MELHORANDO A TOLERÂNCIA DE ESTRESSE A FRIO EM *CORYLUS AVELLANA* L. USANDO VÁRIOS COMPOSTOS QUÍMICOS

IMPROVING COLD STRESS TOLERANCE IN *CORYLUS AVELLANA* L. USING VARIOUS CHEMICAL COMPOUNDS

بهبود مقاومت به تنش سرما در *CORYLUS AVELLANA* L. با استفاده از ترکیبات شیمیایی مختلف

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RESUMO

Corylus avellana L., avelã comum, é uma árvore de nozes cultivada globalmente e economicamente valiosa que tem uma sensibilidade a estresses abióticos, especialmente a baixas temperaturas, cuja intensidade varia com base no crescimento e estágio de desenvolvimento. As consequências comerciais do estresse pelo frio na definição de nozes de avelãs são significativas e abordagens práticas para resolver o problema são altamente exigidas. Para enfrentar a questão da intolerância ao estresse pelo frio em *C. avellana* e fornecer aos produtores soluções práticas, este estudo teve como objetivo investigar os efeitos de várias substâncias químicas em árvores de *C. avellana* com dez anos de idade sob estresse pelo frio e suas respostas nos níveis fisiológico e bioquímico em folhas e flores femininas e masculinas. As avelãs foram tratadas com ácido salicílico (3 mg L⁻¹), potássio (2 mg L⁻¹), tiofer (5 mg L⁻¹), biobloom (66 mg L⁻¹) e aminoácido (0,5 mg L⁻¹) em tratamentos separados utilizando a pulverização foliar sob temperatura média de 4,13°C. Pôde-se observar que a aplicação exógena dos compostos afetou a regulação osmótica (aumento do teor de proteína e prolina) e enzimas antioxidantes (superóxido dismutase, catalase, ascorbato peroxidase e glutathione peroxidase). No caso da antocianina e dos pigmentos fotossintéticos, o teor de clorofila a, b, e total nas folhas das árvores tratadas com potássio com 217,38 ± 6,13, 66,23 ± 6,21, 150,66 ± 4,32 e 19,01 ± 2,20 mg g⁻¹ de peso fresco, apresentou as maiores quantidades. As folhas tratadas com ácido salicílico apresentaram o maior teor de carotenóides (289,62 ± 2,41). O mesmo padrão quase pode ser aplicado à gama dessas substâncias em flores masculinas e femininas. Embora todos os cinco usados ajudem as plantas de *C. avellana* a superar melhor o estresse causado pelo frio, o ácido salicílico e o potássio foram os mais eficazes. Pode-se concluir que a ampla aplicação comercial desses compostos químicos para conferir resistência ao frio às avelãs é altamente recomendável.

Palavras-chave: Enzimas antioxidantes, H₂O₂, Prolina, Proteína, Resistência à baixa temperatura

ABSTRACT

Corylus avellana L., common hazelnut, is a globally cultivated economically valuable nut crop tree with a sensitivity to abiotic stresses remarkably low temperature. Its intensity varies based on the growth and developmental stage. The commercial consequences of cold stress on nut setting of hazelnuts are significant and practical approaches to address the problem are highly demanded. To tackle the issue of cold stress intolerance in *C. avellana* and provide producers with practical solutions, this study aimed to investigate the effects of various chemical substances on ten years old *C. avellana* trees under cold stress and its responses at the physiological and biochemical levels in leaves and female and male flowers. Hazelnut trees were treated with salicylic acid (3 mg L⁻¹), potassium (2 mg L⁻¹), thiofer (5 mg L⁻¹), biobloom (66 mg L⁻¹), and amino acid (0.5 mg L⁻¹) in different

treatments using the foliar spray under an average temperature of 4.13°C. It could be observed that the exogenous application of the compounds affected osmotic regulation (enhancement of protein and proline content) and antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, and glutathione peroxidase). In the case of the anthocyanin and photosynthetic pigments, the content of chlorophyll a, b, and total in leaves of trees treated by potassium with 217.38 ± 6.13 , 66.23 ± 6.21 , 150.66 ± 4.32 , and 19.01 ± 2.20 mg g⁻¹ fresh weight, showed the highest quantities. The leaves treated by salicylic acid had the highest content of carotenoid (289.62 ± 2.41). The same pattern can almost be applied to the range of these substances in male and female flowers. Although all five used helps *C. avellana* plants to overcome cold stress better, salicylic acid and potassium were the most effective. It can be concluded that the widespread commercial application of these chemical compounds to confer cold resistance to hazelnut trees is highly recommended.

Keywords: Antioxidative enzymes, H₂O₂, Proline, Protein, Resistance to low temperature

چکیده

Corylus avellana L., فندق معمولی یک درخت آجیلی با ارزش اقتصادی بالا و سطح کشت و کار جهانی بوده که دارای حساسیت به تنش‌های محیطی بخصوص تنش دمای پایین می‌باشد که شدت آن وابسته به رشدی متفاوت است. اثرات اقتصادی تنش سرما به درخت فندق قابل توجه است و ارائه راهکارهای عملی برای رفع این مشکل بسیار مورد توجه و تقاضا قرار دارند. در جهت مقابله با عدم تحمل سرما در *C. avellana* و ارائه تولید کنندگان با راهکارهای عملی، در این مطالعه سعی بر بررسی اثرات ترکیبات شیمیایی مختلف رو درختان ده ساله *C. avellana* تحت تنش سرما داشته و واکنش‌های آن را در سطح فیزیولوژیکی و بیوشیمیایی در برگ، گل‌های نر و ماده را مورد مطالعه قرار گرفت. درختان بطور جداگانه با سالیسیلیک اسید (3 mg L^{-1})، پتاسیم (2 mg L^{-1})، تیوفر (5 mg L^{-1})، بیوبلوم (66 mg L^{-1})، و آمینو اسید (0.5 mg L^{-1}) با اسپری روی برگ تیمار گردیدند. قابل مشاهده است که اعمال خارجی پنج ترکیب تنظیم اسمز (افزایش محتوای پروتئین و پرولین) و آنزیم‌های آنتی‌اکسیدانی (سوپر اکسید دیسموتاز، کاتالاز، آسکوربات پروکسیداز، گلاپکول پروکسیداز) را تحت تاثیر قرار داد. در مورد محتوای آنتوسیانین رنگ‌دانه‌های فتوسنتزی، محتوای کلروفیل a, b و کل در برگ درختان تیمار شده با پتاسیم به ترتیب با 217.38 ± 6.13 , 66.23 ± 6.21 , 150.66 ± 4.32 و 19.01 ± 2.20 mg g⁻¹ وزن تر، از بیشترین میزان ممکن برخوردار بودند. برگ‌های تیمار شده با سالیسیلیک اسید از بیشترین محتوای کارتنوئید (289.62 ± 2.41) برخوردار بودند. الگوی مشابهی را تقریباً می‌توان نسبت به محتوای این ترکیبات در گل‌های نر و ماده درختان تیمار شده اعمال نمود. اگرچه همه ترکیبات اعمال شده رو درختان فندق به آن‌ها در غلبه بر تنش سرما کمک نمود، سالیسیلیک اسید و پتاسیم به‌عنوان موثرترین ترکیبات مشاهده شدند. نتایج به دست آمده از این آزمایش اطلاعات تازه‌ای را در مورد نقش فیزیولوژیکی و بیوشیمیایی این ترکیبات در ایجاد مقاومت به سرما ارائه می‌دهند. می‌توان نتیجه گرفت که استفاده گسترده تجاری این ترکیبات برای ایجاد مقاومت به سرما در درختان فندق به شدت توصیه می‌گردد.

کلیدواژه‌ها: آنزیم‌های آنتی‌اکسیدانی، H₂O₂، پرولین، پروتئین، مقاومت به دمای پایین

1. INTRODUCTION:

Low-temperature stress is one of the principal limiting environmental stresses that can reduce the productivity of crops. Low-temperature stress is classified into chilling (temperatures above 0°C) and freezing (temperatures under 0°C). Chilling stress causes cellular membrane injury, oxidative stress, and a decrease in plant growth. Freezing stress leads to creating ice crystals in the cells (Xin and Browse, 2000; Huang *et al.*, 2014). Cold stress disturbs cellular metabolism in plants. Membrane damage is one of the adverse impacts of cold stress in plants that eventually reduces germination, aging of leaves, and a decrease in crops' growth (Suzuki *et al.*, 2008). Plants have enzymatic and non-enzymatic antioxidant mechanisms to deal with ROS's oxidative stress. These solutes contribute to enzymes, membranes, and other cell constituents' stability, adjust cellular water content, and reduce cellular desiccation (Farooq *et al.*, 2009).

Generally, environmental stresses such as drought, salinity, heavy metals, and high or low-temperature more often than not impose their negative effect by increasing the generation of reactive oxygen species (ROS). Moreover, an imbalance between primary and secondary photosynthesis reactions leads to ROS accumulation in chloroplast and mitochondria under cold stress (Li *et al.*, 2009). These products can directly degrade proteins, amino acids, nucleic acids, and lipids' peroxidation in membranes (Gwozdz *et al.*, 1997). By transferring an electron to oxygen molecular radical superoxide (O²), with the transfer of two electrons to molecular H₂O₂, and by moving three electrons to oxygen, radical hydroxyl oxygen (OH⁻) is generated. Plants have enzymatic and non-enzymatic antioxidant mechanisms to deal with ROS's oxidative stress (Malecka *et al.*, 2012). Examples of the antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) that involve in the defense system and maintain cells from oxidative damage (Mittler, 2002). In plants,

SOD plays a significant role in defending against oxidative stress. This enzyme belongs to metalloenzymes that convert superoxide radicals into oxygen and hydrogen peroxide. GPX is also a protein that preferentially oxidizes electron-giving aromatic compounds in exchange for hydrogen peroxide (Jebara *et al.*, 2005; Sharma *et al.*, 2012). The critical role of CAT in eradicating ROS, in particular, H_2O_2 in mitochondria that are produced during electron transport, β -oxidation of the fatty acids, and, most importantly, photorespiratory oxidation, has been proven by numerous reports (Li *et al.*, 2009; Malecka *et al.*, 2012).

Corylus avellana L. is a monoecious and wind-pollinated broadleaf species that belongs to Betulaceae, which comprises 25 species that 9 are economically cultivated and essential for breeding. Mostly in the form of shrubs and rarely seen as a tree (Qaderi *et al.*, 2012). Hazelnut is one of the most important nut products globally, which is used as a fruit and has various applications in the food industry and benefits health. Hazelnut fruit is an essential source of vitamin B6 and oleic acid (83 %). It's a rich source of other critical components, including protein, carbohydrates, fiber, vitamins (vitamin E), minerals, phytosterols, mainly cytosterols and antioxidant phenols that can protect the body against diseases (Seyhan *et al.*, 2007; Oliveira *et al.*, 2008; Alasalvar *et al.*, 2012;). Traditionally, the public often views hazelnuts as an unhealthy food because of their high-fat content. However, recent epidemiological and clinical studies have shown that nuts' consumption leads to optimal plasmid lipid levels (Gürcan *et al.*, 2010).

As in the ancient Chinese scripts, the history of its cultivation has shown it goes back to 8000-5000 years ago. In Europe, hazelnut is of the predominant species; however, its exact origin is not clear. This species has a wide distribution range from Portugal's coast to Ireland, and its northern extension goes from Norway to Russia. Still, the main areas of cultivation are proximity to large water bodies with mild winters and cool summers. The main areas of cultivation are Turkey, which is under the influence of the Black Sea, Italy, and Spain due to being close to the Mediterranean Sea and the United States under the Pacific Ocean's influence, owing to geographical features. The countries mentioned above are significant producers of hazelnut (Lunde *et al.*, 2006; Mehlenbacher and Smith 2006; Boccacci and Botta 2009; Gürcan *et al.*, 2010), and Iran, with 14,000 tons production from 18,000 hectares, ranked eighth in the world.

Because *C. avellana* has a low tolerance to heat, cold, drought, and wind stress (Larcher, 2003; Salimi and Hoseinova *et al.*, 2012; Arzanlou *et al.*, 2018). To date, studies on reducing the effect of abiotic stress, particularly cold stress, as one of the critical limiting factors, can increase the yield per hectare.

Plants respond to environmental and biotic stresses by synthesizing signaling molecules. These signal molecules activate the signaling pathways. Several signals or signaling molecules have been identified in plants, including calcium, jasmonic acid, ethylene, salicylic acid (SA), and hydrogen peroxide (H_2O_2). The application of several compounds, such as SA or Potassium (K) is an essential tool for increasing crop production under abiotic stress. Numerous works exhibited that utilizing various compounds reduced the negative impacts of the abiotic stresses in plants (Kaczmarek *et al.*, 2017; Shaki *et al.*, 2017; Rezayian *et al.*, 2018). The role of SA as a defense signal in plants is now proven. SA has also been introduced as a plant hormone due to its substantial roles in the plant (Wilson 1996; Zhang *et al.*, 2007). Over the past 20 years, researchers have paid particular attention to the ability of SA to induce protective substances in plants under environmental stress. There are reports of the role of SA in increasing salinity resistance in wheat, rice, and cucumber, heat and cold in tomatoes and beans, and heavy metals in rice (Zhang *et al.*, 2007; Taşgîn *et al.*, 2003). SA regulates the development and expression of senescence genes in Arabidopsis (Morris *et al.*, 2000). The impact of SA in resistance to cold stress and possibly through its effect on enzymatic antioxidants and metabolism of H_2O_2 , reducing cold stress damage and increasing plant tolerance to cold stress. Efficacy of SA in induction stress tolerance, depending on plant type or concentration of SA. exogenous application of SA, increases low-temperature tolerance (Khan *et al.*, 2012; Miura and Tada, 2014).

Potassium (K) has a significant effect on plant responses and tolerance to environmental stresses (Hasanuzzaman *et al.*, 2018). Zhang *et al.* (2014) showed that exogenous K application reduced the adverse effects of stress in *Zea mays* L. Proline application diminished stress-induced inhibitory impact on wheat growth (Bekka *et al.*, 2018). Proline, as an essential osmolyte, plays a crucial role in modulating the osmotic pressure of cells under stresses such as soil salinity, drought, low temperatures, nutrient deficiencies, heavy metal exposure, and high acidity. High production of proline inhibits cold stress damage on the

normal cellular process by increasing intracellular osmotic pressure. This increase in proline levels persists for about a month, even after the stress conditions have been resolved. The positive role of proline in modulating osmotic pressure relative to low-temperature, drought, and salinity has been reported by researchers in various plants such as maize (Chen and Li 1979), alfalfa (Liu *et al.*, 2009), and Arabidopsis (Khavari-Nejad *et al.*, 2013). Proline influenced cryoprotectant under chilling stress (Gleeson *et al.*, 2004). α -aminobutyric acid treatment mitigated the injury induced by chilling stress in *Lycopersicon esculentum* L. (Shang *et al.*, 2011). Given K's significant effect on protecting plants against cold stress, there is fertilizer so-called Bio bloom contains more than 85% of organic matter, high amounts of phosphorus, and potassium. In addition to affecting the rate of metabolism and the structure of living membranes, organic Bio bloom regulates the membrane's fluidity by creating stability and balance of certain types of fatty acids so that proteins and enzymes can function correctly. Potassium is involved in the regulation of osmosis and the prevention of ice formation that can damage energy-carrying molecules (Rezayian *et al.*, 2018).

One of the essential organic antifreeze is called Thiofer. It contains various Thiobacillus bacteria, minerals, and proteins and can increase the resistance of plants to stress such as heat, cold, and frost (Stipešević *et al.*, 2014). The application of this substance at the right time causes the plant to produce antifreeze protein (AFP) and antifreeze amino acids (AAA). It increases the plant's resistance to cold and frost. This substance is systemic and penetrates plant tissues and prevents ice formation (Balesini *et al.*, 2013).

Therefore, this study aimed to evaluate the ameliorating influence of K, SA, thiofer, amino acids, and Bio Bloom on the physiological and biochemical responses of hazelnut trees. The primary purpose was to provide a practical solution to a very limiting environmental condition that jeopardizes the hazelnut orchards of Iran located in mountainous areas. Considering the extent of this study, this is the first comprehensive research on improving low-temperature tolerance in hazelnut to the best of our knowledge.

2. MATERIALS AND METHODS:

2.1. Plant materials

This research was conducted at the Agricultural and Natural Resources Research

Center of Qazvin Province (East of Alamut) in 2016 (October)-2017 (March). The average temperature in this area was 4.13 °C. Various compounds [SA (3 mg L⁻¹), potassium (2 mg L⁻¹), thiofer (5 mg L⁻¹), biobloom (66 mg L⁻¹), and amino acid (0.5 mg L⁻¹)] were sprayed uniformly on 10 years old hazelnut trees using an atomizer four times for 18 months (Figure 1). After treatments, the leaves were harvested and stored at -70 °C and analyzed the following parameters (Figure 2).

2.2. Antioxidant enzymes assay

For the measurement of enzymes activities, fresh material was extracted at 4 °C in 1 M Tris-HCl (pH 6.8) and centrifuged at 13000 rpm for 30 min. Supernatants were kept at -70 °C and used for enzymes assay. The content of protein was measured by Bradford (1976) using bovine serum albumin as the standard.

SOD activity was assayed based on the Giannopolitis and Ries (1977) method. Reaction solution comprised sodium phosphate buffer (50 mM), 0.1 mM EDTA, 13 mM methionine, 75 μ M nitroblue tetrazolium (NBT), 75 μ M riboflavin and 100 μ L of enzyme extract. The reaction solution was placed in front of the light for 18 minutes, and then absorbance was measured at 560 nm.

CAT activity was determined by the method of Aebi (1984). Assay mixture contained 50 mM phosphate buffer (pH 7.0), H₂O₂; 3% and 10 μ L enzyme extract. The decline in absorption was followed for 180s, and CAT activity was expressed as units per mg of protein.

Ascorbate peroxidase (APX) activity was determined by Jebara *et al.*, (2005) method. Reaction mixture comprised ascorbic acid (0.5 mM), 50 mM potassium phosphate buffer (pH 7.0), H₂O₂ (0.1 mM) and 10 μ L of enzyme extract. In this reaction, ascorbate was oxidized and the concentration of oxidized ascorbate was determined by the decrease in absorbance at 290 nm. One unit of APX was defined as 1 μ M oxidized ascorbate per min per mg protein.

Glutathione peroxidase (GPX) activity was assayed using the method of Lin and Kao (1999). Reaction mixture (1.0 mL) comprised 50 mM sodium phosphate buffer (pH 7.0), 9 mM glutathione, 19 mM H₂O₂ and protein extract. After the addition of protein extract, the increase in absorbance at 470 nm was recorded for 1 min.

2.3. Hydrogen peroxide

The H₂O₂ content was estimated via Velikova *et al.* (2000) method. Leaf tissue (0.3 g)

was homogenized in 0.1% Trichloroacetic acid (TCA) then was centrifuged at 12000 rpm for 15 min. The supernatant (0.5 mL) was added to 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml potassium iodide (1 M), and absorbance was recorded by spectrophotometer at 390 nm.

2.4. Determination of Proline

Proline content was measured, according to Bates *et al.* (1973). Fresh tissues were homogenized in 5 mL sulfosalicylic acid (3%) and then centrifuged at 13000 rpm for 20 min. Two mL of supernatant was mixed with acid ninhydrin (2 mL) and glacial acetic acid (2 mL) and then was boiled at 100°C for one hour. The reaction mixture was extracted with 4 mL toluene, and the absorbance was recorded at 520 nm.

2.5. Measurement of anthocyanin and pigments content

Anthocyanin content was determined in 0.3% HCl in methanol at 25°C using the extinction coefficient ($33 \text{ cm}^2 \text{ mol}^{-1}$) at 550 nm (Wagner, 1979). Chlorophyll a (Chl a), chlorophyll b (Chl b) chlorophyll total (Chl T), and carotenoids were extracted by 80 % acetone and quantified spectrophotometrically (UNFCO-2100 model) according to Lichtenthaler and Wellburn (1983) and calculated based on the following formula:

$$\text{Chl. a } (\mu\text{g/g}) = 12.25 A_{663} - 2.79 A_{646} \times V/W \quad (1)$$

$$\text{Chl. b } (\mu\text{g/g}) = 21.50 A_{646} - 5.1 A_{663} \times V/W \quad (2)$$

$$\text{Chl. T. } (\mu\text{g/g}) = \text{Chl. a} + \text{Chl. b} \quad (3)$$

$$\text{Carotenoid } (\mu\text{g/g}) = (1000A_{470} - 1.82\text{Chl.a} - 85.02\text{Chl.b})/198 \times V/W \quad (4)$$

Where A_{663} , A_{646} , A_{470} are absorptions at wavelengths of 663, 646, and 470 nm. The final results were expressed as mg g^{-1} fresh weight (FW).

2.6. Protein extraction and 2-DE

Proteins were extracted using the method described by Damerval *et al.* (1986). 500 mg of fresh material was homogenized in liquid nitrogen using a mortar and pestle. The resulting powder was placed in a 2-mL microtube, and 1 mL of extraction buffer [10% TCA in acetone] was added. The samples were incubated for 60 min at 4°C and then centrifuged at 16000 rpm for 30 min. The supernatant was discarded, and the pellet was washed three times in cold acetone with 20 mM DTT. Subsequently, the pellets were resuspended in 1 mL of buffer containing 7M urea, 2M thiourea, 1% DTT, 2% Triton-100, and 1mM

phenylmethanesulfonyl fluoride and stirred for 60 min at 4°C until the samples were resuspended entirely. The samples were incubated on ice for 30 min and then centrifuged at 16000 rpm for 10 min. Gels were visualized by Coomassie Brilliant Blue G-25. The gels were scanned using a GS-800 densitometer (BioRad) and analyzed using Melanie 7 software (GeneBio).

2.7. Statistical analysis

The experiment was designed in a six-factorial complete randomized experiment with three replications per treatment. The experimental data were subjected to one-way ANOVA, and significant differences between means were determined by Duncan multiple rang ($P < 0.05$).

3. RESULTS AND DISCUSSION:

Plants need to an optimum temperature for appropriate growth and development. Low temperature is one of the environmental stresses that lead to a reduction in yield (Bray *et al.*, 2000). Various physiological parameters were measured in male and female flowers and leaves. SA and potassium treatment increased protein content in leaf, but the other four treatments declined these parameters. While treatments enhanced protein content in male and female flowers (Table 1).

Proteomics analysis was carried out for leaf and two types of flowers under SA treatment which the results of this analysis indicated a fluctuation in protein in trees treated by SA. Foliar application of SA enhanced the proteins 122, 147, 117, 34, 113, 31, 114, 16 and 89 in leaf, but reduced the proteins 38, 4, 74, 3, 58, 71, 57, 64, 102, 46, 110, 137 and 177. The proteins 103, 47, 75, 32, 180, 25, 115 and 37 showed a significant increase in female flowers under SA treatment, whereas the proteins 157, 38, 3, 4, 41, 30, 73, 106, 46, 71, 65, 62, 63 and 179 declined. SA treatment-induced protein 32 and decreased in proteins 41 and 62 (Figures 3 to 8). One of the key responses in increasing freezing tolerance during cold acclimation is the synthesis of particular proteins (Antikainen *et al.*, 1996). Our results showed that SA and potassium application improved the protein content of *C. avellana* and enabled the possibility of overcoming cold stress. SA prevents protein destruction by neutralizing free radicals and leads to an increase in protein content (Mellouk *et al.*, 2016). These findings are in agreement with Tasgin *et al.* (2003) and Farooq *et al.* (2008). By its involvements in osmoregulation, energy source, osmoprotectant, and ROS scavenger, proline plays an important role in

tolerance to abiotic stresses (Trovato *et al.*, 2008). Proline accumulation is a response in plants under cold stress that maintains plants from dryness, causing cold stress by decreasing plant cells' water potential (Szekely *et al.*, 2008). Liu *et al.* (2008) showed that proline content increased in *Avena nuda* L. under cold stress. In our study, all treatments enhanced proline accumulation in *C. avellana* plants. This compound is a compatible osmolyte that causes osmotic regulation and osmoprotectants under stress. These data show that these compounds help to *C. avellana* to overcome stress via osmolytes accumulation better. These results are in agreement with Misra and Saxena (2009), Ali *et al.* (2014), and Bekka *et al.* (2018). Apoplastic water losses in plants due to freezing under freezing stress lead to dehydration, and an appropriate K amount regulates the osmotic potential and declines dehydration under freezing (Wang *et al.*, 2013).

The alteration in the activities of antioxidant enzymes, including SOD, CAT, APX, and GPX, showed different trends under various treatments. SOD activity was not changed in the treated three tissues. Potassium, SA, and biobloom application improved CAT activity in leaf; however, the effect of SA was more prominent (3-fold). Five types of treatments induced CAT activity in female flowers, but potassium, thiofer, and amino acid increased this enzyme activity in male flowers. Biobloom treatment only enhanced (1.23-fold) APX activity in leaf. Exogenous application of thiofer, biobloom, and amino acid promoted APX activity in male flowers, whereas potassium caused its reduction. All treatments decreased or did not affect APX activity in female flowers. GPX activity remarkably increased in leaf by SA, potassium, and amino acid treatments. Exogenously applied all chemical compounds induced GPX activity in male flowers, and this increase by amino acid was more than other treatments. In contrast to male flowers, most treatments declined GPX activity in female flowers (Table 1).

Potassium, thiofer, and amino acid application lessened H₂O₂ content in leaf. A relatively higher accumulation of H₂O₂ occurred in the plants exposed to biobloom. SA, potassium, and biobloom declined H₂O₂ content in male flowers by 12.4, 30.7, and 30.71%, respectively. All treatments significantly affected the content of H₂O₂ in female flowers (Table 1).

Proline showed a sharp increase in the leaf of trees received the foliar application of chemical compounds. SA and biobloom application induced the accumulation of proline (58.33%) when compared to control. Proline content significantly

increased by SA, thiofer, and amino acid treatments in female flowers, but other treatments had little effect on this parameter. All treatment, except potassium, caused proline accumulation in male flowers (Table 2). Abiotic stresses increase the ROS production in plants, comprising superoxide anion radicals, hydroxyl radicals, H₂O₂, alkoxy radicals, and singlet oxygen. ROS causes adverse effects on proteins, lipids, and DNA, thus disrupts cell function (Foyer and Fletcher, 2001; Munné-Bosch and Penuelas, 2003). The enzymatic antioxidant defense system leads to ROS degradation and has a primary role in maintaining cell homeostasis. SOD leads to the dismutation of superoxide radicals to oxygen and H₂O₂. APX is an enzyme that plays a prominent role in the alteration of H₂O₂ into H₂O by ascorbate, such as an electron donor (Rosa *et al.*, 2010). Cold stress boosted CAT activity in some wheat cultivars (Javadian *et al.*, 2010). Fahimirad *et al.* (2013) indicated that antioxidative enzymes enhanced in canola under cold stress. High activities of antioxidant enzymes are essential for abiotic stress tolerance. The treatments caused a reduction of H₂O₂ and an increase of antioxidant enzymes in *C. avellana*; however, the oxidative injury was increased by the application of compounds simultaneously. According to our results, these treatments' enhancement of antioxidant enzymes may play an important role in cold tolerance. Similar results have been published in barley (Mutlu *et al.*, 2013), Lettuce (Shams *et al.*, 2016), and *Triticum aestivum* L. (Jan *et al.*, 2017).

Anthocyanin content substantially enhanced using potassium, thiofer, and amino acid treatments in leaf. The highest anthocyanin content in leaf was observed in potassium treated plants, of which 31.92% higher as compared to the control. Anthocyanin content was not shown a significant change in male and female flowers under different treatments (Table 2).

Carotenoid content notably increased by 36.9% in leaves exposed to SA, but it did not change in treated leaves with other treatments. All five compounds applied in this study affect carotenoid content in male flowers, and the highest increase was (81%) in SA-treated plants (Table 2).

The exogenous application of thiofer, K, SA, and amino acid enhanced Chl a, Chl b, and Chl T contents in leaf. Chl content was much higher in SA-treated plants than the plants exposed to other treatments. SA application only triggered a significant increase (1.23%) in Chl content in female flowers. In contrast to Chl a, Chl b and Chl

T contents improved by five treatments, and the thiofer was more effective than the other compounds. Chl a and Chl T content were not induced in male flowers under all treatments, whereas Chl b enhanced by potassium and biobloom (Table 2). Anthocyanins are types of water-soluble pigments derived from flavonoids that synthesis of the shikimic acid pathway. Plants generate anthocyanins under stress for cell defense (Chutipaijit *et al.*, 2009). Potassium, amino acid, and thiofer induced accumulation of anthocyanin in *C. avellana*, which was positively related to antioxidant capacity. So, these treatments can help this plant to better cope with cold stress. Our results are in agreement with other previous reports (Amira *et al.*, 2017; Kocira, 2019).

Low temperature leads to disruption in the photosynthetic apparatus by photo-inhibition (Huang *et al.*, 2014). Li *et al.* (2018) displayed that cold stress declined Chl content in zoysiagrass genotypes. Tewari and Tripathy (1998) described that a decline in Chl biosynthesis in plants under cold stress is due to 5-aminolevulinic acid biosynthesis inhibition. The applied compounds mainly enhanced the Chl content in *C. avellana*. An increase in Chl content as the critical photosynthetic pigment in plants could improve photosynthetic activity. The present research data were in agreement with the results of previous studies (Hussein *et al.*, 2014; Fayez and Bazaid, 2014; Shaki *et al.*, 2018). Carotenoids are the first line of defense against $^1\text{O}_2$ toxicity and can stabilize membranes and preventing lipid peroxidation by their antioxidant activity (Niyogi, 1999). SA treatment alleviated the effect of cold tolerance in *C. avellana* by the increase of carotenoid content.

4. CONCLUSIONS:

Mitigating compounds can alleviate the adverse effects of cold stress on *C. avellana* by improving antioxidant capacity, accumulating proline, and increasing the content of photosynthetic pigments. Although all five applied compounds in this study have the potential to be stress protectants, SA and potassium were the most effective in which spraying K markedly enhanced the content of protein, proline, antioxidant enzymes (SOD, CAT, APX, and GPX). While SA was found to have the highest positive impact on increasing the content of anthocyanin, chlorophyll a, b, and total in leaves and male and female flowers. Additionally, 2-D gel results for the influence of SA on the protein profile of leaves, and male and female flowers indicated enhancing

some specific proteins while caused a decrease in some others. This study revealed applicable critical results to protect hazelnut trees against cold stress. However, give the complicated biological mechanisms involved, further research using a broad spectrum of low-temperature stress treatments are highly recommended to uncover the physiological responses of *C. avellana*.

5. REFERENCES:

1. Aebi H. (1984). Catalase *in vitro*. *Methods Enzymology*, 105:121-126.
2. Ali, M; Bakht, J; Khan, G. D. (2014). Effect of water deficiency and potassium application on plant growth, osmolytes, and grain yield of Brassica napus cultivars. *Acta Botanica Croatica*, 73(2), 299-314.
3. Alasalvar, C; Pelvan, E; Bahar, B; Korel, F; Ölmez, H. (2012). Flavour of natural and roasted Turkish hazelnut varieties (*Corylus avellana* L.) by descriptive sensory analysis, electronic nose and chemometrics. *International journal of food science and technology*, 47(1), 122-131.
4. Antikainen, M; Griffith, M; Zhang, J; Hon, W. C; Yang, D. S; Pihakaski-Maunsbach, K. (1996). Immunolocalization of antifreeze proteins in winter rye leaves, crowns, and roots by tissue printing. *Plant Physiology*, 110(3), 845-857.
5. Arzanlou, M; Torbati, M; Golmohammadi, H. (2018). Powdery mildew on hazelnut (*Corylus avellana*) caused by Erysiphe corylacearum in Iran. *Forest Pathology*, 48(5), e12450.
6. Bates, L. S; Waldren, R. P; Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, 39, 205-207.
7. Bekka, S; Abrous-Belbachir, O; Djebbar, R. (2018). Effects of exogenous proline on the physiological characteristics of Triticum aestivum L. and Lens culinaris Medik. under drought stress. *Acta agriculturae Slovenica*, 111(2), 477-491.
8. Bray, E. A. (2000). Response to abiotic stress. *Biochemistry and molecular biology of plants*, 1158-1203.
9. Boccacci, P; Botta, R. (2009). Investigating the origin of hazelnut (*Corylus avellana* L.) cultivars using chloroplast microsatellites. *Genetic Resources and Crop Evolution*, 56(6), 851-859.
10. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram

- quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254.
11. Chen, W. P., and Li, P. H. (2002). Membrane stabilization by abscisic acid under cold aids proline in alleviating chilling injury in maize (*Zea mays* L.) cultured cells. *Plant, Cell and Environment*, 25(8), 955-962.
 12. Chutipaijit, S; Cha-Um, S; Sompornpailin, K. (2009). Differential accumulations of proline and flavonoids in indica rice varieties against salinity. *Pakistan Journal of Botany*, 41(5), 2497-2506.
 13. Damerval, C; De Vienne, D; Zivy, M; Thiellement, H. (1986). Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. *Electrophoresis*, 7(1), 52-54.
 14. Farooq, M; Basra, S. M. A; Rehman, H; Saleem, B. A. (2008). Seed priming enhances the performance of late sown wheat (*Triticum aestivum* L.) by improving chilling tolerance. *Journal of Agronomy and Crop Science*, 194(1), 55-60.
 15. Farooq, M; Wahid, A; Kobayashi, N; Fujita, D. B. S. M. A; Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. In *Sustainable agriculture* (pp. 153-188). Springer, Dordrecht.
 16. Fayez, K. A; Bazaid, S. A. (2014). Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. *Journal of the Saudi Society of Agricultural Sciences*, 13(1), 45-55.
 17. Foyer, C. H; Fletcher, J. M. (2001). Plant antioxidants: colour me healthy. *Biologist (London, England)*, 48(3), 115.
 18. Giannopolitis, C. N; Ries, S. K. (1977). Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. *Plant physiology*, 59(2), 315-318.
 19. Gürcan, K; Mehlenbacher, SA; Botta, R; Boccacci, P. (2010). Development, characterization, segregation, and mapping of microsatellite markers for European hazelnut (*Corylus avellana* L.) from enriched genomic libraries and usefulness in genetic diversity studies. *Tree Genetics and Genomes*, 6(4), 513-531.
 20. Gwóźdz, EA; Przymusiński, R; Rucińska, R; Deckert, J. (1997). Plant cell responses to heavy metals: molecular and physiological aspects. *Acta Physiologiae Plantarum*, 19: 459-465.
 21. Hasanuzzaman, M; Bhuyan, M. H. M; Nahar, K; Hossain, M; Mahmud, J. A; Hossen, M; Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*, 8(3), 31.
 22. Hegazi A. M; El-Shraiy, A. M (2017). Stimulation of Photosynthetic Pigments, Anthocyanin, Antioxidant Enzymes in Salt Stressed Red Cabbage Plants by Ascorbic Acid and Potassium Silicate. *Middle East Journal*, 6:553-68.
 23. Huang, B; DaCosta, M; Jiang, Y. (2014). Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Critical reviews in plant sciences*, 33(2-3), 141-189.
 24. Hussein, M. M; Mehanna, H; Zaki, S. N; Nagwan, F. A. H. (2014). Influences of salt stress and foliar fertilizers on growth, chlorophyll and carotenoids of jojoba plants. *Middle East Journal of Agricultural Research*, 3, 221-226.
 25. Hughes, M. A; Dunn, M. A. (1996). The molecular biology of plant acclimation to low temperature. *Journal of Experimental Botany*, 47(3), 291-305.
 26. Javadian, N; Karimzadeh, G; Mahfoozi, S; Ghanati, F. (2010). Cold-induced changes of enzymes, proline, carbohydrates, and chlorophyll in wheat. *Russian Journal of Plant Physiology*, 57(4), 540-547.
 27. Jan A. U; Hadi, F; Nawaz, M. A; Rahman, K. (2017). Potassium and zinc increase tolerance to salt stress in wheat (*Triticum aestivum* L.). *Plant Physiology and Biochemistry*, 116:139-149.
 28. Jebara, S; Jebara, M; Limam, F; Aouani, M. E. (2005). Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. *Journal of plant physiology*, 162(8), 929-936.
 29. Kaczmarek, M; Fedorowicz-Strońska, O; Głowacka, K; Waśkiewicz, A; Sadowski, J. (2017). CaCl₂ treatment improves drought stress tolerance in barley (*Hordeum vulgare* L.). *Acta Physiologiae Plantarum*, 39(1), 41.
 30. Kang, G; Li, G; Xu, W; Peng, X; Han, Q; Zhu, Y; Guo, T. (2012). Proteomics reveals the effects of salicylic acid on growth and

- tolerance to subsequent drought stress in wheat. *Journal of Proteome Research*, 11(12), 6066-6079.
31. Khavari-Nejad, RA; Band, RS; Najafi, F; Nabiuni, M; Gharari, Z. (2013). The role of Pro-P5C Cycle in chs mutants of Arabidopsis under cold stress. *Russian journal of plant physiology*, 60(3), 375-382.
 32. Kocira, S. (2019). Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chilean journal of agricultural research*, 79(1), 17-25.
 33. Larcher, W. (2003). *Physiological plant ecology: ecophysiology and stress physiology of functional groups*. Springer Science and Business Media.
 34. Li, Z; Wakao, S; Fischer, B. B; Niyogi, K. K. (2009). Sensing and responding to Excess light. *Annual review of plant biology*, 60, 239-260.
 35. Li, S; Yang, Y; Zhang, Q; Liu, N; Xu, Q; Hu, L. (2018). Differential physiological and metabolic response to low temperature in two zoysiagrass genotypes native to high and low latitude. *PLoS One*, 13(6), e0198885.
 36. Lichtenthaler, H. K; Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 603, 591-593.
 37. Lin, C. C; Kao, C. H. (1999). NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings. *Plant and Soil*, 216(1-2), 147.
 38. Liu, W; Yu, K; He, T; Li, F; Zhang, D; Liu, J. (2013). The low temperature induced physiological responses of *Avena nuda* L., a cold-tolerant plant species. *The Scientific World Journal*, 12, 451-457.
 39. Liu, L; Chen, LB; Li, ZY; Wang, MZ; Guo, SJ. (2009). Effect of temperature in late autumn on free proline, soluble sugar and POD in alfalfa. *Pratacultural Science*, 10: 326-335.
 40. Mehlenbacher, SA; Smith, DC. (2006). Self-compatible seedlings of the cutleaf hazelnut. *HortScience*, 41(9), 482-483.
 41. Malecka, A; Piechalak, A; Mensinger, A; Hanć, A; Baralkiewicz, D; Tomaszewska, B. (2012). Antioxidative defense system in *Pisum sativum* roots exposed to heavy metals (Pb, Cu, Cd, Zn). *Polish Journal of Environmental Studies*, 21, 16-25.
 42. Mellouk, Z; Benammar, I; Hernandez, Y. (2016). Effects of foliar application with salicylic acid on the biochemical parameters and redox status in two canola plant varieties exposed to cold stress. *International Journal of Agronomy and Agricultural Research*, 8, 77-87.
 43. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, 7(9), 405-410.
 44. Misra, N; Saxena, P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science*, 177(3), 181-189.
 45. Miura, K; Tada, Y. (2014). Regulation of water, salinity, and cold stress responses by salicylic acid. *Frontiers in plant science*, 5, 4.
 46. Morris, K; Mackerness, SAH; Page, T; John, CF; Murphy, AM; Carr, JP; Buchanan-Wollaston, V. (2000). Salicylic acid has a role in regulating gene expression during leaf senescence. *The Plant Journal*, 23: 677-685.
 47. Munne-Bosch, S; Penuelas, J. (2003). Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta*, 217(5), 758-766.
 48. Mutlu, S; Karadağoglu, Ö; Atici, Ö; Nalbantoğlu, B. (2013). Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. *Biologia Plantarum*, 57(3), 507-513.
 49. Niyogi, K. K. (1999). Photoprotection revisited: genetic and molecular approaches. *Annual review of plant biology*, 50(1), 333-359.
 50. Oliveira, I; Sousa, A; Morais, JS; Ferreira, IC; Bento, A; Estevinho, L; Pereira, J. A. (2008). Chemical composition, and antioxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L.) cultivars. *Food and Chemical Toxicology*, 46(5), 1801-1807.
 51. Rezayian, M; Niknam, V; Ebrahimzadeh, H. (2018). Improving tolerance against drought in canola by penconazole and calcium. *Pesticide biochemistry and physiology*, 149, 123-136.
 52. Rosa, S. B; Caverzan, A; Teixeira, F. K; Lazzarotto, F; Silveira, J. A; Ferreira-Silva, S. L; Margis-Pinheiro, M. (2010). Cytosolic APx knockdown indicates an ambiguous redox responses in rice. *Phytochemistry*, 71(5-6), 548-558.

53. Seyhan, F; Ozay, G; Saklar, S; Ertaş, E; Satır, G; Alasalvar, C. (2007). Chemical changes of three native Turkish hazelnut varieties (*Corylus avellana* L.) during fruit development. *Food Chemistry*, 105(2), 590-596.
54. Shaki, F; Ebrahimzadeh Maboud, H; Niknam, V. (2017). Central role of salicylic acid in resistance of safflower (*Carthamus tinctorius* L.) against salinity. *Journal of plant interactions*, 12(1), 414-420.
55. Salimi, S; Hoseinova, S. (2012). Selecting hazelnut (*Corylus avellana* L.) rootstocks for different climatic conditions of Iran. *Crop Breeding Journal*. 2(2), 139-144
56. Shang, H; Cao, S; Yang, Z; Cai, Y; Zheng, Y. (2011). Effect of exogenous γ -aminobutyric acid treatment on proline accumulation and chilling injury in peach fruit after long-term cold storage. *Journal of agricultural and food chemistry*, 59(4), 1264-1268.
57. Shams, M; Yildirim, E; Ekinçi, M; Turan, M; Dursun, A; Parlakova, F; Kul, R. (2016). Exogenously applied glycine betaine regulates some chemical characteristics and antioxidative defence systems in lettuce under salt stress. *Horticulture, Environment, and Biotechnology*, 57(3), 225-231.
58. Sharma, P; Jha, A. B; Dubey, R. S; Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*.
59. Stipešević, B; Brozović, B; Jug, D; Jug, I; Ranogajec, L; Šego, D. (2014). Economic comparison of different cropping systems for niger (*Guizotia abyssinica*) in Croatia. In *Proceedings of TEAM 2014 6 th International Scientific and Expert Conference of the International TEAM Society 10–11 th November 2014, Kecskemét, Hungary* (p. 81).
60. Suzuki, K; Nagasuga, K; Okada, M. (2008). The chilling injury induced by high root temperature in the leaves of rice seedlings. *Plant and Cell Physiology*, 49(3), 433-442.
61. Szekely, G; Abrahám, E; Cséplő, A., Rigó, G; Zsigmond, L; Csiszár, J; Strizhov, N. J; ásik, J; Schmelzer, E; Koncz, C; Szabados, L. (2008). Duplicated P5CS genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant Journal* 53, 11-28.
62. Taşgín, E; Atıcı, Ö; Nalbantoğlu, B. (2003). Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. *Plant Growth Regulation*, 41(3), 231-236.
63. Trovato, M; Mattioli, R; Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei*, 19(4), 325-346.
64. Qaderi, A; Omid, M; Etminan, A; Oladzad, A; Ebrahimi, C; Dehghani, M. M; Mehrafarin, A. (2012). Hazel (*Corylus avellana* L.) as a new source of taxol and taxanes. *Journal of Medicinal Plants* 1:66-77.
65. Velikova, V; Yordanov, I; Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant science*, 151(1), 59-66.
66. Wang, M; Zheng, Q; Shen, Q; Guo, S. (2013). The critical role of potassium in plant stress response. *International journal of molecular sciences*, 14(4), 7370-7390.
67. Wilson, JM. (1996). The Mechanism of chill and drought hardiness. *New Physiologist*, 97: 257-270.
68. Xin, Z; Browse, J. (2000). Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant, Cell, and Environment*, 23(9), 893-902.
69. Zhang, L; Gao, M; Li, S; Alva, A. K; Ashraf, M. (2014). Potassium fertilization mitigates the adverse effects of drought on selected *Zea mays* cultivars. *Turkish Journal of Botany*, 38(4), 713-723.
70. Zhang, HQ; Zou, YB; Xiao, GC; Xiong, YF. (2007). Effect and Mechanism of Cold Tolerant Seed-Coating Agents on the Cold Tolerance of Early *Indica* Rice Seedlings. *Agricultural Sciences in China*, 6: 792-801.

Figure 1. The emergence of male (long catkins) and female (small reddish-purple florets) flowers of treated *Corylus avellana* tree.

Figure 2. Assays for the content of enzymatic antioxidants after bain-marie.

Figure 3. 2-D gel of proteins extracted from the leaf in the control condition.

Figure 4. 2-D gel of proteins extracted from leaf under salicylic acid (SA) treatment.

Figure 5. 2-D gel of proteins extracted from male flowers in the control condition.

Figure 6. 2-D gel of proteins extracted from male flowers under salicylic acid (SA) treatment.

Figure 7. 2-D gel of proteins extracted from female flowers in the control condition.

Figure 8. 2-D gel of proteins extracted from female flowers under salicylic acid (SA) treatment.

Table 1. Effects of different treatments on protein, H₂O₂, and antioxidative enzymes in *Corylus avellana*.

Treatment	Tissue	Protein	H ₂ O ₂	SOD	CAT	APX	GPX
Control	Leaf	1±0.02 b	0.58±0.23 b	98.29±2.12 a	0.01±0.00 c	1.85±0.1 bc	0.82±0.09 d
Potassium		1.10±0.01 a	0.30±0.022 e	98.26±4.32 a	0.01±0.00 c	1.75±0.1 cd	1.66±0.11 a
Thiofer		0.92±0.11 d	0.53±0.056 c	98.36±2.13 a	0.01±0.00 c	1.65±0.02 d	0.67±0.30 e
Biobloom		0.98±0.04 c	0.96±0.02 a	98.38±2.11 a	0.03±0.00 b	2.28±0.14 a	0.44±0.01 f
Amino acid		0.97±0.04 c	0.47±0.036 d	98.34±1.12 a	0.009±0.001d	1.43±0.11 e	1.12±0.30 b
Salicylic acid		1.10±0.05 a	0.60±0.04 b	98.53±2.21 a	0.04±0.01 a	1.91±0.14 b	1.02±0.40 c
Control	Male flowers	1.17±0.01 c	0.50±0.02 b	98.51±2.22 a	0.14±0.01 b	1.54±0.3 bc	0.96±0.6 1d
Potassium		1.38±0.11 b	0.35±0.021 d	98.56±1.14 a	0.16±0.01 a	1.32±0.45 c	1.66±0.10 a
Thiofer		1.44±0.13 ab	0.61±0.034 a	98.54±3.46 a	0.17±0.07 a	2.02±0.06 a	1.66±0.20 a
Biobloom		1.40±0.04 b	0.35±0.012 d	98.59±4.90 a	0.12±0.06 c	1.69±0.09 b	1.20±0.30 c
Amino acid		1.53±0.05 a	0.51±0.06 b	98.93±2.56 a	0.18±0.05 a	1.76±0.12 ab	1.73±0.21 a
Salicylic acid		1.42±0.06 b	0.44±0.045 c	98.57±2.13 a	0.13±0.01 b	1.62±0.03 bc	1.41±0.22 b
Control	Female flowers	0.94±0.13 b	0.15±0.08 a	98.35±3.23 a	0.03±0.01 d	0.97±0.09 a	1.05±0.09 c
Potassium		0.97±0.24 b	0.01±0.08 a	98.30±2.78 a	0.04±0.01 c	0.92±0.02 a	2.14±0.12 a
Thiofer		1.02±0.06 ab	0.93±0.09 b	98.38±2.67 a	0.05±0.01 c	0.43±0.09 ab	1.83±0.14 b
Biobloom		1.08±0.45 a	0.01±0.01 a	98.46±4.86 a	0.09±0.05 a	0.62±0.08 ab	1.88±0.32 b
Amino acid		1.08±0.09 a	0.01±0.02 a	98.30±3.213 a	0.06±0.01 b	0.31±0.021 b	2.32±0.43 a
Salicylic acid		1.10±0.08 a	0.04±0.01 a	98.47±2.124 a	0.09±0.08 a	0.34±0.05 b	1.05±0.32 d

Notes: Values are means ± SE of three replicates. Different letters indicated significant ($P < 0.05$) differences. Protein (mg g⁻¹ FW), Hydrogen peroxide (H₂O₂ μM g⁻¹ FW), superoxide dismutase (SOD U mg⁻¹ protein), catalase (CAT U mg⁻¹ protein), peroxidase (POX U mg⁻¹ protein) and Glutathione peroxidase (GPX U mg⁻¹ protein).

Table 2. Effects of different treatments on proline, anthocyanin, chlorophyll (Chl a, Chl b, and Chl T) and carotenoid (mg g⁻¹ FW) in *Corylus avellana*.

Treatment	Tissue	Proline	Anthocyanin	Chl a	Chl b	Chl T	Carotenoid
Control	Leaf	0.04±0.01 c	14.41±2.31 d	127.41±2.12 d	41.50±3.10 e	168.70±5.80 e	192.07±6.09 c
Potassium		0.07±0.01 ab	19.01±2.20 a	150.66±4.32 a	66.23±6.21 a	217.38±6.13 a	195.33±8.12 c
Thiofer		0.06±0.01 b	16.26.60 b	135.29±2.13 c	56.52±1.31 d	192.80±4.32 d	287.41±3.33 a
Biobloom		0.07±0.01 a	10.63±2.11 e	98.54±2.11 e	40.44±1.51 f	138.64±3.14 f	268.83±4.01 b
Amino acid		0.07±0.04 a	15.69±3.61 bc	145.35±1.12 b	59.57±1.81 c	204.58±5.11 c	258.27±3.39 b
Salicylic acid		0.07±0.05 a	15.05±4.11 cd	144.48±2.21 b	63.66±3.70 b	208.42±3.14 b	289.62±2.41 a
Control	Male flowers	0.06±0.01 d	9.09±2.07 a	35.34±2.22 a	37.62±2.11 c	72.96±4.38 a	24.98±1.61 c
Potassium		0.07±0.01 d	8.15±1.32 a	3.63±0.14 a	60.58±3.11 a	64.21±1.45 b	40.02±2.11 b
Thiofer		0.32±0.01 b	8.69±2.11 a	8.74±1.46 a	12.67±0.98 e	21.41±4.06 d	62.02±1.21 a
Biobloom		0.11±0.03 c	7.19±1.21 a	30.97±2.90 a	44.64±0.06 b	75.61±2.09 a	44.10±1.33 b
Amino acid		0.12±0.045 c	7.26±2.61 a	17.81±1.56 a	22.423±2.55 d	40.23±1.12 c	47.84±4.21 b
Salicylic acid		0.39±0.06 a	8.57±0.52 a	24.76±3.13 a	22.6±2.19 d	47.36±5.03 c	63.34±2.22 a
Control	Female flowers	0.06±0.01 d	6.32±0.11 a	127.79±3.93 b	103.71±7.12 c	232.48±7.11 d	80.76±2.41 a
Potassium		0.06±0.04 d	5.14±0.80 a	94.63±1.78 f	224.54±5.80 a	319.81±5.02 ab	77.67±2.12 a
Thiofer		0.07±0.06 c	4.73± 1.01 a	120.51±3.67 e	231.17±4.40 a	350.65±3.19 a	89.64±2.15 a
Biobloom		0.06±0.00 cd	4.30±0.90 a	124.38±4.86 d	174.35±5.55 b	298.37±5.89 bc	83.25±3.32 a
Amino acid		0.08±0.01 b	5.16±0.20 a	126.54±3.21 c	151.58±6.78 b	277.59±6.02 c	85.34±5.13 a
Salicylic acid		0.09±0.02 a	6.08±1.09 a	158.29±2.40 a	152.50±2.80 b	311.42±3. 54 bc	85.35±1. 21 a

Notes: Values are means ± SE of three replicates. Different letters indicated significant (P<0.05) differences.