells may be due to the reduction of cell wall microfibrils, as well as the increased cambium activity, as expressed above. In the current study, the toxicity of zinc increased in the same way with increased concentrations in 0.1, 0.3, 0.5 and 1 ppm zinc series.

Cambial zone cell line, cambial zone thickness and radial and tangential extent of cambial zone cells decreased, (inversely proportional) with increased zinc concentrations. Each of the 0.1, 0.3, 0.5 and 1 ppm zinc serrations were separately treated with 10, 20 and 30 ppm H.M. The toxic effect of zinc was reduced and was directly proportion to the increased hormone concentration. As in our study, Fatima and Chaudry (2004) applied 50 ppm Pb (NO<sub>3</sub>)<sub>2</sub> (Lead dinitrate), 200 ppm IAA and 200 ppm GA<sub>3</sub> to *C. arietinum* as separate, double and triple combinations. The IAA increased trunk diameter, GA<sub>3</sub> increased trunk extension and IAA + GA<sub>3</sub> increased branching in the trunk. Pb (NO<sub>3</sub>)<sub>2</sub> inhibited the increase in length of the plant when applied alone or in double and triple combination with IAA and GA<sub>3</sub>. In another study, with an increase in the concentration of sodium arsenate (5 μM, 10 μM and 20 μM) there was a significant decrease in seedling length, water content and primary leaf area. The vital pigments like chlorophyll, carotenoid content as well as Hill activity were reduced appreciably in sodium arsenate treated seedlings which indicates poor photosynthetic metabolism. The use of GA<sub>3</sub> and kinetin may, to some extent, help to resist arsenic toxicity in the seedling stage, in arsenic contaminated areas (Swarnakar 2017). In our study, the toxic effect of zinc heavy metal decreased gradually with 10, 20 and 30 ppm H.M. applications (IAA + GA<sub>3</sub> + kinetin), and even completely disappeared. In that study, the toxic effect of Pb (NO<sub>3</sub>)<sub>2</sub> did not disappear by 200 ppm IAA + 200 ppm GA<sub>3</sub>. The reason for this could be the fact that Pb was more toxic than zinc or was applied in greater amounts than zinc.

As a result, hormone mixtures showed positive effects, while zinc showed negative effects on the cambium.

Hormones significantly reduced the harmful effects of zinc. This was due to the positive effects of hormones and the negative effects of zinc on cell division and cell expansion. The reason of the reduced cell line in the cambial zone in the plants treated with zinc solutions can be the stopping of cell division and the continuing differentiation. It has been observed that cell division in zinc + hormone mixtures continues. This suggests

that hormone mixtures are more effective than the harmful effects of zinc. The reason of the reduced radial and tangential wall thicknesses in the cambium cells of the plants with hormone mixtures may be the fact that the hormone loosens the wall structure and prepares the cell to divide. The reason of the increased thickness in the walls may be the fact that zinc stops cell division and causes the cell to differentiate. In the current study, it is difficult to determine if cells that differ from the cambial zone in all plants were added to the flotation or xylem. More detailed research for this is necessary.

## Conclusions

Zinc is an essential micronutrient for plants and humans. It is involved in protein, nucleic acid, carbohydrate, and lipid metabolism. In addition, Zn is critical for the control of gene transcription and the coordination of other biological processes (Bashir *et al.* 2012). Zinc is especially required for the growth of young tissues. Deficiency causes stunting and the formation of small leaves. But if the zinc concentration is  $>120 \text{ mg} \cdot \text{kg}^{-1}$  in plants, zinc has toxic effects. In recent years, zinc has been described as a neurotransmitter. Zinc finger proteins are transcription factors that require zinc. In the current study, the cambial zone cell line in the 10, 20, 30 ppm H. M. series increased by 9, 28 and 49%, respectively, compared with the control. In 0.1, 0.3, 0.5 and 1 ppm Zn concentration series, the cambial zone cell line decreased by 6, 23, 43 and 59%, respectively, compared with control. Thirty ppm H.M. increased the cambial zone thickness by 65, 15, 5% in the 0.1, 0.3 and 0.5 Zn, respectively, compared to the control. The use of zinc in industry and fertilizer is increasing day by day. Considering that zinc is necessary for living, it is understood that this use will continue. From this study it is clear that the uses should be carefully balanced. For this reason, more work is needed to determine the effects of zinc on humans.

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