SECTIONS FROM LIFE AND AGRICULTURE

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PREFACE

Life and agriculture go hand in hand. They are pieces that complement each other. Because life cannot continue without agriculture, animal husbandry and crop production. Sometimes it is possible to replace the missing parts with other components, but if agriculture and livestock are missing or if the work places are insufficient, life will also be interrupted.

In this book, the sections where life and agriculture affect and integrate each other are mentioned. I would like to thank our authors for presenting their experiences and sharing their knowledge with the readers, with examples from all sections of life and agriculture.

Sincerely Yours,

ARZU ÇIĞ

CHAPTER 1

SUGENO FUZZY INFERENCE ALGORITHM AND APPLICATION IN LIVESTOCK

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INTRODUCTION

Seen as a technique that can express linguistic uncertainty mathematically, fuzzy set theory is a mathematical criterion for a wide variety of uncertain events, including the concept of probability. Here, the uncertainty is modeled with the logical system. Due to the different structures of fuzzy systems, fuzzy inference methods are also different. Frequently used fuzzy inference techniques are Mamdani and Sugeno methods. The Mamdani method is largely used in fuzzy expert systems due to its capacity to reflect expert information in fuzzy rules. Mamdani type fuzzy inference requires a significant computational load. In Sugeno fuzzy system, linear functions are formed instead of fuzzy sets in the results of fuzzy rules. The output of this system is a "fuzzy" combination of a set of linear functions. The Sugeno method works well with optimization and adaptive techniques, making it easy to model especially for dynamic nonlinear systems.

In 1985, Takagi and Sugeno (Mallows, 1973) modified the consequence of implication from fuzzy sets to linear functions and developed the so-called "Takagi-Sugeno fuzzy systems" which were applied to parking control of a model car (Zhang et al., 1994; Chak et al., 1998). The format of their fuzzy rules is

If x_1 is A_1 and x_2 is A_2 and … x_n is A_n, then $y = a_0 + a_1x_1 + \ldots + a_nx_n$.

The structure of these systems varies significantly from that of the conventional one. As a consequence of implication, they contain a linear function by which the output can be computed. The aim of the

linear function in Takagi-Sugeno fuzzy systems is to describe the local linear behavior of the system. Fuzziness, which appears only in the premise part of the fuzzy rule, indicates the uncertainty about which the output range of the linear function varies. Takagi-Sugeno fuzzy systems have a number of advantages by their nature. The systems can be easily understood and the local system equations can be directly related to the local behavior of the system. Takagi-Sugeno fuzzy systems include two kinds of knowledge: one is the qualitative knowledge represented by the if-then rules, and the other is the quantitative knowledge represented by the local functions. The systems allow us to formulate these two kinds of knowledge into a unified mathematical framework (Chak et al., 1998). Behind the best-decision making and modeling process within the automation and atmosphere of uncertainty underlies fuzzy logic proposition and reasoning through smart and expert systems, which are common in today's technology. Today fuzzy logic has taken place widespread not only in designing and manufacturing but also in practice with what we call smart robots as integral parts of our life within the area of technology. Recently in Türkiye fuzzy logic has an important place in learning and application of system and control principles, at least in scientific and research areas. Research and Development units of many international businesses have been needed fuzzy system and control mechanisms. This necessity has emerged in Türkiye as well (Şen, 2001).

The aim of this study is to estimate the live weight of hair goats by applying the Sugeno fuzzy inference method. Different body measurements will be used as input.

1. FUZZY EXPERT SYSTEM APPLICATIONS IN LIVESTOCK

Fuzzy logic based expert systems recently has begun to be implemented in livestock; some examples of the studies are given below.

Wade et al. (1998), stated that in their study based on monthly production data, if the cows have lower milk yield, longer birth interval and older ages then culling can be done easily but it is difficult to decide when the milk yield and age are high and the birth interval is long. In the fuzzy logic model, yield index, parity and reproductive efficiency evaluated as input and culling as an output. Consequently, they decided which animals will be culled from the herd according to the results.

De Mol & Woldtf (2001) in their study, tried to estimate which cow are in estrus, which cow is infected with mastitis by considering activity, milk temperature and electrical conductivity properties for each cow. They stated that the mastitis is the basic problems of dairy cattle farms and a very costly disease, therefore early diagnosis of the mastitis is very important. Also, they stated that the chance of visual diagnosis of udder infections in automatic milking systems is very low, but in these automatic systems, diseases can be detected based on some data such as milk yield, milk temperature and electrical conductivity.

Firk et al. (2002a) have benefited from fuzzy logic for the correct detection of estrus in the farms having herd management software. For this purpose, they used cow activity (number of steps) and the period lasts from the latest estrus. As a result, they decided that the cow is not in estrus when the following situations: the activity is low; the period from the latest estrus is short, normal, longer than normal and long; activity is medium; the period from the latest estrus is short and longer than normal; activity is high and the period from the latest estrus is short. In the other combinations, they agreed that the cow is in estrus. In another study, Firk et al. (2002b) stated that the accuracy of fuzzy logic model is enhanced and its error is reduced when the activity, milk yield, milk flow rate and electrical conductivity evaluated all together with adding the period lasts from the latest estrus.

Wang & Samarashinge (2005) tried to envisage a model which can detect mastitis online in the farms using automatic milking system. They stated that it is possible to develop systems which can diagnose also mastitis during milking in automatic milking system thanks to measuring electrical conductivity, milk yield and milk temperature.

Cavero et al. (2006) studied to determine mastitis by a fuzzy expert system based on the data of electrical conductivity, milk production rate and milk flow rate. The researchers evaluated the model according to sensitivity, specificity and error ratio and reported that the specificity of mastitis diagnose changes between 75.8% and 93.9% and the error ratio varied from 41.9% to 95.5% when the sensitivity ratio is at least 80%.

Alizadeh et al. (2008) in their study introduced a novel application of fuzzy logic based expert systems for type judging of dairy cattle.

Filho et al. (2011) in their work developed a system based on fuzzy rules, which indicates the body mass index of ruminant animals in order to obtain the best time to slaughter. The performance validation of the system was based on a statistical analysis using the Pearson correlation coefficient of 0.923, representing a high positive correlation, indicating that the proposed method is appropriate.

Memmedova et al. (2011) have demonstrated a sample model for culling the animals by using a ranking list prepared from first calving age, calving interval and lactation milk yield properties.

In a study carried out by Memmedova & Keskin (2011) in Holstein cows to detect estrus correctly, it was reported that the cows in estrus can be determined with an accuracy of 98% that can be accepted as high ratio by using a fuzzy logic model which evaluates cow activity feature, cow type for this property and the period lasts from the latest estrus together.

Taşdemir et al. (2011), in their study aimed at determining body sizes of Holstein cows through image analysis (IA) and estimating live weights (LW) of them using body sizes along with a fuzzy logic based model, created an image atmosphere in a huge cattle farm, stating that 0number of cows were determined. During the first stage, digital images of each animal was taken by cameras and body sizes, such as wither height (WH), hip height (HH), body length (BL) and hip width

(HW), were measured manually and through meter, laser and test strip. LWs of cows were available at a weighing scale and data were recorded automatically in a computer. During the second stage, images were analyzed through IA method and Delphi programming language and body sizes were calculated. Values measured manually were determined to be very close to IA results. As a result, a fuzzy system was developed by using body sizes. This Fuzzy System was developed via MATLAB software. Weights estimated via methods developed were compared with those in Information Based System and platform scale. Correlation coefficient ($R^2 = 0.99$) was calculated. A statistically significant relation was determined among the compared data.

Neto et al. (2014) in their paper tried to develop a fuzzy inference based on expert system to help preventing lameness in dairy cattle. Hoof length, nutritional parameters and flor material properties (roughness) were used to build the fuzzy inference system.

Mikail & Keskin (2015) in their study showed that, subclinical mastitis can be diagnosed at an early stage with the help of fuzzy logic-based expert systems which interpret the data like daily milk yield, electrical conductivity, automatic milking duration and season in dairy farms using herd management software.

2. MATERIAL AND METHOD

2.1. Material

2.1.1. Hair Goats

Today goat breeding is a production branch which is becoming more important. The number of goat in Türkiye is close to the number of goat in whole Europe. The quality of goat meat and particularly goat milk plays a crucial role in attracting more attention in goat breeding in the world. Goat milk is distinctive as it is the closest milk to the breast milk among the consumable milk products. With 34 times more calcium content than breast milk and digestive system problems related to cow's milk, goat milk is considered more advantageous. This milk is valued as it is preferred more when dairy products are made through goat milk and some special products are produced depending on the milk.

2.1.2. The Climatic and Geographic Structure of Siirt

This study was performed between 2015-2017 at Siirt University. Siirt Province is geographically located between 41˚-57' East longitude and 37˚-55' latitude. The Province is surrounded by Şırnak and Van provinces in the East, Batman in the West, Batman and Bitlis in the North and Şırnak and Mardin in the South. Dicle Valley and some of the city's mountains which are of great importance are located in the east of the town, and the highest point is 2838m. The highest valley called Cemikari (Botan valleys), and high mountains such as Ceman and Herekol are also situated within the borders of this town. The Southeastern Mountains of the city are called Yassi and Şeyh Omar

mountains. The streams and rivers within the province are namely Reşinan, Garzan, Kezer, Başur, Botan (Uluçay). The altitude of the city is between 600-1600m (Anonymous, 2017a).

Siirt Province with its wide pastures and plateaus is very suitable for cattle grazing. Animal breeding in the area is the main means of living. Mainly pasture animal breeding is performed in the city. However, livestock is not so developed here. There are especially small cattle such as sheep and goat bred. The people called nomads generally deal with animal breeding in the region. These are the ones who do not settle in one area instead go to highlands in the summers, come back to their winter quarters in the winters, wandering around with their herds almost the whole year. The nomads, who spend the summers in Siirt, Bitlis, Hakkari, Van and Muş highlands and winters in some calmer places make use of the animal products they get to get profit in these towns. The dense population of nomads in Siirt causes an increase in the animal products in the area (Anonymous, 2017b).

Siirt has generally got a continental climate. The winter is very harsh in the northern and eastern parts and wet whereas in the southern and southwestern parts. Winters are mild however in the summers the province is very dry and hot. The average rainfall is about 698.6 mm The hottest days in Siirt (on average 46.0 °C) are in August and the coldest days are in January (-19.3 °C). The average heat in the town is 15.9 °C according to the last 60 years average. The average number of overcast days in the town is 60.5, clear days 154 and finally cloudy days 151.2. The average of relative humidity is 51% and the months which

has the highest rate of relative humidity are January and August with a percentage of 70% (Anonymous, 2017c).

2.1.3. The Animals Used in the Research

The material of the study was provided from a private farm. The data were collected from 81 female hair goats. Heart girth (HG), Body depth (BD), Body length (BL) and Live body weight (LBW) parameters were recorded after 8 hours of feed restriction. Body measurements were taken by a tape measure and body weight was taken using a digital scale (Chacon et al., 2011).

1. Heart Girth: is a circumferential measure taken around the chest just behind the front legs and withers. While the measurement, the animal kept stable and the accuracy of the reading of the meter was double checked.

2. Body Depth: For this parameter, the height between the very end of the goat's front leg and its back was measured. The stability of the goat is very important during these measurement processes.

3. Body Length: refers to the distance from the base of the ear to the base of the tail. It can also be measured as the distance from base of tail to the base of neck (first thoracic vertebrae), or to front of the chest or to tip of the nose (Mahmud et al., 2014).

4. Body Weight: The goat which body parameters were measured was weighed according to its body weight by digital scale and the values were noted down.

2.2. Method

2.2.1. Fuzzy Logic

Fuzzy logic is not logic that is fuzzy, but logic that is used to describe fuzziness. Fuzzy logic is the theory of fuzzy sets, sets that calibrate vagueness. Fuzzy logic is based on the idea that all things admit of degrees. Temperature, height, speed, distance, beauty - all come on a sliding scale. Fuzzy logic reflects how people think. It attempts to model our sense of words, our decision making and our common sense. As a result, it is leading to new, more human, intelligent systems.

In 1965 Lotfi Zadeh, Professor and Head of the Electrical Engineering Department at the University of California at Berkeley, published his famous paper 'Fuzzy sets'. In fact, Zadeh discovered fuzziness, identified and explored it, and promoted and fought for it. Zadeh extended the work on possibility theory into a formal system of mathematical logic, and even more importantly, he introduced a new concept for applying natural language terms. This new logic for representing and manipulating fuzzy terms was called fuzzy logic, and Zadeh became the Master of fuzzy logic. As Zadeh said, the term of fuzzy is concrete, immediate and descriptive; we all know what it means. However, many people in the West were repelled by the word fuzzy, because it is usually used in a negative sense (Negnevitsky,

2005).

Figure 1: Range of Logical Values in Boolean and Fuzzy Logic: (a) Boolean Logic; (b) Fuzzy Logic (Negnevitsky, 2005)

Unlike two-valued Boolean logic, fuzzy logic is multi-valued. It deals with degrees of membership and degrees of truth. Fuzzy logic uses the continuum of logical values between 0 (completely false) and 1 (completely true). Instead of just black and white, it employs the spectrum of colors, accepting that things can be partly true and partly false at the same time. As can be seen in Fig. 1, fuzzy logic adds a range of logical values to Boolean logic. Classical binary logic now can be considered as a special case of multi-valued fuzzy logic.

2.2.2. Fuzzy Sets

The concept of a set is fundamental to mathematics. However, our own language is the supreme expression of sets. For example, *car* indicates the set of cars. When we say *a car,* we mean one out of the set of cars. Let X be a classical (crisp) set and x an element. Then the element x either belongs to X ($x \in X$) or does not belong to X ($x \notin X$). That is, classical set theory imposes a sharp boundary on this set and gives each member of the set the value of 1, and all members that are not within

the set a value of 0.

Crisp set theory is governed by a logic that uses one of only two values: true or false. This logic cannot represent vague concepts, and therefore fails to give the answers on the paradoxes. The basic idea of the fuzzy set theory is that an element belongs to a fuzzy set with a certain degree of membership. Thus, a proposition is not either true or false, but may be partly true (or partly false) to any degree. This degree is usually taken as a real number in the interval [0.1].

Let *X* be the universe of discourse and its elements be denoted as x. In classical set theory, crisp set *A* of *X* is defined as function f_A (x) called the characteristic function of *A*

$$f_A(x) : \mathrm{X} \rightarrow 0.\ 1$$

where,

$$f_A(x) = \begin{cases} 1, & if \quad x \in A \\ 0, & if \quad x \notin A \end{cases}$$

This set maps universe *X* to a set of two elements. For any element *x* of universe X, characteristic function f_A (x) is equal to 1 if *x* is an element of set A, and is equal to 0 if *x* is not an element of A.

In the fuzzy theory, fuzzy set *A* of universe *X* is defined by function μ_A (x) called the membership function of set *A*

$$\mu_A\,(\mathrm{x}) : \mathrm{X} \rightarrow [0,\ 1]$$

where,

μ_A (x) = 1 if *x* is totally in A;
μ_A (x) = 0 if *x* is not in A;
$0 < \mu_A(x) < 1$ if *x* is partly in A.

This set allows a continuum of possible choices. For any element *x* of universe X, membership function μ_A (x) equals the degree to which *x* is an element of set A. This degree, a value between 0 and 1, represents the degree of membership, also called membership value, of element *x* in set A.

The membership function must be determined first. A number of methods learned from knowledge acquisition can be applied here. For example, one of the most practical approaches for forming fuzzy sets relies on the knowledge of a single expert. The expert is asked for his or her opinion whether various elements belong to a given set. Another useful approach is to acquire knowledge from multiple experts. A new technique to form fuzzy sets was recently introduced. It is based on artificial neural networks, which learn available system operation data Now assume that universe of discourse *X*, also called the reference super set, is a crisp set containing five elements $X = \{x_1,x_2,x_3,x_4,x_5\}$. Let *A* be a crisp subset of *X* and assume that *A* consists of only two elements, $A = \{x_2, x_3\}$. Subset *A* can now be described by $A = \{(x_1, 0), (x_2,1), (x_3,1), (x_4,0), (x_5,0)\}$, i.e. as a set of pairs $\{(x_i, \mu_A(x_i)\}$, where $\mu_A(x_i)$ is the membership function of element x_i in the subset *A*. The question is whether μ_A (x) can take only two values, either 0 or 1, or any value between 0 and 1. It was also the basic question in fuzzy sets examined by Lotfi Zadeh in 1965 (Zadeh, 1965).

If *X* is the reference super set and *A* is a subset of *X*, then *A* is said to be a fuzzy subset of *X* if, and only if,

$$A = \{(x,\mu_A(x)\} \quad x \in X, \mu_A(x): X \rightarrow [0.1]$$

In a special case, when $X \rightarrow \{0.1\}$ is used instead of $X \rightarrow [0.1]$, the fuzzy subset *A* becomes the crisp subset *A*.

Fuzzy and crisp sets can be also presented as shown in Fig. 2.

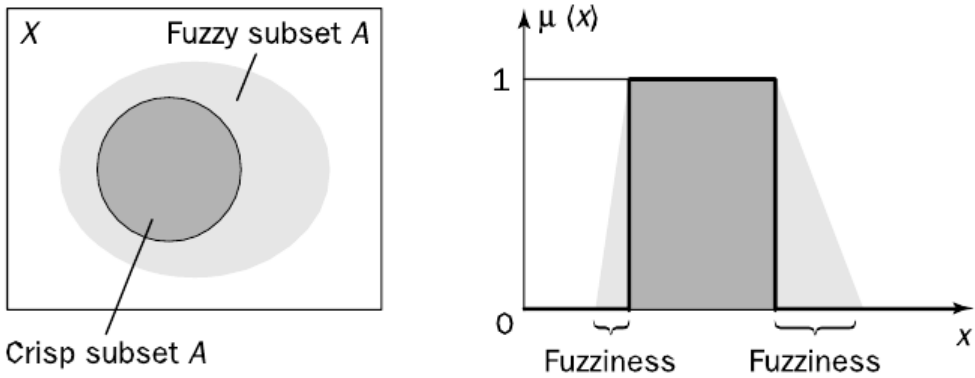

Figure 2: Representation of Crisp snd Fuzzy Subset of X (Negnevitsky, 2005)

Fuzzy subset *A* of the finite reference super set *X* can be expressed as,

$$A = \{(x_1, \mu_A(x_1)\}, \{(x_2,\mu_A(x_2)\}, \ldots, \{\{x_n,\mu_A\{x_n)\}$$

However, it is more convenient to represent *A* as,

$$A = \{\mu_A(x_1)/x_1\}, \{\mu_A(x_2)/x_2\}, \ldots, \{\mu_A(x_n)/x_n\},$$

where the separating symbol / is used to associate the membership value with its coordinate on the horizontal axis.

To represent a continuous fuzzy set in a computer, we need to express it as a function and then to map the elements of the set to their degree of membership. Typical functions that can be used are sigmoid, gaussian and pi.

2.2.3. Linguistic Variables

At the root of fuzzy set theory lies the idea of linguistic variables. A linguistic variable is a fuzzy variable. In fuzzy expert systems, linguistic variables are used in fuzzy rules. For example,

IF wind is strong
THEN sailing is good

IF project _duration is long
THEN completion _risk is high

IF speed is slow
THEN stopping_ distance is short

The range of possible values of a linguistic variable represents the universe of discourse of that variable. For example, the universe of discourse of the linguistic variable *speed* might have the range between 0 and 220 km per hour and may include such fuzzy subsets as *very slow*, *slow*, *medium, fast*, and *very fast.* Each fuzzy subset also represents a linguistic value of the corresponding linguistic variable.

2.2.4. Fuzzy Rules

In 1973, Lotfi Zadeh published his second most influential paper (Zadeh, 1973). This paper outlined a new approach to analysis of complex systems, in which Zadeh suggested capturing human knowledge in fuzzy rules. A fuzzy rule can be defined as a conditional statement in the form:

IF x is A
THEN y is B

where x and y are linguistic variables; and A and B are linguistic values determined by fuzzy sets on the universe of discourses X and Y, respectively.

A classical IF-THEN rule uses binary logic, for example,

Rule: 1
IF speed is > 100 THEN stopping_distance is long

Rule: 2
IF speed is < 40 THEN stopping_distance is short

The variable speed can have any numerical value between 0 and 220 km/h, but the linguistic variable *stopping_distance* can take either value *long* or *short.* In other words, classical rules are expressed in the black-and-white language of Boolean logic. However, we can also represent the stopping distance rules in a fuzzy form:

Rule: 1
IF speed is fast THEN stopping _distance is long
Rule: 2
IF speed is slow THEN stopping _distance is short

Here the linguistic variable *speed* also has the range (the universe of

discourse) between 0 and 220 km/h, but this range includes fuzzy sets, such as *slow, medium* and *fast.* The universe of discourse of the linguistic variable *stopping_distance* can be between 0 and 300 m and may include such fuzzy sets as *short, medium* and *long.* Thus fuzzy rules relate to fuzzy sets.

Fuzzy expert systems merge the rules and consequently cut the number of rules by at least 90 percent.

Fuzzy reasoning includes two distinct parts: evaluating the rule antecedent (the IF part of the rule) and *implication* or applying the result to the consequent (the THEN part of the rule).

In classical rule-based systems, if the rule antecedent is true, then the consequent is also true. In fuzzy systems, where the antecedent is a fuzzy statement, all rules fire to some extent, or in other words they fire partially. If the antecedent is true to some degree of membership, then the consequent is also true to that same degree.

2.2.5. Sugeno-Style Fuzzy Inference

Fuzzy inference can be defined as a process of mapping from a given input to an output, using the theory of fuzzy sets. Sugeno-style inference method was first introduced by Michio Sugeno, the 'Zadeh of Japan', in 1985 (Sugeno, 1985). A singleton, or more precisely a fuzzy singleton, is a fuzzy set with a membership function that is unity at a single particular point on the universe of discourse and zero everywhere else.

Sugeno-style fuzzy inference is very similar to the Mamdani method. Sugeno changed only a rule consequent. Instead of a fuzzy set, he used a mathematical function of the input variable. The format of the Sugeno-style fuzzy rule is

IF x is A
AND y is B
THEN z is $f(x, y)$

where x, y and z are linguistic variables; A and B are fuzzy sets on universe of discourses X and Y, respectively; and $f(x,$y$)$ is a mathematical function.

The most commonly used zero-order Sugeno fuzzy model applies fuzzy rules in the following form:

IF x is A
AND y is B
THEN z is k

where k is a constant.

In this case, the output of each fuzzy rule is constant. In other words, all consequent membership functions are represented by singleton spikes. Fig. 3 shows the fuzzy inference process for a zero-order Sugeno model. The similarity of Sugeno and Mamdani methods is quite noticeable. The only distinction is that rule consequents are singletons in Sugeno's method.

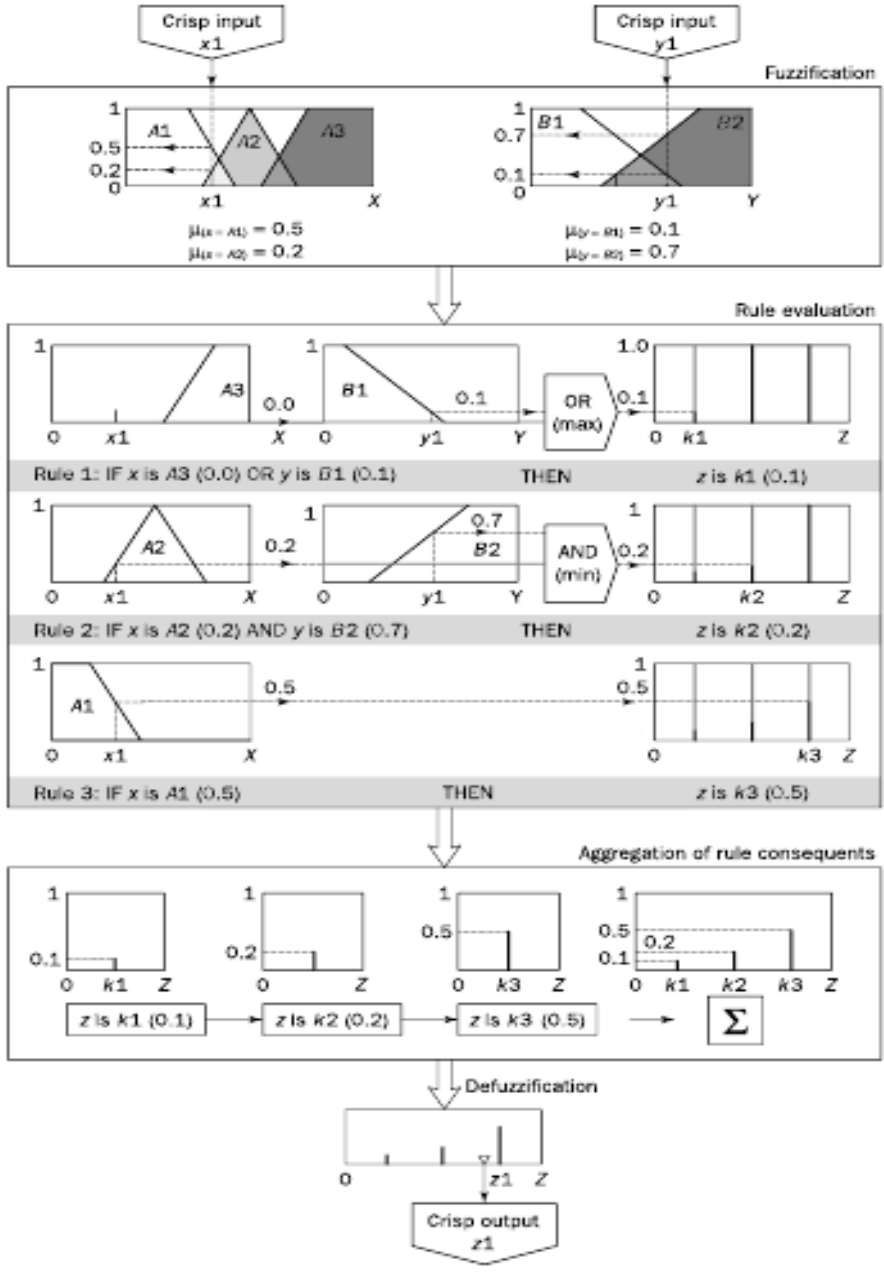

Figure 3: The Basic Structure of Sugeno-Style Fuzzy Inference (Negnevitsky, 2005)

As we can see from Fig. 3, the aggregation operation simply includes all the singletons. Now we can find the weighted average (WA) of these singletons:

$$WA = \frac{\mu(k1) \times k1 + \mu(k2) \times k2 + \mu(k3) \times k3}{\mu(k1) + \mu(k2) + \mu(k3)}$$

$$= \frac{0.1 \times 20 + 0.2 \times 50 + 0.5 \times 80}{0.1 + 0.2 + 0.5}$$

$$= 65$$

Thus, a zero-order Sugeno system might be sufficient for our problem's needs. Fortunately, singleton output functions satisfy the requirements of a given problem quite often.

The Mamdani method is widely accepted for capturing expert knowledge. It allows us to describe the expertise in more intuitive, more human-like manner. However, Mamdani-type fuzzy inference entails a substantial computational burden. On the other hand, the Sugeno method is computationally effective and works well with optimization and adaptive techniques, which makes it very attractive in control problems, particularly for dynamic nonlinear systems (Negnevitsky, 2005).

2.2.6. Building a Fuzzy Expert System

A typical process in developing the fuzzy expert system incorporates the following steps:

1. Specify the problem and define linguistic variables.
2. Determine fuzzy sets.

3. Elicit and construct fuzzy rules.
4. Encode the fuzzy sets, fuzzy rules and procedures to perform fuzzy inference into the expert system.
5. Evaluate and tune the system.

The centroid technique appears to provide consistent results. This is a well- balanced method sensitive to the height and width of the total fuzzy region as well as to sparse singletons. Therefore, unless you have a strong reason to believe that your fuzzy system will behave better under other defuzzification methods, the centroid technique is recommended (Negnevitsky, 2005).

2.2.7. Performance Criteria

The estimated performance was calculated with coefficient of determination (R^2). The results of FES predicted LBW were compared with the actual weighed values.

$$R^2 = \frac{\sum_{i=1}^{n}\left(Y_i - \overline{Y}\right)^2}{\sum_{i=1}^{n}\left(Y_i - \overline{Y}\right)^2}$$

Where,

Y_i – observed value,

$\widehat{Y}_i$ – predicted value,

$\overline{Y}$ – Arithmetic mean,

n – the total number of observations.

3. RESULTS

3.1. FES Design for Live Body Weight Prediction

Descriptive statistics concerning body measurements and live body weight of hair goats grown in Siirt are shown in Table 1 (Ameen & Mikail, 2018).

Table 1: Descriptive Statistics of the Obtained Data

Morphological Traits	N	Mean ± S	Minimum	Maximum
Heart Girth (cm)	81	85.84 ± 5.605	74	96
Body Depth (cm)	81	88.43 ± 7.586	73	105
Body Length (cm)	81	67.64 ± 4.293	56	82
Live Body Weight (kg)	81	39.16 ± 7.131	22.40	56.60

The general structure of the developed FES is shown in Fig. 4.

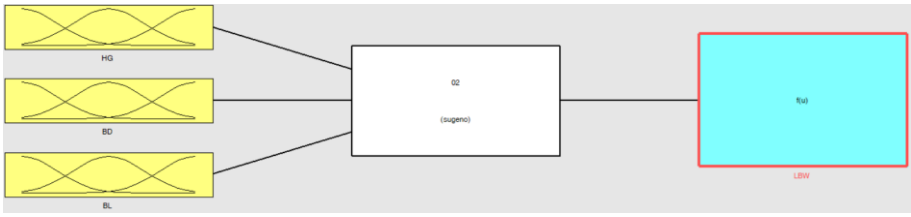

Figure 4: Structure of the Developed FES

Plots representing the relations between inputs and output were shown in Fig. 5-7.

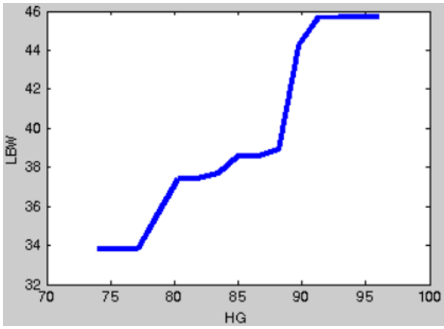

Figure 5: Relation Between HG and LBW

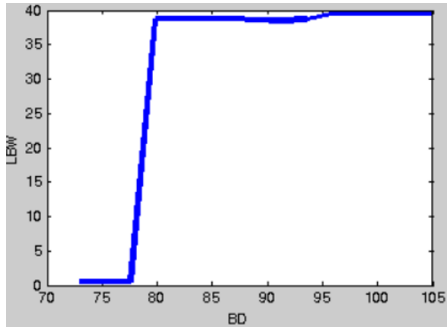

Figure 6: Relation Between BD and LBW

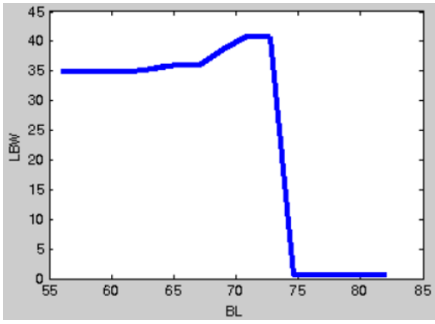

Figure 7: Relation Between BL and LBW

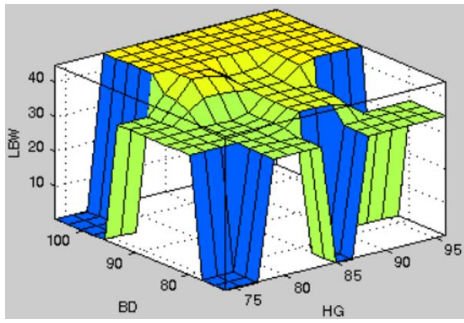

Figure 8: 3D Representation of LBW by BD and HG

Three dimension graphics explaining the FES were shown in the Fig.8-10.

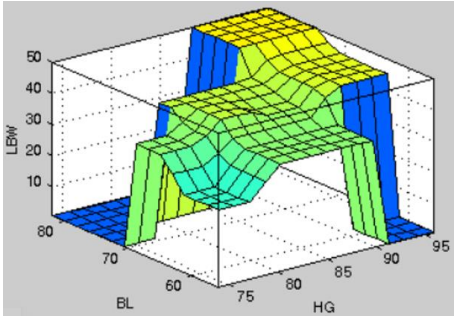

Figure 9: 3D Representation of LBW by BL and HG

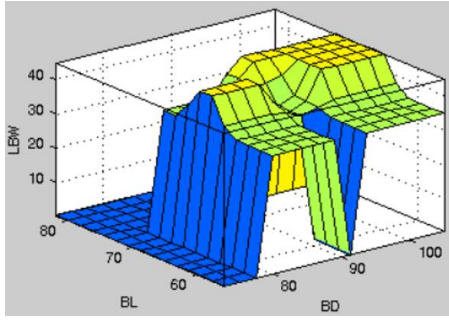

Figure 10: 3D Representation of LBW by BD and BL

3.2. Fuzzification

Input crisp numerical data were fuzzified and converted into linguistic variables (Table 2).

Table 2: Linguistic Terms Used for the Body Measurements

HG range (cm)	Linguistic terms	Membership functions
74 – 80	Short	trapezoidal
78 – 85	Medium	triangular
83 – 90	Long	triangular
88 – 96	Very Long	trapezoidal
BD range (cm)	Linguistic terms	Membership functions
73 – 80	Short	trapezoidal
78 – 90	Medium	triangular
88 – 95	Long	triangular
93 – 105	Very Long	trapezoidal
BL range (cm)	Linguistic terms	Membership functions
56 – 65	Short	trapezoidal
63 – 70	Medium	triangular
68 – 74	Long	triangular
72 – 82	Very Long	trapezoidal

It was set up triangular and trapezoid membership functions for the fuzzy variables as inputs (Ameen & Mikail, 2018).

3.3. Fuzzy Rules

The system knowledge base was constituted from 29 rules. Part of rules was demonstrated in Fig.11.

For example, Rule 10 from the table can be explained as follows: If HG is medium and BD is very high and BL is long then LBW is 41.5 kg.

1. If (HG is short) and (BD is medium) and (BL is short) then (LBW is 23) (1)
2. If (HG is short) and (BD is short) and (BL is short) then (LBW is 27.6) (1)
3. If (HG is short) and (BD is medium) and (BL is medium) then (LBW is 29.7) (1)
4. If (HG is short) and (BD is long) and (BL is medium) then (LBW is 36.2) (1)
5. If (HG is medium) and (BD is medium) and (BL is long) then (LBW is 36.2) (1)
6. If (HG is medium) and (BD is medium) and (BL is medium) then (LBW is 34.9) (1)
7. If (HG is medium) and (BD is medium) and (BL is short) then (LBW is 39.5) (1)
8. If (HG is medium) and (BD is long) and (BL is medium) then (LBW is 34.9) (1)
9. If (HG is medium) and (BD is long) and (BL is short) then (LBW is 34.9) (1)
10. If (HG is medium) and (BD is very_long) and (BL is long) then (LBW is 41.5) (1)
11. If (HG is medium) and (BD is short) and (BL is medium) then (LBW is 34.9) (1)
12. If (HG is medium) and (BD is short) and (BL is long) then (LBW is 37.9) (1)
13. If (HG is medium) and (BD is long) and (BL is long) then (LBW is 41.5) (1)
14. If (HG is long) and (BD is long) and (BL is medium) then (LBW is 34.9) (1)
15. If (HG is long) and (BD is medium) and (BL is short) then (LBW is 34.9) (1)
16. If (HG is long) and (BD is medium) and (BL is medium) then (LBW is 37.9) (1)
17. If (HG is long) and (BD is very_long) and (BL is long) then (LBW is 39.5) (1)
18. If (HG is long) and (BD is long) and (BL is long) then (LBW is 41.5) (1)

Figure 11: Part of Rule Set for the FES

The fuzzy expert system result obtained for given inputs is given in Fig.12.

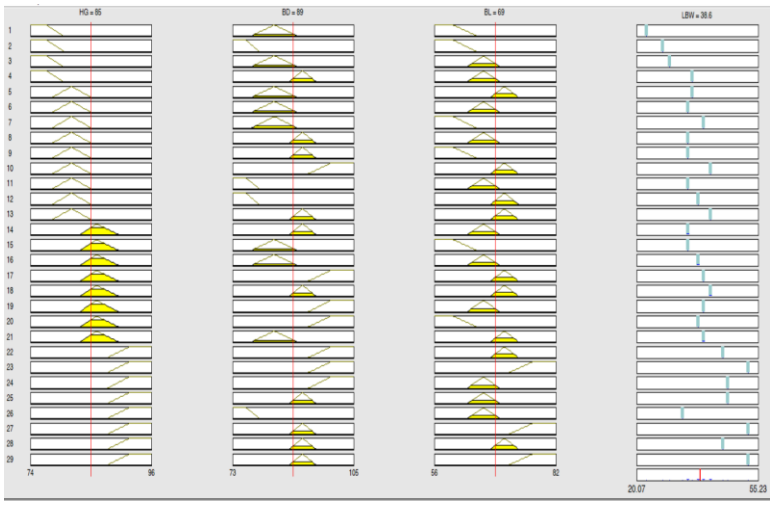

Figure 12: Result of FES

For the inputs 85 cm HG, 89 cm BD and 69 cm BL 38.6 kg LBW was calculated. After performing this simulation for all goats, it was possible to compare values obtained from FES with the weighed LBW (Fig.13).

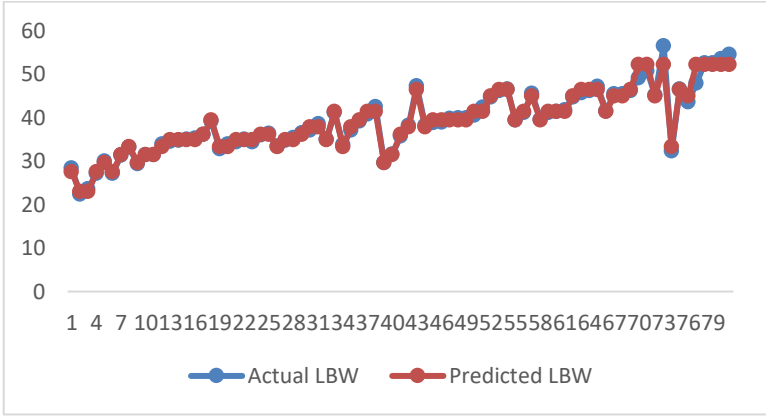

Figure 13: Comparison of Actual and FES Predicted LBWs

Pearson correlation coefficient between the actually and predicted data calculated as 0.99, which represents a high positive correlation between these sets and indicates that the proposed method is suitable for the LBW prediction. Determination coefficient of the model was calculated as 0.98.

CONCLUSION

In this study, general information of fuzzy logic and expert systems was given and various applications in livestock were mentioned in the frame of this information. At the same time, traditional methods for live body weight predictions was investigated. Results obtained from Fuzzy Expert System was better than the results obtained from conventional multiple regression models.

In this study, 81 experimental data were used to estimate live body weight, with the linear body measurements (heart girth, body depth and body length). Experimental data were compared with results obtained with the fuzzy expert system. It was observed that the designed FES results were highly correlated with the experimental data and %98 was confirmed. Another feature of the designed fuzzy expert system is that results can be obtained for input values not included in the experimental data. Much better results can be obtained in the subsequent studies with increasing the number of input and output parameters and the number of linguistic variables.
The usage of FES modeling may be highly recommended to predict LBW instead of time consuming experimental studies.

ACKNOWLEDGEMENTS

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CHAPTER 2

EVALUATION OF SPENT MUSHROOM COMPOSTS AS A PLANT GROWING MEDIA

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INTRODUCTION

Worldwide mushroom production according to FAO's 2019 data it was 11 million 898 thousand 399 tons in an area of 104.342 thousand hectares. The highest producer countries are respectively; China with 8,938,814 million tons, Japan with 470 thousand tons, USA with 383,960 thousand tons and Poland with 362.400 thousand tons. Mushroom production in Türkiye has also been reported as 34.494 tons (FAO, 2019).

The most commonly produced species in the world are *Agaricus bisporus*, different *Pleurotus* species, *Lentinula edodes*, *Ganoderma lucidum* and *Hericium* species. Regardless of the mushroom species, it is reported that 5 kg of Spent Mushroom Compost (SMC) is released for each kg of mushroom produced (Semple et al., 2001; Williams et al., 2001; Lau et al., 2003). Large amounts of released SMC can be removed from enterprises by incineration, garbage or dumping in agricultural fields. These applications do not economical for enterprises and create a significant environmental pollution problem. However, SMC can be used in many different areas and can be brought into the economy. In several studies, it has been found that SMC application to a degraded soil significantly increases soil organic matter and total nitrogen contents and helps to improve other soil physicochemical properties (Gümüs & Seker, 2017; Lou et al., 2017).

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural waste, have been defined as Spent Mushroom Compost (SMC). It has also been suggested to use the term "waste fungal substrate" for this material because it includes biotic components such as fungal and bacterial biomass and extracellular enzymes as well as abiotic components in recent years (Phan & Sabaratnam, 2012).

These products are valuable organic matter sources left over from mushroom cultivation. After the mushroom harvest, deposition of these substrates may cause some environmental problems. Spending waste mushroom compost as an organic fertilizer is one of the best solutions to evaluate it beneficially. SMC has high organic matter content, neutral pH, rich with nutrient contents and good for microbial activity. It has also been reported that SMC has the potential to biologically improve various agricultural grade insecticides (Ahlawat et al., 2010; Ahlawat et al., 2011). Due to these properties, SMC can be turned into a quality fertilizer for crops. In addition, the use of SMC as an organic conditioner contributes to the use of residues in an environmentally friendly way and increases soil sustainability. In this review availability of SMC as a plant growing media is discussed.

1. PROPERTIES OF SPENT MUSHROOM COMPOST

The most produced edible mushroom species in the world is *Agaricus bisporus*, and the most released AMS belongs to *Agaricus* species (Figure 1). The properties of SMC materials depend on the materials used in compost preparation and changes with some factors such as; SMC age, properties of compost material used, composting method, soluble salt content and pH of SMC, species of mushroom cultivated, fraction size of SMC, nutrient contents of SMC and microbial diversity in SMC (Pathak et al., 2021). Some chemical properties of SMC reported in different sources are given in Table 1.

Figure 1: *Agaricus bisporus* Cultivation (URL-1)

Chorover et al. (2000) reported that SMC is a good source of C, N and other nutrients. Nitrogen content in SMC varies between 0.4% and 13.7% and C:N ratio varies between 9:1 and 15:1. SMC has high organic matter, rich with nutrient content, has a neutral pH and high microbial activity.

Table1: Properties of Some Spent Mushroom Compost (Özgüven, 1998; Jordan et al., 2008; Holozlu, 2013; Medina et al., 2009; Ünal, 2015; Umor et al., 2021; Pathak et al. 2021)

	Özgüven, 1998	Jordan et al., 2008	Medina et al., 2009	Holozlu, 2013	Ünal, 2015	Umor et al., 2021	Pathak et al., 2021
Dry matter, %		31.20		31.30		30.00	
Org. matter, %		64.50		60.27	36.85	62.00	60.97
pH		6.80	7.40	6.99	6.80	8.70	
EC, dS/m		10.00	5.90	7.04	2.95	2.30	
P, %	0.12	1.80	0.068	1.23	0.02	0.01	0.69
K, %	1.00	2.00	0.83	2.05	0.11	0.55	2.44
C, %		37.85		34.96			33.42
N, %	1.63	2.10	2.28	2.33	1.84	0.53	2.65
C/N ratio		18.00		15.44			
Ca, %		2.80	9.29	3.97	0.18	0.25	5.38
Mg, %		1,80	0.65	0.68	0.06	0.03	0.83
Na, %		0.17	0.12	0.32		0.13	0.28
Cu, ppm	44.00	54.00	29.00	140.90	3.98		200.00
Zn, ppm	77.50	143.00	145.00	210.00	35.56		200.00
Mn, ppm	384.80	164.00	302.00	346.30	39.76	2.70	500.00
Fe, ppm	2989.90	4.70	3117.00	4871.00	36.17	6.60	4300.00
Pb, ppm		10.40			0.57		
Cd, ppm		6.20			0.17		
Cr, ppm		0.21			0.002		
Ni, ppm		5.80			1.35		

It also contains biotic components such as fungal and bacterial biomass and extracellular enzymes as well as abiotic components (Phan & Sabaratnam, 2012). Debosz et al. (2002) reported that SMC, which is one of organic matter sources, is rich in plant nutrients, and increases soil microbial activity. It also promotes the colonization of a wide variety of intracellular and intercellular fungal communities in the roots of most plants. It has been reported that leached spent compost contain less salt content than the weathered compost and most of the nutrients as well as the biological properties are the same as SMC (Riahi & Arab, 2004; Riahi & Azizi, 2006).

Pekşen et al. (2011) used the mixtures of hornbeam and oak sawdust supplemented with wheat bran and tea waste in different ratios to prepare the substrates for *Ganoderma lucidum* cultivation. They studied the chemical compositions of the spent substrates after the cultivation and reported that initial mineral composition of the materials affected to composition of spent substrates. *Ganoderma lucidum* spent substrates were rich with nitrogen, phosphorus, potassium, magnesium and trace elements (Fe, Mn, Zn). The EC values of the spent substrates ranged from 1.13 dS/m to 2.33 dS/m while their pH ranged from 5.14 to 5.69.

According to results of different studies, SMCs generally have high organic matter contents, lower C:N ratios and rich of the most essential plant macro-micro nutrients with low salinity and toxic element contents (Table 1). Therefore, SMC can be used as a plant growth media in agricultural production or a good source of soil conditioner in especially degraded soils.

2. USE OF SPENT MUSHROOM COMPOST IN PLANT GROWTH

There are some advantages of using SMC for different cultivars such as, decreasing inorganic fertilizer demand and increasing yield with a low cost, increasing soil quality by restoring soil structure, improving soil microbiological activity and increasing soil nutrient contents, increasing soil permeability and aeration, increasing yield higher than traditional fertilization in drought years (Pathak et al., 2021).

Gülser & Pekşen (2003) reported that application of tea waste, spend mushroom compost as an organic source increased soil organic matter content and brought pH level around neutral. In different studies, it was found that the spent mushroom compost application into growth media increased lettuce production (Polat et al., 2004), germination and growth of garden plants (Medina et al., 2009) and *Chrysanthemum* production (Çiçek et al., 2012).

Benito et al. (2005) used six different growth media prepared as mixing peat, leaves, sand and SMC with the compost material of pruning wastes. Test plants were grass (*Lolium perenne* L.) and cypress (*Cupressus sempervirens* L.) in their experiment. They found that SMC is a good media component and the optimum cultivation media were generally obtained by mixing 25% or 50% of spent mushroom compost into growing media.

Polat et al. (2009) studied the effects of SMC as an organic matter source for greenhouse soil on cucumber (*Cucumis sativus* L.) yield. They found that application of SMC in greenhouse had positive effects on yield and other nutritional and plant growth parameters and the highest fruit yield observed at 40 ton/ha SMC application.

In another study by Medina et al. (2009), SMC used as a seedling growth media mixing with peat in different ratios (25%, 50%, 75% and 100%). They found that the mixing rate of 75% SMC with peat could be used in tomato seedling production effectively, and also all SMC

mixture media had greater effect on vegetable seedling growths than peat media used alone. They concluded that spent mushroom compost was an eco-friendly utilization way with contributing to vegetable production.

Roy et al. (2015) investigated the effect of spent mushroom substrate of different edible mushroom as biofertilizer on the growth and biochemical changes of *Capsicum annuum* L. Spent mushroom substrates were used directly as well as in leached form and weathered compost was also applied either singly or in combination. The results revealed that treated plants with spent substrate of fresh oyster mushroom, button mushroom leachate and weathered compost of button mushroom showed highest increase in growth. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both oyster mushroom and button mushroom leachate, and in fresh oyster mushroom substrate.

In another study, it has been studied on to use of SMC in tomato seedling production and to develop alternative seedling growth media instead of peat resources. Some seedling parameters such as hypocotyl length, seedling root length, shoot wet weight and root wet weight showed the highest values for the tomato seedlings at 100% SMC. At the end of this study, it was reported that utilization of spent mushroom compost in seedling growth media is an alternative to the other growth media such as peat and perlite, which are about to extinct and expensive (Ünal, 2015).

Akşahin & Gülser (2019) studied effects of tea waste, spent mushroom compost and inorganic fertilizers on nutrient contents of fenugreek (*Trigonella foenum-graecum*). In this study, N, Mg, Ca, Fe and Zn contents of fenugreek plant obtained from waste mushroom compost applications were found as higher than those obtained from tea waste applications.

Karaca et al. (2019) investigated effects of tea waste and spend mushroom compost on germination time of fenugreek (*Trigonella foenum-graecum*) and some soil properties of growth media. Tea waste and spend mushroom compost doses increased organic matter content and salinity while tea waste and spend mushroom compost applications decreased pH value of growth media. Germination ratio and germination time were negatively affected by increasing tea waste, spend mushroom compost and inorganic fertilizer doses. Akşahin & Gülser (2020) reported that plant length, plant wet weight, plant dry weight, root wet weight and root length of fenugreek (*Trigonella foenum-graecum*) were significantly ($P<0.01$) affected by spent mushroom compost and inorganic fertilizer applications. Tea waste and spent mushroom compost applications had generally positive effects on plant growth. Guo & Chorover (2006) reported that the most important factor limiting the use of waste mushroom compost is its high soluble salt content. Erkel & Isık (1990) reported that it is appropriate to use the waste mushroom compost if it is left open for 1-1.5 years and then washed 5-6 times. NH_4, NO_3, Ca, Mg, Na and K can be found in significant amounts in waste mushroom compost washing water, while

P, Fe, Cu, Mn and Zn can be found in lesser amounts (Aydın, 2009). Holozlu (2013) washed the waste mushroom compost to reduce the salinity and environmentally harmful compounds in it. It was reported that the zeolite column washing could be preferred over the normal washing due to the ion-retaining property of the zeolite and nutrient losses were less than the normal washing. Applications of washed and unwashed waste mushroom composts to soil significantly differed soil physical and chemical quality parameters affecting crop production.

Michael et al. (2022) used SMC as soil biofertilizer to investigate its effect on soil rhizobial population, nodulation, growth and yield of cowpea plant. They found that dry weight of shoot and root systems significantly increased form 20.6 g and 0.39 g in soil to 43.4 g and 0.75 g in 15% SMC added soil media, respectively. They indicated that the low ratio of SMC (5%-25%) mixing with soil increased plant growth parameters such as; plant height, area and number of leaves, total chlorophyll contents of leaves in cowpea. But the ratio of SMC greater than 25% decreased plant growth, rhizobium population and nodulation.

CONCLUSION

SMC is a valuable input in agricultural production due to its low heavy metal content, being an important source of P and K from plant nutrients, and most importantly, high organic matter content near-neutral pH and beneficial microbial population. SMC includes significant amount of macro (NH_4, NO_3, P, Ca, Mg, Na and K) and also some less amount of micronutrients (Fe, Cu, Mn and Zn). Due to these high nutritional properties, SMC can be turned into a quality fertilizer for crops. NH_4 and NO_3, which are source of plant nutrients, have environmental pollutant properties. Using waste mushroom compost as organic fertilizer is one of the best solutions to evaluate it beneficially. It is an important issue to reduce the compounds that may harm to environment while removing the salinity of waste mushroom compost by using in agricultural production purpose. In addition, the use of SMC as organic amendments contributes to the use of residues in sustainable soil management systems and increases soil fertility and crop yields.

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CHAPTER 3

THE SITUATION OF NUTS CULTIVATION IN TÜRKİYE

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INTRODUCTION

Türkiye, which has favorable and different climatic conditions, is located among the homeland or homelands of many fruit species (Davis, 1972). The fact that Türkiye is among the homelands of fruit species has its advantages. These advantages include being located at the crossroads of three regions, having the characteristics of the Mediterranean, Irano-Turanian, and Euro-Siberian areas of Anatolia, the intersection of the Mediterranean and Near Eastern gene centers on Anatolia, the fact that Anatolia has been home to many civilizations throughout history, and the fact that Anatolia is one of the world's known trade centers. Türkiye, which has 0.53% of the world's land area, is ahead of countries with a larger land area in terms of fruit production (Karadeniz, 2021). These advantages of Türkiye have led to an increase in the richness of species and varieties in the country. Of the 138 fruit species cultivated worldwide, more than 75 can be grown in Türkiye, 16 of which are subtropical fruit species (Ağaoğlu et al., 2019). According to FAO (2020) data, Türkiye has a production of approximately 24.153.128 tons in the world fruit production (excluding citrus fruits), which is 887.027.376 tons. With this production amount, Türkiye meets approximately 2.72% of the world's fruit production and is among the top 10 countries in fruit production. Türkiye also has a production share of 29.15% in Europe, with 82.853.809 tons of fruit production (FAO, 2022). Economic production is carried out in almost every region of Türkiye with fruit species and varieties adapted to different climatic and soil conditions. The country's agricultural area is

231.3 million decares, of which 35.6 million decares are used for fruit production. Nuts fruits have the highest share within this production area, with 37.9%. In terms of production amount, nuts fruits have a share of 6.4% (TÜİK, 2021).

1. DISTRIBUTION OF CROP GROUPS AND AGRICULTURAL AREAS IN TÜRKİYE

According to TÜİK (2022), Türkiye has a total agricultural area of 38.063 hectares. Within agricultural areas, grains and field crops have the highest share (42%), followed by meadows and pastures (38%), fruit, beverage, and spice crops (10%), and vegetables (2%) (Table 1) TÜİK (2022). Looking at the last 20 years in Türkiye, it is seen that while the total agricultural area was 41.196 hectares in 2002, this number fluctuated and decreased in the following years. Among the reasons for this may be the increase in the area allocated to fallow land and the occurrence of natural disasters and losses due to rapidly increasing urbanization in parallel with population growth. However, while the area allocated to fruit growing within the total agricultural area was 2.674 hectares in 2002, this amount increased by 34.44% to 3.595 hectares. In terms of vegetable areas, the vegetable production area, which was 930 hectares at the beginning, decreased by 18.82% to 755 hectares in 2021. When the agricultural areas of Türkiye, one of the world's horticultural crops producing countries, are evaluated in terms of fruit growing, it is seen that a significant portion of the agricultural areas are devoted to fruit growing. The area of fruits, beverages, and

spice plants constitutes approximately 9.445% of the total agricultural area.

Table 1: Total Agricultural Areas in Türkiye Between 2002 and 2021 and the Distribution of Plant Groups Within This Area

Years	Total agricultural area (ha)	Area of grains and other crops (ha)		Meadow and pasture land (ha)	Fruits, beverage and spice crops area (ha)	Vegetable gardens area (ha)	Ornamental plants area (ha)
		Cultivated area	Fallow				
2002	41.196	17.935	5.040	14.617	2.674	930	-
2003	40.644	17.408	4.991	14.617	2.717	911	-
2004	41.210	17.962	4.956	14.617	2.780	895	-
2005	41.223	18.005	4.876	14.617	2.831	894	-
2006	40.493	17.440	4.691	14.617	2.895	850	-
2007	39.504	16.945	4.219	14.617	2.909	815	-
2008	39.122	16.460	4.259	14.617	2.950	836	-
2009	38.912	16.217	4.323	14.617	2.943	811	-
2010	39.011	16.333	4.249	14.617	3.011	802	-
2011	38.231	15.692	4.017	14.617	3.091	810	4
2012	38.399	15.463	4.286	14.617	3.201	827	5
2013	38.423	15.613	4.148	14.617	3.232	808	5
2014	38.558	15.782	4.108	14.617	3.243	804	5
2015	38.551	15.723	4.114	14.617	3.284	808	5
2016	38.328	15.575	3.998	14.617	3.329	804	5
2017	37.964	15.498	3.697	14.617	3.348	798	5
2018	37.797	15.421	3.513	14.617	3.457	784	5
2019	37.716	15.398	3.387	14.617	3.519	790	5
2020	37.762	15.628	3.173	14.617	3.559	779	5
2021	38.063	16.031	3.059	14.617	3.595	755	5

TÜİK, 2022

2. TOTAL AREA, NUMBER OF TREES, PRODUCTION QUANTITIES, AND YIELDS OF NUTS FRUIT

When fruit groups are analyzed in terms of species, the number of plants cultivated in Türkiye is relatively high. In addition to these species, there are wide varieties within each fruit. In terms of production amount and area for each fruit group, Türkiye is one of the leading countries in the world and is among the top 10 producing countries in the world for many species. When the regions of Türkiye are analyzed in terms of the amount of fruit production, the highest production is in the Mediterranean Region. It is followed by the Aegean, Central Anatolia, Marmara, Black Sea, Southeastern Anatolia, and Eastern Anatolia Regions (Gerçekçioğlu et al., 2012).

Important species of the nuts fruit group are Hazelnut, Pistachio, Almond, Walnut, and Chestnut. Data on the number of trees and production values of nuts fruit between 2004 and 2021 are shown in Table 2, while total area and yield values are shown in Table 3. In Türkiye, 178 thousand tons of almonds are produced in an area of 577.324 decares, and the number of fruit-bearing trees is approximately 12.5 million, while the number of non-fruit-bearing trees is approximately 6.8 million. Chestnut production is 77.792 tons, and the number of fruit-bearing trees is approximately 2.5 million, while the number of non-fruit-bearing trees is approximately 493 thousand. Hazelnuts are primarily produced as 'ocak,' and single-stem hazelnut cultivation has recently started. The number of fruiting hazelnut trees is approximately 395 million, and there are approximately 12 million non-

fruiting hazelnut trees. Türkiye's hazelnut production was 684 thousand tons on 7.389.201 decares in 2021. Pistachio production is about 119 thousand tons, and the number of fruiting trees is about 55.5 million, while the number of non-fruiting trees is about 24 million. Walnut, whose production in Türkiye increases every year like other nuts, has a production of 325 thousand tons. While the number of fruit-bearing trees in the country is approximately 14 million, the number of non-fruit-bearing trees is approximately 13 million. The yield of chestnuts is 31 kg/fruit-bearing tree, walnut 23 kg/fruit-bearing tree, almonds 14 kg/fruit-bearing tree, and pistachio 2 kg/fruit-bearing tree. In our country, hazelnut gardens are established with the ocak system, and hazelnut yield is 2 kg. Hazelnut has the highest production among nuts fruit and our country, which ranks first in the world hazelnut production, is far behind the world in terms of yield (Bak, 2021). The low yield in hazelnut orchards with the ocak system may be due to the old age of the orchards. Karadeniz & Kırca (2019) reported that fruit weight, kernel weight, yield and oil amount, which are important quality criteria in hazelnut, have the best values in 10-50 years old orchards, but yield and quality decrease significantly in 70 and 90 year old orchards.

There are many areas where nuts fruit are produced on a regional basis. Almonds are produced in all areas of the country except Erzurum-Kars region and the Black Sea coast with its cool summers. Almond production is concentrated in the southern and western parts of the country and around the Southeastern Taurus Mountains. Among these

areas, the Mediterranean coastal region and inland areas, the Aegean coast and the transition regions in the interior, the coastal part of the Marmara Region, the central parts of the South Eastern Taurus Mountains, and the eastern parts of the South Eastern Anatolia Region are the places where production is the most intensive (Durmuş & Yiğit, 2003; Aydoğdu & Şahin, 2020).

The areas where pistachio production is most common are around Şanlıurfa, Gaziantep, Kilis, and Siirt in the Southeastern Anatolia region. Şanlıurfa plateau, Harran and Suruç plains, and Gaziantep plateau are among the important production centers. It is also produced in Çanakkale, Mersin, Muğla, Manisa, Balıkesir, and Aydın provinces located in the coastal regions of the country (Durmuş & Yiğit, 2003; Çoban et al., 2022).

Walnut, which has a high adaptability to different climatic conditions, is mainly grown in cool and humid places in parts of the temperate climate zone with a continental climate. It is grown almost everywhere except the northeast of the Erzurum-Kars region and west of Salt Lake (Durmuş & Yiğit, 2003). The areas where it is intensively cultivated are the West-Central Black Sea region, South Marmara Region, Aegean Region, Büyük and Küçük Menderes valleys (Aksoy & Kaymak, 2021).

When it comes to hazelnut, the Black Sea Region comes to mind. Hazelnut production, which initially started in Giresun region, has

spread to the entire Black Sea Region with an increase in value and importance. Production is intensively carried out in Ordu, Trabzon, and Giresun regions. These regions meet most of the country's hazelnut production (Bars et al., 2018; Karadeniz, 2021).

Table 2: Data on The Number of Trees and Production Values of Nuts Fruit Between 2004-2021

Years	Number of Fruit-bearing Trees (number)				
	Almond	**Chestnut**	**Hazelnut**	**Pistachio**	**Walnut**
2004	3.450.000	1.890.000	325.000.000	26.500.000	4.200.000
2005	3.400.000	1.890.000	321.500.000	28.000.000	4.535.000
2006	3.235.839	1.862.864	337.380.483	28.264.261	4.595.453
2007	3.517.332	1.948.351	357.948.270	28.463.676	4.926.985
2008	3.430.219	1.949.491	340.285.551	28.667.681	5.094.781
2009	3.407.820	1.951.731	347.414.378	30.143.997	5.191.724
2010	3.683.032	1.920.235	356.761.858	29.617.102	5.441.051
2011	4.221.566	1.922.915	354.713.121	30.868.412	5.594.576
2012	4.679.833	1.939.101	348.781.578	37.150.045	5.977.397
2013	5.255.592	1.958.904	348.563.209	38.116.209	6.526.028
2014	5.637.326	1.991.270	349.189.710	39.329.512	7.000.897
2015	5.863.629	2.007.943	358.147.878	40.597.427	7.596.020
2016	6.663.996	1.949.991	360.416.783	42.570.004	8.171.185
2017	6.810.165	1.978.762	362.255.392	47.765.596	8.766.811
2018	8.490.351	1.954.372	378.280.019	49.557.873	9.875.068
2019	9.521.707	2.114.454	384.935.286	52.060.513	11.250.526
2020	10.380.249	2.306.992	386.194.685	54.548.247	12.488.338
2021	12.471.039	2.469.625	394.052.449	55.464.465	13.899.362
Years	**Number of Trees of Non-Fruiting Age (number)**				
	Almond	**Chestnut**	**Hazelnut**	**Pistachio**	**Walnut**
2004	500.000	475.000	20.000.000	16.000.000	2.200.000
2005	543.000	467.000	15.215.000	18.491.000	2.245.000
2006	578.729	447.308	15.135.382	18.462.394	2.353.440
2007	1.014.251	444.237	19.286.768	14.939.052	2.788.405
2008	1.279.101	529.159	16.803.193	14.032.781	2.951.522

2009	1.875.170	442.003	21.852.143	11.461.604	3.200.279
2010	2.589.493	393.760	11.510.803	10.562.487	3.643.380
2011	3.101.231	366.030	8.569.370	10.419.574	4.045.119
2012	3.242.945	306.821	8.210.481	12.428.352	4.541.958
2013	3.602.097	361.505	6.984.836	12.006.181	4.877.669
2014	3.814.999	362.136	6.220.407	11.152.593	5.374.456
2015	4.294.611	365.517	7.864.829	11.632.973	5.560.227
2016	4.964.011	370.664	7.370.865	17.192.812	6.873.271
2017	5.098.562	377.234	7.775.449	19.460.186	7.894.728
2018	5.400.809	405.518	9.818.708	20.529.250	8.896.575
2019	6.333.129	451.613	10.001.697	20.983.692	10.004.317
2020	7.093.395	483.827	10.110.291	22.721.902	11.579.246
2021	6.772.875	492.678	12.363.806	23.698.780	12.719.106
Years	**Production Amount (tons)**				
	Almond	**Chestnut**	**Hazelnut**	**Pistachio**	**Walnut**
2004	37.000	49.000	350.000	30.000	126.000
2005	45.000	50.000	530.000	60.000	150.000
2006	43.285	53.814	661.000	110.000	129.614
2007	50.753	55.100	530.000	73.416	172.572
2008	52.774	55.395	800.791	120.113	170.897
2009	54.844	61.697	500.000	81.795	177.298
2010	55.398	59.171	600.000	128.000	178.142
2011	69.838	60.270	430.000	112.000	183.240
2012	80.261	57.881	660.000	150.000	203.212
2013	82.850	60.019	549.000	88.600	212.140
2014	73.230	63.762	450.000	80.000	180.807
2015	80.000	63.750	646.000	144.000	190.000
2016	85.000	64.750	420.000	170.000	195.000
2017	90.000	62.904	675.000	78.000	210.000
2018	100.000	63.580	515.000	240.000	215.000
2019	150.000	72.655	776.046	85.000	225.000
2020	159.187	76.045	665.000	296.376	286.706
2021	178.000	77.792	684.000	119.355	325.000

TÜİK, 2022

Chestnut, which grows spontaneously in forest areas in the Black Sea, Marmara, and Aegean coastal areas of Türkiye together with various forest trees, grows as a natural forest tree (Durmuş & Yiğit, 2003).

The regions where it grows intensively are as follows; in the areas between the Menteşe Mountains and Aydın, in the Büyük Menderes and Küçük Menderes plains, around Aydın and İzmir, in the region extending from the Küre Mountains to Sinop, in the Balıkesir plain and its surroundings and around Bursa (Tuttu et al., 2021).

Table 3: Data on total area and yield values of nuts fruit between 2004-2021

Years		Almond	Chestnut	Hazelnut	Pistachio	Walnut
2004	Total Area (decare)	78.000	88.500	6.500.000	2.200.000	168.000
	Yield (kg)	11	26	1	1	30
2005	Total Area (decare)	82.000	90.000	6.550.000	2.410.000	197.000
	Yield (kg)	13	26	2	2	33
2006	Total Area (decare)	83.100	85.135	6.662.262	2.414.670	208.967
	Yield (kg)	13	29	2	4	28
2007	Total Area (decare)	99.505	102.415	6.638.174	2.256.846	286.797
	Yield (kg)	14	28	1	3	35
2008	Total Area (decare)	109.130	103.915	6.631.928	2.253.713	328.873
	Yield (kg)	15	28	2	4	34
2009	Total Area (decare)	131.207	117.108	6.428.669	2.144.897	366.736
	Yield (kg)	16	32	1	3	34
2010	Total Area (decare)	171.478	118.533	6.678.649	2.212.229	413.932

	Yield (kg)	15	31	2	4	33
2011	Total Area (decare)	205.039	119.559	6.969.643	2.338.368	468.378
	Yield (kg)	17	31	1	4	33
2012	Total Area (decare)	235.547	121.244	7.014.067	2.835.517	552.019
	Yield (kg)	17	30	2	4	34
2013	Total Area (decare)	254.570	113.069	7.021.437	2.813.553	639.015
	Yield (kg)	16	31	2	2	33
2014	Total Area (decare)	270.203	111.164	7.011.413	2.823.338	693.947
	Yield (kg)	13	32	1	2	26
2015	Total Area (decare)	296.714	111.080	7.026.279	2.914.179	718.196
	Yield (kg)	14	32	2	4	25
2016	Total Area (decare)	333.221	115.704	7.054.451	3.134.316	868.528
	Yield (kg)	13	33	1	4	24
2017	Total Area (decare)	352.017	115.504	7.066.670	3.288.041	920.128
	Yield (kg)	13	32	2	2	24
2018	Total Area (decare)	421.914	118.249	7.283.808	3.545.003	1.117.749
	Yield (kg)	12	33	1	5	22
2019	Total Area (decare)	470.881	127.141	7.344.087	3.662.103	1.245.527
	Yield (kg)	16	34	2	2	20
2020	Total Area (decare)	523.695	135.705	7.345.377	3.818.466	1.417.899
	Yield (kg)	15	33	2	5	23
2021	Total Area (decare)	577.324	136.132	7.389.201	3.894.509	1.535.204
	Yield (kg)	14	31	2	2	23

TÜİK, 2022

3. NUTS FRUIT AND THEIR SUFFICIENCY LEVELS

The Turkish Statistical Institute (TÜİK) annually provides crop production data. Crop balance tables are also included in these data. Crop balance tables reveal the supply sources and utilization patterns of agricultural products in detail by comparing them over a particular reference period. The data on production, imports, exports, domestic utilization, per capita consumption, and sufficiency level in the fruit balance tables allow us to comment on the annual and future status of the relevant fruit while also providing information on the annual sufficiency of that fruit in the country.

Given the fruit balance tables, the degree of sufficiency shows the degree to which available production covers domestic use, expressed as a percentage, and is calculated as follows;

Degree of sufficiency = (Available production / Domestic use) x100

The degree of sufficiency indicates the extent to which a region's available production (domestic production) is able to meet its demand or domestic use (all the needs of people, animals, and industry). A value less than 100 represents a situation where production cannot fully meet domestic demand. Conversely, a value greater than 100 indicates the existence of exportable or stackable quantities that exceed domestic needs (TÜİK, 2022).

Table 4: Nuts Fruit Balance Table (2011-2021)

Product	**Market Year**	**Production**	**Import**	**Export**	**Domestic Use**	**Per Capita Consumption**	**Sufficiency level**
		(Ton)	(Ton)	(Ton)	(Ton)	(Kg)	(%)
Almond	2011	69.838	34.626	19.537	83.670	1.1	82
	2012	80.261	25.774	19.664	84.926	1.1	92.8
	2013	82.850	30.413	22.670	89.102	1.1	91.3
	2014	73.230	18.542	12.636	77.818	1.0	92.4
	2015	80.000	20.921	12.696	86.785	1.1	90.5
	2016	85.000	36.241	19.149	100.562	1.2	83
	2017	90.000	49.415	20.988	116.807	1.4	75.7
	2018	100.000	50.036	28.901	119.335	1.4	82.3
	2019	150.000	58.528	18.562	187.266	2.2	78.7
	2020	159.187	77.313	42.826	190.809	2.2	81.9
Hazelnut	2011	430.000	3.210	411.785	90.972	1.2	468.4
	2012	660.000	8.697	649.211	93.546	1.2	699.2
	2013	549.000	6.053	567.290	92.822	1.2	586.1
	2014	450.000	7.187	492.871	85.266	1.1	523
	2015	646.000	9.710	534.274	108.912	1.3	587.8
	2016	420.000	10.861	519.332	100.749	1.2	413.1
	2017	675.000	14.848	628.545	133.328	1.6	501.7
	2018	515.000	14.842	594.796	114.569	1.4	445.5
	2019	776.046	9.510	730.572	138.383	1.6	563.9
	2020	665.000	16.337	624.412	119.188	1.4	552.9
Walnut	2011	183.240	46.338	13.711	211.469	2.8	84.6
	2012	203.212	40.009	11.998	226.346	2.9	87.6
	2013	212.140	30.479	14.171	223.357	2.8	92.7
	2014	180.807	34.285	8.407	202.346	2.5	87.2
	2015	190.000	63.800	7.917	241.323	3.0	76.8
	2016	195.000	66.008	8.167	248.161	3.0	76.7
	2017	210.000	77.382	7.185	275.157	3.3	74.5
	2018	215.000	103.345	30.330	282.855	3.4	74.2
	2019	225.000	90.525	8.180	301.945	3.5	72.7
	2020	286.706	100.095	33.747	346.173	4.0	80.8
Pistachio	2011	112.000	58	3.172	106.086	1.4	102.9
	2012	150.000	139	15.289	131.100	1.7	111.6
	2013	88.600	277	5.633	81.029	1.0	106.6
	2014	80.000	52	4.971	73.081	0.9	106.7
	2015	144.000	118	13.887	126.631	1.5	110.9
	2016	170.000	150	12.736	153.164	1.8	108.2
	2017	78.000	218	6.575	69.693	0.8	109.1
	2018	240.000	19.417	39.101	214.316	2.5	109.2
	2019	85.000	198	11.986	71.087	0.8	116.6
	2020	296.376	20.132	50.820	258.279	3.0	111.9
	2011	60.270	222	3.750	53.005	0.7	106.7

Chestnut	2012	57.881	82	5.445	48.929	0.6	111
	2013	60.019	340	5.839	50.799	0.6	110.8
	2014	63.762	585	10.567	49.827	0.6	120
	2015	63.750	123	6.473	53.448	0.7	111.9
	2016	64.750	196	7.432	53.500	0.7	113.5
	2017	62.904	956	10.551	49.409	0.6	119.4
	2018	63.580	1.813	12.865	48.586	0.6	122.7
	2019	72.655	2.231	14.025	56.356	0.7	120.9
	2020	76.045	2.238	9.305	64.263	0.7	111

TÜİK, 2022

The balance table showing the production, import, domestic utilization, per capita consumption, and sufficiency level of fruits with nuts is shown in Table 4. According to this table, production, exports, domestic use, and per capita consumption of all fruits in this group have increased in the last ten years, but the fruits have differed in the rate of increase. This group's sufficiency level of fruits was 552.9% for hazelnut, 111.9% for pistachio, 81.9% for almond, 80.8% for walnut, and 111% for chestnut (TÜİK, 2022).

4. PROBLEMS OF FRUIT GROWING AND SOLUTION SUGGESTIONS

When the production, import, domestic utilization, per capita consumption, and sufficiency levels of fruits are analyzed, it is seen that Türkiye has increased self-sufficiency. However, there is a need to plan the production of fruit and vegetable products in the quality required by the markets. In addition to increased production, there is a need for developing product markets, permanent and stable institutions, and activities to regulate the markets.

Production of fruits, beverages, and spice plants, which have a share of over 30 percent in crop production, has shown significant increases over the years. Fruit planting area tends to increase despite the decrease in cultivated area. However, apart from production, quality problems persist. Therefore, it is difficult to provide products suitable for changing market demands which can be supplied to the market for a long time.

Global warming and related temperature changes affect fruit production. Since temperature increases will increase water losses from the soil and plant surface, plants will be exposed to abiotic stresses such as drought and salinity. As a result, losses will occur according to the time, severity, and duration of stress. Water deficiency that may occur during critical development periods of plants, such as flowering, pollination, and fertilization, will cause a significant decrease in yield and quality. This situation will necessitate improving and producing more drought-resistant varieties and the application of tillage and cultivation techniques for more effective containment and storage of rainwater in the soil (Ministry of Development, 2018). Therefore, planning lands for production, cultural practices in plant cultivation, and management of water resources will come to the fore. In this context, issues such as control of water use, storage of water, development of efficient irrigation technologies, and sustainable planning of water resources will become priorities. In addition to these adverse effects of climate change, some positive effects of climate change are also expected, such as increased yields in some crops,

shorter growing periods due to the increase in temperature, shifting cultivation areas further north, the possibility of early planting and harvesting, and the ability to grow some plants that are currently grown in hot regions in temperate regions (Ministry of Development, 2018).

Apart from these general problems, some problems have persisted over the years regarding fruit growing in Türkiye. These can be listed as follows; the fact that fruit cultivation is done with seeds and therefore standardization in production and quality is not ensured, the fact that companies producing saplings have true-to-type breeding plots, problems in the supply of plant material, in other words, in the field of arboriculture, the fact that production is not carried out with true-to-type standard varieties with known yield and fruit quality, scarcity of orchards containing one type of crop, insufficient and incorrect technical and cultural practices, mistakes made in terms of fertilization biology, which is a crucial issue in fruit growing, wrong harvesting practices that lead to significant product losses and marketing problems.

The generally small and fragmented structure of agricultural holdings is a factor that applies to all areas of fruit production. This situation limits the need for modern agricultural methods in orchards established with some species in nuts fruit cultivation. Healthy and true-to-type seedlings should be used in fruit growing. Arboriculture activities should be emphasized in nuts fruit production, and variety and clone rootstock breeders should be established. In order to increase

production and especially to have a say in global trade, gardens should be established with varieties that produce early and high-quality products, have good resistance to diseases and pests and have marketable high quality and high yield per decare. In the gardens to be established, new cultivation methods should be applied, and classical production systems should be abandoned.

Following new cultivation methods and their gradual introduction into production will not only increase the incomes of many producers. However, it will also have the advantage of reducing production costs and, ultimately, making us more competitive in the markets. However, at this point, public and private sector institutions and organizations should cooperate to determine new cultivation methods and varieties and reach the producers.

As in every fruit species, diseases and pests seriously affect production and yield in nuts fruit species. Some of these diseases and pests are caused by nutritional problems. At this point, producers should be primarily made aware of plant nutrition. A fertilization program based on soil and leaf analysis should be implemented, and modern techniques such as fertigation should be encouraged. Pesticides are intensively used in the fight against diseases and pests. This situation closely concerns every segment of society regarding health and environmental pollution. Unconscious spraying and fertilization can indirectly cause environmental pollution and cause financial losses for producers. However, with the widespread adoption of 'Integrated Fruit Production'

techniques and the introduction of ecological agricultural practices, the risk of environmental pollution and financial losses for producers will be considerably reduced. Production quantity, industry quality, infrastructure, finance, and market conditions should be improved to be among the producing countries and compete with other countries in exports with other nuts fruits other than hazelnuts.

In order to avoid early or late harvesting, producers should be informed on how to determine the harvest time correctly and practically. For this purpose, practical training and educational meetings should be organized on how to determine the harvest period and how to harvest.

In order to ensure maximum standardization in production, classification and packaging facilities should be established. Furthermore, a system where all producers can benefit from these facilities should be established.

CONCLUSION

Fruit growth has shown significant developments in recent years. In some fruit species, this increase has been significant both in terms of area and production amount. The essential factors in this development are; developments in the production and marketing process, consumer demands, increased export opportunities, and the use of new techniques and technologies related to the extension of shelf time. In today's world, where agriculture and food safety are becoming more critical, all stakeholders of the relevant sectors must take all necessary measures by following a planned, scientific and rational path for the further development and progress of fruit growing. According to the adequacy of fruits for the country in fruit cultivation, it is noteworthy that some fruits are overproduced, and some are underproduced. It is necessary to increase the per capita consumption of fruits in the country by taking into account the health and nutrition aspects of fruits, to increase new market and export opportunities in fruits where we have surplus production, to bring a new breath and perspective to fruit growing by solving production problems with our universities, trained qualified personnel and farmer training. The potential of our country is suitable for this. With a planned and scientific perspective, fruit growing will continue its development by increasing its importance.

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CHAPTER 4

IN SILICO GENOME WIDE ANALYSIS OF K^+ EFFLUX ANTIPORTER (*KEA*) FAMILY IN *Helianthus annuus* L. AND GENE EXPRESSION UNDER SALT STRESS

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1. INTRODUCTION

Changes in climate affect all living things negatively. The reaction of plants, directly or indirectly, to the consequences of climate change will affect the risk of extinction of plants, and therefore the sustainability of agricultural production (Anderson & Song, 2020). In order to adapt to environmental changes, plants need to adjust their cell pH and ion contents. Potassium ion is a widespread cation with important functions such as protein synthesis, pH homeostasis, enzyme functions, and transport of metabolites in plants. In addition, this ion helps plants survive against various abiotic stresses such as drought, heat, cold and salt stresses (Zhou et al., 2016; Azeem et al., 2022a). Uptake and distribution of potassium in plant cells are carried out by various carrier proteins of several families with different structures and transport mechanisms. The largest of these families, CPA (The Cation: Proton Antiporter), includes the NHX, KEA and CHX antiporters (Ragel et al., 2019). CPAs are responsible for maintaining pH homeostasis and ion concentrations in all living species, and CPA genes have a critical role in many physiological events such as plant growth, development, and stress responses (Hussain et al., 2022). The CPA family consists of 2 main superfamilies, CPA1 and CPA2. KEA (K^+ efflux reverse transporter family) belongs to CPA2 (Wang et al., 2020). *Arabidopsis* has 6 KEA subfamily. Their functions are stated as follows: *AtKEA1* and *AtKEA2* in chloroplast division and membrane formation; *AtKEA3*, in photosynthesis efficiency; *AtKEA4–6*, in plant growth K^+ and pH homeostasis (Kong et al., 2021).

Helianthus annuus L. is an important plant with 38-50% oil content and its seed contains medicinally valuable substances such as vitamins, minerals, alkaloids, glycosides, flavonoids, tannins (Çetin et al., 2021; Jan et al., 2022). *H. annuus* has antimalarial, antiasthma, antimicrobial, antioxidant effects and is used in the treatment of heart disease, pulmonary diseases, cough and cold. In addition, it is used in many different areas from perfume to medicine (Mahamba & Palamuleni, 2022).

H. annuus is a model system for genomic studies in the Asteraceae family (Sharifi Alishah et al., 2022) and this plant constitutes our research material. In a genomic study (Badouin et al., 2017), a sunflower specific whole-genome duplication was found existed 29 million years ago. It was determined that the sunflower genome encodes 52,232 inferred protein- coding genes and 5,803 spliced long non-coding RNAs (lncRNAs). Moreover, 123 microRNA (miRNA) genes belonging to 43 families was reported.

The main objectives of this research are to conduct a genome-wide analysis for *H. annuus KEA* genes, to analyze their genomic structures and functions. In addition, another aim is to simulate the PCR steps by using bioinformatics tools, identify the *KEA* genes suitable for PCR and determine *KEA* gen expression profil under the salt stress.

2. MATERIALS AND METHODS

2.1. Identification of *KEA* Genes in *Helianthus annuus* L. Genome

KEA protein sequences were obtained from the NCBI (The National Center for Biotechnology Information) database (https://www.ncbi.nlm.nih.gov/, accessed on 22.04.2022). KEA and *Helianthus annuus* L. protein sequences were compared using BLASTP tool of QIAGEN CLC Genomics Workbench 22.0.1. However, the conserved region analysis of KEA proteins was performed in the PFAM 35.0 database (URL-1) via the QIAGEN CLC Genomics Workbench 22.0.1. After comparison, the repetitive sequences were removed, and possible KEA proteins were determined. Finally, physicochemical properties of the determined proteins were calculated using the Expasy ProtParam tool (URL-2).

2.2. Chromosomal Localization Distribution and Prediction of Gene Structure

Exon–intron regions were identified by comparing the genomic sequences with the predicted coding sequences (CDS) in the Gene Structure Display Server (GSDS 2.0, URL-3). The genomic sequences of the related proteins were examined in the NCBI database to find the chromosomal localizations of the genes. MapChart 2.32 software was used to visualize chromosomal localities.

2.3. Phylogenetic Tree Construction and Conserved Protein Motif Analysis of *KEA* Family Genes

Amino acid sequences were loaded to MEGA 11 program which enables to construct a phylogenetic tree. Multiple sequence alignments were done by using MUSCLE. Aligned file was used for constructing of phylogenetic tree by Maximum Likehood Tree method with bootstrap analysis for 1000 replications. Jones–Taylor–Thornton (JTT) substitution model was applied to phylogeny reconstruction. MEME (Multiple Em for Motif Elicitation) Suite 5.4.1 version (URL-4) was applied to carry out motif analysis based on the protein sequences of *KEA* genes, and then the MAST (Motif Alignment & Search Tool) was used to view the detailed information of the motifs. In the analysis, using classical mode, the maximum number of motifs was determined as 10 and the optimum width was set between 6 and 50.

2.4. Subcellular Localization and Functional Gene Ontology Analysis of KEA Proteins

The CELLO2GO (URL-5) was used to predict the subcellular location *KEA* genes and to analysis gene ontology.

2.5. Homology Modeling of KEA proteins

Homology modeling for KEA proteins was performed using intensive mode in Phyre2 (Protein Homology/analogY Recognition Engine V 2.0) program; URL-6) and the predictive structure of the proteins was obtained.

2.6. Cis-Acting Elements Located in the *KEA* Gene Promoters

To analyze the cis-regulatory elements of *KEA* genes, the promoter sequences (2Kb sequence upstream of start codons) were taken from NCBI database. The promoter sequences were scanned with the PlantCARE database (URL-7).

2.7. miRNA (MicroRNA) Analysis

The coding sequence (CDS) of *KEA*s was used to recognize possible target miRNAs in the psRNATarget database (URL-8). The interaction network between the miRNAs and *KEA* genes was drawn by Cytoscape software (V3.9.1).

2.8. Determination of Expression Profiles of *H. annuus KEA* Genes Under Salt Stress Using Transcriptome Data

To obtain *H. annuus* transcriptome data, the Sequence Read Archive database (SRA), which contains raw sequencing data and their access codes, was used on the NCBI website (URL-9). As a result of the scan, access codes SRX11298852 (for root) and SRX11298851 (for leaf) were selected and Illumina HiSeq reads deemed suitable for the study were downloaded in ".fasta" format with clipped for RNA-Seq analysis by NCBI SRA Run Browse. The most suitable primer for PCR was determined using FastPCR Professional 6.8.04 software. FastPCR software is a tool that provides extensive possibilities for designing any type of PCR setup for standard, long distance, reverse, real-time PCR.

SRA files were loaded into this software and in silico PCR was performed. As a result of these procedures, gene expression levels were determined and graphs were drawn.

3. RESULTS

3.1. Identification of *KEA* Genes in *H*. annuus Genome

A total of 6 genes were identified in the *H. annuus* genome. The *KEA* genes were renamed from *HaKEA-01* to *HaKEA-06* based on the gene distribution information on the chromosomes. Gene names included the first letters of the plant's Latin name followed by *KEA* number. When molecular weights and amino acid lengths of the determined *KEA* genes were analyzed, the molecular weights of the *H. annuus KEA* genes were between 82946.87 Da and 126979.59 Da, and their protein lengths varied between 765 and 1173 aa. The predicted range of theoretical isoelectric points (pIs) ranged from to 5.03 to 6.22. Instability index varied between 35.60 and 42.18 (Table 1).

Table 1: The Basic Information About *HaKEA* Genes

ID	NCBI DATABASE	Physical position on sunflower genome			Protein length (aa)	pI	Molecular weight (Da)	Instability index	Stable or unstable
		Chr.	Start position (bp)	End Position (bp)					
HaKEA-01	XP_022030148.1	3	176,089,055	176,096,511	1173	5.03	126680.62	42.18	Unstable
HaKEA-02	XP_022035808.1	4	192,446,788	192,456,311	1166	5.04	125383.61	40.80	Unstable
HaKEA-03	XP_035844615.1	4	192,447,582	192,455,517	1166	5.04	125383.61	40.80	Unstable
HaKEA-04	XP_021969591.1	6	13,058,636	13,707,286	1173	5.05	126979.59	38.45	Stable
HaKEA-05	XP_022002944.1	13	172,043,871	172,058,549	1172	5.11	126163.67	39.37	Stable
HaKEA-06	XP_021977708.1	16	44,608,007	44,612,252	765	6.22	82946.87	35.60	Stable

3.2. Chromosomal Localization Distribution and Prediction of Gene Structure

H. annuus has 17 chromosomes and *KEA* genes are located on chromosomes 3, 4, 6, 13 and 16 (Figure 1). Gene structure analysis was performed to better understand the *KEA* genes in *H. annuus*. The exon-intron configuration was compared to examine the structural features of *KEA* genes in this plant (Figure 2). Gene structure analysis revealed that *HaKEA-06* is intronless and other genes have 21 introns. Moreover, *HaKEA-04* has the longest intron.

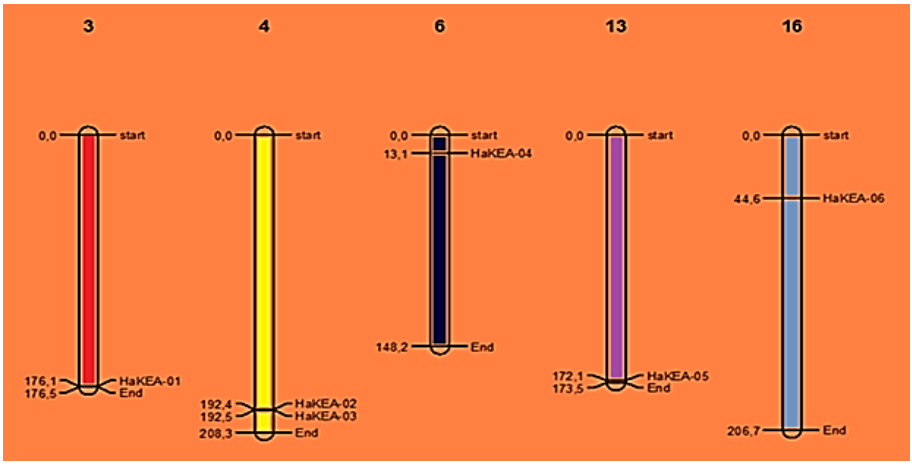

Figure 1: Chromosomal Distribution of 6 *KEA* Genes on *H. annuus* Chromosomes

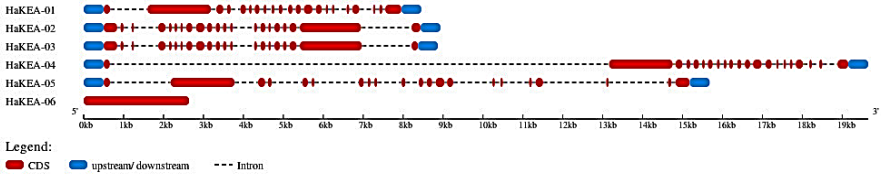

Figure 2: Intron-exon Distribution of *KEA* Genes

3.3. Phylogenetic Tree Construction and Conserved Protein Motif Analysis of *KEA* Family Genes

The *KEA* genes were divided into four categories based on phylogenetic relationships. To examine this distinction in more detail, the conserved motif structures of the KEA proteins were also analyzed. The determined motifs support the classification in our phylogenetic tree. The motifs of the genes *HaKEA-01* and *HaKEA-05*, *HaKEA-02* and *HaKEA-03* were similar. It is also clearly seen in the motif analysis that the *HaKEA-06* gene is different from the other categories (Figure 3). 10 conserved motifs were detected in all proteins except the HaKEA-06 protein.

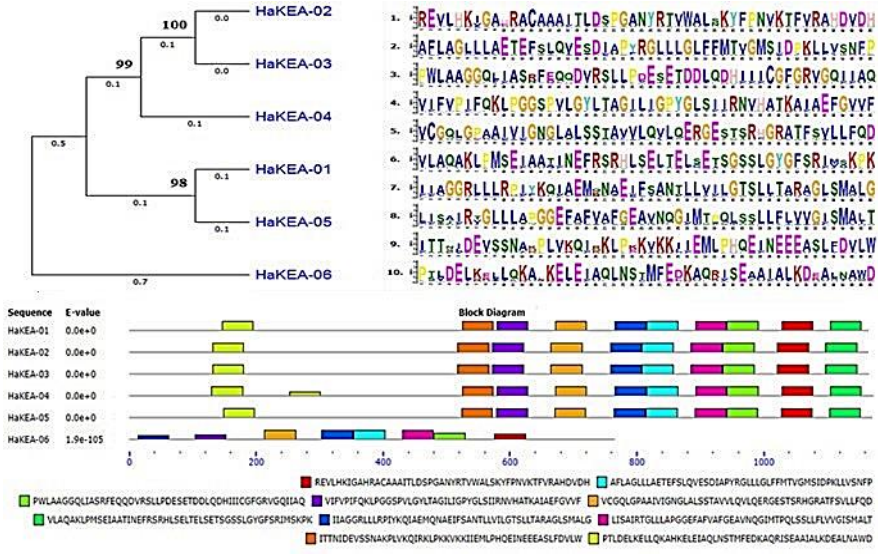

Figure 3: The Phylogenetic Tree of *KEA* Genes and Conserved Motifs in HaKEA Proteins

3.4. Subcellular Localization and Functional Gene Ontology Analysis of KEA Proteins

As a result of the analysis, it was determined that all KEA proteins were found in the plasma membrane and had transmembrane transporter activity. When examined in terms of cellular components, it was concluded that it is located in the intracellular, cell, cytoplasm, plasmid and organelles (Figure 4).

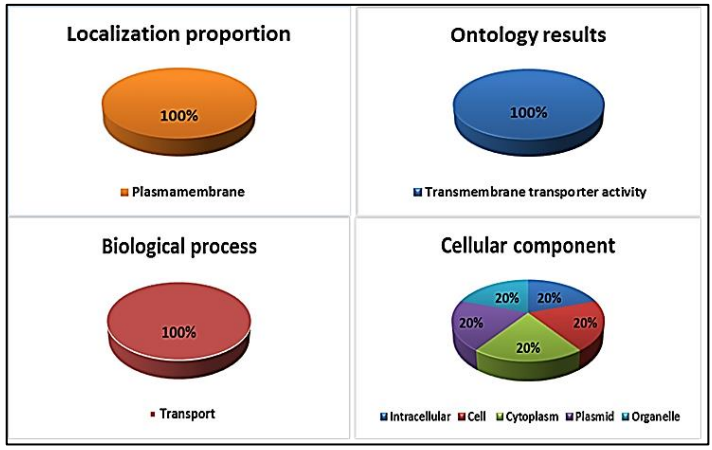

Figure 4: Subcellular Localization, Biological Process, Cellular Component and Gene Ontology Analysis of *KEA* Genes

3.5. Homology Modeling of KEA proteins

The similarity rate for homology modeling was done by selecting the intensive mode from the Phyre[2] database. Its confidence was determined as 90% and similarity between 80% and 100%. Proteins between HaKEA-01 and HaKEA-05 showed high similarity (97%, 97%, 97%, 95%, 96%, respectively).

It was found to be 85% in the HaKEA-06 protein. According to the result obtained, it was determined that the alpha helix structure was more dominant (Figure 5).

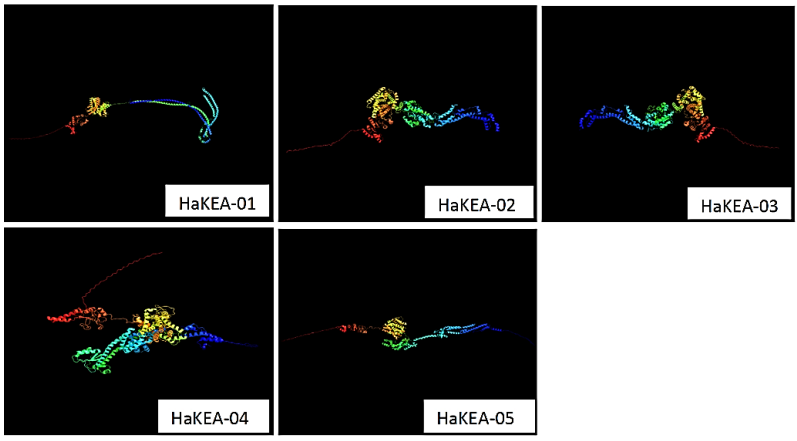

Figure 5: The Predicted Three-Dimensional Structure of Proteins Belonging to the *HaKEA* Genes

3.6. Cis-Acting Elements Located in the *KEA* Gene Promoters

To distinguish the gene functions and regulatory roles, cis-acting elements in *KEA* genes promoter regions were searched a 2000 bp upstream region from each gene's transcriptional activation site. The complete data of cis-elements are offered in Table 2. ABRE, AuxRR-core, TGA-element, GARE-motif, P-box, TATC-box, GARE-motif, CGTCA-motif, TGACG-motif, TCA-element motifs were found to be associated with plant hormones such as abscisic acid, auxin, gibberellin, methyl jasmonate and salicylic acid.

Table 2: Cis-acting Elements in the Promoters of *HaKEA*s

Motif	HaKEA-01	HaKEA-02	HaKEA-03	HaKEA-04	HaKEA-05	HaKEA-06	Function
A-box	0	0	0	2	0	0	cis-acting regulatory element
ABRE	1	4	0	4	1	3	cis-acting element involved in the abscisic acid responsiveness
AE-box	0	0	1	0	0	0	part of a module for light response
ARE	6	3	5	2	1	5	cis-acting regulatory element essential for the anaerobic induction
AT1-motif	0	0	0	0	1	0	part of a light responsive module
ATC-motif	0	0	0	0	0	1	part of a conserved DNA module involved in light responsiveness
AuxRR-core	0	2	0	1	0	1	cis-acting regulatory element involved in auxin responsiveness
Box II	0	0	0	2	0	0	part of a light responsive element
Box 4	0	1	1	2	2	0	part of a conserved DNA module involved in light responsiveness
CAT-box	1	1	1	1	0	1	cis-acting regulatory element related to meristem expression
CAAT-box	25	12	21	24	34	29	common cis-acting element in promoter and enhancer regions
CCAAT-box	1	0	0	0	1	0	MYBHv1 binding site
CGTCA-motif	0	2	0	3	1	1	cis-acting regulatory element involved in the MeJA-responsiveness
chs-CMA1a	0	0	0	2	0	0	part of a light responsive element
circadian	0	1	1	1	0	0	cis-acting regulatory element involved in circadian control
G-box	0	3	0	2	2	4	cis-acting regulatory element involved in light responsiveness
G-Box	1	1	0	2	0	2	cis-acting regulatory element involved in light responsiveness
GA-motif	0	0	0	0	1	0	part of a light responsive element
GATA-motif	0	2	0	0	2	1	part of a light responsive element
GARE-motif	1	2	2	0	0	0	gibberellin-responsive element
GC-motif	1	0	0	0	0	0	enhancer-like element involved in anoxic specific inducibility
GT1-motif	6	0	0	3	6	3	light responsive element
I-box	2	1	0	1	0	0	part of a light responsive element
LAMP-element	0	0	0	0	1	1	part of a light responsive element
LTR	0	3	2	1	1	2	cis-acting element involved in low-temperature responsiveness
MBS	2	1	1	0	0	0	MYB binding site involved in drought-inducibility
MRE	0	0	1	1	1	0	MYB binding site involved in light responsiveness

MSA-like	0	2	2	1	0	0	cis-acting element involved in cell cycle regulation
O2-site	0	0	0	0	1	1	cis-acting regulatory element involved in zein metabolism regulation
P-box	0	3	1	0	0	0	gibberellin-responsive element
Sp1	2	1	0	1	2	1	light responsive element
TATA-box	11	78	81	45	74	23	core promoter element around -30 of transcription start
TATC-box	0	1	0	0	1	0	cis-acting element involved in gibberellin-responsiveness
TCCC-motif	2	0	0	0	0	1	part of a light responsive element
TCA-element	0	0	0	1	0	1	cis-acting element involved in salicylic acid responsiveness
TC-rich repeats	1	2	3	0	1	0	cis-acting element involved in defense and stress responsiveness
TCT-motif	6	0	0	0	0	0	part of a light responsive element
TGA-element	0	0	0	1	0	0	auxin-responsive element
TGACG-motif	0	2	0	3	1	1	cis-acting regulatory element involved in the MeJA-responsiveness

In addition, it has been observed that it has important functions such as anaerobic induction (ARE), meristem expression (CAT-box), circadian control, anoxic specific inducibility (GC-motif), low-temperature responsiveness (LTR), drought-inducibility (MBS), cell cycle regulation (MSA-like), zein metabolism regulation (O2-site), defense and stress responsiveness (TC-rich repeats).

3.7. miRNA Analysis

As a result of the analysis performed to identify miRNAs targeting *HaKEA* transcripts, it was determined that 6 different *HaKEA* transcripts were targeted by 41 different plant miRNAs. Four of these plants (*Arabidopsis thaliana* (L.) Heynh., *Cynara cardunculus* L., *Ricinus communis* L. and *Vitis vinifera* L.) were selected, sources and targets were drawn. The reasons for choosing these plants are as

follows: *A. thaliana* is a model plant, *C. cardunculus* is in the same family as *H. annuus*, *R. communis* is an oil plant, and *V. vinifera* is a perennial plant with economic value.

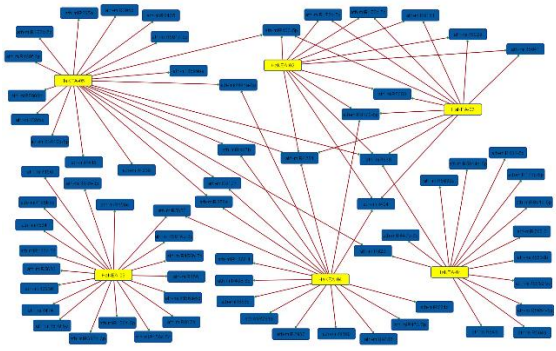

Figure 6: ath-miRNAs Targeting *HaKEA* Genes

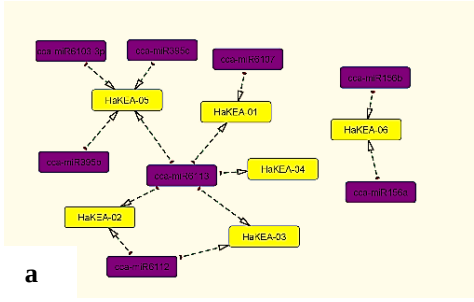

Figure 7: a) cca-miRNAs Targeting *HaKEA* Genes

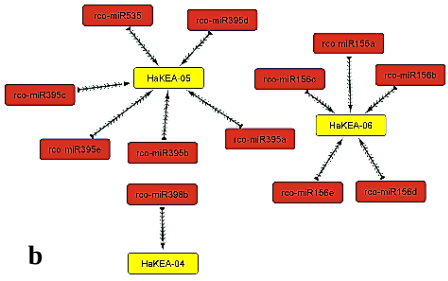

Figure 7: b) rco-miRNAs Targeting *HaKEA* Genes

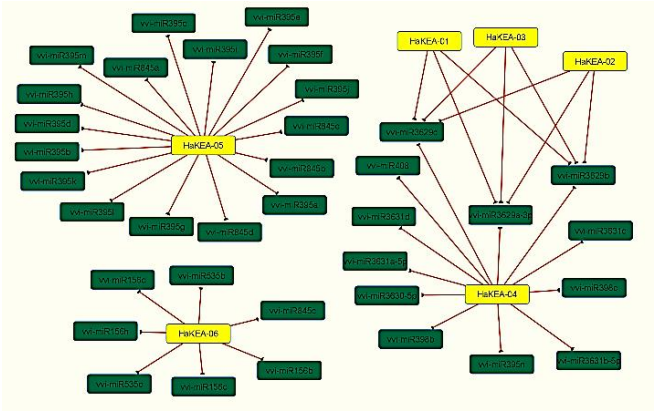

Figure 8: vvi-miRNAs Targeting *HaKEA* Genes

Figure 6 indicates the relationship of *A. thaliana* miRNAs with *HaKEA*. The relationship between *C. cardunculus* miRNAs and *HaKEA* genes is given in Figure 7a. When we examine Figure 7b in detail, it is observed that rco-miRNAs target only *HaKEA-04*, *HaKEA-05* and *HaKEA-06* genes. *V. vinifera* is an important plant with economic value. Figure 8 schematizes which genes the vvi-miRNAs target.

3.8. Determination of Expression Profiles of *H. annuus KEA* Genes Under Salt Stress Using Transcriptome Data

SRA files suitable for the study were downloaded to determine the expression level of *H. annuus* under salinity stress. The accession number is as follows: SRR14986276 (control leaf), SRR14986277 (control root), SRR14986278 (salt stress root), SRR14986279 (salt stress leaf). As a result of SRA analysis, expression levels under salt stress in leaves and roots of the *H. annuus* were examined. First of all, the most suitable primer for PCR was checked with FastPCR software.

As a result of our in-silico PCR analysis, it was determined that the best primer design was in the *HaKEA-04* genome sequence. With this analysis, real-time PCR simulation was performed. As a result of the PCR process, it was observed that up-regulated expression of the *HaKEA-04* gene was observed in the root and leaf, and the expression was higher in the leaf than in the root; but expression in the root is negligible. Obtained results are also graphed. The relative expressions at different plant part were compared with the control (Figure 9).

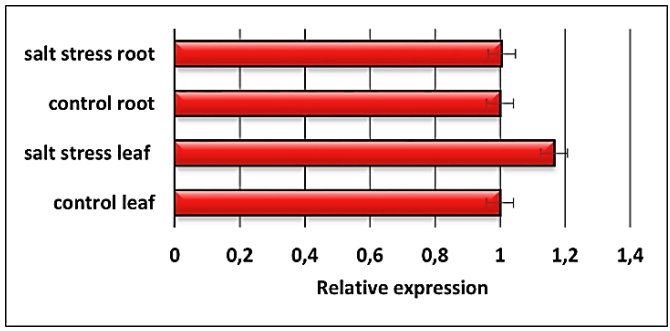

Figure 9: Expression Profiles Under Salt Stress

4. DISCUSSION

In this article, genome-wide analysis was performed on *H. annuus KEA* genes. As a result of the analysis, 6 *KEA* genes were detected. When other studies on the *KEA* gene are examined, 6 in *Cicer arietinum* L. (Azeem et al., 2018), 4 in *Solanum tuberosum* L. (Azeem et al., 2021), 6 in *Vigna radiata* (L.) R. Wilczek (Azeem et al., 2022b), 6 in *Cajanus cajan* (L.) Mill sp. (Siddique et al., 2021) and 7 *KEA* genes were identified in *Gossypium raimondii* Ulbr. (Azeem et al., 2022a). Studies showed that the number of *KEA* genes in plants varies at these levels.

Investigation of the structure of the gene is an important issue to explain the functions of the gene and to determine the phylogenetic relationships (Cao et al., 2022). It was determined that the *HaKEA-02* and *HaKEA-03* genes were on the same chromosome, and when we compared the phylogenetic tree and the exon-intron regions of these genes (Figure 2 and Figure 3), it was observed that the analysis results we obtained supported each other. In a study (Azeem et al., 2022b), it was reported that the average number of introns in *A. thaliana* was nine.

KEA genes are involved in transport, as determined in this article. In a study, it was reported that *AtKEA1–AtKEA3* has a critical function in plant chloroplast function, osmoregulation, photosynthesis and pH regulation, *AtKEA1* and *AtKEA2* are localized in the chloroplast envelope membrane and is localized in the thylakoid membrane and Golgi (Zhou et al., 2016). Moreover, it was determined that the *H. annuus* contains many motifs associated with the light response. When all data are examined, it is thought that *HaKEA* genes may be related to the process of photosynthesis, hormone, defense, abiotic stress, growth and development regulation.

miRNAs are a group of single-stranded, non-protein-coding RNAs containing approximately 18-25 nucleotides. miRNAs are involved in many cellular and metabolic pathways under abiotic stresses such as flowering, morphogenesis and signal transduction (Pervaiz et al., 2022). In a study (Arjmand et al., 2021), it is stated that ath-miR414 can be a stress sensitive miRNA and regulate important target genes under

drought conditions. In another study (Wang et al., 2021), it was reported that ath-miR160c-3p was up-regulated in phosphorus deficiency. It is stated that ath-miR173-5p is the trigger of phasiRNA production during MIGS, which is the simplest of phasiRNA-based gene silencing methods (Han et al., 2015a). In addition, it was indicated that the miR3440s were stress sensitive miRNAs of nutrients (Arjmand et al. 2021). Another study reported that the target function of ath-miR169f-3p is probable phosphoinositide phosphatase (Anna et al., 2019). There are data in another study that ath-miR858a may be involved in zeatin biosynthesis associated with cytokine synthesis (Liu et al. 2019). It is stated that miRNA156 family members are involved in the vegetative and reproductive stages of plants, and function in flower development and coloration of berry (Wang et al., 2019a). There is information that the miRNA named ath-miR393b-3p is associated with defense (Cao et al., 2020). It has also been reported in another study (Huang et al., 2022) that ath-miR395a may be involved in processes related to plant response to low temperature. ath-miR395d is presumed to have a cellular response function to sulfate starvation (Meng et al., 2016). Another miRNA, ath-miR395e, is stated to accelerated seed germination under high salinity/or dehydration (Zhang et al., 2014). It is also included in another study (Verma et al., 2014) that ath-miR404 is associated with cysteine-type endopeptidase inhibitor activity. It was also reported in another study (Arjmand et al., 2021) that ath-miR414 is downregulated under individual and combined drought stress and Pseudomonas syringae infection. Another study (Ahmadizadeh et al., 2020) concluded that ath-miR843 plays a key role in regulating the

post-transcriptional modification of *S-adenosylmethionine* genes in *Arabidopsis*.

One of the *HaKEA*-associated miRNAs, cca-miR398, is thought to play an important role in hickory tolerance to copper sulfate stress (Sun et al., 2020). miR156 has important features. For example, in a study (Zhang et al., 2011), it was stated that miR156 controls developmental timing and flowering in *Arabidopsis*, overexpression of miR156 in rice causes severe dwarfism and delayed flowering, and also increased miR156 causes deformed flower architecture. When we examined the functions of the miRNAs in the Figure 7a, there was no conclusion about most of them. When their interactions with *HaKEA* were examined, we concluded that these miRNAs may have a function in potassium transport and potassium-related functions. cca-miR6113 targets all *HaKEA* genes except *HaKEA-06*. This indicates that this miRNA may have an important role.

We conclude that all rco-miRNAs start at 1 and end at 21. We could not find any findings related to these rco-miRNAs in the literature search. We think that these miRNAs interacting with the *HaKEA* genes have a function in potassium transport.

It has been stated in a study (Wang et al. 2019b) that vvi-miR535a is down-regulated under cold stress, targeting the late embryogenesis abundant (LEA) protein, AP2-like ethylene-responsive transcription factor, and NAC transcription factor gene in cultured grapevines.

Another study reveals that wvi-miR535c is up-regulated in response to UV-B (Sunitha et al., 2019). In addition, it was concluded that wvi-miR3629a-3p in cultured grapes targets the lysosomal beta glucosidase gene associated with D-glucose synthesis (Wang et al., 2019b). Moreover, as a result of the analysis we have obtained, it is observed that vvi-miR3629a-3p is an important miRNA and targets 4 *HaKEA*s (*HaKEA-01*, *HaKEA-02*, *HaKEA-03* and *HaKEA-04*). In another study, anticorrelation was reported between up-regulation of wvi-miR398b-c and down-regulation of serine/threonine-protein kinase and ATP sulfurylase (Bester et al., 2017). Apart from these, it is stated in another study (Leng et al., 2017) that wvi-miR398b has cis-elements responsible for abiotic stress, abscisic acid, anaerobic induction, drought, light and methyl jasmonate; contains cis-elements that respond to low temperature, heat stress and are involved in multiple pathways that react to stress. In the same study, data are presented that vvi-miR398c has cis-acting elements involved in multiple hormones signaling, such as abscisic acid (ABRE), ethylene (ERE), and salicylic acid (TCA element). It is thought that the function of vvi-miR156b/c/d may be closely related to the growth and development of grapevines (Wang et al., 2016). It has been determined that vvi-miR395l and vvi-miR395i play an important role in the copper response (Leng et al., 2017). It has been reported that the miRNA named vvi-miR845c may play a role in the heat tolerance regulated by GABA (Li et al., 2019).

Biotic and abiotic stresses cause negative effects on yield in agriculture, and transcription factors play a role in regulating the plant response to

stress (He et al., 2021). In the study in which we obtained transcriptome data (Barnhart et al., 2021), *H. annuus* seeds were planted in 50 mL Falcon tubes at a depth of 1.5 cm, and sand and turface were used as a growth substrate at 3:1 ratio. The tubes were then observed for 20 days following germination in the plant growing room, under control and stress conditions. The experiment was arranged and repeated in a 16:8 light:dark cycle at 20 °C. Before RNA extraction to determine transcription profiles, leaf and root tissues were collected separately and stored in liquid nitrogen at -80 °C. 100 mM NaCl was used for salinity stress. As a result of the research, it was determined that the upregulated expression was higher than the number of downregulated expressions in the leaf and the downregulated expression was higher than the upregulated expression in the root when compared to the control under salinity stress. When all the data were evaluated, it was observed that the expression was higher in the leaf than in the root. This result supports our result. As a result of the PCR process, it was determined that the *HaKEA-04* gene was most expressed in leaves under salt stress. In another study (Kong et al., 2021), in 100 mM NaCl treatment, *ZmNHX5* and *ZmKEA2* genes were up-regulated in both leaves and roots, while in 100 mM KCl application, *ZmNHX2*, *ZmNHX5*, *ZmNHX6*, *ZmNHX8*, *ZmKEA1*, *ZmKEA2*, *ZmKEA5* and *ZmKEA6* genes were found to be up-regulated in the leaf. In addition, as a result of this research, it was concluded that *KEA* genes have a role in NaCl stress in maize. Likewise, in present study, it was concluded that the *HaKEA-04* gene caused a response to salinity stress in *H. annuus* and that *KEA*

genes might play a role in salinity tolerance. It was determined that *KEA* genes in *A. thaliana* also show a response to salinity (Han et al., 2015b).

CONCLUSION

In this study, 6 *KEA* genes were identified in *H. annuus* and it was determined that *KEA* genes have very important functions and are associated with miRNAs of various plants. The gene expression result shows that in the future, *KEA* genes can be integrated into another plant to provide salinity resistance, and by gene breeding it can also increase the salinity resistance of this plant.

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CHAPTER 5

GROWTH CHARACTERISTICS, CADMIUM TOLERANCE AND PHYTOREMEDIATION CAPACITY OF ORNAMENTAL SMALL SHRUB ROSES (*CUBANA KORDES* ROSE)

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1. INTRODUCTION

Despite of the fact that the climate change event is being mentioned more and more frequently today, and almost negative natural events are attributed to this phenomenon, in fact, it is ignored that the effects of human damage to nature cause these problems. Due to reasons such as urbanization, increase in industrial activities, increase in population, as a result of the random disposal of wastes from various products that make our lives easier; our soils and waters are polluted (Shao et al., 2019; Lu et al., 2020; Seghier & Bouhadjera, 2020). Among these wastes, industrial and domestic wastes, vehicle emissions, mining wastes, high metal wastes, electrical and electronic equipment wastes (Wei & Liu, 2012; Rajarao et al., 2014), computer board wastes (Kiddee et al., 2013), paints, fertilizer use, agricultural irrigation with urban wastewater, treatment sludge applications, pesticides, coal combustion residues, waste batteries, petrochemical spillage and atmospheric accumulations from various sources can be mentioned (Bradl, 2005; Sherameti & Varma, 2010; Alloway, 2013). Depending on the heavy metal pollution problem, it is occurring potential toxic effects on plants, animals, fish and humans (Wei & Liu, 2012; Tchounwou et al., 2012; Rodríguez-Eugenio et al., 2018).

Toxic metal ions enter plant cells through similar uptake processes with micronutrients that are absolutely necessary for plants. Depending on the concentrations and valences of the elements in the soil solution, the movement occurs from the soil to the root surface

and then to the shoot (Patra et al., 2004). One of the heavy metals that has a toxic effect on plants is Cd.

Controlling the uptake, transport, and accumulation of excessive cadmium in the environment in case of soil and water pollution problems, it is of critical importance in terms of food safety for plants and therefore for the people who consume them (Song et al., 2017). It has been stated that Cd in the soil is a great danger to people's health and is the cause of the "Itai-Itai" disease that occurred in Japan in the 1960s (Brown et al., 1994; Pereira et al., 2002; Sawidis, 2008; Wang et al., 2012). Plants exposed to cadmium employ avoidance strategies such as organic acid production, chelation, and sequestration to prevent metal access to their stem cells (Song et al., 2017). Cadmium reduces water and nutrient uptake in plants, causes chlorosis and necrosis in leaves, reduction in height, redness of roots and roll-like curling of leaves. It also reduces growth in plant roots and stems, inhibits chlorophyll biosynthesis, decreases the activities of some enzymes and photosynthetic carbon assimilation (Maksymiec et al., 2007).

Among the methods used to remove various heavy metals from soil and water, which have negative effects on humans and animals when taken with plants and food chain, there is an increasing number of studies on the phytoremediation technique (Cunningham & Berti, 1993; Raskin et al., 1994; Salt et al., 1995; Salt et al., 1998). This technique is environmentally friendly, has an economic effect and has

a high potential about effectiveness. The plants selected for this process should have features such as easy growth and formation of dense vegetative parts.

The disposal of plants having phytoremediation properties after taking up heavy metal is another problem. In this technique, the use of ornamental plants instead of plants consumed as food is beneficial both visually and in terms of improving soil and water pollution. In some studies, it has been reported that ornamental plants have high uptake capacity of heavy metals such as Pb, Ni, Cd, Cu (Liu et al., 2008; Subhashini & Swamy, 2013; Shao et al., 2019; Lu et al., 2020).

Cadmium(Cd) content in root and aboveground parts of ornamental plant *Chlorophytum comosum* at a soil Cd concentration of 200 mg kg^{-1} are 1522 and 865.5 mg kg^{-1} respectively (Wang et al., 2012). In ornamental sunflower (*Helianthus annuus* 'Pacino'), red sage (*Salvia splendens* 'Fuego') and *Tagetes erecta* (*Tagetes erecta* 'Inca Yellow') plants, Cd accumulated mostly in leaves and shoots, and then in flowers (Bosiacki, 2008). It has been determined that *Salix babylonica* can accumulate Cd significantly and can be a phytoextraction plant (Ouyang et al., 2017). In *Osmanthus fragrans* var. *thunbergii* ornamental plant, as Cd applications increased, a tendency to decrease in plant biomass was observed, Cd accumulation in the plant increased, and the highest Cd accumulation was at the dose of 25 mg kg^{-1}. The plant showed high Cd transfer efficiencies when applied to Cd (Wu et al., 2011). Indian mustard (*Brassica juncea*) has Cd

tolerance up to 400 mg kg^{-1}. However, there was an overall decrease in root and stem length, tissue biomass, leaf chlorophyll, and carotenoid content, with the highest accumulation in shoots (10791 µg Cd g^{-1} dry weight), roots (9602 µg Cd g^{-1} dry weight) and leaves (10071.6 µg Cd g^{-1} dry weight) (Goswami & Das, 2015). In *Malva rotundifolia*, another ornamental plant, showed high tolerance to Cd in terms of stem biomass, root biomass, plant height and tolerance index (TI). *Malva crispa, Sida rhombifolia, Celosia argentea* and *Celosia cristata* are moderately tolerant, while *Althaea rosea* and *Abutilon theophrasti* are more susceptible to Cd than other plants. It was found that in the roots and the shoots of *M. rotundifolia* were accumulated >200 mg kg^{-1} Cd and 900 mg Cd kg^{-1} respectively, and BCF and TF values are >1.0. Due to these results, it has been determined that the plant can be classified as a model hyperaccumulator (Wu et al., 2018a). While the Cd concentration in the soil is 30 mg kg^{-1}, maximum Cd accumulation in shoots and roots of *Althaea rosea* Cav. is 131.9 and 67.5 mg kg^{-1}, respectively. When the Cd concentration in the soil was 100 mg kg^{-1}, the maximum Cd accumulation was 178.5 and 135.6 mg kg^{-1}, respectively. *Althaea rosea* Cav. It has been recognized as a potential Cd-hyperaccumulator with chemical enhancement (Liu et al., 2009).

In the study in which the heavy metal uptake capacity of fruit flesh and seeds of rose species were investigated. *Rosa pulverulenta* is more effective for Cd, Al and Si uptake and *Rosa dumalis* subsp. *boissieri* was found to be more effective for Ni uptake. According to the results

obtained, it has been reported that *Rosa pulverulenta* can be useful in phytoremediation applications in areas contaminated with Cd, Al and Si (Turan & Ercisli, 2007). Wang & Zhou (2005) investigated the ecotoxicological effects of Cd on African marigold, scarlet sage and sweet mallow. According to the results, Cd had little effect on seed germination of the three plants ($p > 0.05$) and inhibited root elongation significantly ($p < 0.05$). At the Cd-tolerance indices under the same Cd content, sweet mallow was the most resistant plant, while red sage was the most sensitive.

Leonardite is highly oxidized immature coal, contains high levels of humic acids (40-80%) (Tan, 2014). It is used in plant cultivation like other organic products, it especially increases plant biomass production and soil fertility (Ozkan, 2008; Zeledon-Toruno et al., 2007; Tan, 2014).

In this study, it was aimed to determine the cadmium uptake capacity of Kordes roses, which are used extensively in landscaping studies, at the removal of cadmium from polluted soils with Cd. In addition, it was aimed to determine the effect of leonardite application on plant growth in the growing environment with cadmium pollution. Because of roses are tolerant of pollution, very often it is erected on medians dividing traffic directions (Akay, 2022). *Rosa rugosa, Rosa rugotida* and *Rosa nitida* varieties were recommended for this purpose and it has been determined that *R. rugosa* is a good bioindicator for polluted areas (Calzoni et al., 2007). In the study, the hypothesis that the Cd

accumulation capacity of Kordes roses, which is widely used in medians in the middle of the road in our country is high, was taken as basis on.

2. MATERIALS AND METHODS

In order to determine the effect of leonardite in reducing abiotic stress on the development of rose plant grown in soil contaminated with Cd, this study was carried out as a pot trial. Before planting the experimental plants, fine stream sand (6 kg/pot) was filled in each pot on the basis of dry weight and leonardite was added to the pots at 0% - 3% - 6% doses. The chemical properties of leonardite and soil used in the study are shown in Table 1.

Table 1: Physical and Chemical Properties of the Soil and Leonardite Used in the Experiment

Soil		**Leonardite**	
pH	8.25	**pH**	3.60
EC (µS cm^{-1})	70	**EC** (µS cm^{-1})	1385
$CaCO_3$ (%)	0.93	**Available Fe** (mg kg^{-1})	0.190
Field capacity (%)	12.89	**Available Zn** (mg kg^{-1})	7.97
Texture	Sand	**Available Cu** (mg kg^{-1})	0.14
Available Fe (mg kg^{-1})	4.70	**Available B** (mg kg^{-1})	1.57
Available Zn (mg kg^{-1})	0.15	**Available Mn** (mg kg^{-1})	46.90
Available Cu (mg kg^{-1})	0.36	**Ni** (mg kg^{-1})	0.44
Available B (mg kg^{-1})	0.38	**Pb** (mg kg^{-1})	0.03
Available Mn (mg kg^{-1})	2.70	**Co** (mg kg^{-1})	0.91
Ni (mg kg^{-1})	0.05	**Cr** (mg kg^{-1})	42.30
Pb (mg kg^{-1})	0.97	**Cd** (mg kg^{-1})	0.08
Co (mg kg^{-1})	18.70	**Humic fulvic acid (%)**	29.01
Cr (mg kg^{-1})	8.50		
Cd (mg kg^{-1})	0.45		

After leonardite was added, increasing doses of Cd (0-25-50-100-200 mg kg^{-1}) were applied in the form of $3CdSO_4.8H_2O$ and mixed

thoroughly with the growing medium to be homogeneous than it was incubated for 30 days. The experiment was set up according to the randomized plots factorial design with 5 replications. Cubana Kordes roses, which are used extensively by the Metropolitan Municipality for landscaping and supplied from a private company, at the end of these incubation period, root and branch pruning were done without delay and planted in pots.

The experimental plant Cubana Kordes roses were introduced in Germany, Canada and Australia in 2007 and 2009 (Anonymous, 2019a; Anonymous, 2019b). Cubana Kordes rose is an ornamental plant that has a long-term flowering feature, provides a good surface cover, it is disease resistant and can tolerate drought (Anonymous, 2019c).

After the experiment was established, the developmental status of the roses was followed and the study continued for 4 months, including the intensive flowering period. Throughout the experiment, the plants were regularly watered according to the soil moisture condition and the Hoagland solution was applied to the plants every week together with the irrigation water(The solution contained Fe, B, Mn, Cu, Zn, Mo, Ca, P, K, N, and Mg). Plastic plates were placed under each pot to prevent the loss of nutrients and trace elements during the experiment, and the drained water was returned to the pots. The chemical properties of the soil used in the experiment are shown in Table 1.

2.1. The Data Obtained at The End of The Trial

During and at the end of the trial, data on the number of flowers, bud diameter, bud length, shoot diameter, number of buds, flower diameter, flower color and flower shoot length were obtained from roses. At the end of the trial, root, flower and shoot fresh and dry weights of roses were recorded. Then, the separately ground plant parts were subjected to wet burning with HNO_3 about 6-8 hours (Zheljazkov & Nielson, 1996; Hseu, 2004). Cd concentrations in the filtered solutions obtained after the necessary procedures were determined with a 5100 model ICP-OES spectrometry.

Translocation factor (TF) was calculated according to the following formula (Su et al., 2014).

TF = (Cd content in the flower + Cd content in the shoot) / Cd content in the root

Cd received by plant parts ($\mu g\ kg^{-1}$) = ((Cd content of plant parts (mg/kg) x dry weight of plant parts (g/pot))/1000)*1000

At the end of the trial, soil samples were taken from all pots, and the available Cd content of soils was determined ($mg\ kg^{-1}$) (Lindsay & Norvell, 1978). The bioaccumulation factor (BCF) was calculated according to Zayed et al. (1998).

2.2. Statistical Analysis

Measurements were made in five repetitions in the experiment. Statistical data were analyzed using the Minitab 18 statistical package program, and Tukey multiple comparison tests were used (between mean at 95% level of confidence).

3. RESULTS

3.1. Effect of Cadmium on Some Growth Parameters

In the study, it was observed that Cubana Cordes rose's development and flower formation were not adversely affected by Cd applications. According to the variance analysis results, only the mean flower diameter values showed a significant difference with the Cd doses, while the leonardite applications had significant effect on the number of buds and bud diameter, and average length of flower shoots and bud length ($p<0.05$). The effects leonardite x Cd interaction on shoot diameter, number of buds, flower diameter, flower color, shoot length, flower number and bud diameter were statistically insignificant. The effect of these interaction only on bud length was significant ($p<0.05$) (Table 2).

According to variance analysis results, the dry weight of flowers, shoot, and roots did not differ with increasing doses of Cd application and leonardite x Cd interaction. Only the dry weight of flower and shoot showed significant difference with leonardite application

($p<0.05$). In the study, it is noteworthy that the dry weight of root is higher than the above-ground parts. Increasing doses of cadmium did not have a negative effect on the plant biomass (Figure 1).

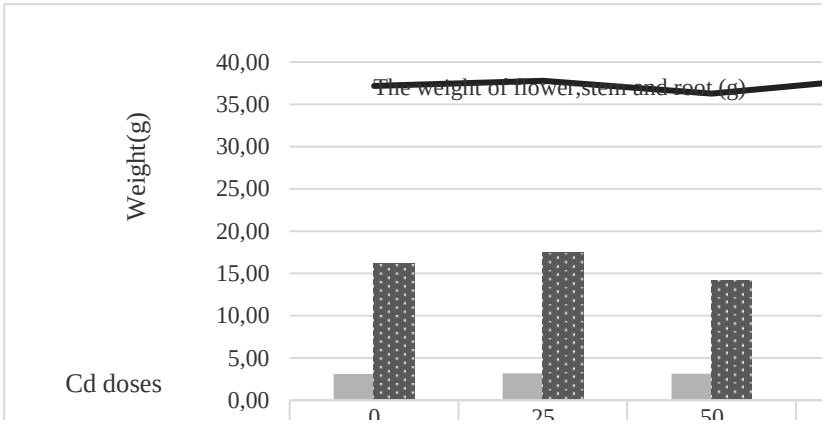


Figure 1: The Effect of the Application of Cd and Leonardite on the Dry Weight of the Flower, Stem, and Root of Cubana Kordes Rose

3.2. Cd Content of The Plant Parts

The effect of Cd and leonardite applications and leonardite x Cd interaction on the Cd contents of the flower, shoot and root of rose was significant at the $p<0.05$ level. 6% application of leonardite compared to 3% application decreased the Cd contents of flower, shoot and root and the highest average Cd contents were in 3% leonardite applications ($p<0.05$). When taken together with Cd applications, the highest values of Cd content of flower, shoot and root were observed at 3% leonardite - 200 mg Cd kg^{-1} application (4.65 - 115.04 - 449.53 mg kg^{-1}, respectively) (Table 3). The Cd

contents of flower, shoot and root increased significantly with increasing of Cd doses ($p<0.05$). The highest Cd contents were in 200 mg kg^{-1} application (Figure 2). Cd content in plant parts is as follows: root > shoot > flower. In other words, the cadmium content is the highest in the plant root parts.

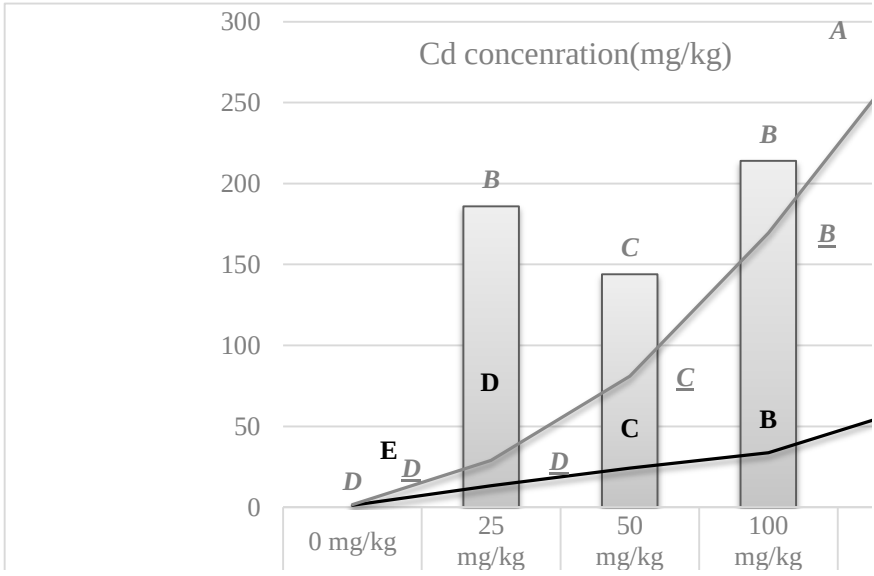


Figure 2: The Effect of the Application of Cd on the Cd Concentrations of the Flower, Stem, and Root of Cubana Kordes Rose
(Letters on the bar plots indicate comparison of mean values according to Tukey test; $p < 0.05$)

It was found that Pb, Ni, Cd and Zn accumulations on leaf of *Rosa gallica* roses were significantly higher than the roots (Esringu et al., 2015). In the study conducted to measure the phytoremediation capacity with woody plant species, all plants has shown high bioconcentration factor for metals (Fe > Cr > Mn > Ni > Cd > Pb > Zn

> Cu) (Shukla et al., 2011). Also in the another study with ornamental plants, it has been stated that *Dendranthema morifolium* species has the highest Ni, Cu, As, Pb, Cd and Cr concentrations compared to the others (Dai et al., 2006).

Table 2: The Effects of Cd and Leonardite (Leo) Applications on the Average Stem Diameter(SD), Number Of Flower Buds (FB), Flower Diameter(FD), Flower Color (FC), the Mean of Flower Stem Length (FSW), Number of Flower (FN), Flower Bud Diameter (FBD) and Flower Bud Length (FBL) of Cubana Kordes Rose

Leo (%)	Cd (mg kg^{-1})	SD (cm)	FB (pcs/ plant)	FD (mm)	FC	FSW (cm)	FN (pcs/ plant)	FBD (mm)	FBL (mm)
0	0	0.72 ± 0.13	3.75 ± 1.26	54.75 ± 3.53	3.58 ± 0.50	22.13 ± 4.46	7.75 ± 4.50	9.11 ± 0.74	20.94 ± 1.01abc
	25	0.61 ± 0.03	3.50 ± 2.08	53.50 ± 1.67	3.27 ± 0.36	23.28 ± 3.72	8.00 ± 2.83	9.30 ± 1.81	22.07 ± 3.41ab
	50	0.60 ± 0.09	3.75 ± 2.75	52.75 ± 4.17	3.00 ± 0.00	21.75 ± 2.26	10.25 ± 3.59	9.47 ± 1.25	22.03 ± 2.11ab
	100	0.66 ± 0.07	3.25 ± 1.26	53.44 ± 1.85	3.63 ± 0.94	20.13 ± 5.09	6.00 ± 2.00	8.75 ± 0.94	19.61 ± 0.63a-d
	200	0.55 ± 0.04	3.25 ± 0.96	50.25 ± 1.34	3.26 ± 0.65	24.31 ± 1.71	8.50 ± 4.79	9.93 ± 1.09	20.41 ± 1.56a-d
	Mean	0.63	3.50 AB	52.94	3.35	22.32 B	8.10	9.31 A	21.01 A
3	0	0.53 ± 0.17	2.25 ± 1.50	53.56 ± 2.59	2.94 ± 0.66	23.94 ± 2.72	7.25 ± 3.30	9.84 ± 1.37	20.46 ± 1.91a-d
	25	0.57 ± 0.15	4.25 ± 3.20	50.38 ± 2.33	2.83 ± 1.13	28.47 ± 4.25	7.50 ± 1.92	8.19 ± 1.97	15.49 ± 4.17cd
	50	0.58 ± 0.06	2.75 ± 1.71	50.13 ± 1.97	3.38 ± 0.48	25.28 ± 2.42	7.00 ± 3.16	8.09 ± 1.20	16.66 ± 0.10bcd
	100	0.58 ± 0.18	2.75 ± 1.26	54.94 ± 1.55	3.25 ± 0.50	27.63 ± 2.05	6.25 ± 3.86	7.63 ± 1.29	17.25 ± 1.75a-d
	200	0.51 ± 0.03	3.25 ± 1.26	51.00 ± 0.20	2.43 ± 0.15	23.24 ± 1.66	6.25 ± 2.75	8.18 ± 0.46	15.31 ± 2.76d
	Mean	0.56	3.05 B	52.00	2.96	25.71 A	6.85	8.38 B	17.03 B
6	0	0.57 ± 0.13	5.00 ± 2.16	54.13 ± 0.83	2.71 ± 0.51	27.56 ± 3.18	8.00 ± 3.65	8.42 ± 0.88	18.36 ± 1.71a-d
	25	0.59 ± 0.02	6.75 ± 4.35	49.69 ± 2.29	3.31 ± 1.14	27.19 ± 4.58	12.50 ± 6.76	9.15 ± 0.48	20.28 ± 1.95a-d
	50	0.55 ± 0.04	6.00 ± 2.82	46.69 ± 3.02	3.71 ± 1.06	22.75 ± 0.74	9.00 ± 3.56	10.27 ± 1.36	22.61 ± 1.59a
	100	0.58 ± 0.16	4.00 ± 1.41	53.81 ± 1.97	3.93 ± 0.56	25.00 ± 3.48	9.00 ± 2.45	8.94 ± 0.56	19.36 ± 1.22a-d
	200	0.60 ± 0.15	3.50 ± 1.92	48.88 ± 7.20	2.71 ± 0.58	23.88 ± 1.19	7.75 ± 4.27	8.71 ± 0.68	20.55 ± 2.97a-d
	Mean	0.58	5.05 A	50.64	3.27	25.27 A	9.25	9.09 AB	20.23 A
Means	**0**	0.61	3.67	54.15 A	3.08	4.54	7.66	9.12	19.92
	25	0.59	4.83	51.19 AB	3.14	26.31	9.33	8.88	19.28
	50	0.58	4.17	49.85 B	3.36	23.26	8.75	9.28	20.43
	100	0.61	3.33	54.06 A	3.60	24.25	7.08	8.44	18.74
	200	0.56	3.33	50.04 B	2.79	23.81	7.50	8.94	18.75

(The values indicated with lower case letters showed the comparison of Cd applications together and the values indicated with capital letters showed the comparison of leonardite applications, according to the Tukey test, $p<0.05$. Statistical analysis results are insignificant in non-letters)

Table 3: The Effects of Cd and Leonardite (Leo) Applications on the Cd Concentration of Flower, Root and Stem, on Residual Cd Concentration in the Soil, TF and BCF Values of Cubana Kordes Rose

Leo (%)	Cd (mg kg^{-1})	Flower Cd (mg kg^{-1})	Stem Cd (mg kg^{-1})	Root Cd (mg kg^{-1})	Soil Cd (mg kg^{-1})	TF	BCF
0	**0**	0.25±0.08 g	0.77±0.09 f	0.91±0.22 f	0.3±0.25	1.18±0.43 abc	8.63±7.4 g
	25	0.63±0.22 g	6.65±0.79 ef	17.53±4.10 f	15.8±5.6	0.43±0.07 cd	8.51±0.9 g
	50	0.69 ±0.04 g	14.99±1.48 def	25.43±0.99 f	18.3±9.7	0.62±0.08 cd	17.51±2.4 efg
	100	1.69±0.35 cd	35.62±2.57 bc	134.33± 25.08 cd	44.0±20.2	0.28±0.05 d	40.69±2.0 bcd
	200	2.62±0.36 b	46.00±3.32 b	256.92±23.20 b	81.3±14.3	0.19±0.02 d	51.82±3.6 b
Mean		1.18 B	20.81 B	87.03 B	31.94	0.54	25.43 B
3	**0**	0.86±0.03 efg	1.49±0.39 f	2.27±0.24 f	0.2±0.1	1.06±0.28 abc	14.82±3.9 fg
	25	4.23±0.68 a	26.70±2.89 cd	22.27±4.18 f	13.3±3.4	1.41±0.19 ab	32.65±3.2 cdef
	50	2.22±0.27 bc	13.21±1.16 def	151.97± 23.06 c	19.1±3.5	0.10±0.02 d	23.71±1.9 defg
	100	2.50±0.28 b	34.35±2.68 bc	313.01± 12.06 b	43.5±11.2	0.12±0.01 d	44.40±4.6 bc
	200	4.65±0.14 a	115.04± 22.35 a	449.53± 98.39 a	76.8±16.1	0.27±0.04 d	125.74±23.8 a
Mean		2.89 A	38.16 A	187.81 A	30.6	0.59	48.26 A
6	**0**	0.24±0.13 g	1.61±0.39 f	1.46±0.62 f	0.2±0.1	1.57±1.00 a	9.49±5.12 g
	25	0.71±0.10 fg	6.75±1.47 ef	47.20±21.04 f	14.6±6.7	0.20±0.12 d	10.95±1.5 g
	50	1.40±0.16 def	44.26±2.59 b	65.53± 10.32 def	16.6±3.4	0.72±0.18 bcd	49.73±1.9 bc
	100	2.22±0.20 bc	31.21±5.74 bc	61.189± 2.34 ef	35.1±5.8	0.55±0.09 cd	35.21±5.9 bcde
	200	1.45±0.24 de	20.26±2.18 cde	128.77± 15.65 cde	63.9±35.9	0.17±0.03 d	24.56±1.3 defg
Mean		1.21 B	20.82 B	60.83 C	26.1	0.64	25.99 B

(*The values indicated with lower case letters in lettering show the comparison of leonardite and Cd applications together, and those with capital letters show the comparison of the average values of the applications according to the Tukey test; $p<0.05$. Statistical analysis results are insignificant in non-letters)*

3.3. Cd Content Taken from Soil by Plant Parts

Cd and leonardite applications and leonardite x Cd interaction significantly affected the content of Cd taken from the soil with flower, shoot and root parts of rose ($p<0.05$). Cd uptake increased with increasing doses of Cd. The 3% leonardite application increased the content of Cd taken from the soil by the flower, shoot and root compared to the control and 6% leonardite application. The highest Cd contents taken from the soil by the flower, shoot and root were in 3% leonardite-200 mg kg^{-1}Cd applications (values are 18.0-1534.6-15561.0 µg Cd kg^{-1}, respectively) (Table 4). Considering the average values, the maximum cadmium uptake with plant flowers, shoots and roots is at 200 mg kg^{-1} Cd application.

Among the plant parts, the highest content of Cd taken from soil was through the roots. This value was 59.2 µg kg^{-1} in the control application and 9485.8 µg kg^{-1} in the 200 mg Cd kg^{-1} application. These findings showed that Cd can cause accumulation, especially in roots without toxicity in plants up to 200 mg Cd kg^{-1} concentration the application of Cd in Cubana Kordes rose plant. Cd transport to the upper part of the plant is less. The results indicated that the Cd content in the rose, reveals that it differs according to other ornamental plant species (Liu et al., 2008; Shao et al., 2019; Lu et al., 2020). This shows that the metal accumulation in the plant is not only related to the metal concentrations in the growing medium (Mahar et al., 2016).

3.4. The Available Cd Content Remaining In The Soil

At the end of the trial, the available Cd contents remaining in the soil after being used by the plant showed a significant increase with increasing doses of Cd ($p<0.05$) (Figure 3), but it did not differ statistically with leonardite applications and leonardite x Cd interaction. The available Cd content values remaining in the soil was between 0.2 and 81.3 mg kg^{-1} (Table 3).

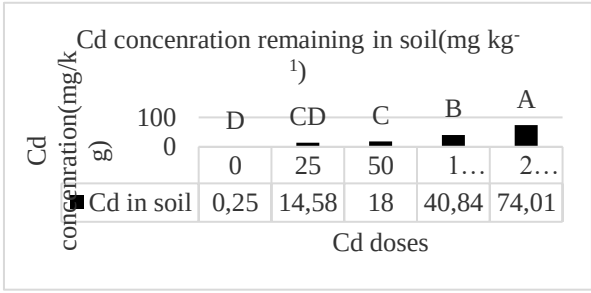


Figure 3: The Effect of Increasing Doses of Cd Application on Cubana Kordes Rose on Remaining Cd Concentrations in the Soil at the End of the Trial *(Letterings show comparison of mean values according to Tukey test; $p<0.05$)*

3.5. Transfer Factor (TF)

The TF parameter is used to determine the transfer capacity of the metals in the soil to the above-ground parts of the plant (Lubben & Sauerbeck, 1991). The effect of Cd application and leonardite x Cd interaction on TF was found to be significantly ($p<0.05$), while leonardite applications did not change the TF value (Table 3). The highest TF value is 1.57 at 6% leonardite-0 mg kg^{-1} Cd dose.

Considering the average values, the TF value decreased significantly with increasing doses of Cd (Figure 4). The TF of *Panax notoginseng*, *Chlorophytum comosum* and *Calendula ofcinalis* ornamental plants were found to be 0.02-0.19, 0.05-0.32 and 0.03-0.27, respectively (Shao et al., 2019). The TF obtained from our study are higher than these plants.

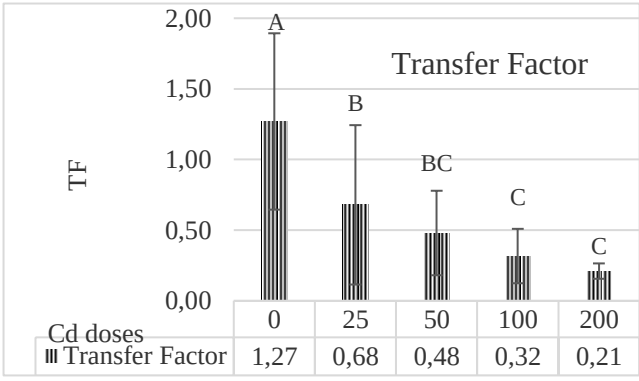


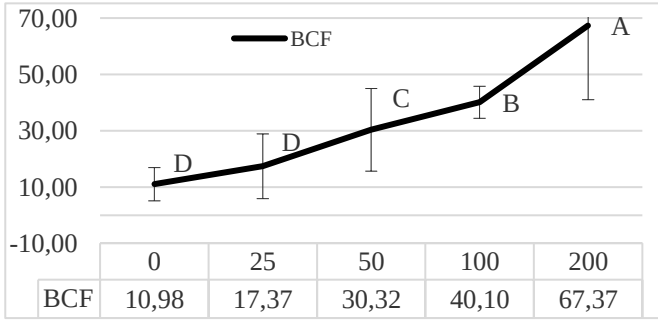
Figure 4: The Effect of Cd Applications on Transfer Factor of Cubana Kordes Rose *(The lettering shows the comparison of the mean values of the applications according to the Tukey test; p<0.05)*

3.6. Bioaccumulation Factor (BCF)

The BCF, which calculated by proportioning the total plant metal content to the soil metal content, showed significantly differences ($p<0.05$) with the applications of Cd and leonardite, and leonardite x Cd interaction (Table 3 and Figure 5). BCF values ranged from 8.51 to 125.74. The highest BCF value was found with 125.74 in 3% Leo-200 mg kg^{-1} Cd application. BCF increased with increasing Cd doses,

and higher values were obtained in 3% leonardite application compared to control and 6% leonardite application.

Hyperaccumulatory plants have an extraordinary ability to accumulate (BCF> 1) and transfer (TF> 1) to heavy metal ions without showing toxic symptoms (Rascio & Navari-Izzo, 2011). It was determined that TF and BCF values increased up to 200 mg kg^{-1} Cd dose in *Catharanthus longifolius* ornamental plant (Yasin et al., 2019). Considering these values, it can be said that Cubana Kordes rose is a candidate to be a hyperaccumulator for Cd.



	0	25	50	100	200
BCF	10,98	17,37	30,32	40,10	67,37

Figure 5: The Effect of Cd Applications on BCF Value of Cubana Kordes Rose
(The letters show the comparison of the mean values of the applications according to the Tukey test; p<0.05)

Table 4: The Effects of Cd and Leonardite (Leo) Applications on Cubana Kordes Rose on the Amount of Cd Removed by Flowers, Roots and Stems (*The values indicated with lower case letters show the comparison of leonardite and Cd applications together, those with capital letters show the comparison of the average values of the applications according to the Tukey test; p<0,05)*

Leo (%)	**Cd doses (mg kg^{-1})**					
	The amount of Cd removed by flowers (µg kg^{-1})					**Means**
	0	**25**	**50**	**100**	**200**	
0	0.7 e	2.5 e	1.6 e	5.1 cde	7.6 bcd	3.5 B
3	3.3 de	12.2 b	7.5 cd	8.3 bc	18.0 a	9.9 A
6	0.6 e	2.0 e	5.2 cde	5.1 cde	2.5 e	3.1 B
Mean	1.5 C	5.6 B	4.7 B	6.2 B	9.4 A	
	The amount of Cd removed by stems (µg kg^{-1})					
0	8.5 e	97.2 cde	220.2 b-e	504.0 bc	607.2 b	287.4 B
3	29.4 de	512.9 bc	211.2 b-e	456.5 bcd	1534.6 a	548.9 A
6	30.0 de	125.2 cde	521.4 bc	570.7 b	318.1 b-e	313.1 B
Mean	22.6 D	245.1 C	317.6 BC	510.4 B	820.0 A	
	The amount of Cd removed by roots (µg kg^{-1})					
0	31.4 g	623.2 fg	903.8 fg	5403.1 cde	8526.8 bc	3097.7 B
3	99.4 g	967.6 fg	5823.2 cd	10906.7 b	15561.0 a	6671.6 A
6	46.9 g	1568.6 efg	2245.8 d-g	2373.8 d-g	4369.7 def	2120.9 B
Mean	59.2 D	1053.1 D	2990.9 C	6227.9 B	9485.8 A	

4. DISCUSSION

According to the results obtained from the present study, the development and growth parameters of rose were not adversely affected by Cd up to 200 mg kg^{-1} Cd dose. According to the variance analysis results, the dry weights of flower, shoot and root did not differ with increasing doses of Cd. The highest averages values of Cd content of flower, shoot and root were observed in 3% leonardite - 200 mg Cd kg^{-1} application (4.65-115.04-449.53 mg kg^{-1}, respectively).

In a similar study, it was reported that *Tagetes erecta* L. and *Tagetes patula* L. ornamental plants strongly tolerate 100 mg kg^{-1} Cd concentration, and Cd accumulation was 166.07 and 231.72 µg kg^{-1} (Liu et al., 2018). The Cd content of the flower, shoot and root parts of the rose and the Cd content taken from the soil increased significantly with increasing doses of Cd ($p<0.05$). The maximum Cd contents were observed in 3% leonardite-200 mg kg^{-1}Cd applications. The available Cd concentrations remaining in the soil after being used by the plant also increased with rising doses of Cd ($p<0.05$) and was 74.01 mg kg^{-1} at a dose of 200 mg kg^{-1} Cd. Cd uptake by the plant is quite high.

Plants grown in soils polluted with heavy metals have to develop various adaptation strategies including chelation, metal uptake, transport, storage, biochemical detoxification and tolerance

mechanism (van Der Ent et al., 2013). The data obtained from this study showed that Cubana Kordes rose has the ability to accumulate Cd. At high Cd concentration (200 mg kg^{-1}), Cd accumulated mainly in the roots (81.5%) and with lower concentrations in shoots and flowers. In the definitions made about the hyperaccumulator properties of the plants, the heavy metal concentration of the above-ground parts was mostly based on. The plants with a shoot concentration of 0.01% (w/w) for Cd were considered as hyperaccumulators (Baker et al., 2000). Accordingly, Cubana Kordes rose can also be considered as a plant with an effective phytoextraction ability. These results are similar to the findings of various researchers (Baker & Brooks, 1989; Turan & Ercisli, 2007; Calzoni et al., 2007).

TF has been used in various studies to evaluate the plant's ability to transport from root to shoot, to tolerate heavy metals such as Pb, Cd (Liu et al., 2018; Shao et al., 2019; Lu et al., 2020). The results obtained in this study, the average TF value for 200 mg kg^{-1} Cd application is 0.21 and is lower than >1.

The bioconcentration factor (Saraswat & Rai, 2018), which is used as a measure of the potential metal accumulation of plants, was much higher than >1 in our study. BCF values vary between 8.51-125.74. Considering that the BCF average values, it is 67.37 for 200 mg kg^{-1} Cd application in our study. According to these results, Cubana

Kordes shrub roses have a high tolerance ability to Cd and also Cd can be transported effectively in the plant.

In case of exposure of *Abelmoschus manihot* ornamental plant to 100 mg Cd kg^{-1}, the plant growing increased without showing signs of damage. The BCF values of the plant exceeded the reference value of 1.0 in all Cd treatments and it has been reported that *A. manihot* can be considered as a Cd-hyperaccumulator (Wu et al., 2018b). The seed germination, root and stem growth of sunflower (*Helianthus annuus*), which is used as an ornamental plant, were significantly affected from heavy metal concentrations such as Cd, Cu, Ni, Pb and Zn at higher than 40 and 50 mg kg^{-1} (Jadia & Fulekar, 2008).

Cd contents of the three *Tagetes erecta* L. cultivars (Mann im Mond, Hawaii and Titania) growing in soil contaminated with Cd and Pb were detected in leaf>stalk>flowers, respectively. *Tagetes erecta* cultivars were reported to be a cultivar that can be used for the reclamation of soils polluted by heavy metals (Bosiacki, 2009). As it is known, leonardite has a metal adsorption capacity. However, if the humic acid contained in leonardite is applied to the soil in high doses, bioavailable metals can shift to less bioavailable stable phases (Stewart & Janin, 2014).

In this study, the highest Cd content taken from the soil by root, shoot and flower considering the mean values was in the 3% Leo application compared to the control and 6% Leo application ($p<0.05$). It is

thought that, Cd may be retained in 6% application of leonardite and accordingly its Cd uptake by the plant is reduced. There was a significant increase in the number of flowering and total flowers per plant of *Arnica montana* L. with increasing doses of leonardite applied to the growing medium. In other study, the activity of enzymes that catalyze the mineralization of soil organic matter was positively affected (Sugier et al., 2013). It was stated by Park et al. (2012) that humic acid increased the accumulation of Pb, Cu, Cd and Ni in the shoots and roots of herbaceous plants. The bioconcentration factor (BCF) in the shoot of *Festuca arundinacea* increased from 0.30 to 1.10, and the BCF increase rate was 264.7% in shoots.

CONCLUSION

In the process of removing metal and organic chemical pollutants that cause pollution in soils and waters with plants, by choosing ornamental plants that are not included in the food chain, will both provide a beautiful visuality and ensure the removal of pollutants without harming humans and animals. In this study, the effectiveness of Cubana Kordes shrub roses, which are ornamental plants, was investigated in terms of their usability in phytoremediation studies due to their aesthetic appearance and abundant blooming. According to the results obtained, Cd did not adversely affect plant growth and development. Cd accumulation was particularly high in the root compared to the stem and flower. The highest amount of Cd removed by the root is at 3% leonardite and 200 mg Cd kg^{-1} application dose.

BCF values are also quite high. According to these results, Cubana Kordes shrub roses can tolerate Cd at a high rate. It has been determined that this plant has the potential to be used in soils with Cd pollution and can be a candidate hyperaccumulator plant.

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Authors' contributions

AA wrote the original draft of the manuscript. HA and AA conducted the greenhouse trial and laboratory studies of the article together.

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CHAPTER 6

EFFECTS OF SEAWEED AND DIFFERENT MOLYBDENUM APPLICATIONS ON GERMINATION AND SEEDLING GROWTH PARAMETERS IN TOMATO (*Solanum lycopersicum*)

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1. INTRODUCTION

The quality of the seed, which is the starting material, is very important for the yield and quality obtained per unit area. Achieving high yield and quality is related to the quality of the seed. In the cultivation of cultivated vegetables, seed health, short-term germination and good performance are associated with seed quality (Sivritepe et al., 2015). However, seeds sown in the first stage of vegetable cultivation may encounter germination problems due to technical, ecological and poor seed quality. This situation causes serious losses in yield and quality. It is very important to ensure homogeneous and rapid germination of seeds in species with small embryos, such as pepper, where germination may be difficult and irregular in cultivation carried out in unsuitable conditions. For this reason, pre-sowing applications to increase seed performance are increasing day by day (Ashraf & Foolad, 2005; Sivritepe et al., 2015). Keeping seeds in osmotic solutions before sowing is one of the applications to increase seed strength and vitality. The basis of this technique is called priming; It is based on the ability of seeds to be kept for a long time without germination by reaching high humidity in solutions with adjusted osmotic potential. Physiological improvement of seeds is provided by priming technique. Many water-soluble organic and inorganic substances are used in priming applications. Some of these substances are NaCl, KNO_3 and $Ca(NO_3)_2$, polyethylene glycol, mannitol, abscisic acid (Sivritepe, 1999), salicylic acid, zinc, iron, molybdenum and similar chemicals.

Molybdenum (Mo), one of the chemicals used in priming applications, is a very important and essential micronutrient for plants, animals and bacteria (Rana et al., 2020a; Ismael et al., 2018). Many soils in the world are damaged by the deficiency of microelements such as Mo (Rana et al., 2020b; Imran et al., 2019). Therefore, Mo deficiency in soil is a comprehensive agricultural problem that causes quality and yield losses in various crop types worldwide (Liu et al., 2000; Liu, 2001). The availability of molybdenum for plant growth is strongly dependent on soil pH, concentrations of adsorbed oxides (such as Fe oxides), water drainage, and organic compounds found in soil colloids. In alkaline soils, molybdenum becomes more soluble and accessible to plants, mainly in the form of anions such as MoO_4. In contrast, in acidic soils, molybdenum availability decreases as the adsorption of anions to soil oxides increases (Reddy et al., 1997). Mo is usually found in a highly soluble form and is rarely prone to leaching in the soils that compose it. Although molybdenum is generally thought to be bound to the mineral surface in acidic soils, it can prevent seepage, but it can also inhibit the uptake of microbes. It is usually found in most agricultural soils with Mo content between 0.6 and 3.5 ppm, with an average Mo and 0.2 ppm, with an average available molybdenum of about 2.0 ppm. As an oxycomplex (MoO_4^{-2}) Mo occurs largely in soil. Therefore, in its behavior in soil, Mo is more like phosphate or sulfate. Molybdate is adsorbed on these two anions by soil minerals and colloids. This adsorption is very closely dependent on soil pH (Xu et al., 2006; Vistoso et al., 2012; Murtaza et al., 2013; Shaaban et al., 2018). It increases as the pH decreases, but is very low in neutrality. The

availability of Mo for plants is weakest for plants in acid soils and can be increased by foliar application, seed preparation, seed coat pelleting and liming if the soil is not characteristically molybdenum deficient (Mengel & Kirkby, 2001). When plants are grown under molybdenum deficiency, they develop various phenotypes that inhibit plant growth. Many of these phenotypes are associated with reduced activity of molybdenum enzymes. These enzymes include primary nitrogen assimilation enzymes such as nitrate reductase (NR) and the nitrogen-fixing enzyme nitrogenase found in bacteroids of legume nodules (Mendel & Hänsch, 2002; Williams & Frausto da Silva, 2002; Rana et al., 2020c). Mo-deficient plants show poor growth (Rana et al., 2020d) and less chlorophyll and ascorbic acid content (Liu, 2002). Soluble MoO_4 can form ionic complexes with various ions such as Na, K, Ca and Mg in solution, as well as complexes with organic substances, especially humic and fulvic acids (Jenne, 1977; Kaiser et al., 2005). Molybdenum deficiencies are quite prominent in the Brassicaceae family. Visual effects on young plants include mottling, pitting of leaves, gray discoloration and drooping leaves often found on seedlings that remain dwarf until death (Hewitt & Bolle-Jones, 1952). Deficiency symptoms may also be masked by the indirect effects of molybdenum on nitrogen assimilation enzymes. Many horticultural, cereal and leguminous crops grown with insufficient molybdenum levels in the presence of nitrate fertilizers develop pale green leaves with necrotic areas at the leaf margins, and overall plant growth reduction is also seen (Hewitt & Bolle-Jones, 1952; Agarwala et al., 1978; Chatterjee et al., 1985; Chatterjee & Nautiyal, 2001; Kaiser et al., 2005). In the studies,

molybdenum deficiency causes necrotic areas on leaf blades and underdeveloped and wrinkled seed formation in oat and wheat (Anderson, 1956; Chatterjee & Nautiyal, 2001), while in maize it causes lack of node, reduced leaf area and chlorosis in leaves (Agarwala et al., 1978). Molybdenum deficiency in reproductive tissues in maize can alter phenotypes in developing flowers, including delayed emergence of tassels, small anthers, poorly developed stamens, and reduced pollen grain development (Agarwala et al., 1979; Kaiser et al., 2005). Pollen released from anthers has been shown to be shriveled and germination rates are low (Agarwala et al., 1978, 1979; Kaiser et al., 2005). Because molybdenum is involved in a number of different enzymatic processes, a defined plant response to molybdenum deficiency can be complex and therefore difficult to causally assign to specific enzyme systems. This is particularly evident in molybdoenzymes involved in nitrogen metabolism, where general reductions in plant growth and health can alter plant growth, susceptibility to pest damage, and fruit or grain development (Graham & Stangoulis, 2005; Rana et al., 2020d). It has been reported that in spinach plants grown under conditions of molybdenum deficiency, leaf NR activity is reduced and total final plant yield is lower than control plants grown at adequate molybdenum levels (Witt & Jungk, 1977). In many studies, seed coating, processing and pelleting with molybdenum have shown the effectiveness of Mo seed coating. It was determined that the application of Mo (80 g/ha) to the seeds had a positive effect on the grain yield, chlorophyll index, seed weight and number of pods. It shows that pelleting soybean seeds with 250 mg/kg ammonium molybdate is highly effective for improving dry

matter production, yield, plant height, growth rate and leaf area index (Ramesh & Thirumurugan, 2001; Biscaro et al., 2009). For increasing soybean and cowpea yields in acidic soils, the benefits of applying Mo with or without rock phosphate were greater or comparable to liming evaluating the effectiveness of a range of pelleting materials (Rhodes & Nangju, 1979). However, no toxicity or improvement was reported from the Mo seed coat.

However, in recent years, human and environmental health has been put at risk due to incorrect chemical applications for high efficiency. This situation ensures that environmentally friendly production methods that protect the ecological balance as well as seedling yield and quality gain importance today. New breeding systems that eliminate or minimize the yield and quality loss experienced in aquaculture with environmentally friendly production techniques are being researched. In this context, the technique used to increase the quality and performance of seedlings in solutions prepared with organic preparations is called "organic priming". Seaweed (*Ascophyllum nodosum*) extract, which is used as a priming agent in organic priming applications, is one of the organic preparations (Sivritepe et al., 2015). In the content of seaweed, macro elements (N, P, K, Ca, Mg and S) and micro elements (Mn, Fe, Cu and Zn) (Senn, 1987), growth regulators such as cytokinin, auxins and abscisic acid (Tarakhovskaya et al., 2007), amino acids (betaines) (Mackinnon et al., 2010), vitamins such as B12, vitamin E, vitamin K, sugars (mannitol and alginic acid), proteins, oils, phenols and antibiotics are known to contribute to the growth and development of

the plant (Craigie, 2011). It has been reported that seaweed extracts increase the respiratory activity of seeds due to the cytokinins and betaines they contain and their hygroscopic properties, and increase the germination rate and rate thanks to the metabolism it accelerates (Senn, 1987). In priming studies with seaweed, it has been reported that seaweed increases the germination power of tomato, pea, turnip and radish seeds. (Sivritepe, 2000; Demir et al., 2006; Demirkaya, 2010; Sivritepe et al., 2015). It is stated that as a result of priming applications made with seaweed extracts in pea, turnip and radish seeds, increases in germination power occur (Demir et al., 2006). It has been reported that the effects of seaweed (*Ascophyllum nodosum*) extracts on seed viability and strength were investigated in pepper (Sivritepe et al., 2015), tomato (Demirkaya, 2012), pepper and onion (Demirkaya, 2010) seeds.

Tomato (*Solanum lycopersicum* L.) is one of the most important cultivated vegetables in the world. The cultivation of tomatoes is by direct seed sowing or by planting the seedlings in the fields. Environmental stress conditions limit direct seed sowing. Many commercial tomato cultivars are susceptible to environmental stress conditions during the germination and seedling stages (Maas, 1986; Foolad & Lin, 1997). Depending on the stress intensity and duration, the water potential of the soil, and the genetic structure of the seed, there are delays and obstacles in germination in unsuitable soils where direct seed sowing is made. It is known that this situation reduces the seedling emergence in the field and accordingly the number of plants, as a result

of which economic efficiency is at risk (Cuartero & Fernandez-Munoz, 1999; Foolad et al., 2007).

When we look at many studies, it was determined that molybdenum and algae applications were separately applied in seed pre-applications and there were no studies in which they were applied together. In the light of all these reasons, we think that the planning and execution of this study will lead to the future studies of using chemical substances and organic fertilizers together.

2. MATERIAL AND METHOD

This study was carried out in the laboratory of the Department of Horticulture at Siirt University. H2274 and Rio Grande tomato cultivars were used as plant material and these cultivars were purchased from Bursa Tohumculuk. These varieties are widely used in the market and commercially important varieties. The application included 3 different concentrations of molybdenum (25 ppm, 50 ppm and 75 ppm), algae (6 ml/lt), Mo concentrations (25 ppm, 50 ppm and 75 ppm) + algae (6 ml/lt) and control. Molybdenum concentrations were determined by making preliminary experiments. While determining the algae concentration, the dose recommended by the company was taken into account. Solutions according to the concentrations of Mo applications to germinate the seeds, algae solution for algae application, Mo concentrations+algae and control (tap water) applications were made.

The study was planned according to the randomized plot design with 3 replications and 25 seeds in each replication.

Filter paper was placed above and below the inside of the petri dishes used for germination of tomato seeds. Petri dishes are labeled according to the applications. After placing tomato seeds between filter papers, 3 different concentrations of molybdenum (25 ppm, 50 ppm and 75 ppm), algae (6ml/1lt), Mo concentrations (25 ppm, 50 ppm and 75 ppm)+algae (6ml/lt) and control The irrigation amount was determined as 5 ml according to the (tap water) applications. After irrigation, the petri dishes were placed in a growing cabinet set at 25±1ºC. Controls were made every day from the beginning to the end of the study and irrigation was done according to the application contents if needed. From the 1st day of the establishment of the experiment, germination controls were made and the germinated seeds were counted. Counting was done under the same conditions and at the same time each day. After counting the germinated seeds, they were kept in the same petri dish. The study was terminated on the 9 day when the maximum germination number was reached. After the counting process was completed, the percentages of the seeds were calculated. The germination criterion was taken as the condition that the radicula was clearly protruding from the testa (İşlek et al., 2010).

At the end of the study, germination parameters such as germination percentage (%), average germination time, germination uniformity,

germination speed parameter, seedling length, hypocotyl diameter, root length, fresh weight and dry weight parameters were evaluated.

2.1. Germination Percentage Parameter

The germinated seeds were counted at 24-hour intervals and the germination percentage was calculated.

The germination percentage was determined according to Scott et al. (1984). The germination percentage was calculated using the formula below.

$GP=((NGSN)(TSN)^{-1})*100$

GP: Germination percentage

NGSN: Number of germinated seeds normally

TSN: Total number of seeds

2.2. Average Germination Time Parameter

Average germination time is used to determine the germination day of seeds. The average germination time was determined based on the formula below (Ellis & Roberts, 1981).

$AGT= \sum(SiGi)(Gi)^{-1}$

Si: The number of seeds germinated on the Gi day.

Gi: Refers to the number of days since the beginning of germination.

2.3. Germination Uniformity

Germination uniformity was calculated according to the method determined by Bewley & Black (1994).

$GU= \sum s \sum [(AGT-g)^2 s]^{-1}$

g: The time in days starting from day 0, which is the day the seed is planted.
s: is the number of seeds that complete germination on day g.

2.4. Germination Speed Parameter

Germination rate was determined according to the method determined by Abazarian et al. (2011). The germination rate was calculated according to the formula below.

$GSP= (N1D1^{-1})+(N2D2^{-1})+……..+(NnDn^{-1})$

N: The number of germinated seeds.
D: The number of days required for germination.

2.5. Measurements Made in Seedling

2.5.1. Seedling Height, Hypocotyl Diameter and Root Length Measurements

For observations, the height, diameter and root samples of the seedlings were scanned with a color scanner (Iscan Color Mini Portable Scanner) at 300 DPI resolution. Precise and detailed measurements of seedling

height, hypocotyl diameter and root length parameters were made using ImageJ software (Rueden et al., 2017; Özyazıcı & Açıkbaş, 2021).

2.5.2. Seedling Fresh and Dry Weight Measurements

The fresh and dry weights of the seedlings were determined with precision scales.

In order to determine the dry weight, the wet seedlings were dried in an oven at 65^{o}C for 36 hours.

2.6. Statistical Analysis

Statistical analysis of the research data was made using the JMP5.0.1 package program. Before analysis of variance, ArcSin transform was applied to the data expressed as percentage. The data obtained as a result of the research were analyzed based on the randomized plots experimental design, and the differences between the averages were checked with the TUKEY HSD multiple comparison test (Açıkgöz & Açıkgöz, 2001).

3. RESULTS AND DISCUSSION

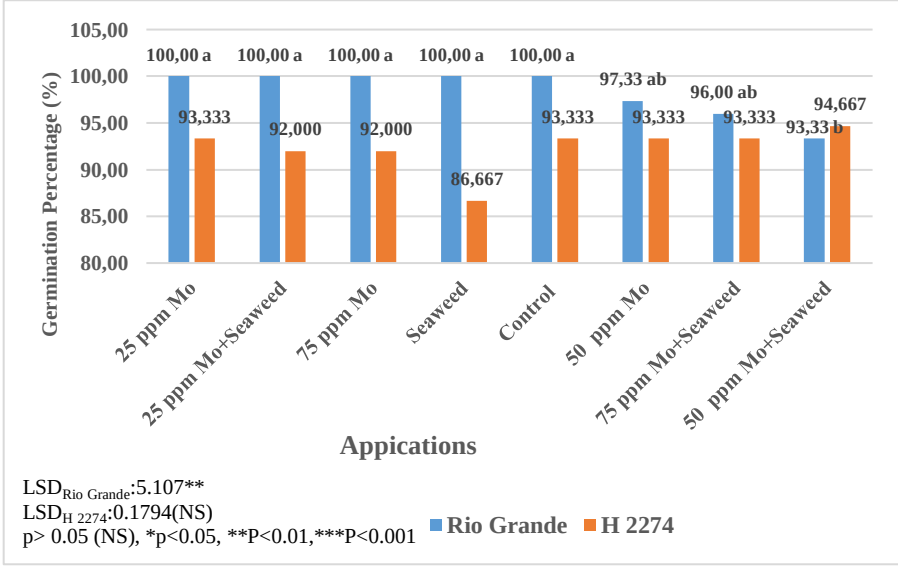
3.1. Seed Germination (%)

The effects of different doses of molybdenum and seaweed applications on seed germination of Rio Grande and H 2274 cultivars are presented

in Figure 1. When the two tomato cultivars were compared, the highest germination percentage was in Rio Grande cultivar and germination percentage was as 100% in control, seaweed, 25 ppm Mo and 25 ppm Mo+seaweed applications. The lowest germination percentage was in seaweed application with 86.667% in H 2274 variety. It was determined that the germination percentage in the Rio Grande cultivar with 50 ppm Mo and 75 ppm Mo application was higher than 50 ppm Mo + seaweed and 75 ppm Mo + seaweed application, that is, seaweed application decreased the germination percentage in the presence of molybdenum. In H 2274 cultivar, the highest germination percentage was 94.667 and 50 ppm Mo+seaweed application. It was determined that 50 ppm Mo+seaweed application in H 2274 cultivar was effective in increasing the germination percentage. It was stated that the pre-application of ammonium molybdate (0.1%) increased the germination percentage and 0.2% ammonium molybdate treatment as a preliminary application had no effect on the germination percentage (Majda et al., 2019). It has been reported that the application of seaweed extract to leek seeds significantly increased the seed germination rate and speed in salty conditions compared to the control, and reduced the negative effects of salinity (Yıldırım & Güvenç, 2005). It has been reported that osmotic conditioning with 1:500 and 1:1000 concentrations of seaweed extract and water treatments increase the seed germination rate in pepper (Sivritepe, 2000). It has been determined that the application of lettuce seeds with seaweed extract for 24 hours has a positive effect on seed germination at normal and high temperatures (Möller & Smith, 1998). It has been reported that seaweed extract is rich with cytokinins and

other growth promoting substances and has a positive effect on germination in many plant species (Wilczek & Ng, 1982). It has been reported that keeping the cauliflower seeds in water at 10, 20 and 30°C significantly increased the germination rate and speed compared to the control application (Fujikura et al., 1993).

Figure 1: Germination percentage values in Rio Grande and H 2274 Cultivars under Different Molybdenum Concentrations and Seaweed Applications

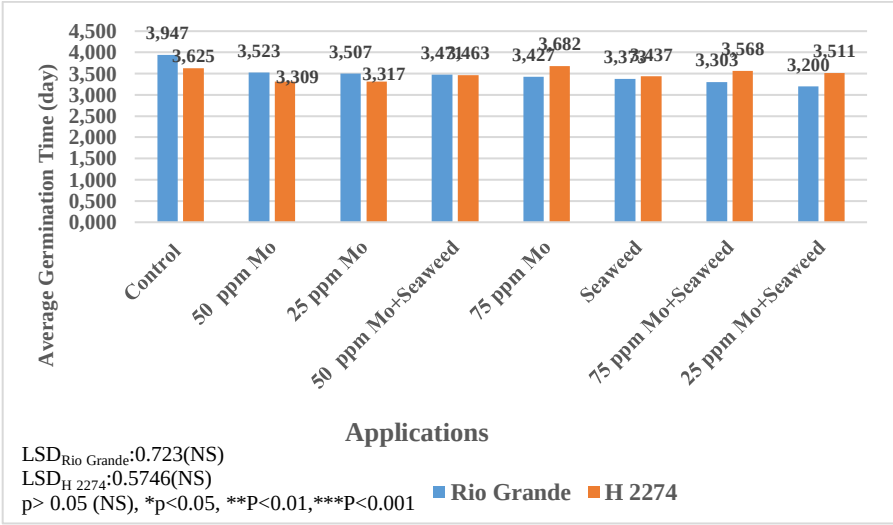


3.2. Average Germination Time (day)

The effects of different doses of molybdenum and seaweed applications on average germination time of Rio Grande and H 2274 cultivars are presented in Figure 2. It was determined that the longest average germination time was in the control application of Rio Grande variety, and the lowest in 25 ppm Mo+seaweed application. It was determined that seaweed application with molybdenum decreased the average

germination time in Rio Grande cultivar. In H 2274 cultivar, it was determined that, apart from 75 ppm molybdenum + seaweed application, 25 ppm molybdenum + seaweed and 50 ppm molybdenum + seaweed application were ineffective in reducing the average germination time. It has been reported that the germination rate of tomato seeds, which were applied controlled hydration under 100 mM NaCl salt stress, increased compared to the control application (Demir & Ermis, 2003). Controlled hydration application and osmotic conditioning with KNO_3 showed that 18.4 dS m^{-1} salt stress in sunflower seeds increased germination and emergence rates and decreased germination times compared to control (Kaya et al., 2006). It has been reported that osmotic conditioning with NaCl with controlled hydration application in tomato (Demir & Ermis, 2003) and melon seeds (Sivritepe et al., 2003) increased germination and emergence rates and decreased germination times compared to control. The 25 and 50 ppm doses of molybdenum decreased the mean germination time compared to the control. Germination speed is significant for successful crop establishment (Finch-Savage & Bassel, 2016). The acceleration of germination at this stage is also associated with increased respiration, which contributes to rapid ATP production. The acceleration of germination is also associated with increased respiration, which contributes to rapid ATP production. This energy source then allows the mobilization of seed reserves and macromolecules, resulting in embryo elongation. Therefore, the increased germination rate is attributed to early mobilization of reserves due to activation of α and β amylases (Lee & Kim, 2000).

Figure 2: Average Germination Time in Rio Grande And H 2274 Cultivars Under Different Molybdenum Concentrations and Seaweed Applications



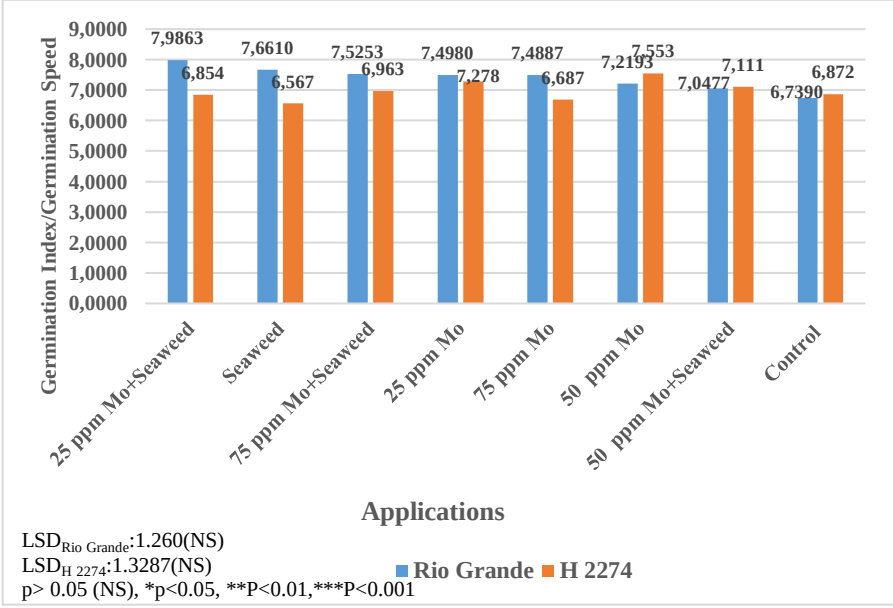
3.3. Germination Index/Germination Speed

The germination index/germination speed of tomato cultivars was shown in Figure 3. The lowest germination index was in the H 2274 variety in seaweed application. The highest germination index was determined in Rio Grande variety. Molybdenum doses increased the germination index in Rio Grande cultivar compared to the control. Seaweed application with molybdenum decreased germination index in H 2274 variety compared to molybdenum doses. It has been reported that the germination rate and speed of seeds of many vegetable species treated with seaweed extract before sowing were increased (Zodape, 2001). It was determined that Mo considerably reduced the viability of the cauliflower seed. Also, artificial seed treated with Mo exposed to -2°C showed the highest viability. Mo application increased the cold

tolerance of cauliflower micro shoots (Rihan et al., 2014). It has been reported that the negative effect of the highest molybdenum concentration on rapid germination can be explained by ionic toxicity with Mo ions and/or excess nitrogen. The toxic effect of Mo may also be related to increased abscisic acid (ABA) levels in seeds, which is responsible not only for drying and maturation of seeds, but also for inhibition of germination in dormant seeds. Mo is an aldehyde oxidase cofactor that catalyzes the final step of abscisic aldehyde oxidation to ABA (Mendel & Hänsch, 2002). Therefore, the highest dose of Mo presumably increased the ABA position to a sufficient dose that would delay but not inhibit germination (Majda et al., 2019). As a result of the study, in which cowpea seeds with low germination power exposed to high temperature and relative humidity were treated with ammonium molybdate (10-3M) and water (hydro-priming), pre-treatments were effective in shortening the germination time and average germination time by 50%, and germination in seeds with low germination power. It has been reported that the germination strength and germination percentage increase slightly in normal seeds, and it increases the electrical conductivity, cell membrane stability, total protein content, α-amylase activity, peroxidase activity and dehydrogenase activity of the seed leachate (Arun et al., 2017). It has been reported that leek seeds kept in water germinate more and faster than the control, and it is important to keep the seeds in seaweed extract or water for 24 hours in order to increase the seed germination rate and speed in leeks under both salt stress and normal conditions (Yıldırım & Güvenç, 2005). It has been reported that seed applications with plant growth promoting

substances and seaweed increase the germination rate and speed in stress conditions such as salinity and drought, and this increase may be due to plant hormones such as stokinin in its content (Zhang, 1997).

Figure 3: Germination Index/Germination Speed in Rio Grande and H 2274 Cultivars Under Different Molybdenum Concentrations and Seaweed Applications

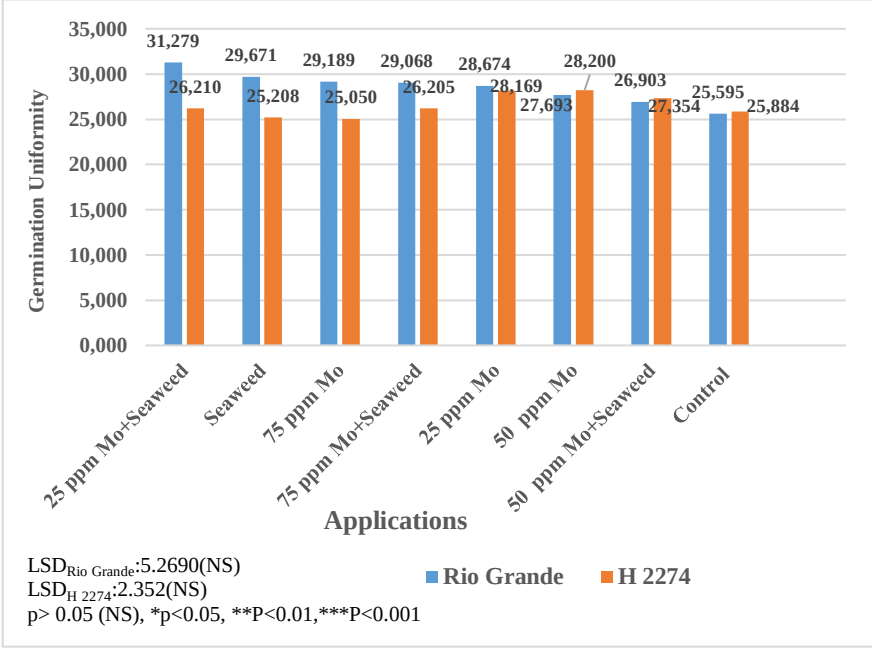


$LSD_{Rio\ Grande}$:1.260(NS)
$LSD_{H\ 2274}$:1.3287(NS)
p> 0.05 (NS), *p<0.05, **P<0.01,***P<0.001

3.4. Germination Uniformity

The germination uniformity of tomato cultivars was shown in Figure 4. The germination uniformity of Rio Grande cultivar increased in all other treatments compared to the control. Germination uniformity in seaweed application was higher than in control. Germination uniformity was increased in the H 2274 cultivar with 25 ppm molybdenum+seaweed, 50 ppm molybdenum+seaweed and 75 ppm molybdenum+seaweed applications compared to the control.

Figure 4: Germination Uniformity in Rio Grande and H 2274 Cultivars Under Different Molybdenum Concentrations and Seaweed Applications



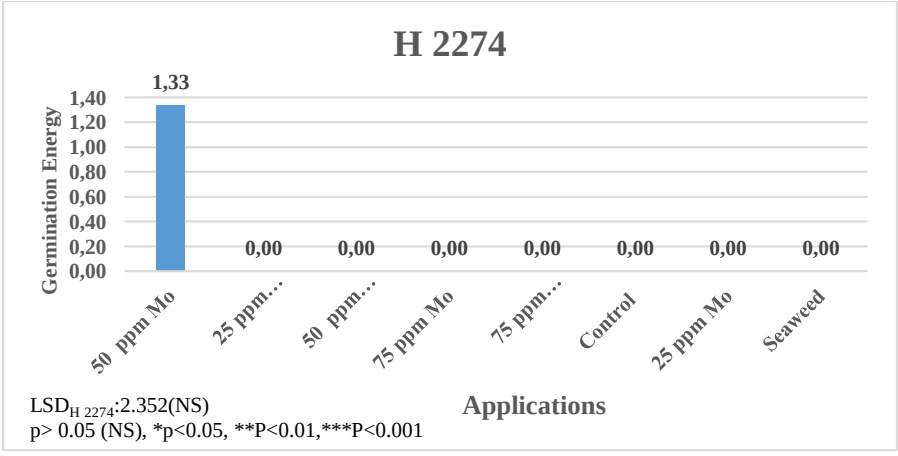
$LSD_{Rio\ Grande}$:5.2690(NS)
$LSD_{H\ 2274}$:2.352(NS)
p> 0.05 (NS), *p<0.05, **P<0.01,***P<0.001

It has been reported that seaweed extract concentrations, especially 1:250 and 1:500 applications, significantly increased both the germination rate and the germination rate index compared to other applications (Yıldırım & Güvenç, 2005). It has been reported that seaweed extracts contain the hormones stokinins, amino acids betaines and their hygroscopic properties cause physiological improvement in seeds and increase their performance (Sivritepe, 2000).

3.5. Germination Energy

Seed viability indicators are a key factor in determining the number of plants per hectare. Seed quality affects emergence rate and uniformity as well as initial plant growth rate (Mrđa et al., 2011). The germination energy of the H 2274 variety was shown in Figure 5. As a result of different preliminary applications, the germination energy was observed as 1.33 in only 50 ppm Mo application. It was reported that the germination energy used to determine the germination rate did not differ between genotypes at day 9 (Charity et al., 2015). It has been reported that in different (10, 30, 50, 70, 90) sulfuric acid concentration pre-treatments, the highest germination capacity (93.8%) was observed in the 90% sulfuric acid concentration application, and it had the highest germination energy 7, 10 and 12 days after sowing (Asiedu et al., 2012).

Figure 5: Germination Energy in H 2274 Cultivar under Different Molybdenum Concentrations and Seaweed Applications

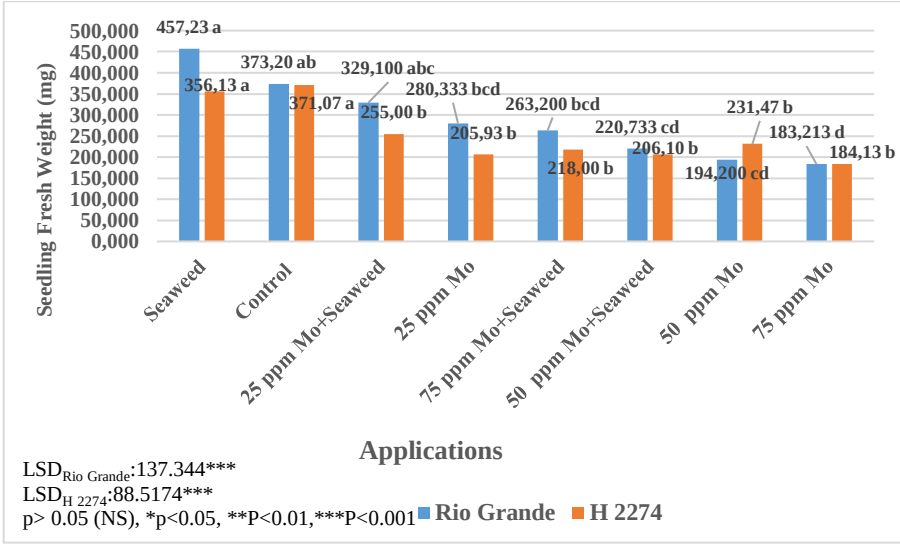


3.6. Seedling Fresh Weight (mg)

The seedling fresh weight of the cultivars was shown in Figure 6. In Rio Grande variety, the highest seedling fresh weight was in seaweed application and the lowest in 75 ppm Mo application. It was determined that seaweed application together with molybdenum increased the seedling fresh weight. The highest seedling fresh weight in the H 2274 tomato cultivar was in the control application, and the lowest in the 75 ppm Mo application. It has been reported that seaweed extract applied to bean seeds positively affects seed germination and root and shoot development in later periods (El-Sheekh & El-Saied, 2000). It was determined that the wet weight of the seedlings decreased at all doses of molybdenum and this decrease was prevented by the application of seaweed together with molybdenum, except for the application of 50 ppm Mo. In non-letral freezing, plantlet fresh increased in Mo application (Rihan et al., 2014). It has been stated that molybdenum pre-application, 0.1% Mo priming for 10 hours improves the net CO_2 assimilation rate, chlorophyll content and biological nitrogen fixation (Majda et al., 2019). Cowpea seeds were treated with ammonium molybdate at 15 °C for 24 hours and it was determined that pre-treatments increased plant height, trifoliate number of leaves, total dry matter, number of pods, number of seeds per pod, 1000 seed weight, grain yield and biological yield compared to control (Arun et al., 2020). It has been reported that different doses of molybdenum significantly increased plant fresh weight and root fresh weight. It has been reported

that plant fresh weight and root fresh weight increase with increasing molybdenum dose in tobacco (Chen et al., 2021).

Figure 6: Fresh Weight in Rio Grande and H 2274 Cultivars under Different Molybdenum Concentrations and Seaweed Applications

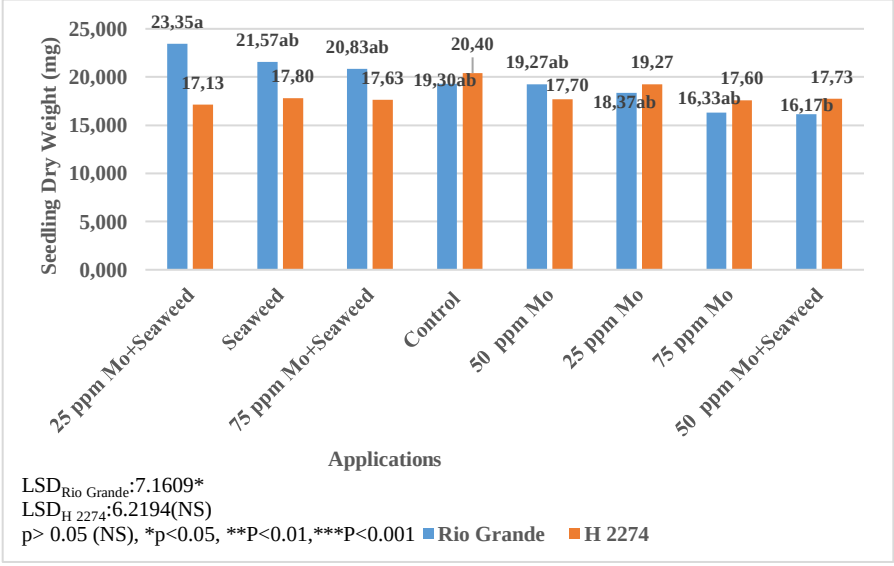


3.7. Seedling Dry Weight (mg)

The seedling dry weight of Rio Grande and H 2274 cultivars was shown in Figure 7. In Rio Grande cultivar, the highest seedling dry weight (23.35 mg) was in 25 ppm Mo+seaweed application, and the lowest (16.17 mg) was in 50 ppm Mo+seaweed application. Molybdenum application was effective in reducing the dry weight of the seedlings and reducing the negative effect of molybdenum on the seedling dry weight at the doses of 25 and 75 ppm Mo of seaweed. The seedling dry weight of the Rio Grande variety was higher in seaweed application compared to the control. In the H 2274 cultivar, the highest seedling dry

weight (20.40 mg) was in control application, the lowest (17.13 mg) was in 25 ppm Mo + seaweed application. The seedling dry weight of the H 2274 cultivar was determined to decrease in other applications compared to the control. Molybdenum application in hemp has been reported to reduce shoot dry weight in bean (Brandelero et al., 2020). It has been reported that the dry weight of the plant increases with the application of molybdenum in drought stress (Hayyawi et al., 2020). It was determined that mung beans grown in Mo field conditions increased plant dry matter (Brkić et al., 2004). It has been reported that chickpea root nodule dry weight increased as the molybdenum dose increased (Singh et al., 2014). It has been reported that storage dry matter increases in potato when Mn was applied (Malakouti & Tehrani, 1999). It was stated that rhizobia and sodium molybdate/100 g seed and 0.2, 0.4 and 0.8 g inoculation applications in peanut increased the dry matter yield (Haque & Amara, 1978). Rhodes & Kpaka (1982) reported that Mo application increased the dry matter, seed yield and pod weight of cowpea. It was stated that the yield was increased by 21% by pelleting the seed with nitro-molybdenum at a seed treatment rate of 0.4 g/100 g. It has been reported that different doses of molybdenum significantly increased plant dry weight and root fresh weight. Applying Mo by this method may be attractive to smallholder farmers because it is inexpensive, simple, less exposed to the variables of wind and rain, and does not require any spraying equipment. There are reports showing that Mo seed coating is effective in improving crop yield, and bacterial strains used for inoculation may have toxic effects (Chen et al., 2021).

Figure 7: Seedling Dry Weight in Rio Grande and H 2274 Cultivars under Different Molybdenum Concentrations and Seaweed Applications

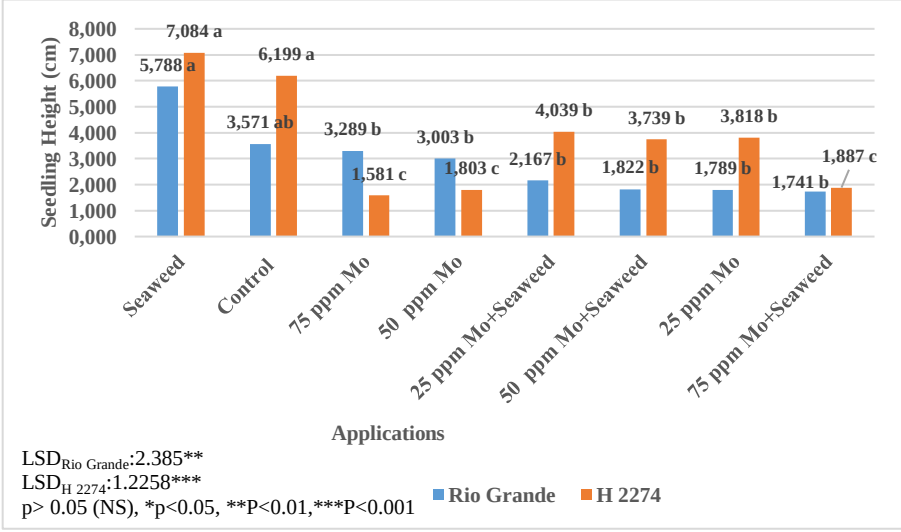


3.8. Seedling Height (cm)

The effects of seaweed and different doses of molybdenum applications on seedling height in Rio Grande and H 2274 cultivars are presented in Figure 8. The highest seedling length of Rio Grande and H 2274 cultivars was in seaweed application. Molybdenum application reduced the seedling height of Rio Grande cultivar. The application of seaweed + 25 ppm Mo increased the seedling height compared to the application of 25 ppm Mo. Seaweed application was determined effective in reducing the negative effect of molybdenum on seedling height in H 2274 cultivar. It was reported that Mo did not have a significant effect on plant height in drought stress (Hayyawi et al., 2020). Seed soaking and foliar application have been shown to increase pod length (Brkić et

al., 2004). Mo application has been reported to increase plant height under drought stress (Wu et al., 2014). It has been reported that Mo application ameliorates the negative effect of drought stress (Ghafarian et al., 2013). It has been reported that molybdenum 500 ppm dose, which was a pre-application in chickpea, increases plant height (Singh et al., 2014). Application of Mo has been reported to significantly increase the yield of Chinese cabbage in both control and salinity treatments. Application of Mo under salt stress increased fresh weight of Chinese Cabbage (Zhang et al., 2014). It has been reported that molybdenum application does not affect plant length and stem diameter in sunflower (Steiner & Zoz, 2015). In common bean, it was stated that the lowest stem height was 43.9 cm, with an average of 0.2% molybdenum dose, and the highest value (51.4 cm) was 0.05 molybdenum dose (Majda et al., 2019). Different concentration pre-treatments of Cu and Mn have been reported to significantly increase shoot length. It was reported that the maximum shoot length (2.96 cm) was obtained in the pre-application of Mn with 450 ppm, and the minimum shoot length (2.33 cm) in the control application (Mehra et al., 2022). It has been reported that different doses of molybdenum significantly increased stem length. It has been reported that plant length increased with increasing molybdenum dose in tobacco (Chen et al., 2021).

Figure 8: Seedling Height in Rio Grande and H 2274 Cultivars under Different Molybdenum Concentrations and Seaweed Applications



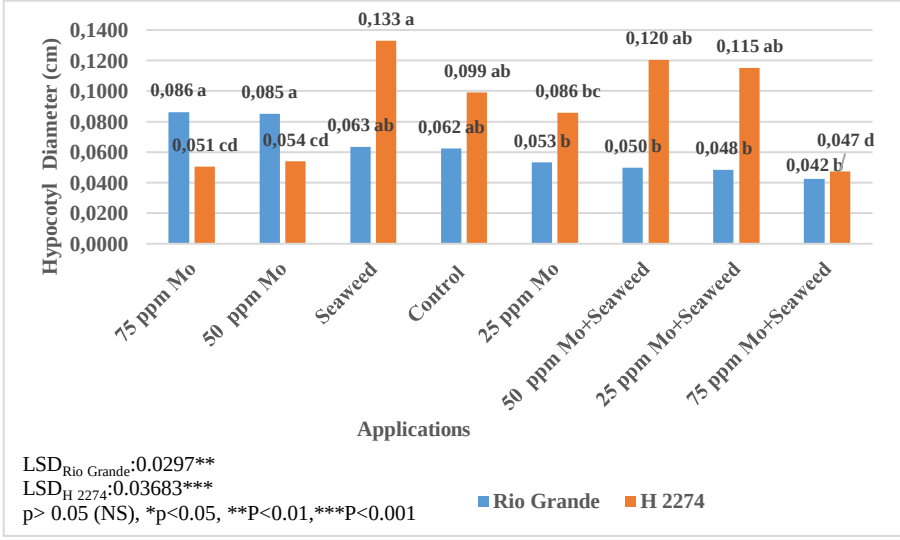
3.9. Hypocotyl Diameter (cm)

The effects of different doses of molybdenum and seaweed applications on hypocotyl diameter in Rio Grande and H 2274 cultivars are presented in Figure 9. The highest hypocotyl diameter (0.086 cm) of Rio Grande variety was in 75 ppm Mo application, the lowest (0.042 cm) was in 75 ppm Mo + seaweed application.

The application of seaweed in combination with molybdenum doses reduced the hypocotyl diameter. In the H 2274 cultivar, the highest hypocotyl diameter was in the seaweed application, and the lowest was in the 75 ppm Mo+seaweed application. Seaweed application with 25 and 50 ppm Mo doses increased the hypocotyl diameter compared to 25 and 50 ppm doses of molybdenum in H 2274 cultivar. In common

bean, it was stated that the pre-applications of 0, 0.025, 0.05, 0.1 and 0.2% ammonium heptamolybdate did not differ on the number of leaves, stem diameter, total fresh biomass and total dry weight (Majda et al., 2019).

Figure 9: Hypocotyl Diameter in Rio Grande and H 2274 Cultivars under Different Molybdenum Concentrations and Seaweed Applications

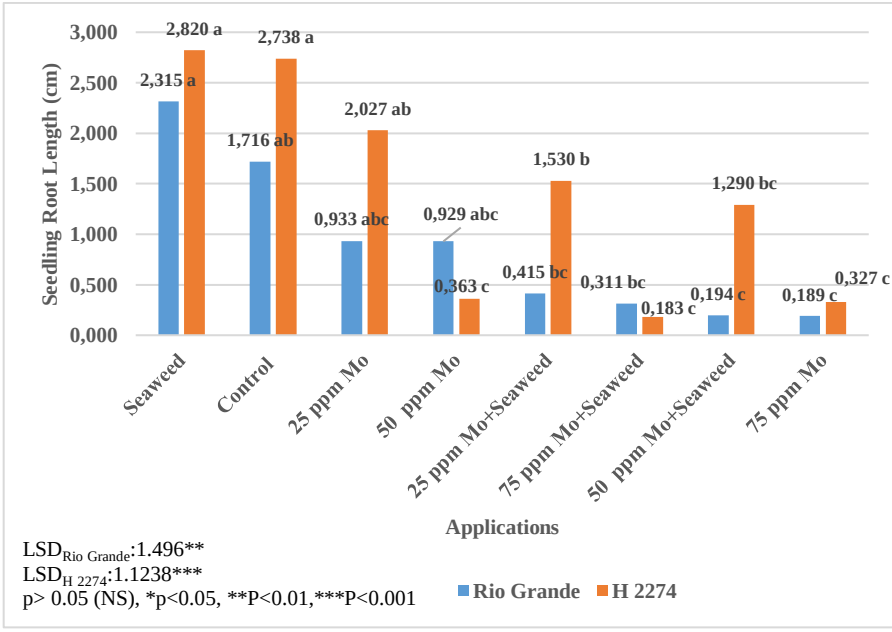


When the percent change in hypocotyl diameter compared to the control was evaluated, the highest percent change was determined as the decrease in the pre-application of 4 M salt with 80.64 in the variety H2274. In H2274, hypocotyl diameter decreased in all salt pretreatments except 2 M salt pretreatment (Dere, 2021). It has been reported that different doses of molybdenum significantly increased stem diameter in tobacco (Chen et al., 2021).

3.10. Seedling Root Length

The effects of seaweed and different doses of molybdenum applications of Rio Grande and H 2274 cultivars on seedling root length are shown in Figure 10. In the cultivar H 2274, the highest seedling root length was 2,820 cm in seaweed application, and the lowest seedling root length was 0.183 cm in 75 ppm Mo+seaweed application. The seedling root length of both cultivars decreased in molybdenum applications compared to the control. The highest seedling root lenght (2.315 cm) of Rio Grande cultivar was in seaweed application, the lowest (0.189 cm) was in 75 ppm Mo application.

Figure 10: Seedling Root Height in Rio Grande Cultivar under Different Molybdenum Concentrations and Seaweed Applications



The positive effect of seaweed application with molybdenum in Rio Grande variety was seen only at 75 ppm Mo dose. It was determined that seaweed application increased seedling root length in both cultivars compared to control. It has been reported that tomato seedling root length decreases under salt stress (Çavuşoğlu, 2012). Root length was an important criterion in salinity stress, as the root was the part in direct contact with the soil. Maximum root length (9.21 cm) was obtained with 450 ppm Mo application. The minimum root length was observed as 5.81 cm in the seeds without pre-treatment (Mehra et al., 2022).

CONCLUSION

When the effects of seaweed and different molybdenum applications on Rio Grande and H 2274 tomato cultivars were evaluated, there was determined a difference between the applications, but these differences were not statistically significant among some applications. The effects of the treatments on the seedling fresh weight, seedling height, hypocotyl diameter, seedling root length parameters of Rio Grande and H 2274 cultivars were observed significant. In Rio Grande cultivar, the highest seedling dry weight (23.35 mg) was in 25 ppm Mo+seaweed application, and the lowest (16.17 mg) was in 50 ppm Mo+seaweed application. In the H 2274 cultivar, the highest seedling dry weight (20.40 mg) was in control application, the lowest (17.13 mg) was in 25 ppm Mo + seaweed application. The highest seedling length of Rio Grande and H 2274 cultivars was in seaweed application. The highest hypocotyl diameter (0.086 cm) of Rio Grande variety was in 75 ppm

Mo application, the lowest (0.042 cm) was in 75 ppm Mo + seaweed application. In the H 2274 cultivar, the highest hypocotyl diameter (0.133 cm) was in the seaweed application, and the lowest (0.047 cm) was in the 75 ppm Mo+seaweed application. In the cultivar H 2274, the highest seedling root length was 2,820 cm in seaweed application, and the lowest seedling root length was 0.183 cm in 75 ppm Mo+seaweed application. The highest seedling root lenght (2.315 cm) of Rio Grande cultivar was in seaweed application, the lowest (0.189 cm) was in 75 ppm Mo application. While the positive effect of seaweed was determined when applied alone, it was determined that it was insufficient when applied together with molybdenum. In order to determine the molybdenum tolerance of these two tomato varieties and the effect of seaweed application, it is recommended to reduce the molybdenum dose and to use different concentrations of seaweed in applications in future studies.

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CHAPTER 7

MECHANICAL WEED MANAGEMENT IN VINEYARDS

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INTRODUCTION

Mechanical treatments are a widely used technique to control weeds in vineyards. Several vineyard vine cultivators are available on the market. Weed control efficacy relies on plant species and operating schedule, but no single piece of equipment will control all weed species or be suitable with all vineyard layouts. Viticulture is grown in irrigated and dry farming in different regions, in cultures ranging from traditional tapestry to modern wire dressing systems (cordon, guyot, Australia, double T, big T). Weed control tactics can vary in these different viticulture systems. Compared to traditional vineyards, foreign ore competition can be done more effectively in stringed finishing vineyards. It is a common tactic to apply soil tillage between rows or mowing weeds in vineyard areas, and to spray weeds on rows with herbicides in the form of strips. In other words, the herbicide is applied in strips to keep the vine rows free of weeds, the weeds in the middle space are done by tillage or mowing. Care should be taken to ensure that tillage is outcropped and away from the roots. Using improperly set equipment risks damaging vine roots, which can make root and vine diseases more likely.

Depending on how the fruit will be used commercially, weed control in the vineyard may vary. Weeds can reduce the quality of wine grapes, and seeds mixed with seedless grapes can contaminate the harvested product. Prior to vineyard establishment, perennial weeds should be controlled using post-emergence herbicides and hand and tractor

hoeing, as they are particularly competitive for vines during the first five years of establishment. Herbicides used before emergence are ineffective against perennial weeds such as bermudagrass (*Cynodon dactylon*), johnsongrass (*Sorghum halepense*) or nutsedge (*Cyperus rotundus*). Even mechanical techniques such as tillage and mowing may not have sufficient effect on these weeds and these weeds may take over the vineyard. It is usually possible to control seedlings of these weeds by applying foliar successive herbicides but controlling mature plants can be much more difficult. For better management, weeds should be sprayed after they germinate and early when they are actively growing. Pre-emergence or post-emergence herbicides can be used together to reduce weed competition in established vineyards and to increase the yield and quality of the product. Herbicide applications and mechanical methods should be supported by alternative techniques such as cover crops.

1. MECHANICAL WEED MANAGEMENT

Mechanical weed control has been regularly employed in grape growing for many years (Clements et al., 1995). First-class tillage equipment including plows, chisels, bottom boilers, cultivators, harrows, and soil mills, as well as second-class soil cultivation instruments like rollers, harrow-cultivators, harrow-rollers, cultivators-toothed harrows, spring harrows, and rotary harrows, are used for these procedures. There are available tool combinations and other mechanized tools (Jordan, 1996). Close to a vine's trunk, hand hoeing

is employed for finer labor and to get rid of weeds that defy machine management (Cloutier et al., 2007; Scienza & Miravalle, 2021). Tillage, mowing, and thermal methods are the most common techniques for mechanical weed control in vineyard areas.

1.1. Tillage

Mechanical practices commonly referred to as tillage are used to increase soil organic matter, promote warmer soil temperatures in the spring, and distribute irrigation water more effectively (Azimzadeh, 2012). In addition to chopping up and incorporating the remains of larger weeds, timely tillage helps eliminate small weeds (Pala, 2020). When the soil is dry enough to sustain the machine without compacting it, this should be done in the spring (Travlos et al., 2018). This is typically done a few weeks prior to many deciduous vineyards blooming and the opening of the grape buds. In young vineyards, bi-vertical tillage or cross tillage can be employed to control most weeds, however irrigation systems and dense plantings frequently limit this method.

Use of discs, rakes, and rototillers for shallow tillage can be used to suppress weeds between mature tree rows and vine plants (Melander et al., 2017). With the aid of more specialized processing instruments, such as crowbars or knives, annual weeds in furrows and on the edges of beds can be eliminated. A hoe plow can be used to remove weeds from the vine's base when working with vines. There are more

revolving cultivators and other row planters available (Olmstead et al., 2012). Another option is cross-disk. Furrow irrigation and basin flood irrigation with levees require occasional regrooving or excavating to make irrigation easier and cut down on weed biomass. While tweens in vineyards can still be treated, other applications like mulches and irrigation systems used in the rows lessen the effectiveness of control (Cabrera-Pérez et al., 2022).

1.2. Mowing

Combining timely mowing, strip herbicide spraying, and the use of cover crops, the triple technique is commonly used to control weeds in the middle of vines in mature vineyards. Many weeds can be controlled economically and effectively by mowing outside the weed-free lane (Ryschawy et al., 2021). However, routine mowing might promote the development of perennial weeds and grasses. To keep weeds under control, newly established vineyards could require mowing multiple times a year for the first two or three years. At ripening age, mature vineyards often need less regular mowing. The cutting time is the most crucial factor when mowing (Reiser et al., 2019). To guarantee that the targeted species can develop seeds for the cover crop that will grow the following year, it is crucial to choose when to mow native vegetation or annual cover crops (Travlos et al., 2018). Knowing the nature of weeds makes it necessary to mow them before they reproduce in order to control invasive species (Manzone et al., 2020). When mowing, it is

best to stay away from delicate times like flowering when some pests can transfer from weeds between or during the vine to the vines.

Research has shown that the freshly planted vineyards receive irrigation two to three times year. Irrigation in the vineyards should receive extra attention right after flowering and at the start of grain harvest. In vineyards that produce just dry grapes and wine, irrigation is discontinued three to four weeks before the fruit ripens, and the cover crop is mowed closely to the ground to reduce harvest disturbance. The frequency of mowing is influenced by irrigation systems. Drip irrigation often requires less summer mowing than sprinkler or flood irrigation since unirrigated areas do not support much weed growth during the dry part of the growing season. Early spring field labor can be complicated by rutting and soil compaction issues brought on by excessive field work, such as mowing. The same weed management techniques and strategies are always employed in vineyards with a long history of profitability.

1.3. Flaming

Using herbicides for weed control in vineyards is a widely preferred method. Because it is simple to implement, it has a quick effect and is often less expensive than other approaches. Weed control is an important agricultural activity (Mainardis et al., 2020). Due to the rising expense of labor globally, herbicides are the pesticide class that are employed the most frequently. The hunt for more environmentally

friendly, human health-safe alternatives to utilizing harmful chemicals in agriculture has quickened, though, as society has started to understand the detrimental consequences of pesticides on both the environment and human health. One of the biggest issues with plant production is weed management, which has given rise to a variety of alternative techniques (Travlos et al., 2018). Of them, one is on fire. Flaring is a method based on the premise of injuring the growth sites of freshly grown weeds, especially on the soil surface, by applying heat. The fundamental idea behind this is that, in contrast to burning, applying strong heat for a brief period causes weeds to expand, burst their cell walls, and eventually wither and die. Most utilized for this purpose are propane and other combustible gases. For use in vast regions, there are versions built into the tractor as well as handheld or backpack-carrying instruments that were specifically created for this purpose. Contrary to widespread belief, aggravation is not a particularly new technique for controlling weeds. Before the development of herbicides, the first flamethrower for agricultural usage, which was developed in 1852, was widely utilized. However, it lost its significance and appeal after herbicides were discovered in the 1940s. With the discovery of pesticide side effects, it is now available to us as a significant replacement for herbicides. Flaring is utilized in both agricultural and non-agricultural settings. It is used to suppress weeds that develop along the edges of courts, runways, pavements, buildings, and other similar structures in non-agricultural regions, particularly on roadsides, parks, and recreation areas.

There are two techniques to apply post-exit flaming: cross and parallel. Flame guns positioned at an angle from both sides of the cultivar row are utilized for cross-flaming (Diver, 2002; Ascard et al., 2007). When the plant body is robust enough to survive heat or when the variety is resistant to high heat due to structural characteristics, this form of application is made. The flame gun should be adjusted to target around the root collar of the variety, the guns on both sides of the row should not face each other. Otherwise, turbulence will ensue, and the growing warmth may injure the planted plant. Never let the flame from the cannon encounter the plant being cultivated. On the other side, parallel burning is a technique that is usually used with cultivars that are early or hot temperature sensitive. This technique effectively eradicates weeds between rows by positioning flame guns parallel to the rows of cultivated plants (Abdulgalimov et al., 2021). Equipment for parallel expansion and cross burning can both be fitted into interrow tillage vehicles. This approach is an alternative to pesticides, especially for organic viticulture, because it selectively eliminates interrow and above-row weeds despite the presence of cultivated plants in the area.

Weeds that have recently emerged, are 3-5 cm tall, or are in the phase with 2-4 leaves are more flammable (Kitiş, 2010). Additionally, flame is more likely to affect broad-leaved weeds than those with grasses (Mainardis et al., 2020; Morselli et al., 2022). because most grass weeds have underground growth points in the initial stages and their growth points are effectively shielded. For this reason, to get a satisfactory result against such weeds, it may be essential to perform a second

treatment by waiting for the growth sites to be exposed. Some weed species, particularly perennial ones, are flame-resistant (Kitiş, 2010). Again, depending on the stages of development, weed responses to exacerbation vary. The length of the weed's growth cycle, the rate of application, and the pressure of the applied gas, or the temperature, are all crucial factors in getting successful flaming outcomes. The drawbacks of this approach include a higher cost than chemical management and the elimination of dormancy in weed seeds near to the soil surface because of the high temperature.

Burning with a burner is a substitute for tillage, mowing, and spraying herbicides. Rather than the flame itself, heat created by the flame kills weeds (Travlos et al., 2018). Exacerbation works better on weeds that have just sprouted than on established ones (Ascard et al., 2007). Additionally, it works better on weeds with broad leaves than those with grasses. Due of their capacity to continually reappear from underground vegetative structures, perennial weeds such as bermudagrass, Canadian thistle, field bindweed are not vulnerable to flare-ups. When the weed species with successful exacerbation according to the developmental periods are examined, it is seen that a) wild mustard, common knotgrass, purple archangel and purple amaranth are in the cotyledon period, b) chamomile, shepherd's purse, black nightshade and groundsel are in the two-leaf period and lamb's quarters, common chickweed, cleavers, common fumitory, cranesbill and speedwell are susceptible in the four-leaf period (Kitiş, 2010). Weed seeds are not significantly affected by flames because of the sudden high temperature (Mainardis

et al., 2020). Young vines in vineyards with green bark should not be treated with this procedure since it could harm the meristematic tissue, slowing growth. Excessive amounts of dry weeds and leaves around the base of the vines should be cleared away to lower the danger of vine damage and fire. Plastic irrigation equipment can potentially sustain heat damage from flame operations. In times of high gasoline costs, flaring might not be economical. In some regions, air quality restrictions may also apply to it.

CONCLUSION

Weeds in vineyard areas compete with the vine for nutrients, water, and light, damaging grape yield, and quality. Indirect harm can also be done by weeds since they serve as a home for numerous pathogens and pests. Crop losses are exacerbated by the presence of perennial weeds, especially in the beginning. The grape variety, environmental factors, weed types and densities, and the vine and weed development times all affect the product losses brought on by direct and indirect interactions between the vineyard and weeds. Additionally, grape harvesting is challenging, and labor expenses rise in regions with high weed densities. Plowing is the most advised mechanical control technique in vineyard areas. Both weeds are eliminated with this process, and the soil can also be softened and aerated. There are drawbacks to the version in addition to its advantages, such as damage to vine roots. Mowing is one of the suggested techniques for weed management in regions when plowing is not an option. Weeds are removed using this

technique at regular intervals before they blossom. Both animal feed and the prevention of weeds from establishing seeds, overgrowing, and harboring various diseases and insects are acquired in this approach. One of the additional mechanical weed control techniques that can be used locally is flame. As a result, appropriate methods for mechanical weed management, including as tillage (or hoeing), mowing, and flaming, should be chosen, and used in coordination with other weed control approaches.

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CHAPTER 8

CULTURAL WEED MANAGEMENT IN VINEYARDS

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INTRODUCTION

The weed problem differs according to the agricultural crops grown and the maintenance procedures applied. Weeds are an important problem in viticulture, as in other agricultural production systems. The problem of weeds varies according to the place of viticulture, production method and maintenance work. Cultural weed management includes techniques that reduce weed competition. Approaches should be adopted in the vineyards to increase the competitiveness of the vines for water, mineral matter, light and space and to minimize the disruption of maintenance activities. Establishing a vineyard can be started by choosing areas with no or fewer weed problems. In the first planting of vines, spacing between rows and rows may be more sensitive to competition by allowing weeds to develop in the first few years, but in later years, after the vines grow and create cover and shade, they become more competitive with weeds. Excessive weed populations in yielding vineyards can make harvesting significantly more difficult. In addition to complicating maintenance and harvesting by competing for resources, weeds can exacerbate other pest problems and reduce the efficiency of the grape production system. Weeds can create alternative habitats for pathogens, insects, nematodes, mites, and rodents. With effective cultural treatments, crop losses caused by these pests in vineyards can be significantly reduced. Weeds can make irrigation less effective. Uncontrolled weeds can potentially reduce the effectiveness of these agricultural processes by hindering the application of other pesticides, growth hormones, foliar and soil fertilizers. Weeds can slow

or hinder workers during maintenance operations such as fertilizing, watering, pruning, and grafting. Even dried weeds can pose a severe fire risk.

Weeds, which are an important problem in the establishment, production and harvesting of vineyards, are controlled with various methods. Farmers use various weed management techniques suited to their vineyard production system (Ingels et al., 1994). This is called integrated weed management. Among the factors that determine the preference of farmers in integrated control, conditions such as weed type, incidence, density, coverage area, irrigation systems, fertilization, time and financial situation of the grower, equipment, and labour pool come to the fore (Monteiro & Lopes, 2007). Preventive measures, cultural processes, physical, chemical, and biological control techniques are applied in the fight against weeds. As a preventative measure, the entry of weed seeds or parts from the outside into the vineyard area should be prevented. For this purpose, all agricultural tools and machines used in the weed-infested area should be cleaned before being used in the vineyards. Weeds on the edge of the vineyard should be tackled before setting seeds. It should be noted that the animal manure to be used in the vineyard areas is well burned. Weeds in and around irrigation canals should be cleaned, weed seeds accumulated in the sediment in irrigation canals should be collected, and sieves or grids should be used to hold weed seeds and other propagation materials. As cultural processes, sowing/planting norms according to the condition of the soil, planting/planting time suitable for the region should be

considered, competitive varieties, crop rotation, appropriate fertilization and irrigation system should be selected. Weeds that develop after ploughing should be destroyed by collecting tools such as a crowbar cultivator with tip iron and harrow. Underground organs of perennial weeds emerging during tillage should be collected, removed, and destroyed. Cultural processes to be used to manage weeds that are a problem in vineyard areas have a prominent place in long-term sustainable viticulture, as they include non-chemical weed control methods.

1. CULTURAL WEED CONTROL

Cultural processes applied to weed management in vineyards are applied to prevent weed growth and to encourage the growth of vines (Monteiro & Santos, 2022; Travlos et al., 2018). Cultural weed control includes preventing or minimizing weed infestation through careful site selection, field preparation, and weed control in adjacent areas (Blackshaw et al., 2007). Weeds can also be affected by changing irrigation patterns and fertilizer rates, location or timing (Pala, 2020). Systems such as vine shading or wire dressing aid cultural weed control so that weeds can be somewhat controlled by planting plans and row orientation (Dastgheib & Frampton, 2000). Mulching in vineyards (such as plastic, fabric, green waste, and live cover crops) is often used to physically inhibit weeds, suffocate emerging weeds, remove weeds, and prevent seeds from germinating.

1.1. Training Methods

Planting spacing and training systems affect canopy and shading in viticulture. In well-established vineyards, most weed species struggle in the heavy shade (Byrne & Howell, 1978). For example, johnsongrass, common reed, nutsedge, pampas grass and bermudagrass can be a challenging issue in new vineyards, while they are less of a problem in mature vineyards. In mature vineyards, vines can suppress weeds in their own shade areas. In wire-seasoned or pruned vineyards, since the vineyard ground is usually lighter shaded and receives more light, weeds find their way out here (Shaulis & Steel, 1969). Tightly planted ties can create more shade and affect weeds (Zabadal & Dittmer, 2001). For this reason, if the emergence of weeds in large spaces is not desired, vineyards should be established at appropriate intervals. In traditional vineyards, a 2-3 m distance between rows and a 1.5-2 m distance between vines on the row is sufficient. The direction of the rows should be in the North-South direction for a good sunbathing.

The vine shape pruning systems preferred in viticulture tend to adopt a certain finishing method based on climate and soil characteristics (Clingeleffer, 2012). Early January is the primary time for pruning grape vines, but frequent pruning and upkeep are required all through the growing season to keep them in check and yield fruit. The Guyot system and the rod and spur (cordon) system are the two primary pruning techniques. Choosing a guyot growing method or cordon

method for vineyards depends on the grape variety and region. Common vine finishing systems are shown in Figure 1.

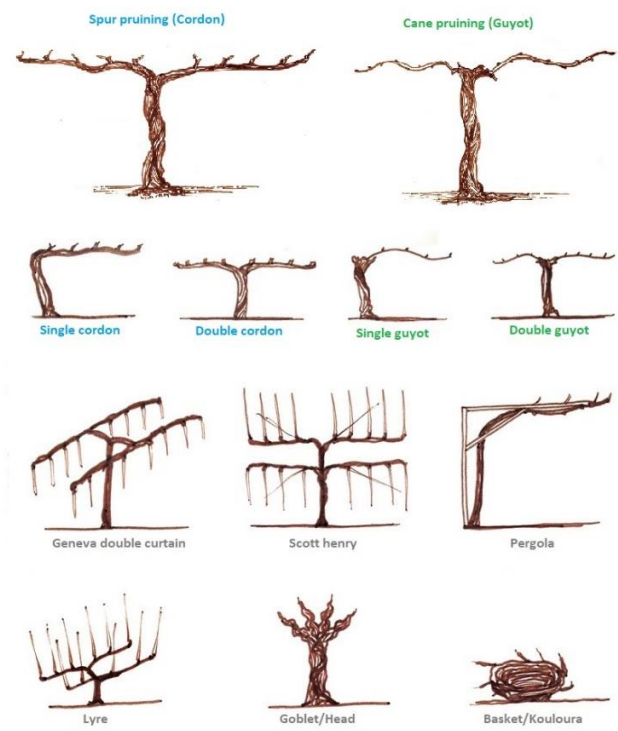

Figure 1: Grape Vine Training Methods (WineFolly, 2022)

Guyot, or cane pruning, is frequently used in growing locations with milder climates. The vine is more protected against frost than spur-pruned vines since only the trunk of the plant receives lignified growth (the hard, brown component of the growth). Cane pruning needs an important level of skill because it entails manually removing all of the vine's previous growth and carefully choosing one (or two) canes that will be in charge of the crop's production the following season. Numerous of the top wine-growing regions in the globe have embraced

cane pruning. Growing regions with warmer climates frequently practice spur pruning (cordon). Spur-pruned training techniques come in a variety of types and are employed for everything from table grapes (high productivity) to quality wine (low production). In general, spurs are simpler to prune than cane stubs with one to three buds, and some training techniques, like the goblet method, are best for drought-prone regions. Spur pruning, a more conventional training technique, is renowned for producing exceptional old-vine wines. Vineyard pruning also causes diversity in weeding. While pruning, care should be taken that the vine forms shading on the soil and suppresses weeds.

1.2. Mulching

Weeds cause yield and quality losses in vineyards and also complicate maintenance and harvesting processes. One of the methods of controlling weeds in grape production is mulching. Mulches block light from reaching the soil surface, which prevents weed seeds from germinating. In general, as the thickness of the mulch materials increases, the weed densities and dry weights decrease (Steinmaus et al., 2008). Mulches made from green waste such as straw, grass, sawdust and leaves for weed control work best when placed under vines at a depth of 10-15 cm (Figure 2). Paper, cardboard, textile cloth or plastic are other materials used as mulch (Hegazi, 2000). It is difficult to maintain as scraps of paper, cardboard and landscape fabric can be flung around and damaged during windy weather or typical vineyard activities. For mulches to be effective in windy conditions, they must

be placed in a uniform layer of weed-free soil and frequently replenished (Varga & Májer, 2003). The mulch particle size affects how long it lasts (DeVetter et al., 2015). Large or coarse mulches last longer than fine mulches, but they need to be buried deeper to hide the light. Planting or irrigation techniques that compromise the mulch barrier shorten the useful life of the mulch.

Figure 2: Straw Mulching (LGVS, 2022)

Organic mulches will break down because of typical microflora and microfauna activities. Some follow-up with hand-picking or spot herbicide sprays is often necessary to destroy weeds using mulch (Jiang et al., 2008). Perennial weeds such as field bindweed are susceptible to regrowth with mulch, but the effect is negligible. Mulches can promote vertebrate pest activity or host them for concealment. Mulching can be preferred in dry and irrigated vineyard areas with a traditional wire

finishing system. It is an important component, especially in areas where integrated or organic farming is carried out (Hammermeister, 2016).

1.3. Grazing

Weeds can be managed by grazing animals, such as cattle, sheep, and goats (Figure 3). In general, grazing (herbivory) for weed management varies more by the plants being grazed than the animals grazing them, albeit there are differences in effectiveness among grazers based on weed species and settings being targeted. Although grazing can be a helpful tool in an integrated management strategy, there are a variety of factors to consider, such as toxicity, plant growth stage, palatability, etc. (Popay & Field, 1992).

Grazing animals are sometimes used to control weed growth in vineyard areas (Gonçalves et al., 2021). In some vineyards, sheep, cows, chickens, and geese are used to control weeds. The frequency of grazing may vary depending on the number of animals, feeding needs, weed germination status and the development period of the vines (Nóbrega et al., 2017). Goats can be used to manage shrubs around vineyards, but they can seriously damage vines if used inside the vineyard. To reduce the possibility of damage to young shoots, animals should be removed from the vineyard before buds open (Popay & Field, 1996).

Figure 3: Grazing (Anderson, 2022)

In vineyards, geese are occasionally used to destroy weeds, but they will also consume other weeds once the narrow-leaved weeds are gone. Young geese eat more weeds than older geese. Grazers should not be allowed to feed on poisonous plants such as foxtails, horsetail, milkweed, poison hemlock, ryegrass, star thistle etc. Many plants can be toxic to animals (UCANR, 2022).

It is difficult to control weeds such as johnsongrass and bermudagrass, which reproduce by rhizomes, and nutsedge, which multiplies by tubercles, in organically grown vineyards because even if the above-ground green parts of these species are destroyed, they reappear with underground production materials. Animals used to suppress weeds in vineyards will typically need some form of protection from predators (dogs, coyotes, etc.). Before integrating animals into the system, it is

necessary to verify that its addition will not compromise the marketability of the grape or pose an unacceptably considerable risk to food safety. In recent years, grazing should be considered not only in terms of livestock viticulture and weed management for grape production but also as a way of agroecological sustainability and health control.

1.4. Cover Cropping

Weeds can be suppressed by planting cover crops (especially winter species) in vineyards (Steinmaus et al., 2008). Cover crops also reduce soil erosion caused by wind and water, increases the water-holding ability, bind the free nitrogen of the air to the soil, create a green walking area in the vineyard without sinking into the mud, and contribute to biodiversity (Baumgartner et al., 2008). It can be said that this technique reduces soil compaction since heavy machinery is not used in cover crops, and these plants are mixed into the soil (Miglécz et al., 2015). Perennial cover crops can be used in vineyards where rainfall is insufficient and can be irrigated (Monteiro & Lopes, 2007). Cover crops suitable for use in vineyard areas should be selected (Figure 4). According to the usage criteria, suitable cover plants listed below can be used in Table 1 (WineAustralia, 2022).

As cover crops, many kinds of plants can be used. The most often used plants are grasses (including cereals) and legumes, but brassicas

(including rape, mustard, and forage radish) are gaining popularity. Others, like buckwheat, continue to do so as well.

Table 1: Cover crops suitable for use in vineyards

Weed suppression	**Biological nitrogen**
Wheat (*Triticum aestivum* L.)	Faba beans (*Vicia faba*)
Triticale (*Triticosecale* spp.)	Field peas (*Pisum sativum*)
Barley (*Hordeum vulgare* L.)	Vetch (*Vicia* spp.)
Oats (*Avena sativa* L.)	Lupins (*Lupinus* spp.)
Grain rye (*Secale grain* L.)	Clovers (*Trifolium* spp.)
Forage radish (*Raphanus sativus* L.)	Medics (*Medicago* spp.)
Oilseed mustard (*Brassica juncea* L.)	**Insectary / habitat**
Canola (*Brassica napus* L.)	Allysum (*Lobularia maritama*)
Creeping salty bush (*Atriplex semibaccata* R.Br.)	Phaselium (*Phacelia tanacetifolia*)
Green manure	Buckwheat (*Fagopyrum esculentum*)
Wheat (*Triticum aestivum*)	Creeping saltbush (*Atriplex semibaccata*)
Triticale (*Triticosecale* spp.)	Ruby saltbush (*Enchylaena tomentosa*)
Barley (*Hordeum vulgare*)	**Grazing**
Oats (*Avena sativa*)	Wheat (*Triticum aestivum*)
Cereal rye (*Secale cereale*)	Triticale (*Triticosecale* spp.)
Faba beans (*Vicia faba* L.)	Barley (*Hordeum vulgare*)
Field peas (*Pisum sativum* L.)	Oats (*Avena sativa*)
Vetch (*Vicia* spp.)	Cereal rye (*Secale cereale*)
Lupins (*Lupinus* spp.)	Fodder radish (*Raphanus sativus*)
Fodder radish (*Raphanus sativus*)	Perennial ryegrass (*Lolium perenne*)
Oil-seed mustard (*Brassica juncea*)	Annual ryegrass (*Lolium rigidum*)
Canola (*Brassica napus*)	Tall fescue (*Festuca arundinacea*)
Annual ryegrass (*Lolium rigidum* L.)	Cocksfoot (*Dactylis glomerata*)
Green manure	Clovers (*Trifolium* spp.)
Clovers (*Trifolium* spp.)	Medics (*Medicago* spp.)
Medics (*Medicago* spp.)	**Devouring vines**
Permanent cover	Perennial ryegrass (*Lolium perenne*)
Perennial ryegrass (*Lolium perenne* L.)	Tall fescue (*Festuca arundinacea*)
Tall fescue (*Festuca arundinacea* Schreb.)	Cocksfoot (*Dactylis glomerata*)
Cocksfoot (*Dactylis glomerata* L.)	Chicory (*Cichorium intybus* L.)
Weeping grass (*Microlaena* spp.)	**Undervine cover crop**
Windmill grass (*Chloris truncata* R.Br.)	Annual ryegrass (*Lolium rigidum*)
Creeping saltbush (*Atriplex semibaccata*)	Clovers (*Trifolium* spp.)
Ruby saltbush (*Enchylaena tomentosa* R.Br.)	Medics (*Medicago* spp.)

Cover crops are more expensive and often require more attention than native plants. In many cases, it is possible to manage the local vegetation to reduce the need for increased water consumption, provide a habitat for beneficial insects, and improve water penetration. Regular mowing is required to maintain local vegetation, which can also be controlled to favour more aesthetically pleasing species. Plants like annual bluegrass and common chickweed can be promoted by adjusting the time of herbicide application and/or mowing, for example. Using native vegetation, on the other hand, may increase the likelihood of unwanted plants invading the vineyard.

Figure 4: Cover Crop (Boyd, 2020)

Cover crops have the advantage of being able to be chosen to enhance integrated pest control techniques, aid in soil improvement, or reduce field machine activity (Miglécz et al., 2015). Annual cover crops, which are more competitive than resident vegetation during the winter and

spring months, can help to prevent the establishment of more unfavourable species (Bordelon & Weller, 1997).

Cover crops are grown as a crop within a crop and rotated regularly to prevent the accumulation of perennial weeds and soil-borne illnesses. It is possible to choose cover crops that naturally die before competing with the crop for nutrients and water. Selecting a cover crop is critical and should be done with the overall cropping strategy in mind. Bug and disease problems may become more severe in pistachio and cherry trees, where insects and diseases can accumulate on mustards, vetches, and legumes and spread to tree flowers and growing nuts.

Weeds that cause problems in vineyards include johnsongrass, bermudagrass, dodder, and field bindweed. Due to competition, cover crops can more easily harm young vines. Vetch and cloves can grow into vines if not carefully controlled. It is critical to strike a balance between the need for cover crops in new vineyard areas and delaying planting until the vineyard has established a solid foundation. Vineyards may occasionally encounter additional pest problems because of cover crop systems (Reiff et al., 2021). Disease, pest, and rodent host status are one of the specific challenges with cover crops, and more research is needed in this area. Increased moisture within the crop cover can lead to an increase in crop diseases in some cover crop conditions. Diseases like *Elsinoe ampelina, Uncinula necator, Plasmopara viticola, Botrytis cinerea, Stereum hirsutum, Phellinus igniarius, Agrobacterium vitis, Phomopsis viticola*, Rugose Wood

Complex, Grapvein Leaf Roll Virus, and Grapvein Fan Leaf Virus can be found in some species (ZMMAE, 2022). Cover crops hide and protect crops and irrigation systems from predators such as voles and squirrels. Squirrels and other rodents may eat cover crops. Snails can become a significant problem in some crop environments. Growing different cover crops in vineyards varies depending on the host, but *Lobesia botrana, Tetranychus urticae, Otiorhynchus* spp., *Anaphothrips vitis, Colomerus (=Eriophyes) vitis, Viteus vitifolii, Planococcus citri, Asymmetrascavil (=Emmetrascavil, Polyphyllascavil,* Arcpo Insect pests like *Klapperichicen (=Chloropsalta) viridissima, Theresimima ampelophaga, Arboridia (=Erythroneura) adanae,* and *Spargano pilleriana* can be a major issue (ZMMAE, 2022). It is difficult to grow beneficial insects (parasoid or predator) in the same vineyard locations as pests that attack the vine. Some cover crops are hosts for vine-damaging nematodes and mites, promoting pest population growth. Cover crops shade the soil in colder vineyards, resulting in lower soil temperatures throughout the spring and potentially delaying early spring growth. Frost is more likely in vineyards with cover crops due to the vegetation's ability to prevent heat absorption and reradiation. Some grasses and shrubs may contain ice-forming bacteria, making crops more susceptible to freezing in colder weather.

CONCLUSION

Plants known as weeds are opportunistic and rely on nutrients, moisture, and light for growth. When weeds compete with vines for soil moisture and nutrients, interfere with vineyard equipment and operations, harbour vine pests and diseases, offer a fire danger, or damage grapes—from the table and dried grapes to fruit and wine grapes—they can be a concern for vineyards. There are both chemical and non-chemical weed control solutions available to fight weeds' undesirable traits. There is no "one size fits all" weed control method because of the wide variety of weeds that can be found in vineyards, as well as regional and seasonal variations. For the best results, many producers use different weed management strategies. The physiological traits and life cycles of various weed species determine the most efficient control strategies. This section discusses cultural practices that help control weeds, which are an issue in vineyard areas, including shade, mulching, grazing, and the use of cover crops. Mulching and the use of cover crops for weed management can sometimes be grouped as physical methods. For sustainable viticulture, it is critical to combine the right cultural practices with various weed control strategies.

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CHAPTER 9

RELATIONSHIP OF EPIGENETICS WITH CANCER, PLANT AND INSECTS

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INTRODUCTION

Every year brings a plethora of new discoveries in the scientific community as a result of the ever-shifting climate and other external factors. The biological sciences, in particular, stand to benefit from these innovations, but they can have far-reaching effects across the scientific spectrum. One of these advances is the field of epigenetics. Waddington (Noble, 2015) popularized the concept that merges the previously distinct fields of genetics and developmental biology. The origins of epigenetics can be traced back to the notions of epi and genetics, however these terms have distinct meanings. The Greek prefix epi means "beyond" in English. Thus, advances in epigenetics might be viewed as transgenetic innovations (İnce & Karaca, 2019; Şahin et al., 2018). Epigenetics refers to the study of heritable modifications to gene function that occur independently of the DNA sequence in a cell's genome. Mitosis and meiosis allow it to be passed on to future generations (Qureshi & Mehler, 2011; Şahin et al., 2018). Because of these characteristics, it can serve as the basis for countless investigations across all of scientific inquiry (plants, insects and various diseases). In 2006, approximately 2500 investigations were undertaken on epigenetics, as determined from published publications; in 2010, over 1300 epigenetics studies were conducted, as determined from data received (Martin & Zhang, 2007; Şahin et al., 2018).

Every cell in our body has its own genetic material. This genetic material is known as DNA. The DNA molecule has the ability to

determine the genotype and phenotype. Genotype-forming genes play a crucial role in phenotypic development by controlling the production of several proteins and enzymes essential to cellular and organismal life. A gene's expression is typically influenced by phenotypic traits. These phenotypic variations do not, however, alter the genetic code in any way. Alterations in gene expression can be heritable and reversible across generations. Thus, epigenetic mechanisms underlying phenotypic differences are been investigated (Noble, 2015; İnan, 2021). Non-coding RNA (ncRNA), DNA methylation, and histone alterations are the three main categories of epigenetic mechanisms (Gürel et al., 2016).

1. EPIGENETIC MECHANISMS

1.1. Histone Modification

Genetic information is stored in the genetic material called DNA (Bahar et al., 2016). DNA is housed in the nucleus alongside histone and non-histone proteins in a complex nucleoprotein structure known as chromatin (Gürel et al., 2016). Genomic organization in eukaryotes is more complicated than in prokaryotes. Therefore, histones are nuclear proteins in eukaryotic organisms that organize and package the chromosomal DNA molecule. Low-molecular-weight basic proteins called histones play an important role in eukaryotic chromosome and chromatin structure formation (Gürel et al., 2016; Şahin et al., 2018). Nucleosomes are the smallest functional unit of chromatin (Yıldız et al., 2021). Nucleosomes are formed by the histone proteins H2A, H2B,

H3, and H4, wrapping around the DNA molecule, which has a 147 base structure, 1.7 times (Uçar, 2019). There are two distinct structural forms of chromatin, euchromatin and heterochromatin. The euchromatin form is in a transcriptionally active form and has a loose structure, while the heterochromatin form is compact, transcriptionally quieter and denser. Histone proteins have N-terminal tails. These tails participate in post-translational gene regulation through at least eight distinct modifications: phosphorylation, methylation, ubiquitination, acetylation, deimination, sumoylation, proline isomerization, and ADP ribolization. These changes to histones can show variations in histone protein-DNA interactions. Dynamic and reversible alterations to histones are known as histone modifications (Gürel et al., 2016). Histone modifications are factors that regulate the transcription stage of the gene by interacting with the proteins associated with chromatin (İzmirli, 2013). Modifications to histones alter chromatin shape and affect transcription (Şahin et al., 2018). It is widely acknowledged that changes in histones and DNA methylation function in tandem to establish the level of gene expression and hence play a crucial role in regulating the future actions of the cell (Qureshi & Mehler, 2011). The alteration of histones has enabled the use of epigenetics in numerous scientific domains. Because alterations to the N-terminal tails of histones are recognized as the second most significant epigenetic mechanism involved in essential biological activities such as chromatin architecture, gene transcription, DNA replication, and DNA (Özgür et al., 2020).

1.2. DNA Methylation

Methylation is a chemical event that ensures the appropriate structure and development of the DNA in biological systems. It is the best known of the epigenetic mechanism (İzmirli et al., 2012). DNA methylation is one of the main mechanisms employed in the study of epigenetics, since it has significant impacts on gene regulation and piques the curiosity of researchers with fresh discoveries. DNA methylation is one of the key mechanisms employed in the study of epigenetics, as it has significant impacts on gene regulation and piques the interest of researchers with fresh discoveries. An irregularity or error that may arise in this system, which produces variations in the expression of genes through the chemical modification of the DNA molecule, may be the root cause of a number of disorders. Therefore, elucidating the unknown features of DNA methylation can assist to the clarification of the partogenesis of illnesses (Güler & Peynircioğlu, 2016). The methyl group is attached to carbon 5 of the pyrimidine ring of the cytosine base (islets called CpG) of DNA (İzmirli et al., 2012). As a result of DNA methylation, the cytosine base is converted into 5 methyl-cytosines (Güler & Peynircioğlu, 2016). DNA methylation has effects on gene regulation. An inactive DNA molecule is more methylated than a normally transcribed DNA molecule. Comparing genes in various tissues reveals that DNA is more methylated in cells devoid of gene expression. Conversely, demethylation of inactive genes can activate these genes in their inactive form. Conversely, demethylation of inactive genes can activate these genes in their inactive form (Şahin et al., 2018). The DNA

methylation event is crucial for gene expression regulation (Eser et al., 2016). During the regulation of the expression of genes, they are involved in the suppression of gene expression by methylation that occurs especially in the promoter regions of genes, by causing changes in the recognition regions of transcription and by preventing the binding of transcription factors. DNA methylation creates the recognition site for a group of methyl-CpG binding proteins by altering the structure of the DNA molecule (MeCPs) (Qureshi & Mehler, 2011).

1.3. Non-coding RNA

Gene regulation through RNA shows a wide distribution in eukaryotic organisms with complex genomes. Therefore, non-coding RNA molecules have an important role in epigenetics and gene regulation (Bodur & Demirpençe, 2010). Noncoding RNAs exist as molecules of RNA without being translated into proteins. In other words, these are RNA molecules that are transcribed from DNA but not translated to protein (Yoon et al., 2013). These RNAs have been discovered to play crucial regulatory roles in cellular processes. Additionally, these noncoding RNAs function as posttranscriptional regulators. Therefore, it can be utilized in place of unconverted protein (Şahin et al., 2018). Recent breakthroughs in the identification of non-coding RNA have demonstrated that the genome may contain a large number of non-coding RNA (ncRNA) genes in addition to protein-coding genes. These findings suggest that the RNA molecule has a variety of activities, including gene expression regulation, epigenetic processes, and signal

transmission. Viruses also utilize lncRNAs, a variation of the non-coding RNA molecule, for a variety of biological regulation (Tycowski et al., 2015). LncRNAs play a significant role in numerous cancers and can therefore be employed as biomarkers to predict prognosis and disease recurrence (Çoşan et al., 2018). Due to these characteristics, it is recognized as one of the favored processes in the fields of epigenetics.

2. RELATIONSHIP BETWEEN EPIGENETICS AND CANCER

Epigenetic mechanisms are important for normal development and maintenance of tissue-specific expression in mammals. Variable epigenetic pathways exist within the tissue. It is feasible to determine the distinct phenotypes of organisms and cells with the same genome using epigenetics. When epigenetic controls are disrupted, it leads to the realization of various unfavorable events and is involved in the partogenesis of many diseases, including cancer. Cancer is recognized as a complicated disease that results from the accumulation of epigenetic or genetic alterations. Cancer is defined by the loss of function of genes that have a suppressive role in the tumor or the activation of oncogenes, leading in the uncontrolled proliferation of cells and the acquisition of metastatic capabilities. Early on in the progression of cancer, epigenetic regulation is disrupted. With its emergence in these periods, changes occur in gene function and cause further progression of cancer disease (Gürel et al., 2016). Determining the treatment process and predicting the prognosis for early diagnosis in cancer is a difficult and laborious process. Epigenetic variations,

including histone modifications and DNA methylation, are events with pharmacologically important roles. DNA hypermethylation in CpG islets is a biomarker that can be used to detect cancer early on (colon cancer and other cancer types) (Nursal, 2016). In the detection and treatment of cancer, biomarkers based on epigenetic pathways can be utilized (İzmirli, 2013). Epigenetic alterations are not exclusive to cancer alone. Insulin resistance is closely linked to metabolic diseases including Type 2 diabetes and obesity, as well as debilitating neuropsychiatric disorders like depression, autism, and schizophrenia (İnan, 2021).

3. RELATIONSHIP BETWEEN EPIGENETICS AND PLANTS

Plants play an essential role in our lifestyles. For this reason, it is essential to comprehend how plants adapt to their environment and how epigenetics influences phenotypic gene expression variations in plants. Using a variety of regulators, epigenetic changes control the expression of genes. In addition, studies show that non-coding RNA, DNA methylation and histone modifications, which are epigenetic mechanisms, take place in all areas of the plant, such as fruit development, plant immunity, flowering time and responses to environmental factors. Vernalization is one of the epigenetic applications utilized in agriculture. In order to acquire high yields from the cultivated area and quality products from the planted plants, it is necessary to ensure optimal germination in the field at the suitable times. The timing of planting can vary based on the cold tolerance of

the plant and the administration of vernalization. The bulk of agricultural goods are affected by climate in terms of quality or yield. Due to the fact that elements like as humidity, precipitation, and temperature cannot be regulated during production, growers are required to cultivate in line with the prevailing conditions. In this context, epigenetic mechanisms are of great importance in agriculture. The vernalization application used in agriculture was revealed by Lysenko, a Soviet Agricultural Biologist. Lysenko centered his research on how the biological life cycles of plants respond to various temperatures. The mechanisms believed to be beneficial for growing winter wheat in the spring have been the subject of investigation. Therefore, seeds and seedlings were soaked and chilled throughout the winter, and when they were planted in the spring, they were able to complete their life cycles in a shorter amount of time and produce goods without having to wait until autumn. Lysenko referred to this occurrence as vernalization (Kim et al., 2009; Durmuş, 2018). Applications with low temperatures, which promote the emergence of vernalization, do not directly encourage the creation of flowers; rather, they impact the functioning of the systems that allow the flower to emerge. Vernalization is a process that can take months. It demonstrates the epigenetic memory of plant cells through mitosis of cells whose vernalization transmission is meristematic. It includes the constant suppression of the C gene in flowering, the absence of the flowering suppressor, the absence of the active histone marker, the increase in the number of the suppressor histone marker due to the absence of this active histone marker, constant suppression due to its enrichment, and

gradual epigenetic changes.It includes the constant suppression of the C gene in flowering, the absence of the flowering suppressor, the absence of the active histone marker, the increase in the number of the suppressor histone marker due to the absence of this active histone marker, constant suppression due to its enrichment, and gradual epigenetic changes. The vernalization desire of plants, where temperature is crucial, can be either mandatory or facultative. The vernalization desire of plants, where temperature is crucial, can be either mandatory or facultative. For instance, the application of vernalization to commence the flowering event in annual plants is optional, whereas the application of vernalization during the flowering event in biennial plants is mandatory. Therefore, blooming happens if vernalization is not applied to biennial plants. Epigenetic information is mediated by DNA methylation by histone posttranscriptional modifications (FTM). Stable transmission of histone posttranscriptional modifications via mitosis occurs with the initial vernalization process. Some A. thaliana ecotypes and other annual plants require a particular length of cold to bloom during this phase (Kim et al., 2009; Batı Ayaz et al., 2018; Şahin et al., 2018).

Without alterations to the DNA sequence, the arrangements that occur throughout the functioning of the gene result in diverse activities and characteristics. These are used epigenetics to explain and elucidate, for which classical genetics fails to explain. In numerous living organisms, distinct roles of gene control manifest in distinct forms. Although epigenetic controls are viewed as one of the genetic regulation

processes that take place during environment-gene interactions in complex systems, they are distinct from these regulations. The major distinguishing characteristic between epigenetic and other genetic controls is their molecular memory. Due to its molecular memory, it can store and transmit information to subsequent phases (Baulcombe & Dean, 2014). Plants are a useful model for the research of epigenetics. Epigenetics plays a significant role in the formation of a plant's response to stress and in the development of plants (Şahin et al., 2018).

The importance of epigenetic regulation in plants, the evolutionary story of plants, can be understood by the developmental method. During the embryonic phase of animal development, each tissue and organ formation is specialized, whereas in plants, new organs are formed from meristems, which are populations of stem cells that are able to proliferate. After embryonic development, environmental variables and influences have a significant effect on the shape of the plant organ. This impact provides plants with a high degree of phenotypic stability. Unlike animals, plants cannot leave their surroundings in response to changing environmental conditions. Accordingly, it becomes beneficial for them to adapt to their surroundings (Alonso et al., 2019). Epigenetic mechanisms permit metastable changes in gene activity and are capable of fine-tuning gene expression motifs. Thus, plants can successfully adjust to changing environmental conditions and live and reproduce in unpredictably harsh environments. Polyploidization, also known as an increase in the number of chromosomal sets, the development of the specialization of the copied genes, and the duplication of gene families

are among the epigenetic regulators observed in plants (Gallusci et al., 2017).

Arabidopsis thaliana (L.) Heynh., a member of the mustard family, is the first plant to have its genome sequenced (Schmid et al., 2005). The plant species that are most frequently utilized to comprehend the mechanism of epigenetic control are plant species having sequences like these. In these studies, it contributes to seed plants (Pikaard & Scheid, 2014). The function of the plant's genome is regulated by chromatin markers such as post-translational modification of histones and DNA methylation. The techniques used to examine model plants such as *A. thaliana* allow scientists to begin elucidating the processes required to reveal and preserve chromatin changes. Genomic studies allow mapping of comparable alterations, such as DNA methylation, on a genome-wide scale. Small RNA molecules appear to have a significant role in defining the distribution of chromatin changes, and the RNA molecule may also be responsible for complicated epigenetic interactions between homologous sequences. It can be utilized to regulate mechanisms of epigenetic silencing, parent-of-origin imprinted gene expression, and plant development (Henderson & Jacobsen, 2007). The eukaryotic genome is covalently modified by chromatin markers contained in both the linked histones and the DNA molecule. Due to their effect on the initial DNA sequence, these changes can be passed on to multiple generations via continued cell division. Hence, they are categorized as epigenetic markers. Many aspects of chromosomal biology and gene expression are influenced by

epigenetic markers that are conserved. Epigenetic markers that are conserved have characteristic genomic distributions. Genomes are arranged as gene-rich heterochromatin and euchromatin at the chromosomal level. The defining characteristics of heterochromatin are dependent on epigenetic information, such as the methylation of cytosine bases in the DNA molecule and the translation of histones into DNA. The genome's defense mechanism is known as the silencing of transposable element sequences in heterochromatin. Nevertheless, heterochromatin may play crucial functions in chromosome segregation. In addition, it has been demonstrated that both gene silencing and transposons contribute to cis regulatory regions and affect gene expression. In this regard, plant systems are recognized as a valuable source for the study of epigenetic inheritance (İnce & Karaca, 2019).

The epigenetic resources utilized in the study of the model plant *A. thaliana* provide insight into this plant's epigenetics. The genome of *A. thaliana* is 130 megabases (mb) in size, and contains a large amount of heterochromatin (Mai et al., 2016). It has a high concentration of pericentromeric and centromeric repeat regions. Using whole-genome microarrays for high-resolution mapping of cytosine methylation reveals that this change co-localizes with centromeric regions and repeat sequences. Despite the fact that nearly one-third of genes are methylation in open reading frames, less than five percent of active genes have methylated promoters. Methylation in the body of a gene has been discovered to be connected with both constitutively expressed

and highly transcribed genes, although its significance is not entirely understood. In contrast, the expression levels of genes with methylated promoters are lower and their expression patterns are predominantly tissue-specific. This distribution of cytosine methylation is known to be in contrast to that observed in mammalian genomes, which in general have densely methylated but hypomethylated CG islets in gene promoters. To test the overall pattern found in *A. thaliana*, it may be necessary to find the ‘methylome' of other repeat-rich plant genomes, such as those of grasses (İnce & Karaca, 2019).

Epigenetic regulatory mechanisms and chromatin markers play a significant role in controlling the plant's developmental process and determining the plant's phenotypic stability. Approaches based on the process of shaping epigenetic changes are helpful for measuring and analyzing the effects on plant performance. Chromatin markers give a stimulation from the environment and considerably contribute to the phenotypic stability of the plant. Studies using stress factors like as heat, drought, mineral and salt applied to numerous plant species (*Solanum lycopersicum* L., *Oryza sativa* L., *A. thaliana* ve *Zea mays* L.), as well as biotic and abiotic stresses induced by herbivore and pathogen attacks, have led to the discovery of the activity of epigenetic regulators in plants under stress (Şahin et al., 2018). When plants are exposed to several diseases, they develop a long-lived epigenetic memory. First-time pathogen-exposed plants can activate defensive mechanisms including hormone genes and salicylic acid to inhibit the pathogen's proliferation and growth. Initial answers are not continuous. When the

plant is exposed to the pathogen a second time, the genes can be activated more rapidly than the first response. A similar defense effect to this procedure has also been demonstrated in the application with β-amino butyric acid (Dowen et al., 2012).

The primary epigenetic marker is DNA methylation, which is one of the epigenetic mechanisms. Stress application and changes in DNA methylation in plants have been demonstrated, and continuous exposure to cold has been found to have a substantial impact on the stable transcriptional silencing of the flowering gene (FLC) (Şahin et al., 2018). Cold exposure of the root tissues of corn seedlings has been shown to result in DNA methylation in the nucleosome, which is a DNA-wrapped histone complex. Thus, DNA methylation was significantly reduced in frozen tissues. This functions as a stress-stimulated transcriptional switch for many stress-regulated genes due to cold-stimulated de-methylation of the nucleosome core and relaxing of chromatin structure (Steward et al., 2002).

4. RELATIONSHIP BETWEEN EPIGENETICS AND INSECTS

There are many varieties and species of insects. Within the context of intergenerational epigenetic mechanisms and phenomena, insects are intensively investigated. All noncoding RNAs, histone modifications, and DNA methylation are involved in insect phenotypic change. Stress variables such as nutrition, an overcrowded environment, and physicochemical conditions may alter the patterns of epigenetic

markers (Burggren, 2017). Some insects have positive effects on living organisms and their environments, while others have detrimental effects. Some hazardous insects reduce soil productivity. This circumstance results in infertile soil and low crop yields. Several techniques, including epigenetics, are employed to minimize or eradicate pests. RNA interference technology is an example of one of these uses (RNAi). Using RNA interference technology, the target gene areas of dangerous insects are silenced, thereby decreasing the damage they cause and reducing their populations. CRISPR/Cas9 system is another technology used to combat pest insects. This is a microbial immune system that employs RNA-guided nucleases to eliminate foreign genetic material. In contrast to RNAi technology, which disrupts gene expression, the CRISPR/Cas9 system is a system that not only disrupts gene expression, but also targets many genes, alters coding sequences, and reduces the complexity of these investigations (Gümüş & Kaymaz, 2018). In insects, epigenetic mechanisms also play a significant role. Gene expressions can be decreased or increased by epigenetic mechanisms. Epigenetic interactions can target insect-essential receptor genes or pheromones using the CRISPR/Cas9 and RNAi systems. In this manner, the population of dangerous insects can be repressed or managed by altering their behavior in locating food and mates, so stopping them from feeding and reproducing. The CRISPR/Cas9 system has applications including epigenetic editing. In investigations conducted on *Drosophila melanogaster*, it was shown that an increase in DNA methylation and in regions containing CpG islands has a significant impact on gene expression. Moreover,

investigations have shown that the majority of DNA methylation occurs during the early embryonic stage (Ertürk & Koçak, 2017).

CONCLUSION

Due to its diverse mechanisms, epigenetics is utilized in numerous domains, including the scientific community. Thanks to epigenetic mechanisms, it will be able to produce promising outcomes for inherited disorders and insoluble difficulties in cancer research. Epigenetic mechanisms are crucial to the advancement of plant, insect, and human health research. The processes it contains have made it feasible to conduct research on plants and insects, have a big impact on resolving potential issues, and will have a substantial impact on reducing the problems caused by plants and insects in agriculture and many other industries. The mechanisms it contains have made it possible to study plants and insects, have a significant impact on resolving potential problems, and can have a significant impact on minimizing problems caused by plants and insects in agriculture and many other fields; consequently, epigenetic mechanisms will be considered in a great number of future studies.

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CHAPTER 10

THERMOSTABLE α-AMYLASE FROM *BACILLUS* – A REVIEW

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INTRODUCTION

Microorganisms, have been regarded as precious sources of useful enzymes. Among microorganisms, *Bacillus* species that can be found widely in nature are used to produce the enzyme (Agüloğlu Fincan et al., 2021). *Bacillus* are more preferentially in their habitats; They are found in thermophilic areas and geothermal heated waters. (Ercan Kaya & Kıvanç, 2008). In 1888, Miquel reported that the first thermophilic *Bacillus* was isolated from the Seine river in Paris (Nazina et al., 2001). He stated that *Bacillus* lives at 70 ^{0}C. In the following years, *Bacillus*, which can live at approximately 60 ^{0}C, were isolated from soil, sewage and food, and many new thermophilic *bacillus* species were identified in the last 20 years. Today, thermophilic bacteria have gained great importance due to their enzyme properties that are resistant to high temperatures (Sharp at al., 1991).

Due to the ever increasing development of enzyme technology, researches conducted on industrial enzymes gain more importance. Recently, interest in thermostable amylases produced from thermophilic bacteria has intensified. Considering the characteristics of thermophilic bacteria; It has been defined as having rod, disc, or elliptical morphologies that are resistant to temperature, anaerobic, and facultatively viable. (Agüloğlu Fincan & Enez, 2014)

1. *BACILLUS*

Bacillus is a rod or rod-shaped, spore-forming and Gr (+) bacterial. It belongs to the *Bacillaceae* family. Spores are heat resistant (Turnbull, 1996). *Bacillus*, the largest class of bacteria, are often found in plant products, soil, and in places such as animal feces. *Bacillus* species are either obligate aerobes or facultative anaerobes. Cultured species are catalase positive in the presence of oxygen. These bacteria, which are called industrial microorganisms; They are preferred because they grow rapidly, are easy to isolate and define, are not pathogenic, have a short fermentation time and leave the products they produce to the outside environment (Enez, 2021; Agüloğlu Fincan et al., 2021). *Bacillus* are used by developing countries for the production of α-amylase due to their simple structure and suitable nutritional requirements (Sachdev et al., 2016).

2. THERMOSTABLE AMYLASES

The most desirable feature of industrial enzymes is that they are thermostable. Thermostable enzymes are isolated from thermophilic microorganisms and started to be applied in commercial areas (Agüloğlu Fincan et al., 2021; Özdemir et al., 2018; Enez & Agüloğlu Fincan, 2016). Amylases are enzymes that randomly hydrolyze starch molecules, including polymers consisting of glucose and substances such as dextrin (Agüloğlu Fincan & Enez, 2014).

Figure 1: Structure of Thermostable α-amylase **(**Janecek & Balaz, 1992**)**

Saccharification processes are processes that require high temperatures. The produced thermostable amylases are of great interest because they can withstand these temperatures (Agüloğlu et al., 2000; Peixoto et al., 2003). Important advantages of thermostable amylases are increased substrate solubility, reduced cooling costs, and resistance to denaturing agents (Agüloğlu Fincan & Enez, 2014). Enzyme resistance to high temperatures is of great importance in the industry, especially in the starch industry (Leveque et al., 2000; Peixoto et al., 2003).

Thermostable amylases were obtained from various bacterial strains using SmF and SSF media (Agüloğlu Fincan et al., 2014; Couto & Sanroman, 2006). Thermostable α-amylases have important commercial applications such as sugar production, paper industry, baking and detergent industries (Haki & Rakshit, 2003). *Bacillus* is generally utilized for the commercial production of thermostable α-

amylases (Özdemir et al., 2018). Table 1 shows the properties of thermostable amylases obtained from *Bacillus*.

Table 1: Properties of *Bacillus* Thermostable α-amylases

Microorganism	**pH optimal / stabilty**	**Tempature optimal / stabilty**	**Inhibitors**	**Reference**
Geobacillus stearothermophilus	7.0	70 °C	Cu^{2+}, Zn^{2+},Fe^{2+}, Hg^{2+}	Agüloğlu Fincan & Enez (2014)
Geobacillus thermoleovorans	7.0	70 °C	-	Rao & Satyanarayana (2007)
Bacillus licheniformis SO-B3.	7.0	70 °C	Hg^{2+}, Cu^{2+}, EDTA	Agüloğlu Fincan et al. (2021)
Anoxybacillus flavithermus	7.0	55 °C	Cu^{2+}, Zn^{2+}, Hg^{2+} glucose, sucrose	Agüloğlu Fincan et al. (2014)
Geobacillus sp. *Iso5*	8.0	90 °C	EDTA, Zn^{2+}	Gurumurthy & Neelagund (2012)
Bacillus cereus	9.0	65 °C	Zn^{2+}, Cu^{2+}, Mn^{2+}	Priyadarshini et al. (2020)
Geobacillus LH8	5.0-7.0	80 °C	Mg^{2+}, Ba^{2+}, Ni^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+}, EDTA	Mollania et al. (2010)
Bacillus sp. I-3	7.0	70 °C	EDTA, $HgCl_2$	Goyal et al. (2005)
Bacillus subtil	7.0	135 °C	-	Konsoula & Liakopoulou-Kyriakides (2007)
Bacillus caldolyticus DSM405	5.0-6.0	70°C	-	Schwab et al. (2009)
Bacillus sp. IMD 435	6.0	65 °C	glucose, fructose	Hamilton et al. (1999)
Bacillus sp. KR-8104	4.0 - 6.0	70-75 °C	-	Sajedi et al. (2005)
Bacillus amyloliquifaciens TSWK1	7.0	70 °C	Na^{+}, Fe^{++}	Kikani & Singh (2011)
Bacillus mojavensis SO-10.	5.0 - 6.0	70°C	Zn^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+}	Özdemir et al. (2018)
Bacillus sp. PS-7	6.5	60 °C	-	Sodhi et al. (2005)
Bacillus dipsosauri DD1	6.1	60 °C	Zn^{2+}, Cd^{2+}	Deutch (2002)
Bacillus sp. Ferdowsicous	4.5	70 °C	Hg^{2+}, Zn^{2+}, EDTA	Asoodeh et al. (2010)

3. CLASSIFICATION OF AMYLASES

Amylases, which are extracellular enzymes, are found in three subtypes. These are; They are α-amylase, γ-amylase and ß-amylases. The most common types of amylase in animal systems are α-amylase and γ-amylases. These three types of enzymes are; They are produced by bacteria, yeast, fungi and plants. There are four groups that affect starch; transferases, exoamylases, endoamylases and branching enzymes (Agrawal et al., 2005). The enzymes that cleave α-1,4 bonds in amylose or amylopectin are endoamylases (Benjamin et al., 2013). α-Amylases are the best known endoamylases (Agrawal et al., 2005; Akkaya et al., 2012).

3.1. α- Amylase (EC 3.2.1.1)

The metalloenzyme of calcium is α-amylases. α-Amylases move along the starch chain, breaking down long-chain carbohydrates. As a result of this degradation, maltose and maltotriose are formed from amylose or glucose, maltose and limit dextrin are formed from amylopectin (Kandra, 2003; Rajagopalan & Krishnan, 2008). α-Amylases are used in many sectors such as clinical, analytical chemistry and medical fields. Besides these; It is found in industrial areas such as textiles, pulp and distillation, bakery, food (Ortakaya et al., 2017; Pandey et al., 1999; Francis et al., 2002). The microorganisms considered the most important source of α-amylase are *Bacillus* spp. (Babu & Satyanarayan, 1995).

3.2. β- Amylase (EC 3.2.1.2)

1,4-α-D-glucan glycogenase is saccharogen, or maltohydrolase is synonymous with β-amylase. They are generally synthesized by bacteria, fungi and plants. β-Amylase cleaves two glucose units (maltose) at once by acting on the second α-1,4 glycosidic bond starting from the non-reducing end (Bijttebier et al., 2007). These enzymes are enzymes that perform exoaction.

3.3. γ-Amylase (EC 3.2.1.3)

γ-Amylase is also defined as 1,4-α-D-glucan glucohydrolase. This enzyme acts on the α-(1-4)-glycosidic bond at the non-reducing end of amylopectin and amylose. In addition, γ-amylase cleaves α-(1-6)-glycosidic bonds (Tateno et al., 2007; Hsieh et al., 2008).

4. INDUSTRIAL APPLICATON OF α-AMYLASE

α-Amylases have many potential applications in the industrial process. We can list these areas as follows; food, bread making, glucose syrups, textiles, alcoholic beverages, sweeteners and fruit juices (Agüloğlu Fincan & Enez, 2014; Sundarram & Murthy, 2014; Rakaz et al., 2021).

Table 2: Applications of α-amylases from *Bacillus*

Sl. No	Microorganism	Reference
Food industry	*Bacillus licheniformis* SO-B3	Agüloğlu Fincan et al. (2021)
	Geobacillus stearothermophilus	Agüloğlu Fincan & Enez (2014)
	Bacillus mojavensis SO-10	Özdemir et al. (2018)
	Geobacillus LH8	Mollania et al. (2010)
	Anoxybacillus flavithermus	Agüloğlu Fincan et al. (2014)
	B. licheniformis Shahed-07	Rasooli et al. (2008)
	Bacillus sp. *Ferdowsicous*	Asoodeh et al. (2010)
	Bacillus subtilis	Sani et al. (2014)
	Bacillus subtilis JS-2004	Asgher et al. (2007)
	B. licheniformis	Shaw et al. (2009)
	Bacillus. sp. strain SMIA-2	Carvalho et al. (2008)
	B. cohnii	Ghorbel et al. (2009)
Textile industry	*Geobacillus stearothermophilus*	Agüloğlu Fincan & Enez (2014)
	Anoxybacillus flavithermus	Agüloğlu Fincan et al. (2014)
	B. licheniformis	Shaw et al. (2009)
	Bacillus sp. B3	Thippeswamy et al. (2006)
Detergent industry	*Bacillus* strains	Ito et al. (2005)
	Geobacillus sp. Iso5	Gurumurthy & Neelagund (2012)
	Bacillus sp. A3-15	Arıkan (2008)
	Bacillus sp. PN5	Saxena et al. (2007)
	Bacillus subtilis	Sani et al. (2014)
Paper industry	*Bacillus* sp. B3	Thippeswamy et al. (2006)
	Bacillus subtilis KIBGE HAS	Bano et al. (2011)
	B. licheniformis	Ul-Haq et al. (2003)
Pharmaceutical industry	*Bacillus* sp.	Samrot & Vijay (2009)
	B. licheniformis	Kandra (2003)

Bacterial α-amylases are frequently used for medical purposes (Saini, 2017). Amylases are now preferred over chemical hydrolysis in starch processing industries (Ortakaya & Agüloğlu Fincan, 2019; Agüloğlu Fincan et al., 2021. *Bacillus* α-amylases used in many industrial areas; It is understood more clearly when we look at Table 2 that it is used in areas such as food, detergent, paper, medicine and textile.

5. MOLECULAR WEIGHT OF A-AMYLASE

Table 3: Molecular Weight of Amylase Purified from *Bacillus*

***Bacillus* species**	**Purification strategy**	**Molecular weight (kDa)**	**Reference**
Bacillus licheniformis SO-B3.	DEAE-cellulose ion-exchange chromatography	74	Agüloğlu Fincan et al. (2020)
Geobacillus stearothermophilus	Sephadex G-100 & DEAE cellulose columns	63	Agüloğlu Fincan & Enez (2014)
Geobacillus sp. Iso5	Sephadex G-150 & DEAE-Cellulose columns	43	Gurumurthy & Neelagund (2012)
Geobacillus thermodenitrificans HRO-10	DEAE Sephadex A-50 and Superdex - 200 columns.	58	Ezelji & Bahl (2006)
Geobacillus LH8	Q-Sepharose & MonoQ-Sepharose columns	52	Mollania et al. (2010)
Bacillus licheniformis ATCC 9945a	Sephadex G-100 & FPLC Superose 12 columns	31	Božic et al. (2011)
Bacillus sp. YX-1	DEAE-Sepharose Fast Flow & Sephadex G-75 columns	56	Liu & Xu (2008)
Bacillus amyloliquifaciens TSWK1-1	Phenyl Sepharose 6FF	43	Kikani & Singh (2011)
B. methylotrophicus P11-2	DEAE FF & Superdex 75 10/300GL	44	Xie et al. (2014)
B. subtilis KCC-103	DEAE Sephadex A 50	53	Nagarajan et al. (2006)
Anoxybacillus flavithermus	DEAE-cellulose	60	Agüloğlu Fincan et al. (2014)
Lactobacillus amylovorus	Sepharose 6B column	66	Sanoja et al. (2000)
Bacillus subtilis KIBGE HAS	Sepharose CL-6B	56	Bano et al. (2011)
B. licheniformis	Starch column	58	Rao et al. (2005)
Bacillus sp. IMD 435	CFS & a-CD Sepharose 6B affinity chromatography	63	Hamilton et al. (1999)
Bacillus licheniformis NH1	Gel filtration: Sephadex G-100& Anion exchange chromatography: mono Q Sepharose	58	Hmidet et al. (2008)
Bacillus licheniformis	Sephadex G-100 & CM Sepharose CL-6B	58	Ivanova et al. (1993)
Bacillus mojavensis SO-10	DEAE (Diethylaminoethyl) cellulose (DE 32) column	73	Özdemir et al. (2018)

CONCLUSION

Bacillus is an endospore-forming, Gr (+), thermophilic bacterial that is frequently used in the canning industry. The use of thermophilic microorganisms in microbiological fermentation processes have some advantages due to their heat-resistancy: high temperatures increase Many compounds occur where solubility and diffusion capacity are reduced. With a sufficiently high temperature, some volatile products are separated from the environment and cell growth is inhibited. Due to their high resistance to heat, *Bacillus* are suitable organisms for equipment validation studies to confirm the accomplishment of sterilization. Spores used in such studies are called “biological indicators” as they provide evidence for a proper sterilization process.
Enzymes used in industrialised applications are required to have characteristics such as maximum efficiency with lower costs, and high adaptability to different fields. Thermostable enzymes show high tolerance to various denaturing conditions. *Bacillus* α-amylase is a thermostable enzyme which is extensively used in biotechnological processes. Thermostable amylases prevent environmental pollution by acting on biologically degradable and inseparable materials.

In the present review, we report the biochemical properties, classification, and the use of thermostable α-amylase from *Bacillus*.

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CHAPTER 11

MEDICAL, PHARMACOLOGICAL AND INDUSTRIAL USE OF LICHENS

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INTRODUCTION

Lichens are living things that are composed of two supporting parts, called the photobiont and the mycobiont, which are symbiotically linked (Stocker-Wörgötter, 2008). The word lichen means surface growth on the olive peel in Greek (Denton & Karlén, 1973). It is well known that the habitats of lichens are the surface of stones, the inside of cracks and tree trunks. In addition, they can grow in different atmospheres and underwater structures. These creatures need to adapt to harsh environmental conditions in order to survive. To this end, they turn their vegetative parts to the sun and enable the photobiont to use solar energy (Ahmadjian & Reynolds, 1961). Lichens can be found in three different structures: crusty, leafy and branchy. There are also some intermediate forms such as leprosy, placodioid, squamulose and dimorphic. In classification, lichens are located in the plant kingdom epiphytically (Nayaka, 2014). More than 25,000 lichen species are known to inhabit approximately 10% of the land surface, including the poles (Muggia, 2009). Lichen species have a tendency to grow slowly due to their metabolites and can live for hundreds of years without deteriorating their physiological effects (Mitrović et al., 2011).

1. SYSTEMATIC AND MORPHOLOGICAL STRUCTURE OF LICHENS

Lichens are symbiotic plants formed by fungi and unicellular algae, as shown in Figure 1.

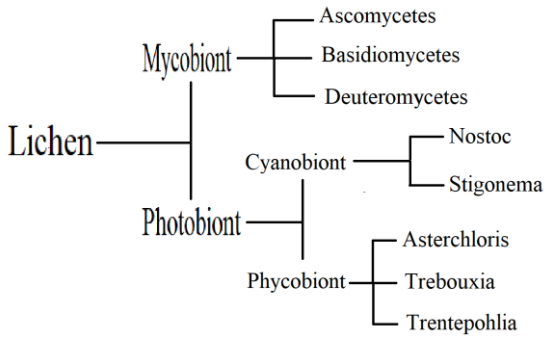

Figure 1: Schematic Illustration of Lichen Partners

The most common growth structure of lichens is shown in Figure 2. Crustacean growth forms depend on habitat surfaces. Leafy growth forms have a leafy thallus that grows horizontally on surfaces.

Figure 2: Lichens Growth Forms (Sachin et al., 2018)

They are mostly gray-green in color and grow in shaded areas. Another growth form, the branched growth forms, has a bush-like appearance with many branching tubes. This growth form is mostly light green or

light yellow in color (Jayanthi et al., 2012). Many of these forms are found in the terrestrial region, but they can also be found in the aquatic ecosystem (Molnár & Farkas, 2010). Apart from these growth forms we have mentioned, lichens can also show different growth forms such as squamulose (cluster-like), leprosy (dust-like), gelatinous (jelly-like) and filamentous (fine hair-like) as shown in Figure 3. Usually small in size, the squamulose growth form appears as a foliate growth form when its thallus, which are loosely attached to the surface on which they live, are lifted. In the Filamentous growth form, which is unique from all other growth forms, the fungal partners of the lichen form layers and wrap around the Cyanobacteria or their photosynthetic members. Leprosy growth forms, which cannot form an organized thallus, are mostly in powder form and their surface consists of granules. These lichens are devoid of cortex. In the form of gelatinous growths, carbohydrates are produced by blue-green algae (Bhattacharyya et al., 2016).

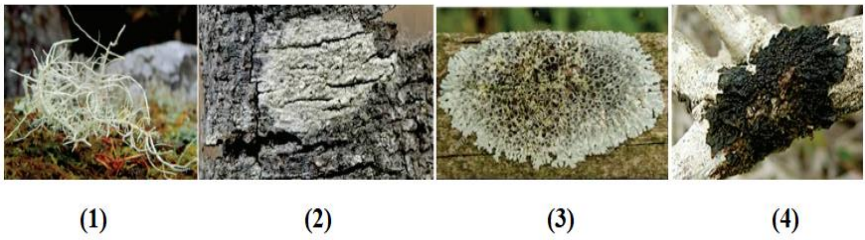

Figure 3: Other Lichens Growth Forms (1) Filamentous Lichen Growth Form (2) Leprose Lichen Growth Form (3) Squamulose Lichen Growth Form (4) Gelatinous Lichen Growth Form (Shahid et al., 2020)

In Figure 4, the chemical formulas of some of these compounds are given.

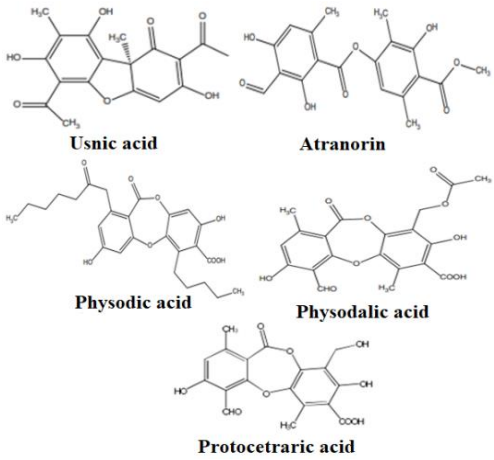

Figure 4: Some Chemical Compositions of Lichen
(Ranković et al., 2014; Tomović et al., 2017)

2. SECONDARY METABOLITES PRODUCED BY LIKENS AND THEIR USE

Metabolites produced by lichens are divided into two groups, called primary and secondary metabolites (Lawrey, 1986). Examples of primary metabolites are carotenoids, amino acids, vitamins, proteins and polysaccharides. On lichens, some of the primary metabolites are produced by the mycobiont and some by the photobiont partner. However, it is not easy to determine which partner is responsible for a particular metabolite, although most primary metabolites are water-soluble and can be separated from the lichen with boiling water. Secondary metabolites are extracted with organic solvents because most of them are insoluble in water (Armstrong, 1983).

Typical cortical lichen substances can usually be distinguished from compounds found only in the medulla. While the most common cortical elements of these compounds are parietin, atranorine and usnic acid (UA), compounds such as protocetraric acid, physodic acid and physodalic acid can be found in the medullary layer (Marques, 2013).

Various methods such as micro crystallography, classical spot tests, high performance liquid chromatography (HPLC), thin layer and paper chromatography, mass chromatography and gas spectrometry, chemical methods can be used for accurate identification of secondary metabolites. Secondary metabolites of lichens are synthesized by three chemical pathways: mevalonic acid pathway, acetatepolymalonate pathway and shikimic acid pathway. Lichen secondary metabolites exhibit a variety of biological activities, including antioxidant, antimicrobial, anticancer, antiviral, and antidiabetic (Ranković & Kosanić, 2019).

2.1. Antioxidant

Hidalgo et al. (1994) found that depsidons such as 10-chloropannarin, pannarin from *Erioderma chilense*, *Protousnea malacea*, *Psoroma pallidum* and *Placopsis* sp., and some depsids such as divaricatic acid and atranorin showed antioxidant activities. Atalay et al. (2011) found that seccaic acid, usnic acid (UA) and salazinic acid isolated from three terrestrial natural lichen species, *Ramalina pacifica*, *R. nervulosa*, *R. celastri*, are highly radical scavenger. They found that pulmonarianin,

rhizonyl alcohol, rhizonaldehyde and isidiophorin retarded lipid peroxidation in emulsion systems and liposome lipid peroxidation tests. They found that UA isolated from *Usnea barbata* and norstitic acid isolated from *Toninia candida* showed very good antioxidant activity. Kosanić et al. (2013) investigated the antioxidant effects of lichen metabolites in their studies on *Pseudoevernia furfuraceae, Evernia prunastri*, and *Hypogymnia physodes* lichens. As a result of all tests, they found that physodic acid, one of the metabolites, was the most effective antioxidant. Kosanić et al. (2014a) evaluated the antioxidant activities of *Cladonia rangiferina, C. furcata, C. pyxidata* lichens and found that atranorin had the highest antioxidant activity with an IC_{50} value of 131.48 µg/mL. When Kosanić et al. (2014b) examined the antioxidant activity of *Acarospora fuscata* lichen, they proved that gyrophoric acid has a strong antioxidant capacity in the DPPH radical scavenging activity experiment with an IC_{50} value of 105.75 µg/mL. Ristic et al. (2016a), in their antioxidant study on *Melanelia fuliginosa* and *Melanelia subaurifera* lichens, found that lichens showed antioxidant activity between 121.52 and 424.51 µg/mL IC_{50} values. In another study, investigated the antioxidant activity of acetone extracts isolated from *R. fastigiata* and *R. fraxinea* and found that they showed antioxidant activity between 285.45 and 423.51 µg/mL µg/mL IC_{50} values (Ristić et al., 2016b). Manojlović et al. (2012) investigated the antioxidant activities of lichen metabolites UA, protocetraric acid and salazinic acid obtained from *Parmelia saxatilis, P. sulcata* and *P. caperata*. As a result, they found that UA showed stronger antioxidant activity than protocetraric acid and salazinic acid. Selvaraj et al. (2015)

investigated the antioxidant activity of salazinic acid isolated from *Parmotrema reticulatum* and their derivative hexaacetyl salazinic acid. Their antioxidant properties, FRAP, DPPH, metal chelation, hydroxyl scavenging, lipid peroxidation, superoxide dismutase and phosphomolybdenum activity were investigated. As a result, they found that the derivative of the lichen metabolite and the metabolite exhibited high antioxidant activity. Melo et al. (2011) showed that atranorine exerts differential effects on reactive species production by increasing nitric oxide and hydrogen peroxide production, and acts as a superoxide scavenger. They also found that atranorin showed lipoperoxidation induced by the developed peroxyl radical in-vitro. Thadhani et al. (2011) evaluated *Parmotrema grayana*, *Cladonia* sp., *Roccella montagnei* and *Heterodermia obscurata* with the SOR scavenging test, the NOR scavenging test, and the radical scavenging test with DPPH. Researchers who achieved significant IC_{50} values reported promising antioxidant activities of lecanic and secicaic acid. Lücking et al. (2014) evaluated the antioxidant effect, total phenol content and flavonoid content of four lichen species in *Cora*, *Peltigera laciniata*, *Thamnolia vermicularis* and *Cladonia* genus and evaluated the relationship between total flavonoid and phenolic content and antioxidant effect statistically.

2.2. Antimicrobial

Honda et al. (2010) investigated the activity of metabolites of *Usnea subcavata*, *Pseudoparmelia sphaerospora*, *Parmotrema tinctorum* and

Parmotrema dilatatum lichens against *Mycobacterium tuberculosis*. They found that diffractic acid, one of the lichen metabolites, reached the most effective MIC value. Candan et al. (2007), in their study investigating the antimicrobial effect of petroleum ether, methanol, diethyl ether, chloroform and acetone extracts of *Parmelia sulcata*, showed that extracts other than petroleum ether extract were effective and salazinic acid also showed activity against *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Ranković & Mišić (2008a) investigated the antimicrobial activities of metabolites obtained from lichens *H. physodes*, *Umbilicaria polyphylla*, *Physcia aipolia*, *Parmelia conspresa*, *P. caperata*, *Ochrolechia androgyna*, and *C. furcata*. They found that physodic acid, gyrophoric acid, UA, lecanoric acid, fumarprotocetraric acid, and atranorin showed relatively strong antimicrobial effects against a variety of bacteria and fungi. In two different studies, found that metabolites obtained from *H. physodes*, *U. barbata* and *T. candida* showed significant antimicrobial effects (Ranković et al., 2008b; Ranković et al., 2012; Ranković et al., 2014). Kosanić et al. (2013) reported that physodic acid and evernic acid obtained from *P. furfuraceae* and *E. prunastri* lichens had antimicrobial effects and found that physodic acid was effective. In another study by Kosanić et al. (2014), they examined the antimicrobial activity of metabolites obtained from the lichen *A. fuscata*. They found that the most active compound in terms of antimicrobial activity was gyrophoric acid. Basile et al. (2015), in their study, found that acetone extracts of *Xanthoria parietina* lichen and parietin metabolite obtained from the lichen showed antibacterial and antifungal activities. Ristić et

al. (2016a) reported that methyl evernate isolated from *R. fastigiata* and obtusatic acid isolated from *R. fraxinea* showed inhibition against bacterial and fungal species. In another study, Ristić et al. (2016b) demonstrated antimicrobial properties for lecanoric acid from *Melanelia subaufera* and 20-O-methyl anziaic acid isolated from *Melanélia fuliginosa*. Sarıözlü et al. (2016) found that the barbatolic acid component of lichen *Bryoria capillaris* exhibited antibacterial and antifungal activities. Oh et al. (2018), in their study, observed that the divaricatic acid compound of *Evernia mesomorpha* has antimicrobial activity and is effective against gram positive bacteria.

2.3. Anticancer

Russo et al. (2006) in this study, the anticancer activities of the metabolites obtained from *Bacidia stipata*, *Protousnea magellanica*, *P. malacea*, *Psoroma dimorphum*, *Rhizoplaca melanophthalma*, *Sphaerophorus globosus*, *Psoroma palladium*, *P. reticulatum*, *P. pulchrum* and *Cornicularia epiphorella* lichen species were examined. They found that atranorin, difractaic acid and divaricatic acid are active against prostate cancer cells at a certain concentration (Russo et al., 2012). Poornima et al. (2016) *P. reticulatum, R. montagnei, P. reticulatum*, and *P. hababianum* they tested lichens on albino wistar rats using different cancer cell lines and investigated their effects. They found that *P. reticulatum* showed the most effective activity against the cervical cancer cell line. Brandão et al. (2013), *U. subcavata*, *P. dilatatum*, *Ramalina* sp., *Usnea* sp., *Cladonia confusa* and *Parmotrema*

lichexanthonicum some metabolites obtained from lichens (atranorin, divaritik, difraktaik, psoromik, perlatolik, and norstitik protosetrarik acids) normal fibroblast 3T3 cells and UACC-62, B16-F10 melanoma cell lines and has been tested against anti-cancer activity have also been reported. Paluszczak et al. (2018) evaluated the effects of depsides (squamatic acid, lecanoric acid, atranorine), depsidones (salazinic acid, physodic acid) and caperatic acid obtained from *Hyposenomic scalaris*, *H. physodes*, *P. sulcata, Cladonia uncialis* and *Platysmatia glauca* lichens on Wnt signaling in HCT116 and DLD-1 colorectal cancer cell lines. Among the compounds tested, they found that physodic acid exhibited the strongest inhibitory activity against HCT116 and DLD-1 cells at 50 μM, while caperatic acid had similar potential cytotoxicity to the reference compound atranorine at 100 μM. Cardile et al. (2017) investigated the cytotoxic activities of atranorine, gyrophoric acid and physodic acid isolated from *B. stipata*, *Ochrolechia deceptionis*, *Hypogymnia lugubris* lichens on A375 melanoma cancer cell line. As a result, they showed that physodic acid has good cytotoxic effect associated with apoptosis. In another study, Šeklić et al. (2018) investigated the effects of acetone, methanolic and ethyl acetate extracts of *Pseudevernia furfuracea* and *Platismatia glauca* lichens on colorectal cell lines HCT 116 and SW-480. They observed that the extracts showed significant cytotoxic effects on cancerous cells and that the highest activity after 72 hours on SW-480 and HCT 116 cells was in ethyl acetate and acetone extracts of *P. furfuracea* with potential apoptotic effects. Suh et al. (2017) evaluated the anticancer activity of *Ramalina terebrata* with cancer cell line HCT116 cells. They found that

ramalin from this lichen species showed anticancer activity at certain concentrations and induced apoptosis in HCT116 cells. They also found that ramalin induces modulation of genes that cause cell cycle arrest in G2/M phase at transcriptional and translational levels. Hong et al. (2018) studied the anticancer activities of lobarstin and lobaric acid obtained from *Stereocaulon alpinum*, an Antarctic lichen. In this study, a time- and dose-dependent significant reduction in proliferation was observed in the treatment of colon carcinoma HCT116 cells and human cervical adenocarcinoma HeLa cells with lobarstin and lobaric acid. As a result, they showed that these metabolites can be used as new agents in the clinical treatment of colon carcinoma and cervical adenocarcinum. Yang et al. (2015), in their study to find new lichen metabolites showing inhibitory activity against lung cancer cells in 13 lichen species they collected, found that physiosporin showed potential inhibition in extracts obtained from *Pseudocyphellaria coriacea*. They observed that physiosporin caused an increase in the metastasis reducing gene KAI 1 and decreased the activity of the 3'-untranslated region of KITENIN. They also saw a decrease in Cdc42 and Rac1 activity. In another study by the same researchers, they investigated similar inhibitory activities using the lichens *Usnea florida*, *Flavocetraria nivalis*, *Alectoria samentosa* and *Alectoria ochroleuca*, and found that the usnic acid metabolite of the lichens used in this study was a potential anti-cancer (Yang et al., 2016). Fernandez-Moriano et al. (2015) investigated the anticancer potential and neuroprotective effects of lichen extracts of *Vulpicida canadensis* and *Cetraria islandica*. In this study, they performed experiments on two CNS-like

cell models, human astrocytoma U373-MG and human neuroblastoma SH-SY5Y cell lines, to investigate the in vitro neuroprotective effects of depsidone fumarprotocetraric acid, an important secondary metabolite of *C. islandica*. Researchers exposed cells to H_2O_2 to mimic cellular oxidative stress, which is an important marker in the detection of neurodegenerative diseases, and the extracts promoted astrocyte survival by reversing the oxidative damage caused by H_2O_2. They found that both extracts have anticancer activity, usnic, pinastric and vulpinic acid in *V. canadensis* and fumarprotocetraric acid in *C. islandica* may cause these biological effects.

2.4. Antiviral

Lai et al. (2013) investigated the viability of 13 phenolic compounds from *Ramalina farinacea* lichen, secaic acid, HEp2 and Vero cell lines, which showed a strong inhibitory effect against A2 respiratory syncytial virus strain and rg respiratory syncytial virus and revealed that secaic acid interferes with viral replication. Vu et al. (2015) evaluated the anti-hepatitis C virus (HCV) activity of the atranorine metabolite from *Stereocaulon evolutum*. To expand the study of atranorine, they synthesized two more analogues. They found that these compounds were active against HCV and that depsids were more potent than monoaromatic phenols. They have proven to have anti-viral activity. In two studies, investigated the inhibitory effect of UA derivatives obtained from *Usnea* sp. and *Cladonia stellaris* lichens against influenza viruses at certain concentrations and saved mice from fatal

influenza infection (Shtro et al., 2014; Shtro et al., 2015). In their study, where Premanathan et al. (1999) examined the activities of the extract obtained from the bark of *Rhizophora mucronata* lichen against human immunodeficiency virus (HIV), they observed that the alkaline extract inhibited viral binding and syncytium formation.

2.5. Enzyme Inhibition

In the study of Hengameh et al. (2016) *Heterodermia leucomelos*, *Ramalina sinensis*, *P. reticulatum* and *Herpothallon* sp. evaluated the extracts of lichens and their α-amylase inhibitory activity. In the studies, the highest inhibition was observed in *H. leucomelos*, and the lowest inhibition activity was observed in *R. siensis* extracts. In the study of Tekale (2018), amylase inhibitory activity of *Parmelia perlata* lichen extracts was tested. They found that methanol extract showed maximum activity and aqueous extract showed minimum activity. Salin Raj et al. (2014) investigated the inhibitory effect of ethyl acetate extracts of *P. tinctorum*, an edible lichen species, against α-glucosidase, α-amylase and aldose reductase (AR) enzymes. They reported that the extract has a significant IC_{50} value and has potential effects against these enzymes. Lee & Kim (2000) determined that lichen *Umbilicaria esculenta* extracts showed an inhibitory effect against β-glucosidase. They also reported that the extracts seriously inhibited mold and mammalian disaccharide hydrolytic enzymes, and that the lichen extracts of *Parmelia praesorediosa* and *P. austrosinensis* showed similar effects.

2.6. Anti-Neurodegenerative

de Paz et al. (2010) tested *Xanthoparmelia* spp. metabolites on U373 MG cells in their study and observed that lichen extracts protected U373 MG cells from hydrogen peroxide-induced damage. Based on the results obtained, they proved that these lichen extracts can be used as agents against oxidative damage-related neurodegenerative diseases such as Parkinson's and Alzheimer's. Pejin et al. (2013) found that the metabolites they isolated *from Lobaria pulmonaria* showed acetylcholinesteresis (AChE) inhibiting activity but did not show any activity in the AChE test of stichic acid on the TCL plate. Similarly, in the acetylcholinesterase tests performed by Luo et al. (2013) in *Cladonia mucilenta*, they found that a compound isolated, biruloquinone, exhibited dose-dependent inhibitory activity against acetylcholinesterase with an IC_{50} value of 27.1 mg/mL. Thadhani et al. (2014) found that lobaric acid extracted from *Heterodermia* sp. showed IC_{50} values of 36.76 μM and 26.86 μM against butyryl-cholinesterase (BChE) and AChE, respectively. Based on these results, they revealed that lobaric acid could be a potential immunomodulatory agent. Emsen et al. (2016) investigated the antitumor potential of psoromic acid (PSA) isolated from *R. melanophthalma* lichen and physodic acid (PHA), olivetoric acid (OLA) lichen secondary metabolites isolated from *P. furfuracea* lichen on PRC C cells and human U87MG-GBM cell lines. As a result of their study, these metabolites showed a positive correlation with the concentration, oxidative DNA damage and LDH

activity, and showed that OLA and PSA have a high potential for use in GBM treatment.

2.7. Anti-genotoxic

In the study of Shibamoto & Wei (1984), which is one of the first studies to investigate the genotoxic effects of secondary metabolites of lichens, researchers tested UA, physodalic and physodic acids isolated from *Hypogymnia enteromorpha* lichen for mutagenicity in the Ames Salmonella microsome test. They found that physodalic acid exhibited dose-dependent mutagenicity against *S. typhimurium* strain TA 100. Koparal et al. (2006) investigated the genotoxic and cytotoxic activities of (+) and (-) UAs isolated from *Cladonia foliacea* and *R. farinacea* lichens in their study. In their study, they used human lung carcinoma epithelial-like A549 and Chinese hamster lung fibroblast-like V79 cell lines. As a result, they found that even low-dose UA had an effect against cancer cells without any genotoxic effect. In the study of Mayer et al. (2005), they investigated the non-genotoxic anticancer activities of important lichen metabolites UA (+ and -) on lung cancer cell lines H1299 and breast cancer cell lines MCF7 and MDA-MB-231. They found that all three cell lines were affected by usnic acid by MTT analysis and IC_{50} values, and that this metabolite could be a non-genotoxic anticancer. da Rosa Guterres et al. (2017), in their study on *Pseudoparmelia sphaerosphora, P. dilatatum, Parmotrema cetratum* and *Usnea jamaicensis* lichens, observed that psoromic and salazinic acid metabolites significantly reduced genetic damage caused by

Doxorubicin. They found that when tested on somatic cells of *Drosophila melanogaster*, it had an antigenotoxic effect and the compounds did not show mutagenicity. Anar et al. (2016) investigated the antigenetoxic and antioxidant activities of *Xanthoria candelaria*, *Anaptychia ciliaris*, *Parmotrema chinensa* and *Bryoria fuscescens* lichen species and found inhibitory mutagenic activities against toxic aflatoxin B1 at a concentration of 20 μg/ml. They also found that these lichen species have strong antioxidant activity.

2.8. Anti-diabetic

Karunaratne et al. (2014) isolated zeorin, methylorellinate and methyl ß-orcinolcarboxylate metabolites from lichen *Cladonia* sp. in their study to discover α-glucosidase inhibitors and develop therapeutics for diabetes treatment. They reported that these three isolated metabolites showed α-glucosidase inhibitory effect and other isolated metabolites (Atranorine and lobaric acid) did not show this effect. In another study, Verma et al. (2012) investigated the α- and β-glucosidase inhibitory effects of the metabolites they isolated from *R. pacifica*, *R. celastri* and *R. nervulosa*. They showed that secicaic acid isolated from *R. nervulosa* and salazinic acid isolated from *R. celastri* had competitive inhibition against α-glucosidase activity and non-competitive inhibition against β-glucosidase activity. They also found that UA isolated from *R. pacifica* showed noncompetitive inhibition of two glucosidase activities. Seo et al. (2009a) tested metabolites isolated from methanol extracts of *Umbilicaria antarctica* for PTP1B inhibition and treatment of type 2

diabetes. They found that gyrophoric acid, lecanoric acid and methyl orsellinate from isolated compounds inhibited PTP1B activity, and gyrophoric acid inhibited PTP1B non-competitively. In another study, the same researchers investigated PTP1B inhibition by using metabolites in methanol extracts of *S. alpinum*. In their study, they found that one of the pseudodepsidon compounds and lobaric acid inhibited PTP1B activity non-competitively. A few years later, the same researchers tried the same experiment, this time with metabolites in methanol extracts of *Lecidella carpathica*, and found that hopane-6α,22-diol and brialmontin 1 metabolites suppressed PTP1B inhibition competitively (Seo et al., 2009b; Seo et al., 2011). Cui et al. (2012) used diterpene furanoid metabolites of methanol extract of Antarctic lichen *Huea* sp. to detect PTP1B inhibition in these studies, they found that the first of the four isolated compounds exhibited inhibitory activity against the targeted protein, and the diterpene furanoids led to non-competitive inhibition of PTP1B activity. They reported that the alkaloid fractions and glycoside they purified showed α-amylase inhibition and could shed light on anti-diabetic studies. In the review of lichen compounds prepared by Thadhani & Karunaratne (2017) as antioxidative and antidiabetic potential agents, they explained that especially *Ramalina* sp. and *Parmotrema* sp. lichen extracts are quite efficient in these matters. Valadbeigi & Shaddel (2016) investigated amylase inhibition of lichens *Ramalina pollinaria*, *Usnea articulata*, *R. hyrcana*, *Flavoparmelia caperata*, *Cladonia rei*, *Punctelia subrudecta*, *Parmotrema chinense*, *P. borreri*, *Peltigera praetextata* and *Hyperphyscia adglutinata*. As a result of their studies, they found the

highest inhibition at 75mg/ml in *U. articulata* extract as 60%. Shivanna et al. (2015) found that methanol extracts of *Flavoparmelia caperata* had the highest inhibition as a result of alpha amylase tests performed on *P. aipolia* and *F. caperata* lichens. Researchers have suggested that the inhibitory properties of both lichen extracts can be used in the development of agents involved in regulating diabetes.

2.9. Insecticidal

In a comprehensive review by Sachin et al. (2018), they reported that lichen metabolites may also have insecticidal activities, and for example, atranorine, usnic acid, vulpinic acid, barbatic acid, fumarprotocetraric acid, diffractic acid and norstictic acid. In the study by Emsen et al. (2015), extracts of *Peltigera rufescens*, *Letharia vulpina*, and *Lecanora muralis* lichen were tested against *Sitophilus granarius* adults. As a result of the study, they concluded that *L. vulpina* and *P. rufescens* lichens are more toxic and have higher insecticidal activities. In their study of the effects of lichen secondary metabolites on *Culiseta longiareolata*, Çetin et al. (2012) reported that gyrophoric acid, (+)-UA, atranorine and 3-hydroxyphysodic acid showed increased toxicity, respectively, and as a result, lichen metabolites could be potentially larvicidal. Martins et al. (2018) investigated the insecticidal activity of secondary metabolites of usnic acid, fumarprotocetraric acid and barbatic acid obtained from the lichens of *Cladia aggregata*, *Cladonia verticillaris* and *Cladonia substellata* against *Nasutitermes corniger*. Kekuda et al. (2015) revealed that the extracts of *Dirinaria*

applanata and *Parmotrema cristiferum* have insecticidal activity against *Aedes aegypti*. In another study, Moreira et al. (2016) found that (+)-UA and 2-hydroxy-4-methoxy-6-propyl-methyl benzoate compounds from methanolic extracts obtained from *Ramalina usnea* lichen had larvicide activity against *Aedes aegypti*. Khader et al. (2018), in their study, tested and found that methanolic extracts of *Parmotrema kamatti, P. reticulatum, P. tinctorum, Leptogium papilosum, Parmelia erumpens* and *Roccella montagnei* lichens showed insecticidal activity against *Anopheles stephensi, Culex quinquefasciatus* and *A. aegypti*.

CONCLUSION

Lichens are used in many areas thanks to their various biological activities. Lichen can grow in many parts of the world thanks to the photobiont and mycobiont diversity it contains. The fact that it can be grown in different parts of the world and its content diversity provides important contributions to both the ecosystems it is in and the studies on human health. Examination of lichens and their metabolites may be important in adding new studies to the literature and improving existing studies.

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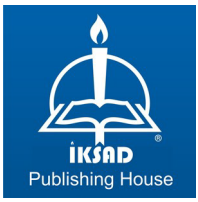

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