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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

EFFECTS OF WATER STRESS ON GROWTH PARAMETERS, ALKALOID CONTENT (GALANTHAMINE AND LYCORINE), AND ANTIOXIDANT ACTIVITIES IN SUMMER SNOWFLAKE (*LEUCOJUM AESTIVUM* L.)

MASTER OF SCIENCE

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APPROVAL OF THE THESIS

EFFECTS OF WATER STRESS ON GROWTH PARAMETERS, ALKALOID CONTENT (GALANTHAMINE AND LYCORINE), AND ANTIOXIDANT ACTIVITIES IN SUMMER SNOWFLAKE (*LEUCOJUM AESTIVUM* L.) submitted by **Yavuz BABA** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 3.01.2023** by

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ABSTRACT

EFFECTS OF WATER STRESS ON GROWTH PARAMETERS, ALKALOID CONTENT (GALANTHAMINE AND LYCORINE), AND ANTIOXIDANT ACTIVITIES IN SUMMER SNOWFLAKE (*LEUCOJUM AESTIVUM* L.)

MSC THESIS

YAVUZ BABA

BOLU ABANT IZZET BAYSAL UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF BIOLOGY

(SUPERVISOR: PROF. DR. ARZU TURKER)

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xiii + 68

Leucojum aestivum L. is a perennial bulbous plant belonging to the Amaryllidaceae family that contains two pharmaceutically significant alkaloids (galanthamine and lycorine). Galanthamine, an acetylcholinesterase inhibitor (AChEI), is an important treatment for Alzheimer's disease. Lycorine has potent antiretroviral, antimalarial, antimitotic, and cytotoxic properties. The aim of this investigation was to establish the effects of various water stress (WS) treatments on growth parameters, galanthamine and lycorine contents, non-enzymatic antioxidant activities (total phenol-flavonoid content and free radical (FR) scavenging activity), and enzymatic antioxidant activities [superoxide dismutase (SOD) and catalase (CAT)] in *L. aestivum*. The plants were grown for 7 weeks under different WS conditions. A control group (C), flooding stress (FS), and two different drought stress (DS) treatments were used: irrigation regime (IR) adjustment (25, 50, and 75%), and PEG 6000 (15, 30, and 45%). According to the obtained results, 50% IR produced the highest levels of galanthamine and lycorine in the bulbs and the highest levels of galanthamine in the leaves. In general, galanthamine and lycorine levels were increased with PEG treatments. On the other hand, galanthamine levels in the leaves and bulbs decreased with the treatment of FS. The best DPPH activity was observed in the 50% IR of the bulbs. The treatment with 50% IR enhanced the total phenolic and flavonoid content of both leaves and bulbs. The IR treatments reduced bulb SOD activity. An increase in CAT activity was detected in all bulb samples. In conclusion, 50% IR treatment has been considered the most effective in terms of pharmaceutical value.

KEYWORDS: *Leucojum aestivum*, HPLC, Galanthamine, Lycorine, Antioxidant, Water stress

ÖZET

SU STRESİNİN GÖL SOĞANINDA (*LEUCOJUM AESTİVUM* L.) BÜYÜME PARAMETRELERİ, ALKALOİD İÇERİĞİ (GALANTAMİN VE LİKORİN) VE ANTİOKSİDAN AKTİVİTESİNE ETKİLERİ
YÜKSEK LİSANS TEZİ
YAVUZ BABA
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
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Leucojum aestivum L. Amaryllidaceae familyasına ait, farmasötik açıdan önemli iki alkaloid (galantamin ve likorin) içeren çok yıllık soğanlı bir bitkidir. Bir asetilkolinesteraz inhibitörü (AChEI) olan galantamin, Alzheimer hastalığı için önemli bir tedavidir. Likorin ise güçlü antiretroviral, antimalaryal, antimitotik ve sitotoksik özelliklere sahiptir. Bu araştırmanın amacı, *L. aestivum*' da çeşitli su stresi (SS) uygulamalarının büyüme parametreleri, galantamin ve likorin içerikleri, enzimatik olmayan antioksidan aktiviteleri (toplam fenol-flavonoid içeriği ve serbest radikal süpürme aktivitesi) ve enzimatik antioksidan aktiviteleri [süperoksit dismutaz (SOD) ve katalaz (CAT)] üzerindeki etkilerini belirlemektir. Bitkiler farklı SS koşulları altında 7 hafta boyunca yetiştirilmiştir. Bir kontrol grubu, su basma stresi (SBS) ve iki farklı kuraklık stresi (KS) uygulaması kullanılmıştır: sulama rejimi (SR) ayarı (%25, 50 ve 75) ve PEG 6000 (%15, 30 ve 45). Elde edilen sonuçlara göre, %50 SR yumruda en yüksek galantamin ve likorin seviyelerini ve yapraklarda en yüksek galanthamine seviyelerini üretmiştir. Genel olarak, galantamin ve likorin seviyeleri PEG uygulamaları ile artmıştır. Öte yandan, yaprak ve yumrulardaki galantamin seviyeleri SBS uygulaması ile azalmıştır. En iyi DPPH aktivitesi yumruların %50 SR' sinde gözlemlenmiştir. %50 SR ile muamele hem yaprakların hem de yumruların toplam fenolik ve flavonoid içeriğini artırmıştır. SR uygulamaları yumru SOD aktivitesini azaltmıştır. Tüm yumru örneklerinde CAT aktivitesinde bir artış tespit edilmiştir. Sonuç olarak, %50 SR uygulaması farmasötik değer açısından en etkili uygulama olarak kabul edilmiştir.

ANAHTAR KELİMELER: *Leucojum aestivum*, HPLC, Galantamin, Likorin, Antioksidan, Su stresi

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LIST OF ABBREVIATIONS AND SYMBOLS

ACh : Acetylcholine

AChE : Acetylcholinesterase

AChEI : Acetylcholinesterase Inhibitor

AD : Alzheimer's Disease

$AlCl_3$: Aluminium Chloride

ANOVA : Analysis of Variance

CAT : Catalase

C : Control Group

DPPH : 2,2-Diphenyl-1-Picrylhydrazyl

DS : Drought Stress

FR : Free Radical

FS : Flooding Stress

GAE : Gallic Acid Equivalent

H_2O_2 : Hydrogen Peroxide

HPLC : High-Performance Liquid Chromatography

HPLC-DAD : High-Performance Liquid Chromatography - Diode Array Detector

IR : Irrigation Regime

MPa : Megapascal

Na_2CO_3 : Sodium Carbonate

$NaNO_2$: Sodium Nitrite

NaOH : Soidum Hydroxide

Nm : Nanometer

PEG : Polyethylene Glycol

QE : Quercetin Equivalents

ROS : Reactive Oxygen Species

SM : Secondary Metabolite

SOD : Superoxide Dismutase

TFA : Trifluoroacetic Acid

TFC : Total Flavonoid Content

TPC : Total Phenolic Content

WS : Water Stress

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1. INTRODUCTION

Humans have started to utilize nature and medicinal plants to find a remedy for their diseases, similar to the instinctive behavior of animals. People have discovered specific plant usage in the cure of various diseases based on their experiences in this process, which has been going on since ancient times. People learned to develop chemical procedures more as the years progressed, and in the early nineteenth century, they identified and separated alkaloids from medicinal plants (1).

Leucojum aestivum L. is an Amaryllidaceae family medicinal plant that contains galanthamine and lycorine (2). Galanthamine, one of the isolated alkaloids, is a natural substance found in various members of the Amaryllidaceae family that is broadly utilized in the cure of Alzheimer's disease (AD) (3). Lycorine, a pyrrolophenanthridine alkaloid, has potent antiretroviral, antimalarial, antimitotic, and cytotoxic properties (2).

Secondary metabolites (SMs) of plants are biologically active molecules like alkaloids. These molecules play a vital role in plants' defence mechanisms for dealing with environmental threats or stressors. SM accumulation is powerfully influenced by various environmental parameters, such as salinity, soil fertility, soil water, temperature, and light (4).

1.1 Characteristics of *L. aestivum*

L. aestivum, summer snowflake, is a bulbous plant of Amaryllidaceae family. It is a protected medicinal and ornamental plant (5) native to the South Europe, Balkans, Caucasus, Mediterranean regions, Northern Iran, Turkey, and Western Asia (6) (Figure 1.1). It is typically found in environments including swamps, marshes, and floodplains that are humid and semi-shaded from sea level to high elevations (7). The bulb diameter is approximately 6 cm in and sub-spherical. It has a brown tunic that protects the plant during the dry summer season. In addition, it has a fleshy scale, basal plate, 1-4 branches, and lateral buds. Grown-up bulbs were located 10.7±1.2 cm underneath the soil's surface. A sympodial branching system exists in the bulb with a lingulate scale and 6-8 foliage leaves, which ends in an inflorescence (8).

During the vegetative stage, the leaves are widely linear and amplexicaule (Figure 1.2), have a 10-110 cm lamina in length and 5-20 cm in width; the bottoms of foliage leaves that wrap and eventually expand around the axis act as a food storage organ (8). Many natural *L. aestivum* habitats have degraded or become threatened during the last three decades as a result of rising pharmaceutical demand. Galanthamine and lycorine are two pharmacologically important alkaloids synthesized by *L. aestivum* (9).

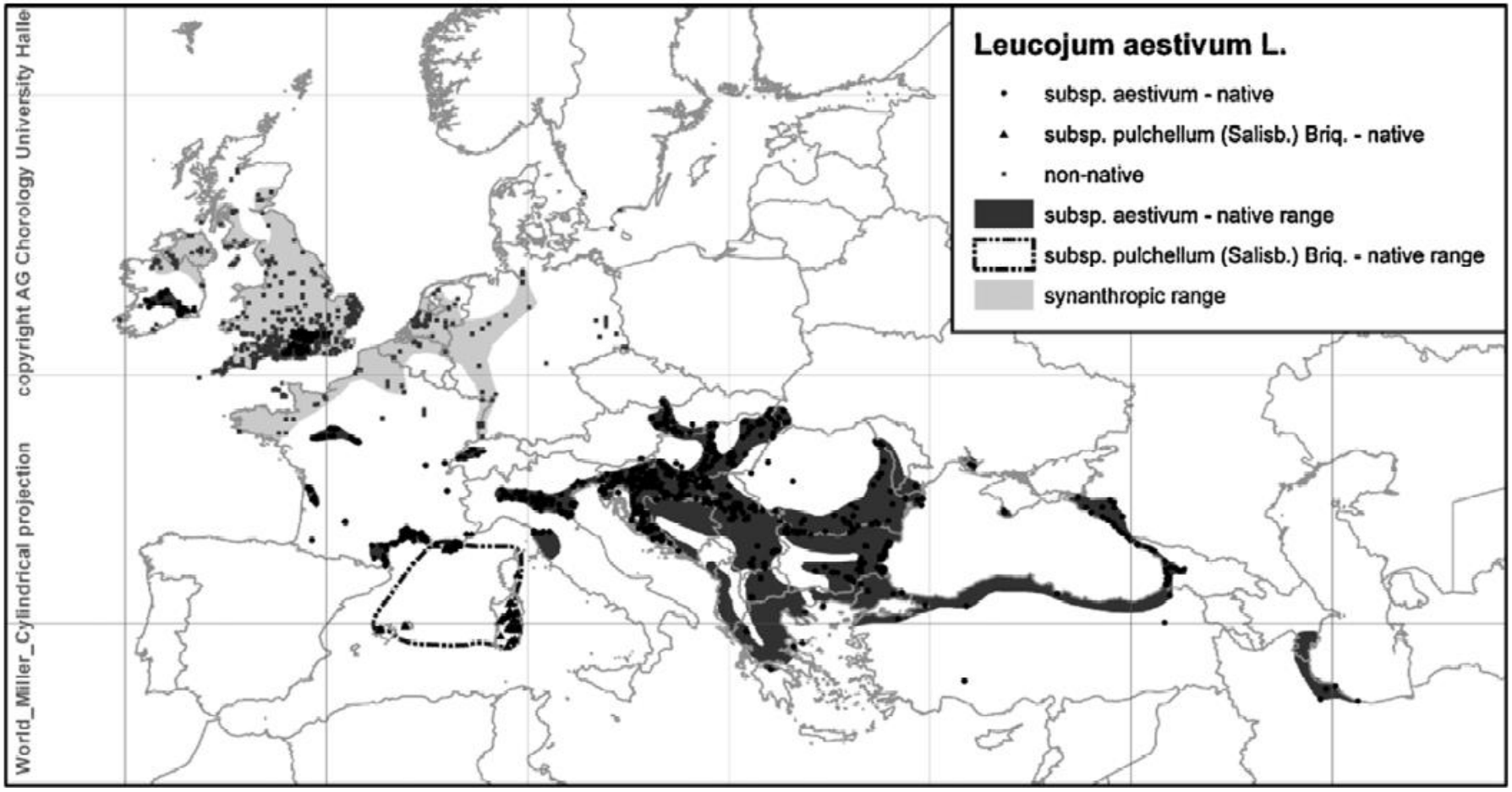

Figure 1. 1. *Leucojum aestivum* distribution (8).

Figure 1. 2. Summer snowflake (*Leucojum aestivum* L.) (by Sina C. Demir).

1.2 Secondary Metabolites Known as Active Constituents

In nature, several secondary metabolism pathways evoke a variety of plant-defense compounds known as secondary metabolites (SMs). SMs in plants refer to metabolic pathways and small molecule byproducts that are inessential to the organism's survival. SMs of plants are often categorized according on their chemical structure. The activation and improvement of plant defense mechanisms have been associated with a number of large molecule types, including terpenoids and steroids, phenolic acids and flavonoids, and alkaloids (4). Alkaloids belong to a large group of SMs that were originally characterized as pharmacologically active nitrogen-based chemicals (10), and this sort of chemical has historically piqued the interest of researchers due to the vast and different physiological impacts that it has on people (11). Based on these SMs, a number of current pharmaceutical medications and herbal medical supplements derived from medicinal plants have been developed (12). The family Amrydaliaceae, which includes *L. aestivum*, generates alkaloids with remarkable pharmacological characteristics. The most significant alkaloid is galantamine, an acetylcholinesterase inhibitor (AChEI) utilized to treat AD. Lycorine, a potent antiviral medication with antimitotic and cytotoxic properties, is another useful alkaloid (13).

1.2.1 Galanthamine

Galanthamine, an isoquinoline alkaloid (14), was discovered in 1952 in the Amaryllidaceae family's perennial herbaceous plant *Galanthus woronowii* (15) (Figure 1.3 A). It is derived from plants of the genera *Leucojum*, *Galanthus*, and *Narcissus* (16). It is a reversible, long-acting, selective, and effective AChEI (17) and an acetylcholine (ACh) allosteric modulator of the neuronal nicotinic receptor (18). It is utilized to treat AD, poliomyelitis, and other neurological disorders (19). Sopharma (Bulgaria) began producing galanthamine with the brand name Nivalin® from the tiny plant *Galanthus nivalis* 1960s. After that, it was isolated from the substantially larger *L. aestivum* plant (20).

Galanthamine inhibits AChE and increases the response of nicotinic receptors to ACh. This increase in nicotinic neurotransmission is a remarkable remedy for AD due to the activation of presynaptic nicotinic receptors, which enhances the release of ACh (21). AChE is an enzyme that, through the rapid hydrolysis of the neurotransmitter ACh, is involved in the peripheral and central

cholinergic synapses, termination of impulse transmission in neuromuscular junctions, and parasympathetic target organs. Inactivation of the enzyme causes ACh accumulation, nicotinic and muscarinic receptor hyperstimulation, and disruption of neurotransmission. AChEI are the solely medicines approved for the AD medication, which is one of the most prominent types of dementia and is identified by continuous memory loss and cognitive and functional impairment (22).

1.2.2 Lycorine

The pyrrolophenanthridine alkaloid lycorine is found in a number of Amaryllidaceae plants (Figure 1.3 B). It was the first biologically active alkaloid isolated from the *Narcissus pseudonarcissus* in 1877. Due to their distinct chemical structures and potent biological effects, lycorine and its derivatives have caught the interest of the field of medicine (23). Along with strong antiretroviral, antimalarial, antimitotic, and cytotoxic effects, it possesses substantial antiviral activity against the measles, poliovirus, and Herpes simplex type 1 viruses (2). Lycorine suppresses viral RNA-dependent RNA polymerase activity and is a strong non-nucleoside direct-acting antiviral against coronavirus infections in development, it may be effective against the COVID-19 pandemic (24).

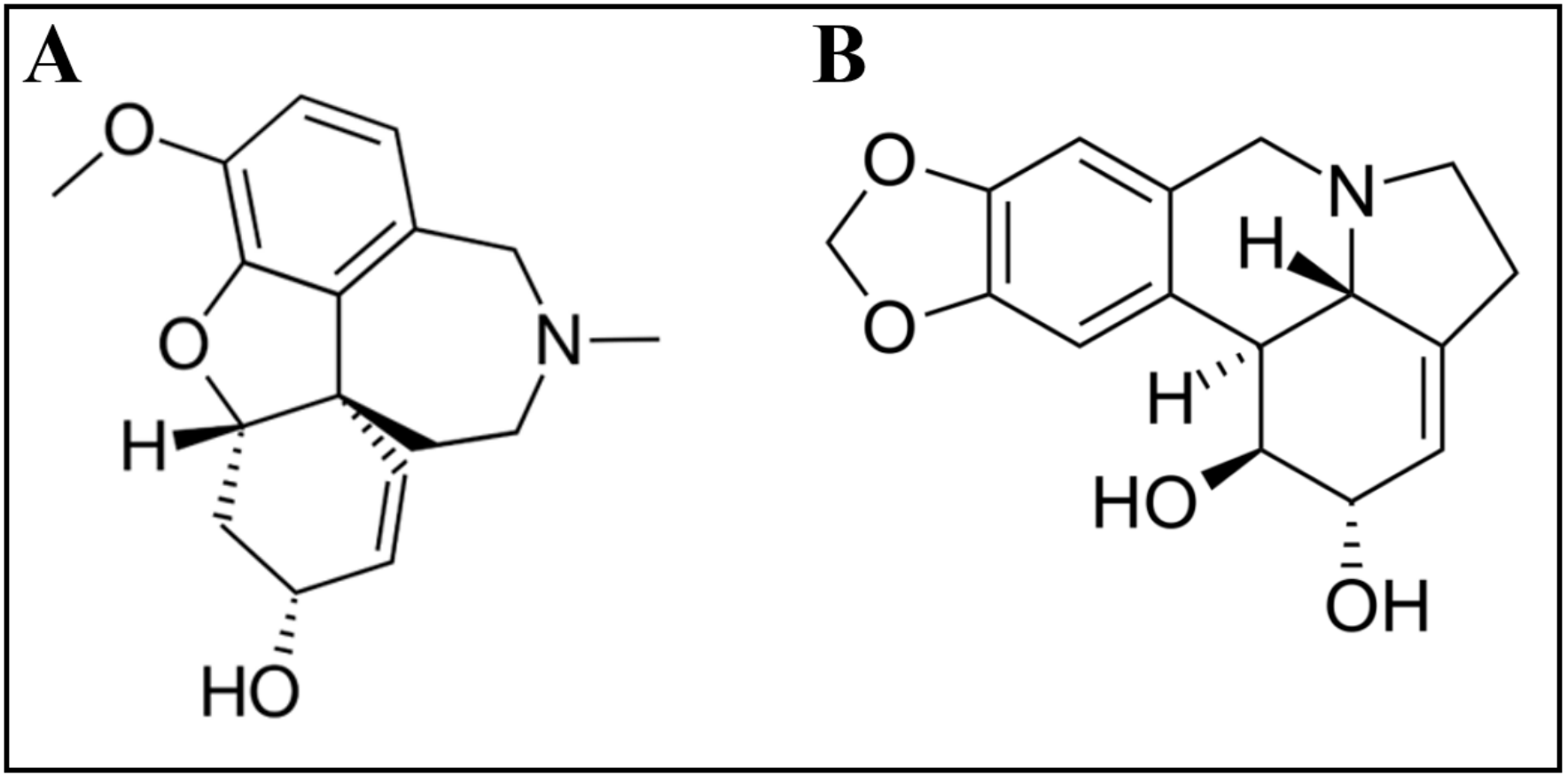

Figure 1. 3. Structure of *Leucojum aestivum* alkaloids; A) Galanthamine, B) Lycorine

1.3 Plant Stress

Stress is an altered physiological state generated by events that seek to upset balance (25). Plants are immobile organisms incapable of evading environmental restrictions (26). Environmental stresses have several implications on plant development. Extreme stresses can cause serious harm during plant biomass development rates (27). The plant environment influences herbage quality mostly through modifying leaf/stem ratios, but it also causes additional morphological changes and alterations in the chemical makeup of the plant components (28). Throughout evolution, plants have developed sophisticated methods for coping with the many stressors that affect them throughout their life cycle (29). In order for plants to survive, they must identify and respond to stress circumstances using a range of biological signals at suitable times and rates. Changes in environmental stress circumstances may be quick or gradual (27). Plants are subject to both biotic and abiotic environmental stress (30).

1.3.1 Biotic Stress

Biotic stress is produced by pathogenic microorganisms that inhibit normal plant growth and have a variety of detrimental impacts on agricultural crops worldwide (31). Certain bacteria, viruses, insects, fungi, weeds, nematodes, and arachnids are responsible for biotic stress in plants (32).

1.3.2 Abiotic Stress

The study of abiotic factors or stressors that may produce stress in a range of species is covered under the field of plant abiotic stress. These stressors contain high and low levels of light, radiation, temperature, water (drought, flooding, and submersion), salinity due to excessive Na+, deficiency or excess of essential nutrients, chemical factors (heavy metals and pH), gaseous pollutants (ozone, sulfur dioxide) (33).

1.3.2.1 Water Stress

One of the most important environmental elements that regulate plant development and production is water stress (WS). By changing their cellular metabolism and activating multiple defensive systems, plants might react to and adapt to WS (34). WS, which generates photosynthesis-related genes that are down-regulated as a result of changes in stomatal openness and leaf water potential and decreased CO_2 availability, has been identified as one of the key drivers of excessive light (EL) stress (35) (Figure 1.4).

Photosynthesis is essential for plant growth; nevertheless, EL can cause serious harm to plants. EL triggers photooxidation, which results in a rise in the generation of highly reactive oxygen intermediates that have a deleterious impact on biological molecules and, if severe, a considerable reduction in plant productivity. (35)

Additionally, the production of reactive oxygen species (ROS) and antioxidant defenses is increased when there is a WS (34). The water restrictions lead to the overproduction of ROS in plants, including hydrogen peroxide (H_2O_2) and superoxide anion radicals (O_2), causing growth inhibition, a reduction in photosynthetic capabilities, lipid peroxidation, and an enhance in the frequency of programmed cell death (36).

The antioxidant mechanism within plants is unique. Antioxidants with small molecular weight make up this system, including ascorbate, glutathione, α-tocopherol, and carotenoids, as well as many enzymes, including superoxide dismutase (SOD) and catalase (CAT) (37).

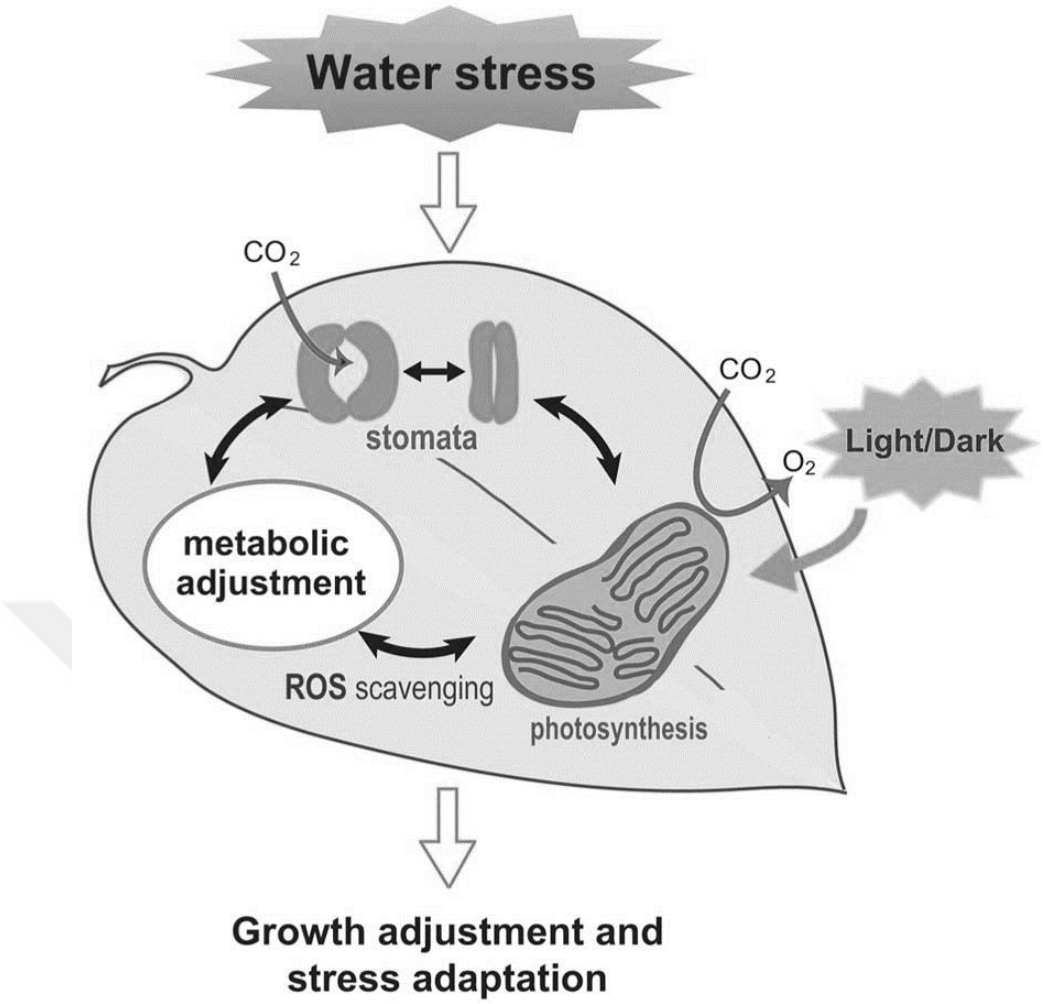

Figure 1. 4. Illustration of how plants react when under stress from water (35).

1.3.2.1.1 Drought Stress

A period without significant rainfall is frequently referred to as a drought according to meteorology. DS results from less water in the soil, and atmospheric conditions cause continual water loss through transpiration or evaporation (25). In water deficit situations, the plant's water potential and turgor are reduced enough to interfere with normal functions and DS triggers changes in physiological and morphological features in the plants. Plants cease growing entirely under extreme WS conditions induced by excessive salt or drought in order to preserve cell volume and turgor against dehydration. This is referred to as "osmotic adjustments" (38). When plants are stressed by drought, the physiological activities of aboveground portions can be regulated by using abscisic acid (ABA) produced in the rhizosphere as a positive signal. The root cells produce ABA first, which is then transmitted to other organs and tissues through vascular bundles. This causes the senescence of leaves and the closing of stomata in an effort to stop water loss (39). DS causes stomatal closure, which restricts CO_2 fixation and decreases NADP+ regeneration through the Calvin Cycle (40).

Due to the decrease in turgor pressure, cell development is a physiological process that is susceptible to dryness. In higher plants under extreme WS, the blockage of water transport from the xylem to the nearby elongating cells can prevent cell growth. Reduced crop growth, plant height, and leaf area occur as a result of decreased mitosis, cell elongation, and expansion under DS (41).

Furthermore, as one of the primary obstacles to plant development, drought can prevent plant respiration, stomatal movement, and photosynthesis impacting physiological metabolism and plant growth. In order to reduce the stress caused by drought, plants respond by activating mechanisms including structural and morphological changes, drought-resistant gene expression, hormone synthesis, and osmotic regulating chemicals (39).

DS frequently leads to the formation of ROS at the cellular level. Excessive ROS generation can lead to oxidative stress in the photosynthetic system and adversely affect cell function. The increased amount of ROS can be considered a danger to the cell. However, ROS can also behave as secondary messengers involved in the stress signal transduction pathway. Plant drought tolerance may be linked to their ability to scavenge ROS and reduce their adverse effects (42).

In response to DS, plants have developed a variety of morphological, biochemical, physiological systems. The biosynthesis and degradation that result from these modifications to the proteome, metabolome, and transcriptome of plants happen at the cellular level. Therefore, to sustain growth and productivity with DS conditions, plants engage a number of mechanisms including the generation of SMs, phytohormones, ROS signaling, osmotic adjustment, and plant hydraulic status. Under various climatic conditions, plant growth requires a balanced production of primary and SMs. Primary metabolites are produced by plants for key functions like growth and development as well as SMs for certain purposes. SMs are abundantly produced by plants and are essential for surviving in hostile environments (43).

1.3.2.1.1.1 PEG Induced Drought Stress

Polyethylene glycol (PEG) is a creamy white flake or free-flowing powder that is utilized as a water-soluble lubricant for metal forming processes and textile fibers (44) (Figure 1.5). PEG compounds have been utilized to imitate DS effect in plants (40). Exposure to PEG solutions have been utilized successfully to simulate DS with limited metabolic interferences, such as those associated with the use of plant-absorbable low molecular weight osmolytes (45). PEG molecules with a Mr≥6000 (PEG 6000) are inert, nonionic, and essentially impermeable chains that are frequently utilized to start WS and keep a constant water potential during the duration of the experiment. PEG 6000 molecules are small enough to affect osmotic potential but big enough to avoid being absorbed by plants. As a result, water is taken out of the cell and the cell wall while PEG is prevented from entering the apoplast. Due to the fact that PEG solutions don't enter the cell membrane like low Mr osmotica solutions do, they more closely resemble dry soil (46).

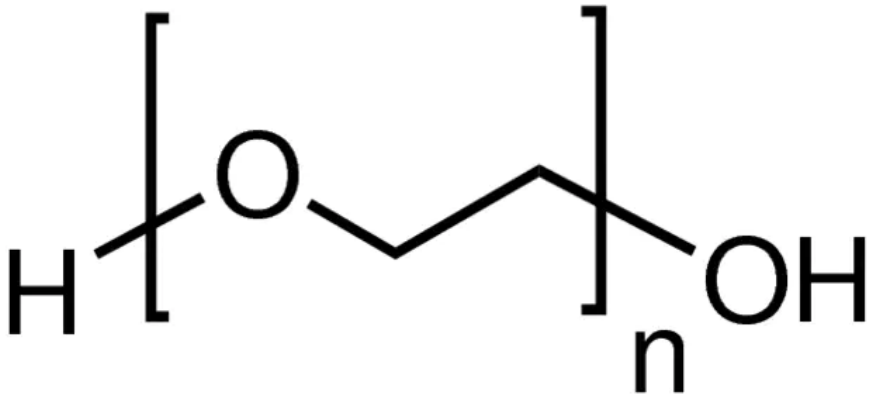

Figure 1. 5. Structure of PEG

1.3.2.1.2 Flooding Stress

Flooding (also known as waterlogged, ponded, saturated, or submerged soil) is a critical stress factor that has a significant impact on crop growth, eventually leading to decreased yield and production of various crop plants (47,48).

It is usually triggered by a climatic difference, particularly when there is unexpected, erratic, or inconsistent rainfall. This stress is most common in rain-fed ecosystems with inadequate drainage (48). When compared to non-flooded soils, there is a restriction in gas diffusion in those environments, and thus the cellular quantity of oxygen can be decreased to levels that adversely impact aerobic respiration, reflecting in low energy with lactate and ethanol formation. Plant growth and production are affected when available energy is restricted (49).

1.4 Antioxidant Activities of Plants

Antioxidants are substances that can slow down or stop the oxidation processes brought on by atmospheric oxygen, or ROS. Antioxidants are used to stabilize petrochemicals, foods, pharmaceuticals, and polymeric materials (50). All organisms, including higher plants, animals, and microbes, produce ROS. They are waste products of normal metabolism, including respiration and photosynthesis, and they are vulnerable to biotic and abiotic stress. Evidence suggests that ROS play a significant role in antioxidant synthesis as well as programmed cell death, stress reactions, pathogen defense in plants, and systemic stress signaling (51).

There are four ROS: singlet oxygen, hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and superoxide ($O_2^{\bullet -}$). In addition, ROS includes nitric oxide (NO) and peroxynitrite ($ONOO^-$). ROS have the ability to create free radicals (FR), which are atoms and molecules with unpaired electrons. FRs are molecules with unpaired electrons that may form in lipids, proteins, DNA, and certain ROS ($O_2^{\bullet -}$, •OH, •NO, and $ONOO^-$) in biological organisms (52).

FRs can cause cell membrane damage, which can result in degenerative diseases and conditions including AD, cardiovascular disease, liver toxicity, aging, nephroblasts, diabetes, inflammation, and DNA damage that can result in carcinogenesis (53).

The substances that can scavenge FRs have a significant capacity for treating these diseases. Therefore, antioxidants play a vital role in protecting the human body against damage by ROS (54).

There are many short, multipurpose peptides that have been found in nature that may stop pro-oxidative metal ions and neutralize FRs. The enzymatic degradation of proteins results in the production of antioxidant peptides. It's possible that vitamin C, vitamin E, polyphenols, carotenoids, and phenolic compounds contain cinnamic acid derivatives, coumarins, tocopherols, flavonoids, and multipurpose organic acids. All of the flavonoid compounds, including flavonols, isoflavones, flavones, chalcones, and catechins have antioxidant properties. There are other derivatives of cinnamic acids include ferulic acid, caffeic acid, and chlorogenic acid. This phenomenon results from the double bond and the hydroxyl group (-OH) (55).

The categorization of antioxidants provides evidence for a variety of perspectives, based on the antioxidant source, the antioxidant action during radical chain reactions, or the antioxidant mechanisms as hydrogen or electron transfer reactions. Antioxidants can be classified as endogenous (internally synthesized, enzymatic ones like SOD and CAT or non-enzymatic (bilirubin, albumin, and glutathione) and exogenous (ascorbic acid, polyphenols, anthocyanins, vitamin E, and carotenoids) (Figure 1.6). They can be characterized as preventative antioxidants as well as chain-breaking antioxidants based on their mechanism of action (56).

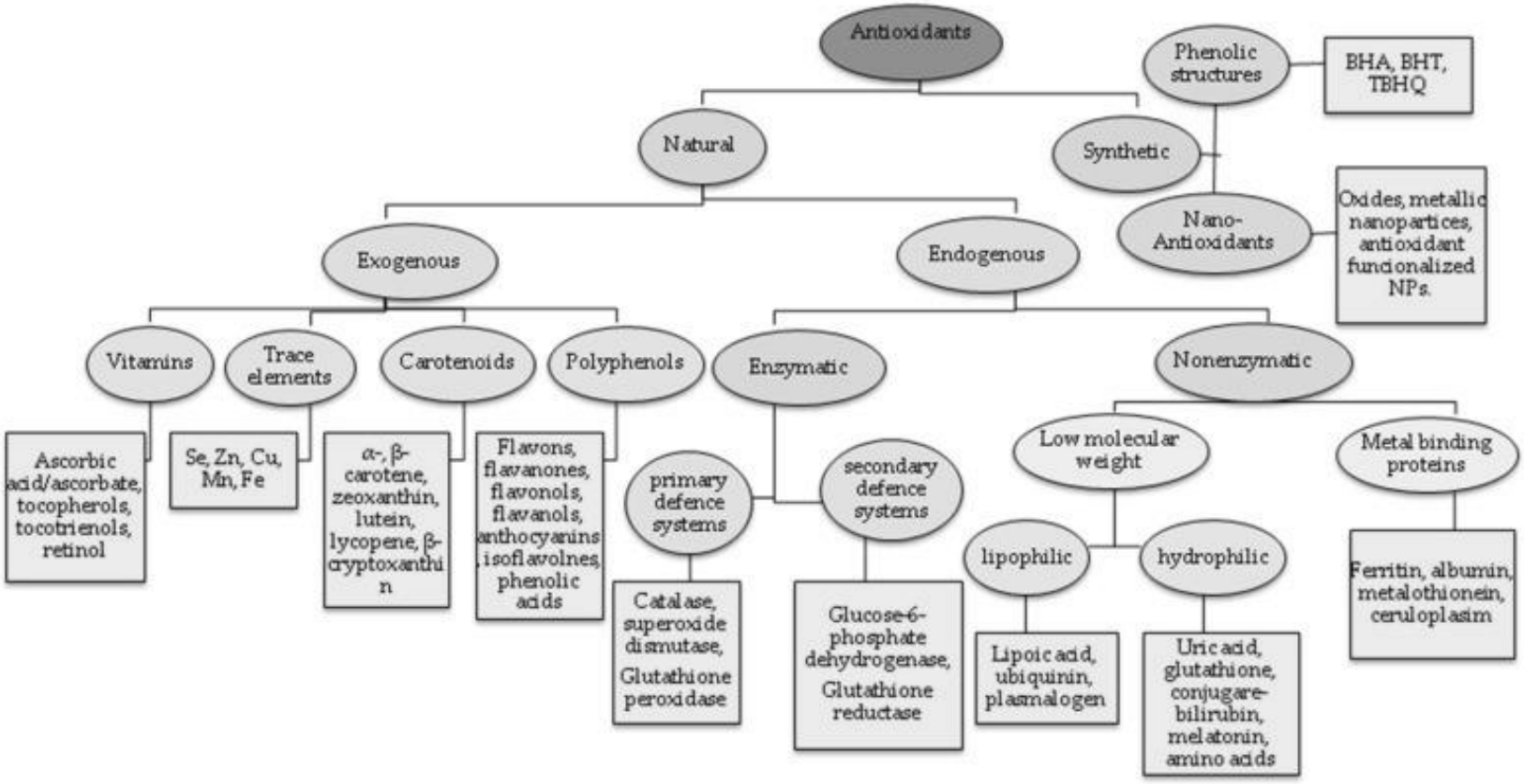

Figure 1. 6. The classification of antioxidants (57)**.**

1.4.1 Non-Enzymatic Antioxidant Activities

The total amount of phenolics and flavonoids, which are in the polyphenols category of exogenous antioxidants, determined by the colorimetric method can also show the non-enzymatic antioxidant capacity.

The DPPH technique is one of the most well-liked and generally utilized techniques for determining a compound's capacity to operate as a FR scavenger or hydrogen donor. Also, it is used for evaluating the amount of non-enzymatic antioxidant activity of the foods (58,59).

The DPPH radical is long-lived natural nitrogen radical with a dark purple color. When a DPPH solution radical is combined with an antioxidant or reducing substance, the resulting hydrazine turns from purple to yellow. The ability of antioxidants to reduce DPPH can be measured using electron spin resonance or by observing a decrease in absorbance at 515-528 nm as the formed hydrazine DPPH gives a yellow solution (59).

1.4.2 Enzymatic Antioxidant Activities

Antioxidant capacity is frequently utilized as a parameter to identify various chemicals and food samples with the capability to scavenge or neutralize FRs. This capacity is linked to the existence of substances capable of protecting a biological system against dangerous oxidation (59). SOD is a cell's primary detoxifying enzyme and most effective antioxidant. It is a vital endogenous antioxidant enzyme and a part of the first line of defense against ROS. It catalyzes the dismutation of two superoxide anion molecules (O_2) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), reducing the potential damage of the superoxide anion. SOD is a metalloenzyme, and due to this feature, it needs a metal cofactor to activate. There are various types of metal ions needed as cofactors by SOD. SOD often binds with the metal ions iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). SODs are divided into three categories in this context: (i) Fe-SOD, a substance generally found in prokaryotes and plant chloroplasts; (ii) Mn-SOD, which is present in prokaryotes and eukaryotic mitochondria; and (iii) Cu/Zn-SOD, which is more prevalent in eukaryotes and is mostly located in the cytosol but may also be found in chloroplasts and peroxisomes, is another kind of antioxidant (60) (Figure 1.7).

A common antioxidant enzyme called CAT is found almost in every living cell that uses oxygen. By utilizing either iron or manganese as a cofactor, the

enzyme catalyzes the breakdown or lowering of hydrogen peroxide (H_2O_2) to water and molecular oxygen, ending the detoxification process imitated by SOD. CAT is extremely effective; it can break down millions of hydrogen peroxide molecules in one second (60).

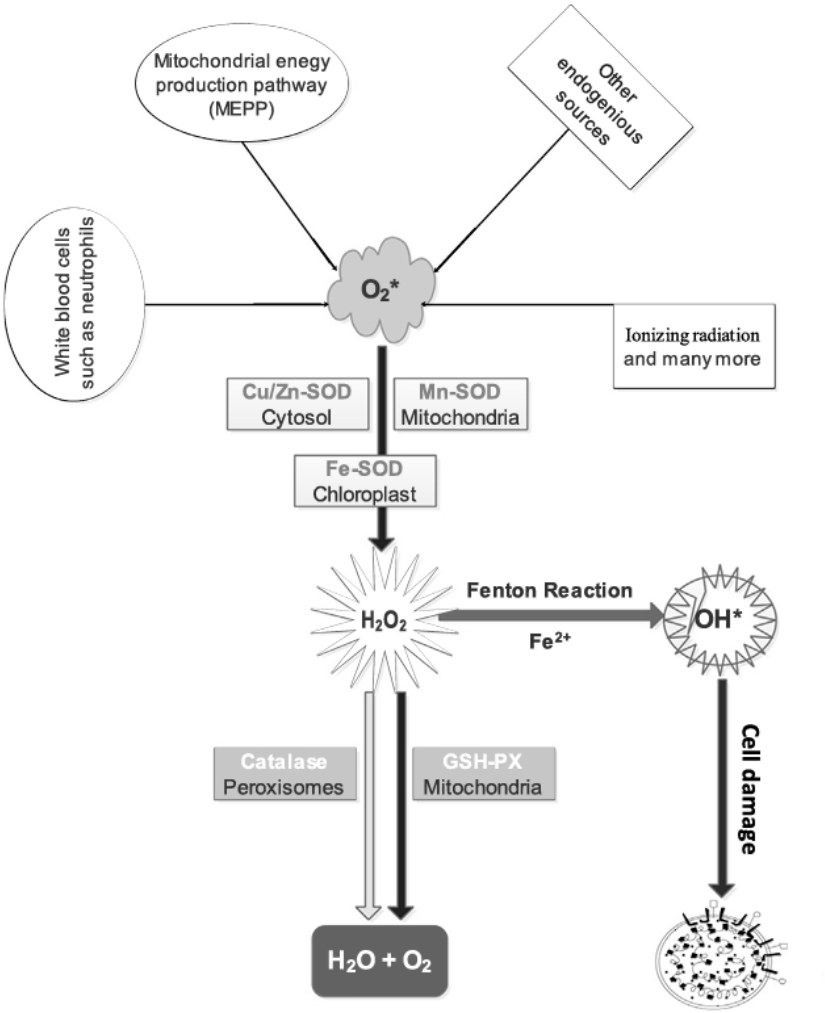

Figure 1. 7. Antioxidants defense against ROS (60).

2. AIM AND SCOPE OF THE STUDY

Water stress (WS) treatments

-To measure and compare the effects of 8 different WS treatments [control group (C) (normal irrigation), flooding stress (FS), and in order to create drought stress conditions: irrigation regime (IR) adjustments of 25%, 50%, and 75%, and PEG6000 treatments of 15%, 30%, and 45%] on the growth of *L. aestivum* (length and width of bulb and leaf, and water content %).

-To measure and compare the alkaloid (galanthamine and lycorine) content of methanolic extracts of bulbs and leaves of *L. aestivum* obtained from 8 different WS treatments by HPLC-DAD system.

-To measure and compare the non-enzymatic antioxidant capacities (total phenol-flavonoid content and radical scavenging activity) of *L. aestivum* bulbs and leaves grown with 8 different WS treatments.

-To measure and compare the enzymatic antioxidant capacities (SOD and CAT) of *L. aestivum* bulbs and leaves grown with 8 different WS treatments.

3. MATERIALS AND METHODS

3.1 Cultivation of *L. aestivum* Under Water Stresses

L. aestivum bulbs were gathered from Bolu-Gölcük when they had reached about 5 cm in length. Nearly the same size of the *L. aestivum* bulbs was chosen randomly and planted into the pots (18.5 cm x 15.5 cm) containing the substrate mix, which consisted of peat (Terradena®), crystal sand, and vermiculite by volume (66.7%, 16.7%, and 16.7%, respectively). The electrical conductivity (EC) and pH (inoLab pH 7110, WTW, Weilheim, Germany) of the substrate mix were measured as 1.62 μc/cm and 7.3, respectively. Eight different WS treatments were created. These treatments include the control group (C) (normal irrigation), flooding stress (FS), and in order to create drought stress conditions: irrigation regime (IR) adjustments of 25%, 50%, and 75%, and PEG6000 treatments of 15%, 30%, and 45%.

3.2 Method for WS treatments

The pot was filled with a certain amount of water, and after the medium was saturated, the drain water was measured. The amount of irrigation water for the pot was calculated as the difference between the initial addition of water and the drain water. Using the appropriate amount of irrigation water, the initial irrigation was made. In accordance with the maximum soil moisture, other irrigations were modified. Using a soil moisture meter (Extech Instruments, model number MO750), soil moisture was measured.

The experimental design was indicated in Figure 3.1. The experiment was conducted twice. Each replication consisted of 32 pots and 32 *L. aestivum* bulbs. Each pot contained 1 *L. aestivum* bulb. Each WS treatment contained 4 pots on its own. In total, two replications contained 64 pots and 64 *L. aestivum* bulbs.

The amount of water to be given to the C was determined according to the amount of irrigation water.

To apply flooding stress, the bottom of the pot was covered, and the amount of water remained 5–6 cm above the soil surface, indicating that the pot was filled with water (flooding).

The irrigation water was gradually reduced by 75%, 50%, and 25% for the experimental groups in order to create the first DS treatment with different IR adjustments: 75% (watered to 75% of irrigation water), 50% (watered to 50% of irrigation water), and 25% (watered to 25% of irrigation water).

For the second DS treatment, irrigation was done once a week with irrigation water containing 3%, 6%, and 9% PEG, and at the end of the 5 weeks, cumulatively, 3 different treatment groups containing 15%, 30%, and 45% PEG were formed. Thus, the effect of 3 different osmotic potentials originating from PEG (-0.30, -1.04, and -2.22 MPa, respectively) was investigated.

The osmotic potential of PEG 6000 was calculated according to Michel and Kaufmann (61) with this equation:

$$\text{Osmotic potential } (\psi_s) = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, C \times T + (8.39 \times 10^{-7})\, C^2 \times T$$

The experiment was carried out for a total of seven weeks, including an initial one-week adaptation period and the last one-week period before harvest, in a plant room at 24±1°C with a 16/8 h (light/dark) photoperiod (cool white fluorescent lights, 22–28 μmol m- $2s^{-1}$) and 40% relative humidity.

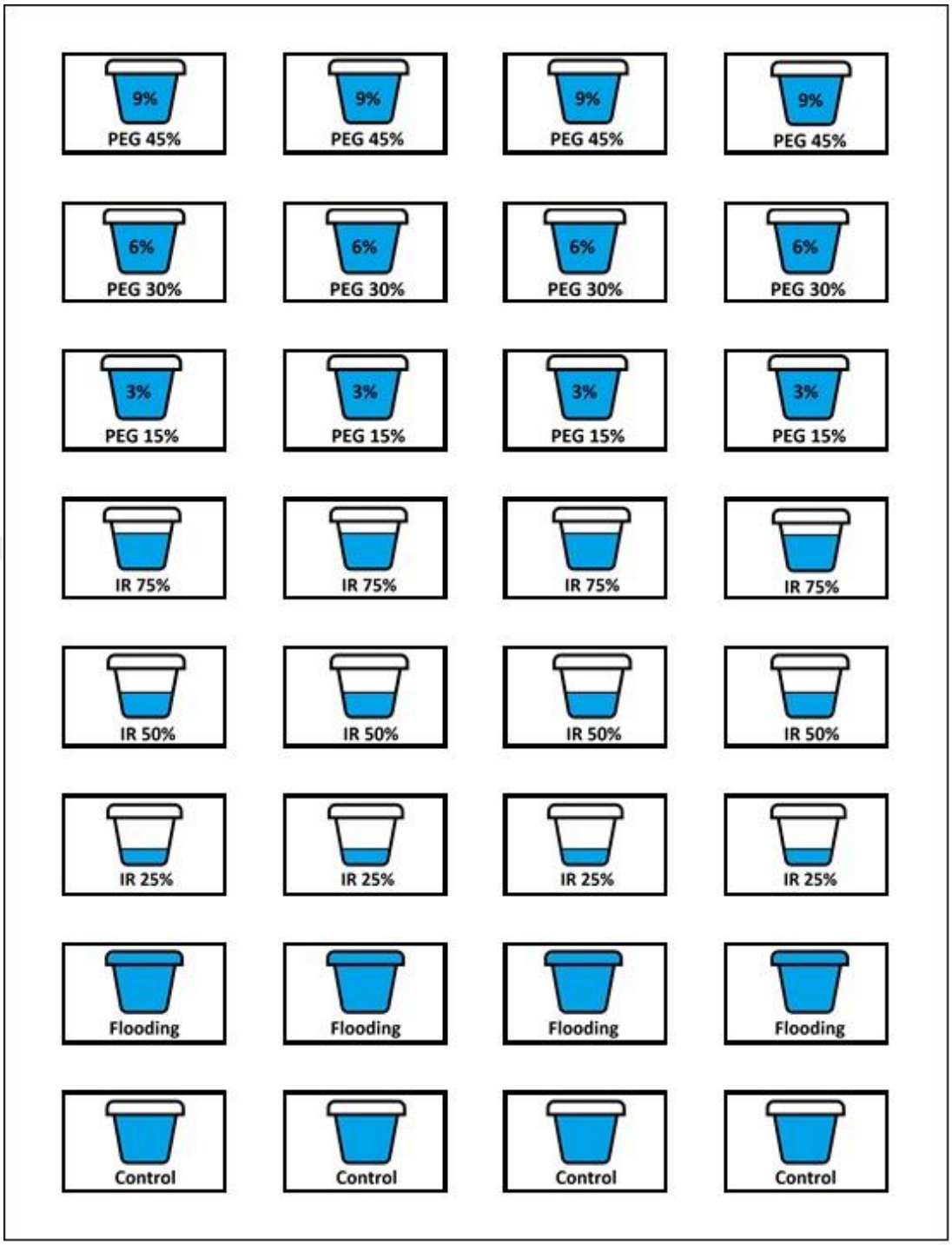

Figure 3. 1. Experimental design of WS treatments.

3.3 Measurement of Growth Parameters

After 7 week, bulbs and leaves have been collected individually at the end of the WS treatments. The length, width, and fresh and dry weights have been determined. Dry weights by drying the bulbs and leaves in a freeze-dryer at -65 ºC (low pressure drying). Then the proportionate water content (WC) in the bulbs and leaves was calculated (Table 4.1). After that, they were kept at -20 °C until their extraction and investigation of biological activity.

3.4 Methanolic Extracts Preparation

L. aestivum leaves and bulbs were freeze-dried (Christ®) before being ground into powder. Plant materials were produced as methanol extracts in a water bath at 40 °C. The extractions were filtered after 24 hours, and then methanol was vacuum evaporated (Buchi® rotary evaporator) at 45 °C. To get the final concentration, the dried extract was dissolved once more in methanol. The yields obtained after extraction were computed using the formula below:

Yield (%) = Obtained extract weight (g) / Initial plant material weight (g) x 100.

3.5 Alkaloid Content Determination Through HPLC

Utilizing an HPLC system (VWR-Hitachi LaChrom Elite®) equipped with a Hitachi L-2455 diode array detector (DAD), a Hitachi L-2130 pump, and a Hitachi L-2200 autosampler, the quantitative analysis of methanol extracts was identified. Two alkaloid standards [galanthamine hydrobromide (TCI America®) and lycorine (Sigma®)] were used as references (in 0.1% TFA), and their calibration curves (6.25, 12.25, 25, 50, 100, and 200 mg/L) were used to determine the amounts of these compounds in plant extracts. Following Arslan et al. (62)'s instructions, the HPLC procedure was done, and an isocratic elution was used for the analysis.

3.6 Investigations of Non-Enzymatic Antioxidant Activity

3.6.1 Total Phenolic Content (TPC) Quantification

According to Turker et al. (63), the total phenolic content (TPC) of *L. aestivum* extracts was measured utilizing a modified Folin-Ciocalteu assay. The TPC of the extracts was calculated using a calibration curve, with Gallic acid (Sigma®) serving as the phenol standard. At 765 nm, the absorbance of each sample was determined against a blank using a UV-VIS spectrophotometer (Hitachi U-1900®). The extracts' TPC was quantified as equivalent to mg of gallic acid (GAE) per 1 g of dried extract. There were three repeats of the experiment.

3.6.2 Total Flavonoid Content (TFC) Quantification

Using Turker et al. (63)'s modified aluminum colorimetric test, the total flavonoid content (TFC) of *L. aestivum* extracts was measured. The flavonoid standard used was quercetin (Sigma®), and a calibration curve was constructed to determine the TFC of the extracts. At 415 nm, the absorbance of each sample was determined against a blank using a UV-VIS spectrophotometer (Hitachi U-1900®). The extracts' TFC was reported in milligrams of quercetin equivalent (mg QE) per one gram of dried extract. There were at least three repetitions of the experiment.

3.6.3 Activity in Scavenging Free Radicals

The antioxidant capacity of *L. aestivum* extracts was identified spectrophotometrically using the 2,2-diphenyl-1-picrylhydrazil (DPPH, Sigma-Aldrich Chemie®, Steinheim, Germany) radical assay, using a modified version of the Blois, (64) method, as published by Basay et al. (65). The DPPH technique, the 0.13 mM DPPH solution, the plant sample, and quercetin (as an antioxidant standard) were all prepared in methanol. 100 mL of plant sample, quercetin at varying doses, and methanol were combined with 1400 mL of DPPH (as a control). After 30 minutes in the dark, the absorbance of the samples was determined using a UV-VIS spectrophotometer (Hitachi U-1900®) against a blank (methanol). There were at least three repetitions of the experiment.

DPPH· Scavenging Effect (% inhibition) = [(A0−A1/A0) x 100] Turker et al. (66) where the absorbance of the control reaction is A0 and the absorbance of the *L. aestivum* extracts is A1.

3.7 Enzymatic Antioxidant Activities

3.7.1 Protein Identification and Extraction of Enzymes

In the beginning, enzymes and proteins were extracted from the bulbs and leaves of *L. aestivum* in order to determine the SOD and CAT enzyme activities. For the enzyme extraction technique, fresh plant samples were completely crushed into a powder in an ice bath using liquid nitrogen. 0.1 g of each powder was then separated and homogenized in 4 mL of 50 mM phosphate buffer (pH 7), which contained 2 mM Na-EDTA and 1% (w/v) polyvinyl polypyrrolidone (PVP).

The homogenate was centrifuged for 15 minutes at 12,000 rpm and 4 °C, and the supernatant was analyzed for SOD and CAT enzyme activity. The Lowry method Lowry (67) has been used to identify the protein amount of plant bulbs and leaves. Bovine serum albumin (BSA) was used as a protein standard.

3.7.2 Activity of the Superoxide Dismutase (SOD) Enzyme

Based on the work of van Rossum et al. (68), a modified technique for detecting SOD activity has been developed. 1425 mL of a reaction mixture including 0.3 mM xanthine, 0.6 mM EDTA, 150 mM nitroblue tetrazolium (NBT), 400 mM sodium carbonate (Na_2CO_3), and 1 g/L of bovine serum albumin (BSA) were put into test tubes. After that, 0.025 mL of xanthine oxidase solution was added to each tube including a reaction mixture. After 20 minutes of incubation at 25 °C, the reaction was stopped by adding 0.05 mL of 0.8 mM copper chloride. Utilizing a UV-Vis Spectrophotometer (Hitachi U-1900®), the absorbances of plant samples were measured relative to distilled water at 560 nm. One unit of SOD is equal to the amount of protein that produces a 50% reduction in NBT in a reaction, and activity is demonstrate as one unit per mg of protein. All analysis has been carried out three times.

3.7.3 Activity of the Catalase (CAT) Enzyme

The CAT activity was evaluated according to the method Lartillot et al. (69) by detecting the reduce in absorbance at 240 nm induced by the catalase enzyme's breakdown of H_2O_2. A combination of 50 mM phosphate buffer and 10 mM H_2O_2 has been added to the test tubes in order to measure CAT activity. To initiate the reaction, 20 mL of enzyme extract have been added to the mixture, which has been incubated at 25 °C for 2 minutes. Lastly, the reaction was stopped by adding 0.5 mL of a 1 M HCl solution. In order to assess the CAT activity in each sample, the consumption of H_2O_2 at 240 nm for two minutes was utilized. The activity was computed using the H_2O_2 extinction value of 0.0392 mM/cm and represented as mmol H_2O_2/mg protein. All analysis has been carried out three times.

3.8 Data Analysis

The investigations have been designed using a completely randomised design. For data analysis, Analysis of variance (ANOVA) and Duncan's Multiple Range Tests using SPSS version 26 (IBM Corp, NY, USA) have been performed. All results in the tables are demonstrated as a mean number ± standard error (SE). Means with the same letter within columns are not significantly different at $P>0.05$.

4. RESULTS AND DISCUSSION

4.1 Cultivation of *L. aestivum*

This study was conducted to investigate the effects of various WS treatments on the alkaloid content (galanthamine and lycorine) and growth, total phenol and flavonoid content, antioxidant capacity, and antioxidant enzymes (SOD and CAT) of the summer snowflake (*Leucojum aestivum* L.).

As WS treatments were made, the C, FS, and two DS treatments [different IR adjustments (25%, 50%, and 75%) and PEG 6000 treatments (15%, 30%, and 45%)] were applied (Figure 4.1).

Figure 4. 1. *L. aestivum* in pots with WS treatments.

4.2 Growth Parameters and Water Content (%) of Water Stress Treatments

Significant findings were found in various parameters of the statistical analyses of the total fresh and dry weight, and percentage of water content (Table 4.1), shoot and bulb width and length results of WS concentrations produced using IR, PEG, and flooding (Table 4.2, Table 4.3).

The treatments of 45% PEG and 15% PEG showed the largest bulb diameter (Table 4.2, Figure 4.2, A; C). On the contrary, bulb diameters were the smallest in 75% IR (Table 4.2, Figure 4.3, C), and C bulbs were close to this treatment. In comparison to the C, it was seen that the results of the other groups produced shorter bulbs. However, there is no significant difference between the C and 75% IR. When weighing the fresh weight of the bulbs, it was found that 45% PEG treatment was significantly different from other treatments and had the highest weight (Table 4.2, Figure 4.2, C). Other groups were marginally less weighted than the C. FS treatment seemed to have the lowest weight compared to the other groups.

25% IR became conspicuous when the leaves were analyzed in terms of width (Table 4.3, Figure 4.3, A). 15% PEG treatment came after it (Table 4.3, Figure 4.2, A), and the C's result was average. The treatment of FS resulted in the lowest leaf width (Table 4.3, Figure 4.4, A). Other treatments' leaf lengths were shown to be shorter compared to the C. PEG treatments produced results that were quite similar to C, while IR treatments-particularly 50% IR—had the lowest results. The leaf fresh weight data found that 50% IR recorded the lowest result (Table 4.1, Figure 4.3, B), which was directly inversely proportional to the leaf lengths. Similar results in the direction of leaf lengths were also produced by other treatment groups. Not much difference was observed in the results of WS treatments on the percentage water content in bulbs and leaves (Table 4.1).

Ates et al. (6) reported that the treatment of salt stress, an abiotic stress factor, did not significantly change the shoot length, bulb size, and water content of *L. aestivum*. In our study, bulb width and fresh weight were significantly increased with 45% PEG treatment. Similarly, leaf width and fresh weight were significantly raised with 25% IR and 15% PEG treatment, respectively. It can be concluded that DS treatments on *L. aestivum* was the most effective than salt stress treatments in terms of growth parameters.

In FS treatments in our study, all growth parameters decreased when compared to the C. The reason may be the plants growing on the waterlogged soil face a stressful condition, including hypoxia (a deficiency of O_2) or anoxia (lack of O_2). Plant growth, development, and survival are severely hindered by these oxygen-deficient circumstances (70).

Many studies have shown that DS treatment had a negative correlation with growth parameters (71–73). Basha et al. (74) investigated the effect of PEG-induced DS on germination and seedling development of tomato germplasm. They applied 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14% and 16% PEG-6000. They found a reduction in germination rate in all the varieties of tomato plants. The shoot length enhanced significantly in Arka Rakshak, Arka Vikas, and PKM-OP at 2% PEG stress condition when compared to the control. They determined a decline in root and shoot length with the increasing PEG concentrations. The highest shoot length was determined in Arka Rakshak at 2% PEG when compared to other germplasm. Similarly, in our study, the bulb and leaf length of *L. aestivum* decreased with PEG and IR treatments. Yosefi et al. (75) determined that at 7% PEG treatment, PEG-induced DS had a detrimental impact on the strawberry plant's fresh and dry shoot weight as well as fresh and dry root weight. Batool et al. (76) indicated that DS with PEG 6000 negatively influenced all studied cultivars of rapeseed. They found that 15 % PEG 6000 treatment decreased the final germination percentage, germination rate, and vigor index. Bilir Ekbic et al. (77) reported that increasing PEG doses had a negative effect on the shoot development of *Vitis labrusca* L. They found that the shoot length, number of nodes and leaves, shoot fresh and dry weight, and leaf fresh and dry weight reduced with increasing PEG doses (1.5, 3.0., 4.5, 6.0 %) gradually. Khodarahmpour (78) investigated the effect of DS caused by PEG on germination indexes in *Zea mays* L. hybrids. As a result, it was observed that the water potential significantly reduced the germination percentage, germination rate, root length, shoot length, seedling length, and seed viability. It concluded that with the decline in the osmotic potential of the PEG solution, the mean germination time and root/shoot length ratio increased.

Manurung et al. (79) indicated DS levels (40, 60, and 80 %) reduced the plant height, leaf number, area, thickness, number of branches, chlorophyll number, and stem diameter of tabat barito plants. Weidner et al. (80) recorded the highest dry matter content (22.7%) in the roots of *Vitis vinifera* L. under severe DS (35 % soil moisture) when compared to the control (14.9%).

Table 4. 1. Effect of WS treatments on total fresh-dry weight and water content (%) of *L. aestivum*.

		L. aestivum					
		Total Fresh Weight (g)		**Total Dry Weight (g)**		**Water Content (%)***	
Water Stress Treatments		**Bulb**	**Leaf**	**Bulb**	**Leaf**	**Bulb**	**Leaf**
Control	-	29.79	30.42	7.14	3.13	76.02	89.70
Flooding Stress	-	18.49	15.16	4.44	1.56	75.97	89.72
Drought stress generated by irrigation regimes	25%	19.73	20.45	4.52	2.02	77.08	90.12
	50%	19.43	13.59	4.93	1.51	74.63	88.92
	75%	22.86	17.54	5.88	1.87	74.30	89.37
Drought stress generated by PEG 6000	15%	20.76	19.89	5.25	2.15	74.73	89.22
	30%	20.10	16.95	5.17	1.81	74.28	89.35
	45%	25.73	21.40	6.21	2.26	75.87	89.44

*Water Content (%) = [Total fresh weight (g) - Total dry weight (g)] / Total fresh weight x 100

Table 4. 2. Effect of WS treatments on width, length, and fresh weights of individual *L. aestivum* bulb.

		L. aestivum		
Water Stress Treatments		**Bulb width (mm)**	**Bulb length (mm)**	**Bulb fresh weight (g)**
Control	-	20.33 ± 0.49^{cd}	37.75 ± 1.18^{a}	6.48 ± 0.17^{bc}
Flooding stress	-	21.50 ± 1.19^{cd}	35.67 ± 0.88^{ab}	3.78 ± 0.14^{e}
Drought stress generated by irrigation regimes	25%	20.40 ± 0.51^{cd}	29.25 ± 0.48^{c}	5.04 ± 0.18^{d}
	50%	22.50 ± 0.87^{bc}	33.50 ± 0.50^{b}	5.87 ± 0.26^{c}
	75%	20.00 ± 1.08^{d}	36.33 ± 2.03^{a}	4.58 ± 0.37^{d}
Drought stress generated by PEG 6000	15%	23.75 ± 0.48^{ab}	30.50 ± 0.50^{c}	6.73 ± 0.34^{b}
	30%	21.00 ± 0.63^{cd}	28.33 ± 0.33^{c}	6.11 ± 0.22^{bc}
	45%	25.25 ± 0.25^{a}	33.33 ± 0.33^{b}	8.85 ± 0.33^{a}

Table 4. 3. Effect of WS treatments on width, length and fresh weights of individual *L. aestivum* leaf.

		L. aestivum		
Water Stress Treatments		**Leaf width (mm)**	**Leaf length (mm)**	**Leaf fresh weight (g)**
Control	-	11.50 ± 0.15^{cd}	438.20 ± 13.15^{a}	8.23 ± 0.69^{ab}
Flooding stress	-	10.08 ± 0.08^{f}	398.00 ± 7.06^{bc}	6.03 ± 0.19^{cd}
Drought stress generated by irrigation regimes	25%	13.13 ± 0.35^{a}	367.20 ± 21.37^{cd}	5.31 ± 0.06^{de}
	50%	10.60 ± 0.22^{ef}	357.50 ± 8.81^{d}	4.17 ± 0.07^{e}
	75%	11.09 ± 0.31^{de}	395.80 ± 4.20^{bc}	7.52 ± 0.63^{ab}
Drought stress generated by PEG 6000	15%	12.50 ± 0.22^{b}	405.75 ± 5.31^{ab}	8.60 ± 0.36^{a}
	30%	11.00 ± 0.19^{de}	402.25 ± 6.30^{bc}	6.80 ± 0.31^{bc}
	45%	12.07 ± 0.16^{bc}	412.60 ± 8.49^{ab}	8.27 ± 0.84^{ab}

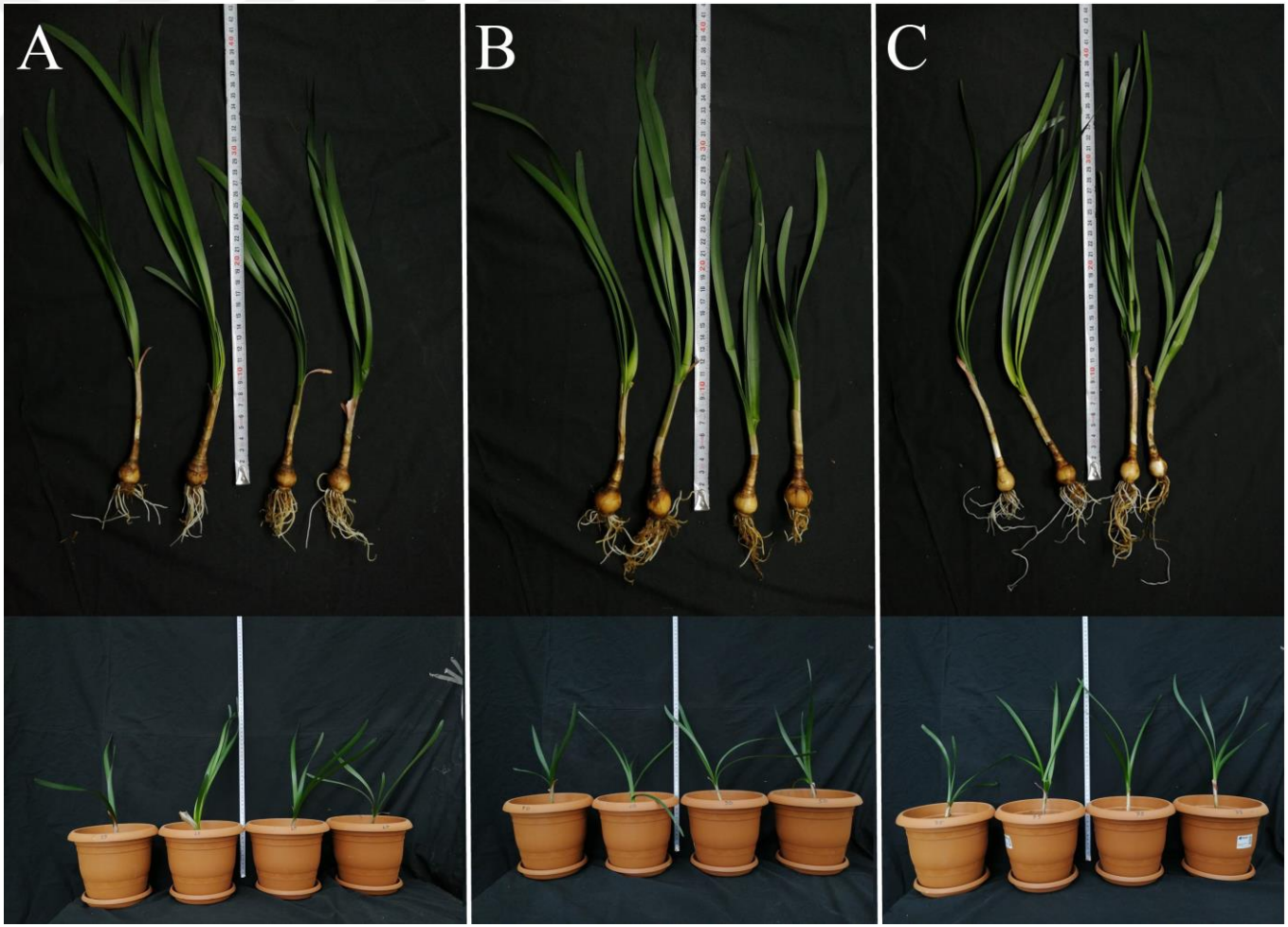

Figure 4. 2. Three different IR treatments: 25% (A), 50% (B) and 75% (C).

Figure 4. 3. Three different PEG treatments: 15% (A), 30% (B) and 45%(C).

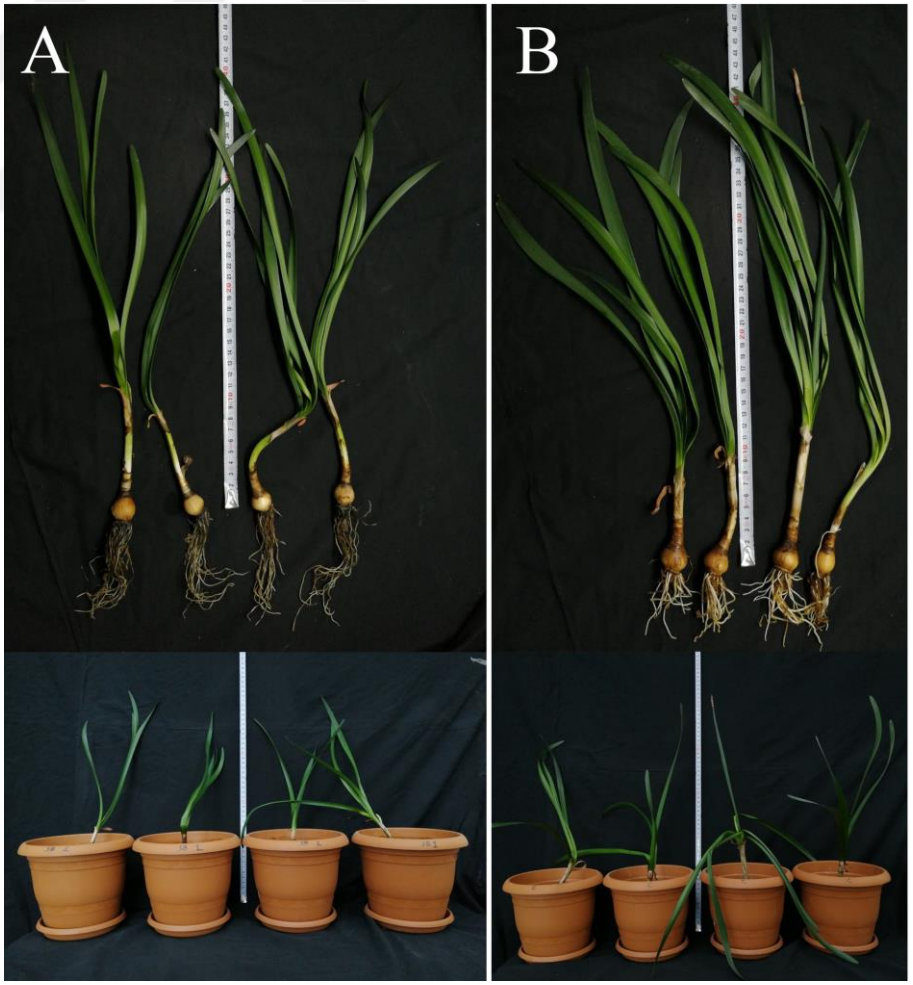

Figure 4. 4. FS treatment (A) and C (B).

4.3 Analyses of Galanthamine and Lycorine Contents by HPLC

For HPLC analysis, methanol extracts of the harvested *L. aestivum* plant's leaves and bulbs were used. The extraction yields were shown in Table 4.4. The results of the HPLC-DAD analysis of 16 different extracts obtained from the *L. aestivum* bulbs and leaves were demonstrated in Table 4.5. The leaf extracts produced a greater yield rate than the bulb extracts when the extract yield percentages were compared. It could be a result of the chlorophyll and larger range of phenolic metabolites contained in the leaves. Figure 4.5 depicts the chromatogram of the utilized standards.

Based on the results of an HPLC analysis of bulb extracts, the treatment of 50% IR resulted in the greatest concentrations of galanthamine and lycorine (Table 4.5, Figure 4.6). 25% IR treatment was very close to the 50% IR in terms of the amount of galanthamine. There was 3.38 mg/g more dry extract in 50% IR treatment than in the C. There was a minor difference in the amount of galanthamine after PEG and FS treatments. It was observed that the 50% IR enhanced lycorine levels by 1.36 times when compared to the C (Table 4.5). It was followed by 15% PEG treatment with a 1.26 time increase rate. 75% IR resulted in the lowest levels of galanthamine and lycorine. It was shown that, despite certain treatment groups having somewhat lower levels of galanthamine and lycorine than the C, stress treatments typically raised the levels of these compounds in the bulbs.

In the HPLC analysis results of the leaf extracts, it was observed that the amount is less than the bulbs. When the leaves were evaluated within themselves, 50% IR for the amount of galanthamine (Table 4.5, Figure 4.7) and a 25% IR for the amount of lycorine came to the fore (Table 4.5). 4.47 mg/g more dry extract was present in 50% IR treatment compared to the C. Galanthamine levels were 1.59 times lower in 25% IR treatment than in the C. In general, it was concluded that the amounts of lycorine in the leaves were increased with stress treatments. While galanthamine contents in the leaves were reduced by FS treatment, lycorine levels rose. There was an increase in galanthamine and lycorine amounts across the board for all PEG treatments (Table 4.5). It was found that there was a distinct increase and reduction in the amount of alkaloids in IR treatments that is independent of the proportion of stress.

There are not many stress studies about *L. aestivum*. In our study, we investigated the WS treatments on the *L. aestivum* plant. Similarly, Ates et al. (6) researched the influence of salt stress treatments on *L. aestivum*. They determined that the amount of galanthamine in the bulbs (28.5 mg/g dry extract) increased 4.13 times, and the amount of lycorine in the bulbs (69.5 mg/g dry extract) increased 1.39 times compared to the control group with the treatment of salinity stress to the *L. aestivum* using 4 g/L $CaCl_2$. They found that the amount of galanthamine in the leaves was elevated with the treatment of 8 g/L NaCl and that the salinity stress they applied had no effect on the amount of lycorine in the leaves. When compared to the WS treatments in our study, 50% IR gave a higher galanthamine amount in bulbs, while it was concluded that there was a greater increase when the salinity stress was compared to the control. Likewise, Ptak et al. (81) determined that the salinity stress applied by using 100 mM NaCl *in vitro* increased 2.6 times compared to the control group. The highest galanthamine and lycorine concentration was seen in Bolu, Turkey, during the driest months of July and August, according to Arslan et al. (62)'s study of the monthly variations of galanthamine and lycorine content in *L. aestivum*. The effect of DS on galanthamine and lycorine was also quite clear in the results of our study. The highest galanthamine contents of the bulb and leaf extracts were observed at 50% IR similar to Arslan et al. (62) results. Likewise, the highest lycorine contents of the bulb extracts were observed at 50 % IR whereas the highest lycorine contents of the leaf extracts were determined at 25 % IR. As a similar study Demir et al. (7) investigated seasonal variation in the alkaloid content of *L. aestivum*. They reported that the highest galanthamine content (29.53 mg/g) in bulbs was seen in the Gölcük-Bolu at the vegetative stage in April. They found that the highest galanthamine content (10.37 mg/g) in the leaves was seen in the Yalova at the vegetative stage in March. They determined the highest lycorine amount (26.17 mg/g) of bulbs in Sakarya during the vegetative growing period in March and the highest lycorine amount of leaves in Yalova (15.80 mg/g) in March during the vegetative growing period.

It is well-established that the production of natural products is highly dependent on growth circumstances, such as temperature, light regime, and nutrient availability. The metabolic pathways responsible for the production of secondary plant products are also affected by more severe environmental impacts, including various stress situations. Numerous tests have shown that plants exposed to DS

accumulated larger levels of SMs (82). The quantities of alkaloids such as trigonelline, pyrrolizidine alkaloids, quinolizidine alkaloids, steroid alkaloids, morphine alkaloids, indole alkaloids, nicotiana alkaloids, and benzylisoquinolines were found to rise in response to DS in many studies (82).

Sahoo et al. (83) investigated how many SMs were present in several arid medicinal plants throughout various seasons. They found that the total alkaloids of *Barleria prionitis*, *Boerhavia diffusa*, *Citrullus colocynthis*, and *Grewia tenax* significantly increased in the summer period when compared to the winter and rainy seasons. Guo et al. (11) investigated that different temperature in short-term and long-term conditions on *Catharanthus rosesus* L. They found that high-temperature treatments increased the alkaloid content. In short-term heat shock, the amounts of vindoline, catharanthine, and vinblastine in the leaves were higher at 40ºC than at 30ºC.

Liu et al. (84) investigated the effects of PEG-induced DS on the regulation of terpenoid indole alkaloid (TIA) biosynthesis in *Catharanthus roseus*. As a result, they observed that vindoline (VIN) and catharanthine (CAT) contents gradually enhanced and then reduced under 35% PEG 6000 stress however, vinblastine (VBL) content gradually increased. The results of the study show that growing *C. roseus* under DS can be used as an effective treatment for the accumulation of TIAs. Jaleel et al. (85) found that the ajmalicine amount enhanced in drought-stressed (water imposed WS of 10, 15, and 20 days interval drought) *Catharanthus roseus* when compared to the control plant. Popović et al. (86) investigated the flavonoid contents and phenolic acid levels of several poplar species. In the B229 genotype, they found an increase in the amount of chlorogenic acid and p-hydroxybenzoic acid in the leaf extracts with 200 mOsm PEG 6000 DS treatment. Likewise, the amount of chrysin, myricetin, and kaempferol increased with 200 mOsm PEG treatment. The amount of salicylic acid, isoferulic acid, chrysin, myricetin, and kaempferol increased with 100 mOsm PEG. For P19/66 genotype, they observed an increasing amount of chlorogenic acid in the leaf under 100 mOsm PEG. In the roots, protocatechic acid, myricetin, and chrysin with 200 mOsm PEG DS increased when compared to the control groups. They observed a significant increase in the flavonoids of B229 genotype roots (chrysin, myricetin, and kaempferol).

Weidner et al. (80) determined that the amount of *p*-coumaric acid, ferulic acid, and caffeic acid increased under severe DS conditions (35% soil moisture) compared to the control in the grapevine root extracts. They observed the highest increase in ferulic acid, *p*-coumaric acid, and caffeic acid, respectively. Sarker and Oba (87) determined the highest content of salicylic acid, vanillic acid, gallic acid, chlorogenic acid, and *p*-hydroxybenzoic acid with moderate and severe DS conditions in *Amaranthus tricolor* L.

Table 4. 4. Effect of WS treatments on extraction yield (%) of *L. aestivum*.

		***L. aestivum* extracts**	
		Extraction yield (%)	
Water Stress Treatments		**Bulb**	**Leaf**
Control	-	6.14	28.01
Flooding Stress	-	5.77	28.07
Drought stress generated by irrigation regimes	25%	6.62	25.98
	50%	6.47	25.02
	75%	6.20	28.16
Drought stress generated by PEG 6000	15%	7.23	28.77
	30%	7.28	29.49
	45%	7.32	26.92

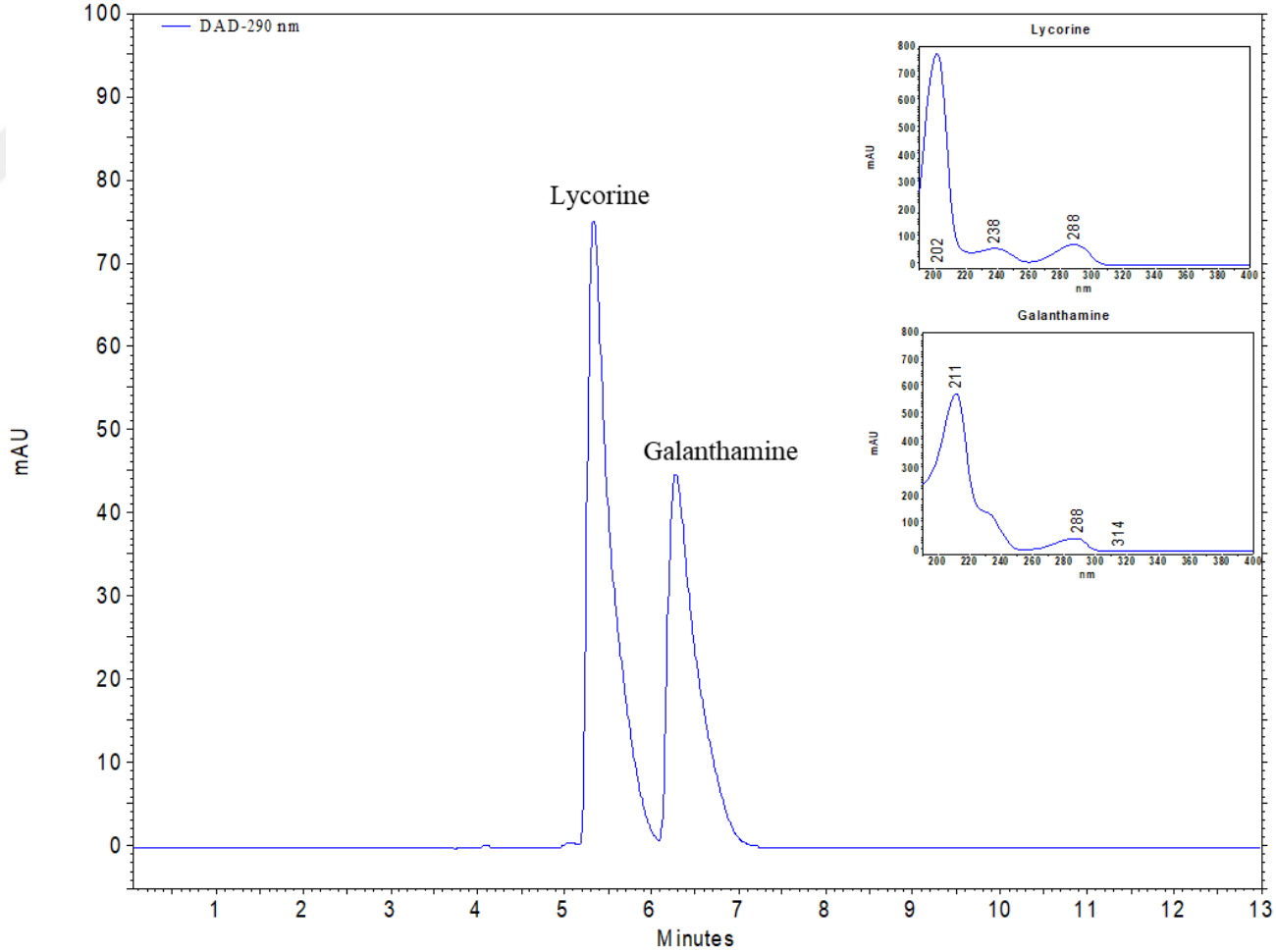

Figure 4. 5. HPLC chromatogram of alkaloid standards and their spectrums. Retention times: 1. Lycorine-5.31 min, 2. Galanthamine-6.29 min.

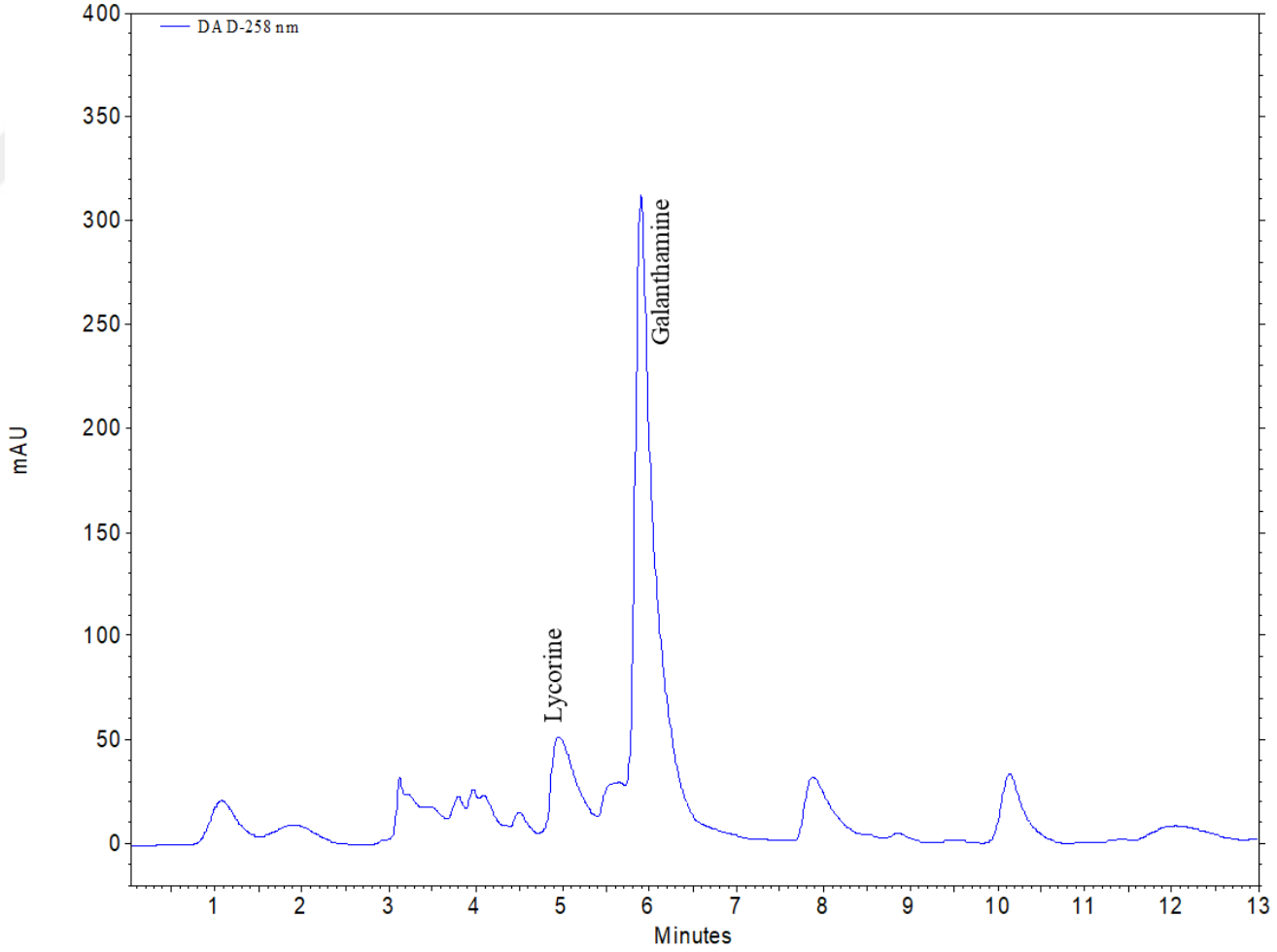

Figure 4. 6. HPLC chromatogram of bulb extract obtained from 50% IR treatment.

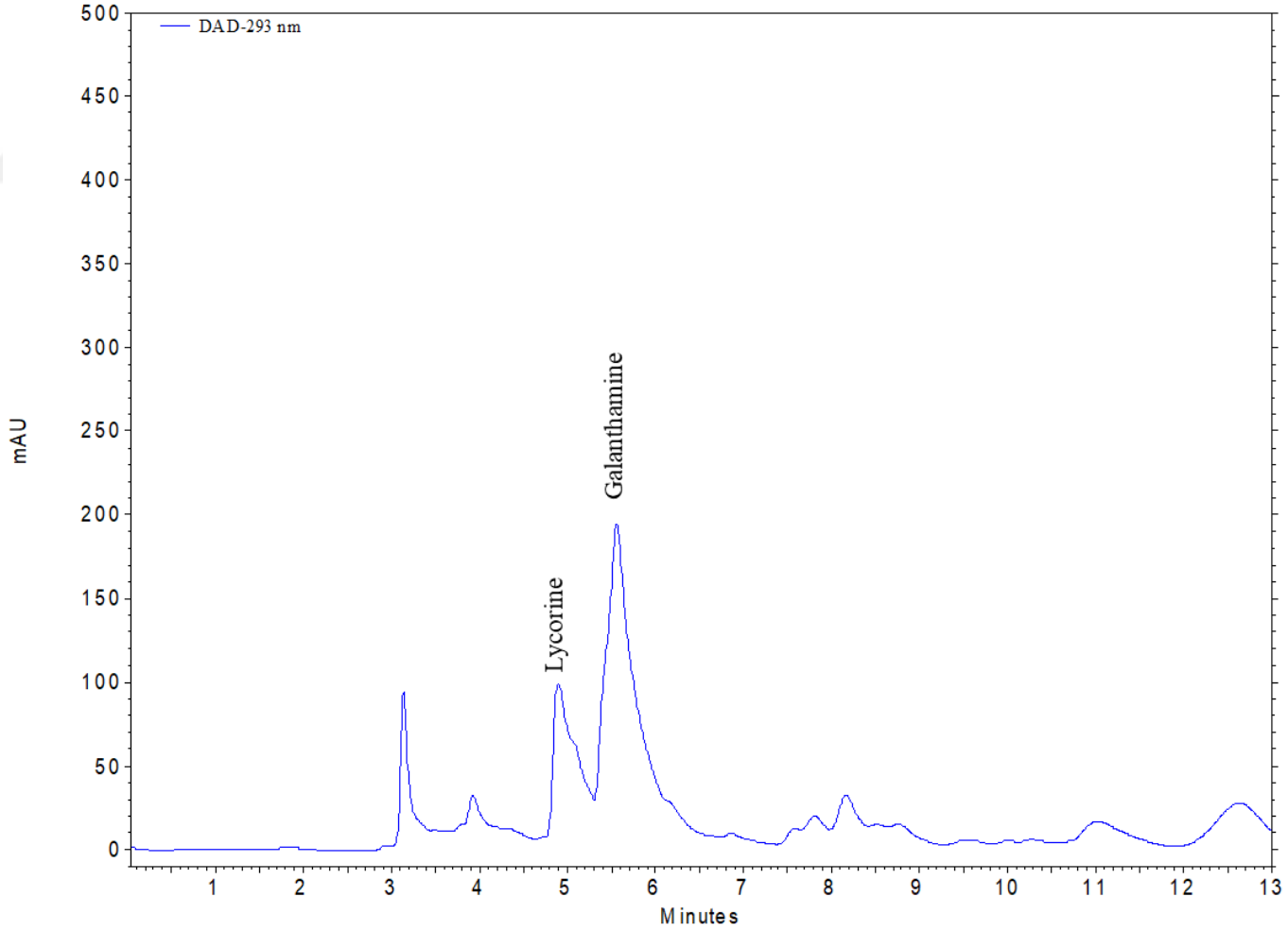

Figure 4. 7. HPLC chromatogram of leaf extract obtained from 50% IR treatment.

Table 4. 5. Effect of WS treatments on alkaloid quantities (galanthamine and lycorine) (mg/g) in the bulb and leaf methanolic extracts of *L. aestivum* with HPLC-DAD analysis.

		Alkaloids in *L. aestivum* (mg/g dry extract)			
		Bulb		Leaf	
Water Stress Treatments		Galanthamine	Lycorine	Galanthamine	Lycorine
Control	-	31.54 ± 0.36^{c}	29.66±0.21^{e}	21.70±0.03^{e}	3.43±0.00^{h}
Flooding Stress	-	31.32±0.11^{c}	31.56±0.09^{d}	20.66±0.25^{f}	4.28±0.05^{f}
Drought stress generated by irrigation regimes	25%	34.11±1.30^{ab}	35.29±0.69^{c}	13.68±0.03^{g}	6.36±0.01^{a}
	50%	34.92±0.06^{a}	40.19±0.01^{a}	26.17±0.02^{a}	5.84±0.00^{b}
	75%	28.76±0.44^{d}	27.54±0.24^{f}	20.97±0.06^{f}	3.67±0.01^{g}
Drought stress generated by PEG 6000	15%	32.79±0.09^{bc}	37.33±0.10^{b}	24.77±0.06^{b}	4.96±0.00^{d}
	30%	31.99±0.25^{c}	29.48±0.10^{e}	24.12±0.10^{c}	4.45±0.02^{e}
	45%	32.16±0.37^{c}	31.25±0.20^{d}	23.43±0.39^{d}	5.06±0.07^{c}

4.4 Non-Enzymatic Antioxidant Activities

4.4.1 Total Phenolic and Total Flavonoid Assay

Due to the hydroxyl groups on their structures, phenols and flavonoids are strong FR scavengers. The total phenolic and flavonoid contents of all methanol extracts are shown in Table 4.6. The calibration curve for gallic acid (R^2 = 0.999) was utilized to calculate the TPC of the *L. aestivum* bulb and leaf extracts (Appendix 1). All treatments had greater TPC than the C, according to an analysis of bulb extract values. Thus, it can be concluded that all treatments resulted in an increase in the TPC of the bulbs. When comparing the results of treatments, 50% IR treatment with 34.30 mg GAE/g had the greatest TPC (Table 4.6). 25% IR (31.73 mg GAE/g) and 75% IR (30.28 mg GAE/g) were observed to have the second and third highest phenolic levels, respectively. In terms of TPC, 15% and 45% PEG treatments were quite close to the C. Upon closer inspection of the leaf extracts, it was clear that 15% PEG treatment with 11.71 mg GAE/g gave the best TPC result (Table 4.6). Other stress treatments produced outcomes that were similar to the C, and the treatments in the C received an average value. With a value of 6.96 mg GAE/g, the flooding treatment had a very low TPC.

The quercetin calibration curve (R^2 = 0.999) was utilized to identify the TFC of *L. aestivum* extracts (Appendix 2). When comparing the TFC of the C (2.59 mg QE/g dry extract) to the WS groups for bulb extracts, 50% IR showed the highest flavonoid concentration (3.74 mg QE/g dry extract) (Table 4.6). The lowest TFC was found in 15% and 45% PEG treatments with 2.21 mg QE/g dry extract, whereas the other treatments were quite similar to the C treatment. Looking at the results of the leaf extracts, it was apparent that the use of 50% IR with 75.17 mg QE/g dry extract had the highest flavonoid concentration similar to the bulbs (Table 4.6). Treatment of 15% PEG was followed by a result of 69.41 mg QE/g dry extract. 30% PEG treatment with 60.02 mg QE/g dry extract had the lowest TFC in the leaves, but the C had a similar average value.

Hundur et al. (88) found total phenol and flavonoid content of methanolic extracts of naturally grown *L. aestivum* bulbs and leaves were 58.92 GAE mg/g and 85 QE mg/g in the bulbs, and 53.93 GAE mg/g and 68.33 QE mg/g in the leaves, respectively. Compared with our study, it was seen that there was a lower amount of total phenol and flavonoid content in the C. However, it was concluded that 50% IR treatment as TFC in the leaves was higher than Hundur et al. (88) results with

75.17 QE mg/g amount. Ates et al. (6) found that with the salinity stress created by 4 g/L $CaCl_2$ applied to the *L. aestivum* plant, a higher amount of total phenol and flavonoid content was obtained in the bulbs compared to the C. These amounts were 27.67 mg GAE/g and 10.95 mg QE/g, respectively. The highest TPC in leaves was determined in the plant grown with 2 g/L NaCl (36.5 mg GAE/g), while the plants grown at the highest NaCl concentration (8 g/L) contained the highest TFC in leaves with 126.23 mg QE/g dry extract. In parallel with our study, it can be concluded that water and salinity stress, which were abiotic stress factors, increased the non-enzymatic antioxidant activity. Resetar et al. (89) reported TPC values as 4.9 mg GAE/g in bulbs and 15.93 mg GAE/g in the leaves of *L. aestivum*. A contrast relationship was observed compared to the C in our study. Demir et al. (7) investigated the total phenolic and flavonoid content of *L. aestivum* in different regions. They determined bulbs collected at ripening stages (30.24 mg GAE/g) in August and leaves collected at vegetative stages (21.03 mg GAE/g) in April in Yeniçağa-Bolu had the best TPC, respectively. They determined bulbs collected at vegetative stages in March (70.70 mg QE/g) in Sakarya and leaves collected at vegetative stages in April (170.85 mg QE/g) in Gölcük-Bolu had the best TFC, respectively. Gharibi et al. (90) determined that the TPC of *Achillea nobilis* L., followed by *Achillea millefolium* L. and *Achillea filipendulina* L., increased under severe DS (25% field capacity). The TFC of *A. filipendulina*, followed by *A. millefolium* and *A. nobilis*, increased under severe DS conditions. Bettaieb et al. (91) determined the TPC of cumin under moderate and severe DS conditions. They found that the DS conditions significantly enhanced the TPC of cumin 26.34 mg GAE/g and 21.12 mg GAE/g, respectively. Popović et al. (86) found that the TPC of *Populus deltoides* L. slightly increased compared to the control under WS by 100 and 200 mOsm PEG 6000 treatment. However, they did not observe a significant difference. Weidner et al. (80) indicated the highest TPC of grapevines (15.7 mg/g FW) in the roots with severe DS conditions (35% soil moisture) compared to the control (9.6 mg/g FW). Sarker and Oba (87) reported that the total phenolic (45%) and flavonoid (60%) content of *Amaranthus tricolor* L. increased with severe DS conditions.

Table 4. 6. Effect of WS treatments on total phenol and flavonoid contents of methanol extracts of the *L. aestivum*.

		***L. aestivum* extracts**			
Water Stress Treatments		**Total Phenol (mg GAE/g extract)**		**Total Flavonoid (mg QE/g extract)**	
		Bulb	Leaf	Bulb	Leaf
Control	-	25.78 ± 0.87^{e}	10.48 ± 0.33^{abc}	2.59 ± 0.05^{bc}	63.58 ± 0.27^{d}
Flooding Stress	-	28.90 ± 0.41^{cd}	6.96 ± 0.19^{d}	2.62 ± 0.08^{bc}	64.64 ± 0.27^{c}
Drought stress generated by irrigation regimes	25%	31.73 ± 0.92^{b}	11.20 ± 0.31^{ab}	2.74 ± 0.05^{b}	62.59 ± 0.35^{e}
	50%	34.30 ± 0.15^{a}	9.85 ± 0.30^{bc}	3.74 ± 0.02^{a}	75.17 ± 0.35^{a}
	75%	30.28 ± 0.37^{bc}	9.58 ± 0.58^{c}	2.62 ± 0.08^{bc}	61.77 ± 0.07^{e}
Drought stress generated by PEG 6000	15%	27.08 ± 0.81^{de}	11.71 ± 0.44^{a}	2.21 ± 0.12^{d}	69.41 ± 0.35^{b}
	30%	30.11 ± 1.11^{bc}	10.28 ± 0.37^{bc}	2.44 ± 0.05^{cd}	60.02 ± 0.50^{f}
	45%	26.21 ± 0.06^{e}	11.19 ± 0.54^{ab}	2.21 ± 0.12^{d}	62.44 ± 0.11^{e}

4.4.2 Free Radical Scavenging Activity - DPPH

The DPPH capacities of the *L. aestivum* plant's leaves and bulbs were reported as the experiment's half-maximum inhibitory concentration (IC_{50}), using quercetin as a reference (Appendix 3).

It is clearly visible that bulbs had a higher capacity when comparing the radical scavenging capacities of bulb and leaf extracts (Table 4.7). In comparison to the C, stress treatments on leaves decreased their capacity to scavenge radicals. 15% PEG treatment in particular showed that leaves had the lowest capability for radical scavenging. 50% IR in the bulbs had the strongest DPPH activity, with an IC_{50} value of 5.94 mg/ml, 1.31-fold bigger than the IC_{50} value of the C (Table 4.7). Having followed 50% IR treatment's ability to scavenge FRs was 25% IR with a 6.73 mg/ml IC_{50} and 15% PEG treatment with a 6.84 mg/ml IC_{50}. In comparison to the C, FS treatment slightly raised the capacity in the bulbs, but it lowered it in the leaves. While IR treatments produced outcomes for leaves that were similar, they produced different results for the bulbs in terms of FR scavenging ability that was not related to stress levels. 30% and 45% PEG treatments provided substantially similar outcomes to the C in PEG treatments (Table 4.7).

Hundur et al. (88) discovered that the IC_{50} values of the *L. aestivum* bulb and leaf methanolic extracts were 317 µg/ml and 345 µg/ml, respectively. Ates et al. (6) determined that the bulb extracts of the *L. aestivum* plant, to which they applied salinity stress, showed low radical scavenging capacity (> 20 mg/mL IC_{50} inhibition). They concluded that only 4 g/L $CaCl_2$ and 8 g/L NaCl treatments from leaf extracts enhanced the antioxidant capacity with IC_{50} values of 14.40 mg/mL. This is a very low activity result compared to the DPPH results in our study. In our study, a 50% IR was determined to be the most effective treatment for the bulbs. However, in the leaf extracts, a significant difference was not observed. Manurung et al. (79) generated 40%, 60%, 80% and 100% DS on tabat barito plants. They reported the highest IC_{50} value (72.47 $\mu g/mL^{-1}$) with 40% DS condition on tabat barito leaves. Bettaieb et al. (91) investigated the antioxidant capacity of the cumin under moderate and severe water deficits. They determined that all extracts had the capacity to scavenge DPPH FRs. They indicated that severe water deficit conditions mostly increased the IC_{50} value of cumin extracts.

Popović et al. (86) applied DS (100 and 200 mOsm PEG 6000) on three poplar genotypes (M1, B229, and PE19/66) for six days. They found a significant increase in antioxidant activity in the B229 (*Populus deltoides* L.) leaf under 100 mOsm PEG 6000 stress treatment. The DPPH ARP (antiradical power) was increased by 19.8% when compared to the control. Weidner et al. (80) found a decline in the DPPH radical scavenging activity of grapevine plants with severe DS conditions (35% soil moisture). Sarker and Oba (87) found that severe DS increased the DPPH scavenging activity (77%) and $ABTS^{+}$ (99%) of *Amaranthus tricolor* L. Gharibi et al. (90) determined the highest DPPH scavenging activity of *Achillea millefolium* L. (70.28 %) followed by *Achillea nobilis* L. (58.86 %) and *Achillea filipendulina* L. (53.21 %) under severe drought conditions (25% field capacity).

Table 4. 7. Effect of WS treatments on DPPH radical scavenging activity of methanol extracts of the *L. aestivum*.

Water Stress Treatments		*L. aestivum* extracts	
		IC_{50} DPPH Inhibition (mg/ml)	
		Bulb	Leaf
Control	-	7.78 ± 0.13^{f}	10.60 ± 0.11^{b}
Flooding Stress	-	7.17 ± 0.10^{d}	13.08 ± 0.23^{e}
Drought stress generated by irrigation regimes	25%	6.73 ± 0.03^{c}	11.30 ± 0.03^{c}
	50%	5.94 ± 0.09^{b}	11.24 ± 0.19^{c}
	75%	7.49 ± 0.00^{e}	11.40 ± 0.12^{c}
Drought stress generated by PEG 6000	15%	6.84 ± 0.02^{c}	14.61 ± 0.37^{f}
	30%	7.88 ± 0.14^{f}	12.29 ± 0.01^{d}
	45%	7.73 ± 0.08^{f}	12.68 ± 0.12^{de}
Quercetin	-	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}

4.5 Enzymatic Antioxidant Activities

4.5.1 SOD and CAT Activities

The alterations in the SOD and CAT activities of *L. aestivum* bulbs and leaves were demonstrated in Table 4.8. Even if there were no significant numerical differences in the results of SOD activity, when we make comments by looking at the Duncan significant letters, the best activity in bulbs was seen in the FS treatment (Table 4.8). Compared to the C, SOD activity was decreased by IR treatments. 25% IR treatment, which has the lowest activity of the treatments, made this condition fairly evident. Treatments of PEG demonstrated average activity comparable to the C (Table 4.8). As compared to the C, WS treatments in the leaves revealed less SOD activity. The treatment of 50% IR was shown to be the lowest activity.

Increased CAT activity was determined in the bulbs with all WS treatments (Table 4.8). 15% PEG treatment which was enhanced 2.51 times in comparison to the C had the best result (Table 4.8). With an increase of 2.50 times against the C, 25% IR treatment had the second-highest CAT activity. FS treatment resulted in the lowest increase in CAT activity as compared to the C, with a 1.43-fold increase rate. When CAT activity results in the leaves were investigated, it was found that 25% IR group had the largest increase and CAT activity value with a 1.25-fold increase rate compared to the C (Table 4.8). It was observed that 45% PEG treatment had the second-highest CAT activity. The result of FS treatment was close to C, and the lowest CAT activity result was seen in 50% IR group.

Ptak et al. (81) found that only 50 and 150 mM NaCl treatments enhanced SOD activity, but all tested doses of NaCl (50, 100, 150, and 200 mM) increased CAT activity in *in vitro* grown *L. aestivum*. Similar to this study, Ates et al. (6) reported increased enzymatic activity in the bulbs and leaves of the *L. aestivum* plant at some concentrations in the salinity stress study they applied using NaCl and $CaCl_2$. Stress-induced variation of antioxidants rely on the severity and duration of the treatment and the species and age of the plant (92). SOD activity decreased in our study except for FS in the bulb extracts, however, increased in Ptak et al. (81) and Ates et al. (6) studies. Likewise, CAT activity increased with 25% IR treatment 2.50 and 1.25 times when compared to the control in the bulb and leaf extracts, respectively in our study.

In FS treatments, the SOD activity of the bulb extracts and CAT activity of the bulb and leaf extracts increased significantly. However, Ahmed et al. (93) determined that SOD, CAT, ascorbate peroxidase (APX), and glutathione reductase (GR) reduced during prolonged waterlogging treatment on mungbean. Yan et al. (94) determined the SOD activity decreased with FS on corn leaves. Contrary to these results, Tang et al. (95) revealed that antioxidant enzymes increased in maize seedlings with different flooding treatments. Kumutha et al. (96) determined a rise in the antioxidant enzymes such as SOD, APX, GR, and CAT increased with FS on pigeon pea plants. Yosefi et al. (75) observed that PEG-induced DS led to higher activity in the SOD and POD (peroxidase) enzymes at 7% PEG treatment on *Fragaria* × *ananassa*. Batool et al. (76) found that 15 % PEG 6000 treatment increased the SOD and CAT activity of rapeseed cultivars JYZ 158, FY 520, YG 2009, and NZ 1838. However, drought-tolerant cultivars (JYZ 158 and FY 520) of rapeseeds more increased than sensitive cultivars (YG 2009 and NZ 1838). Murshed et al. (97) reported that SOD activity increased 35 days after flowering with WS treatments (0%, 25% and 50%) whereas the CAT activity in fruits was raised by WS treatments except 35 days after the flowering stage in tomato plants. Jaleel et al. (85) determined that SOD activity increased in all the WS of 10, 15, and 20 days interval drought on *Catharanthus roseus* roots. In the leaves, 15 and 20 days interval drought increased the SOD activity. The CAT activity was mostly enhanced in the roots, and they did not determine a significant change in the leaf extracts. Pourghayoumi et al. (98) indicated that severe WS significantly enhanced SOD and CAT activity in all pomegranate cultivars. SOD activity was raised by 42.68, 29.96, 19.04, 18.91, and 12.2% in ‘Rabab’, ‘Shishecap’, ‘M-Saveh’, ‘M-Yazdi’, and ‘Gho- jagh’, respectively) when compared to the control. CAT activity was significantly enhanced in all cultivars, except ‘M-Yazdi’.

Chakraborty et al. (99) investigated the effects of water deficit stress on *Arachis hypogaea* plants. They determined the highest SOD activity in ICGS 44, TAG 24, and AK 159 cultivars at the pegging stage compared to the control groups with WS. They discovered the greatest levels of SOD activity in all the cultivars in the pod stage, and they identified the highest increases in ICGS 44 and TAG 24. At the pegging stage, they observed the highest CAT activity TAG 24 and ICGS 44 whereas they observed a reduction in ICGV 86031 and DRG 1. At the pod stage, CAT activity was increased in all cultivars with water deficit stress.

Pan et al. (92) indicated that PEG 6000-induced DS decreased the SOD and CAT antioxidant enzyme activities except for POD activity in *Glycyrrhiza uralensis* L. SOD and CAT activity were decreased in all treatments of PEG-6000 (24h, 48h, 72h, and 96h). Similarly, POD activity was only increased with PEG-6000 treatment with 96h treatment. Yuan et al. (100) generated WS; control (75 to 80% of field water capacity), mild WS (55 to 60%), moderate WS (45 to 50%), and severe WS (35 to 40%) on tomato plant. They found that the SOD activity significantly enhanced in water-stress-treated plants at all developmental stages. POD and CAT activity also increased. Antioxidant enzyme activities were increased with the increasing degree of WS. Slabbert and Krüger (101) reported that the SOD activity of *Amaranthus* species *(Amaranthus tricolor* L., *Amaranthus hypochondriacus* L., and *Amaranthus hybridus* L.) was increased with WS.

Table 4. 8. Effect of WS treatments on SOD and CAT activity of methanol extracts of the *L. aestivum*.

		***L. aestivum* extracts**			
Water Stress Treatments		**SOD Activity (U/mg protein)**		**CAT Activity (mmol/min/mg protein)**	
		Bulb	Leaf	Bulb	Leaf
Control	-	0.096 ± 0.000^{bc}	0.061 ± 0.000^{ab}	6.213 ± 0.732^{d}	29.111 ± 0.945^{cd}
Flooding Stress	-	0.104 ± 0.000^{a}	0.059 ± 0.000^{bc}	8.886 ± 1.451^{cd}	31.456 ± 0.802^{bc}
Drought stress generated by irrigation regimes	25%	0.081 ± 0.000^{f}	0.057 ± 0.000^{c}	15.512 ± 1.242^{a}	36.284 ± 1.833^{a}
	50%	0.085 ± 0.001^{e}	0.050 ± 0.000^{e}	9.105 ± 0.326^{cd}	22.152 ± 0.694^{f}
	75%	0.090 ± 0.001^{d}	0.054 ± 0.000^{d}	13.523 ± 1.041^{ab}	30.285 ± 1.071^{cd}
Drought stress generated by PEG 6000	15%	0.095 ± 0.001^{bc}	0.052 ± 0.000^{de}	15.583 ± 1.113^{a}	26.856 ± 0.725^{de}
	30%	0.097 ± 0.001^{b}	0.063 ± 0.001^{a}	10.576 ± 2.001^{bc}	25.392 ± 1.044^{ef}
	45%	0.094 ± 0.000^{c}	0.057 ± 0.001^{c}	10.272 ± 0.545^{bcd}	34.045 ± 1.442^{ab}

5. CONCLUSIONS AND RECOMMENDATIONS

This study was carried out to investigate the effects of various WS treatments [C, FS, different IR adjustments (25%, 50%, and 75%), and PEG 6000 treatments (15%, 30%, and 45%)] on the growth and development, galanthamine and lycorine content, and non-enzymatic and enzymatic antioxidant activities in *L. aestivum* bulbs and leaves.

Treatment of 45% PEG significantly increased the bulb width and fresh weight. Treatment of 25% IR notably elevated the leaf width, whereas 15% PEG treatment increased the leaf fresh weight. Bulb and leaf lengths did not show a significant change with WS treatments. Under WS treatments, the water content of bulbs decreased slightly. However, bulb and leaf water content were slightly increased with 25% IR.

Among WS treatments, galanthamine and lycorine levels in the bulbs were found to be highest at 50% IR concentration. In the leaves, the highest galanthamine content was determined with 50% IR, whereas the highest lycorine content was determined with 25% IR.

The total phenol-flavonoid content and FR scavenging activity (DPPH) were used to determine the non-enzymatic antioxidant activity. With 50% IR, the TPC of the bulbs were increased significantly. The treatment of 15% PEG enhanced the TPC of the leaves significantly. 50% IR significantly rised the TFC of the bulbs and leaves. Bulbs under 50% IR showed better antioxidant activity with an IC_{50} value of 5.94 mg/ml. Leaf extract did not show a significant difference in antioxidant activity.

The enzymatic antioxidant activity of *L. aestivum* was determined by SOD and CAT. FS treatment resulted in the highest SOD activity in the bulb extracts. Leaves did not demonstrate higher SOD activity under IR treatments. 25% IR and 15% PEG treatments significantly increased CAT activity of the bulbs, whereas the highest CAT activity was determined with 25% IR in the leaves.

Severe (25% IR) and moderate (50% IR) WS conditions improved the alkaloid content and antioxidant defense enzymes significantly, especially 50% IR treatment. *L. aestivum* is a drought- and stress-tolerant plant as a result of the enhanced activity of antioxidant defense enzymes. *L. aestivum* has been established in Turkey in areas that have been authorized for the transport of bulbs to the pharmaceutical sector. It would be easily planted in drought-stricken areas to increase the production of galanthamine. In future studies, different abiotic stress conditions should be used to study how this alkaloid can be improved.

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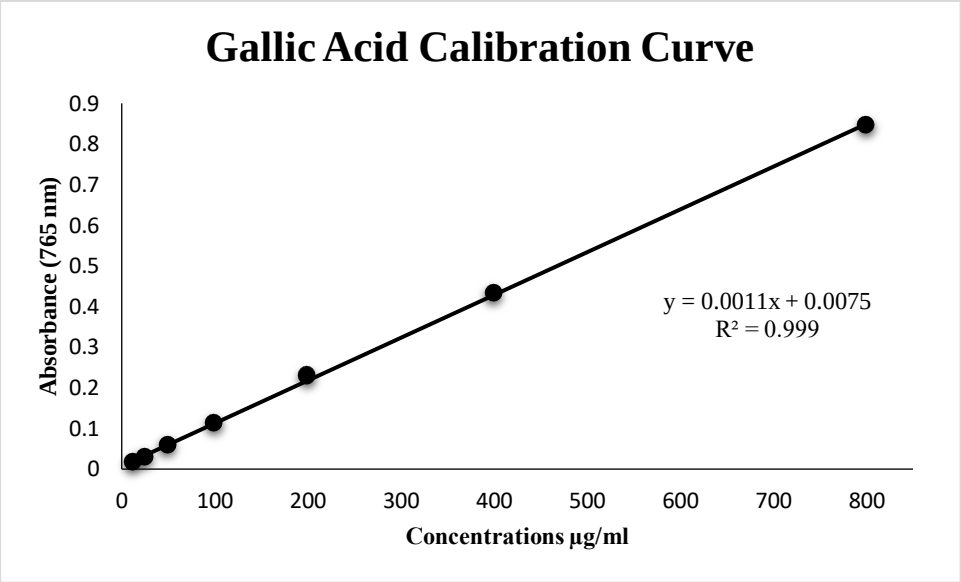
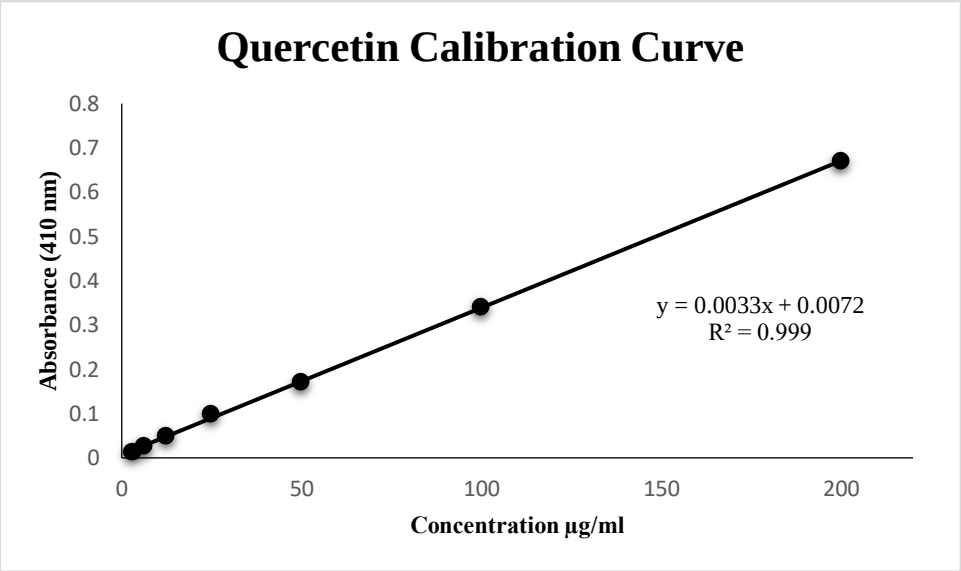
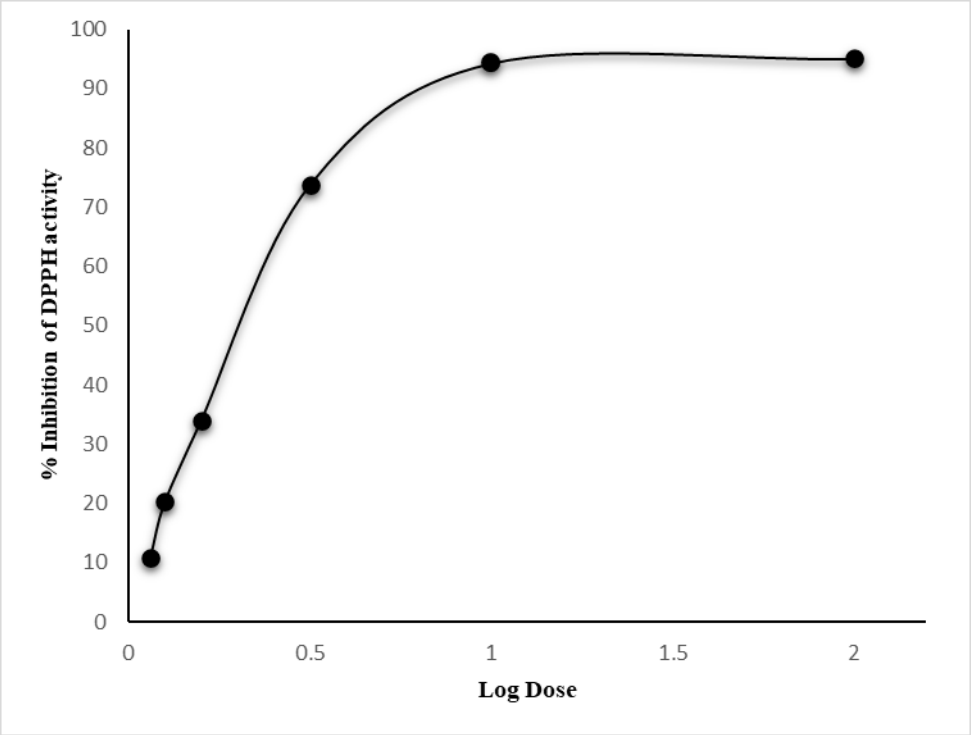
7. APPENDICES



Apendix 1. Calibration curve for gallic acid standard of water stress treatments.



Apendix 2. Calibration curve for quercetin standard of water stress treatments.



Apendix 3. IC_{50} value of quercetin standard for DPPH free radical scavenging effect of water stress treatments.