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Theory, Current Researches and New Trends

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PREFACE

On the global level world is undergoing changes that shape the agricultural systems, and these changes have always had a significant impact on people's lives throughout time. Therefore, agricultural research areas has faced new trends and challenges every year. Agricultural sustainability can be managed by interdisciplinary approaches, suggesting improving the benefits of ecological and agronomic management and redesign. This book was undertaken to lay out the importance of new trends and opportunities in agriculture and food systems.

We thank the authors for contributing and providing an up- to- date reviews and researches in the field of agri-food management. We also thank reviewers, who are our esteemed colleagues, for the evaluation of the manuscripts.

Finally, we expected that this book would present valuable information, data, and perspectives on some agricultural problems and becomes a good source for future studies.

Editors

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CHAPTER I

AN OVERVIEW OF STRESS MANAGEMENT AND KAOLIN PARTICLE FILM IN VITICULTURE

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Introduction

The Eurasian grape is *Vitis vinifera* L. and, this species involves the majority of the varieties used for viticulture. Although there are large differences in the morphological and biochemical characters of the varieties, the aim of grape growing is to obtain an economically sufficient amount of yield in the desired quality, depending on the consumption type. Therefore, the main subject of viticulture is the relationship between the phenological cycle of the varieties and the climatic conditions of a region. Vines are grown in a wide variety of climatic situations. However, the best grape growing areas in the world are between the 35th and the 50th parallels in the Northern Hemisphere and the 30th and the 45th parallels in the Southern Hemisphere. Outside of the given areas, grape growing becomes problematic both production and quality level. On the other hand, even in the appropriate regions, the changing climates depending on the geographic differences, can be a threat for grape growing, such as frost injury or sunburn. In viticulture, it is thus essential to study the relations between the climate, the vineyard, and the grapevine.

Among the climatic factors, light and temperature are determinative in the formation of environmental conditions that affect the growth and yield of grapevines. Besides the optimum effects of climatic factors, recently, the combination of the high sunlight and the high temperature, which are more severe in the summer season and water deficit, which is a close relation with them, is expressed under the term of the summer stress. Some exogenous compounds are experimented to mitigate environmental stress. By the way, that compounds are expected to maintain and even improve productivity and quality characteristics (Dinis et al., 2016; Bernardo et al., 2018). Based on the scientific climate projections worldwide, climate change is a risk on Mediterranean region viticulture due to the stress effects of extreme weather events. It is prompted to search for cost-effective and eco-friendly stress mitigation strategies for economically sustained viticulture. Kaolin, which is a chemically inert mineral with excellent reflective properties, is a promising foliar stress mitigation treatment for sustainable viticulture.

Heat and Light Stress Effects on Grapevine

Solar radiation and temperature are essential for grapevine metabolism. The effects of these two parameters on berry composition and metabolism have been known for many years, and they are considered as primary factors for the production of high-quality table and wine grapes (Bergqvist et al., 2001; Spayd et al., 2002). Although climatic factors affect the entire growth cycle of grapevine, yield and quality are quite sensitive to heat waves at critical phenological stages such as flowering and ripening (Teixeira et al., 2013). Physiological processes of grapevine begin when the temperature reaches to 10ºC. High temperatures over 35ºC during the active growth period cause heat stress and physiological response to heat stress begin within grapevine metabolism (Ferrandino and Lovisolo 2014; Keller, 2010).

Sunlight that reaches the earth has the forms of long, medium, and short-wavelength rays, each with its characteristic effects on plant growth. Long-wavelength rays are infrared (IR) rays with a wavelength longer than 700 nm. Medium wavelength rays are known as "photosynthetic active radiation (PAR)" with a wavelength between 400 - 700 nm. These rays, which are absorbed by the chloroplasts and used as the energy source for photosynthesis, are of vital importance for plants. Short wavelength rays are called ultraviolet (UV) radiation (195-400 nm) that harmful for plant life. They degrade DNA structure, prevent photosystem II and photorespiration in plants (Glenn and Puterka, 2005). However, UV treatments under controlled conditions have highly specific effects for improving secondary plant metabolites production in many plant species. In fact, UV irradiation is an elicitor to increase the synthesis of phenolics in grapevines (Keskin and Kunter 2008, 2017). Mainly short-wavelength UV-C irradiation (254 nm) is described to be a good elicitor for the induction of anthocyanin production (Oğuz et al., 2020) as well as individual phenolics (Çelik et al., 2020) in grape callus cultures.

Intense solar radiation and high temperature, which are typical in arid and semi-arid ecologies, prevent carbon assimilation by increasing the temperature inside the canopy and result, berry quality decrease. Temperatures above 30ºC are associated with a higher transpiration rate and may cause lower berry turgor, lower berry weight, and size via low photosynthetic rate. Also, berry and leaf tissues may be damaged (Fig. 1). It is reported that the optimum temperature for grape berry growth ranges between 25 to 30 °C (Dokoozlian and Kliewer 1996; Bergqvist et al., 2001; Spayd et al., 2002; Teixeira et al., 2013).

Although high temperature alone is a stress factor, its severity increases even more under conditions accompanied by intense sunlight exposure (Glenn and Puterka 2005; Shellie and King 2013a). Therefore, it is necessary to consider the effect of solar radiation and temperature together. It is known that the berry temperature paralleled the diurnal solar radiation curve (Millar, 1972). During the day, short wave radiation has a primary effect on cluster warming. The temperature of sun-exposed leaves and clusters in the canopy was determined to be 5-10ºC higher than those in the shade. As a result of these temperature changes in canopy microclimate, significant differences occur in berry composition (Spayd et al., 2002; Belancic et al., 1997). Under high-temperature conditions, *véraison* occurs earlier; therefore, development and accumulation of primary and secondary metabolites may be reduced. In berries grown under open canopy conditions, compared to berries under shaded canopy conditions, have higher soluble solid, improved acid balance (lower pH and higher titratable acidity). However, while some extent of sunlight may be appropriate, high temperatures resulting from full exposure of berries may inhibit anthocyanin metabolism (Teixeira et al., 2013; Haselgrove et al., 2000). It is stated by many researchers that adequate PAR (100 μmol m^{-2} s^{-1}) in the cluster zone leads to increase soluble solid and decrease total phenols and acidity (Dokoozlian and Kliewer 1996, Bergqvist et al., 2001; Spayd et al., 2002; Teixeira et al., 2013). In warm climates, phenolic and anthocyanin concentrations increase due to high PAR activity and continue until the heat reaches the damaging critical limit for biosynthesis (Tarara et al., 2008; Spayd et al., 2002). The optimum conditions for anthocyanin accumulation in the life cycle are the vines are exposed to mild day (25ºC) and cool night (15ºC) temperatures during the ripening period (Teixeira et al., 2013; Gökçen et al., 2017). Higher temperatures up to 30-35ºC lead to anthocyanin degradation in berries.

High night temperatures reduce the synthesis of UFGT (flavonoid 3-O-glucosyl transferase), which is one of the essential enzymes for anthocyanin biosynthesis (Mori et al., 2007). In warmer years, it is stated that in addition to total anthocyanin content, anthocyanin composition has also changed. Warm climate berries tend to have a higher proportion of malvidin, petunidin, and delphinidin derivatives, while in the cool climate, berries have more peonidin and cyanidin (Downey et al., 2006). Chemical and enzymatic degradation of anthocyanins is also influential in decreasing the berry skin anthocyanin under high-temperature conditions (Ferrandino and Lovisolo, 2014). High temperatures promote oxidative stress that causes the formation of H_2O_2, peroxidase, and other oxidoreduction enzymes responsible for the degradation of anthocyanins, and as a result, total concentration in berry decreases (Mori et al. 2007).

Aromatic volatile compounds in grapes and wines are also sensitive to sunlight and temperature. Although it is known that there is a clear relationship between aroma composition and solar radiation, there are different opinions about the nature of this relationship. In some studies, the authors concluded that different aroma groups react differently to sunlight exposure. It is reported that cultural practices that change the amount of sunlight on grapevines are important in promoting the accumulation of aromatic compounds in grapes (Duan et al., 2014; Clingeleffer, 2009). Ji and Dami (2008), determined that cool temperatures increased the concentration of C_6 compounds, while warmer temperature conditions increased the monoterpene concentration of cv. Traminette.

Kaolin Particle Film as a Stress Mitigation Strategy

To date, several practices have been used in viticulture, such as canopy management techniques and shading nets, to reduce the effect of excessive sunlight exposure and high temperature. The favorable results of these practices, such as reduce plant temperature, increase soluble solid, anthocyanin accumulation, and stomatal conductance, are known (Iacono et al., 1995; Spayd et al., 2002; Chorti et al., 2010). However, the fact that these techniques require much time and labor force, so this situation has forced researchers to find different alternatives (Lobos et al., 2015). On this subject, chemical treatments have also been carried out for many years to reduce yield and quality losses caused by solar radiation or heat stress as well as other environmental stress factors. However, using chemicals is not sustainable due to their harmful effects on the environment and pests that develop resistance to subsequent uses of chemicals. Therefore, it is crucial to apply chemical-free practices to minimize the effects of summer stress (Yazıcı and Kaynak, 2007). There are applications to regulate and reduce solar radiation on leaf and fruit surfaces without preventing photosynthesis and gas exchange. Clay containing reflective films reduce ultraviolet and infrared radiation by forming a white reflective layer on the surfaces similar to the cuticle that is naturally found in leaf structure (Fig. 2) (Glenn and Puterka, 2005). The use of kaolin-based reflective films against biotic and abiotic stress factors is known as "Particle Film Technology." The most widely used particle film is a processed clay mineral "kaolin" that has a light-reflecting property.

Kaolin is white, non-porous, non-swelling, low-abrasive, fine-grained aluminosilicate mineral [$Al_4Si_4O_{10}(OH)_8$], and easily dispersible in water. Aluminum silicate is the secondary mineral formed by the breakdown of feldspar and quartz minerals found in nature. Different formulations of kaolin are generally produced by adding spreading and sticking agents and different herbal extracts to kaolinite, bentonite, and attapulgite (Glenn et al., 1999). It is reported that processed kaolin is more effective in reflecting UV radiation than unprocessed kaolin. The formulation and particle size of kaolin greatly influence its UV reflection ability (Glenn and Puterka, 2005). PAR amount that can reach the chloroplasts without being reflected or absorbed by the particle film during photosynthesis is critical. In this context, particle size and light transmission properties of kaolin are the physical and optical properties to be known. Characteristics of an effective particle film were described by Glenn (2005) as; (1) chemically inert, (2) particle diameter <2 μm, (3) create a uniform film layer, (4) porous structure that does not inhibit gas exchange from the leaf surface, (5) transmits photosynthetic active radiation (PAR) but excludes some extent ultraviolet (UV) and infrared (IR) radiation, (6) effective on insects and pathogens on the plant, and (7) can be removed from the harvested product. Many of these properties are similar to natural plant defense mechanisms. Plants respond to UV-B rays by thickening the cuticle layer and synthesizing compounds that absorb UV-B rays (Glenn 2005; Tevini, 1999). Particle films act as a cuticle layer and artificially increase the leaf thickness, thus increase the distance of the rays to the target cells in leaf tissue, thereby reduce UV radiation damage (Fig. 3). As the particle film layer on leaf and berry increases, the ability to reflect UV rays also increases (Glenn et al., 2002). The studies on grapevine reported that kaolin particle film treatment reduced the leaf and fruit zone temperature between 0.5-5.7 ºC (Lobos et al., 2015; Shellie and Glenn, 2008; Cooley et al., 2008; Coniberti et al., 2013; Canturk et al., 2019; Brillante et al., 2016; Glenn et al., 2010). Kaolin is also reported to improving water use efficiency in plants, and studies above mentioned were mostly integrated with irrigation management. Promising results have been obtained in terms of plant water consumption and water management, especially in arid and semi-arid ecologies with limited water and irrigation potential.

Kaolin particle films have gained a wide range of usage in time. In addition to reducing environmental stress and regulating fruit yield and quality in agricultural products, it is also used to reduce disease and insect damage and prevent frost injury in many plant species (Glenn et al., 1999; Puterka et al., 2000).

The use of stress-reducing materials like kaolin in viticulture has not been attracted much attention due to the adaptation ability of grapevine to dry conditions. Several studies have been conducted about the effects of kaolin particle films on grape cultivars, and promising results have been reported in terms of berry composition and wine quality. Many authors determined that it contributes to the increase of anthocyanin and total phenolic compounds, especially when applied with different cultural practices and different irrigation levels (Song et al., 2012; Shellie and King, 2013b; Canturk et al., 2019; Kök and Bal, 2017; Brillante et al., 2016). Although different effects are obtained in various aroma compounds in grapes and wines, no significant effect has been determined on the aromatic composition generally (Ou et al., 2010; Song et al., 2012, Canturk et al., 2018), few studies have reported that aroma compounds have increased with kaolin treatment (Coniberti et al., 2013; Kök and Bal, 2017). The authors also did not report significant effects on yield, cluster and berry properties and ripening parameters (Cooley et al., 2008; Canturk et al., 2019; Shellie and Glenn, 2008; Ou et al., 2010; Song et al., 2012; Shellie and King, 2013b; Lobos et al., 2015). In contrast to wine grapes, studies on the effects of kaolin particle film on table grapes have been very limited due to the negative effect of white stained clusters on visual quality. According to the results of few studies that have been done, it is concluded that kaolin particle film is also a useful method for table grapes if applied with care not to cover the clusters with kaolin (Canturk et al., 2018, 2019; Kök and Bal, 2017; Bedrech and Farag, 2015).

Conclusion

Evaluation of climatic evidence has pointed us that the tendency of the temperature increase will continue in the worldwide viticultural regions. Within the climate change another related factor is the increased incoming radiation. However, grapevine has a wide adaptation ability to different climatic conditions; the recent past has shown us to plan strategies in order to continue to produce high yield and quality in the vineyards. Among the adaptation strategies, solar protectants like kaolin are clearly eco-friendly applications to mitigate climate change effects by interaction with viti-viniculture practices. Researches have shown that particle film treatments induce metabolic pathways of secondary metabolites, which are responsible for organoleptic characteristics associated with better grape berry quality. Additionally, it will not wrong to say; foliar kaolin application improves the nutraceutical value of grapes when mitigation practices are used.

Figure 1. Solar injury in grapevine leaves (Photo: Sevil Cantürk)

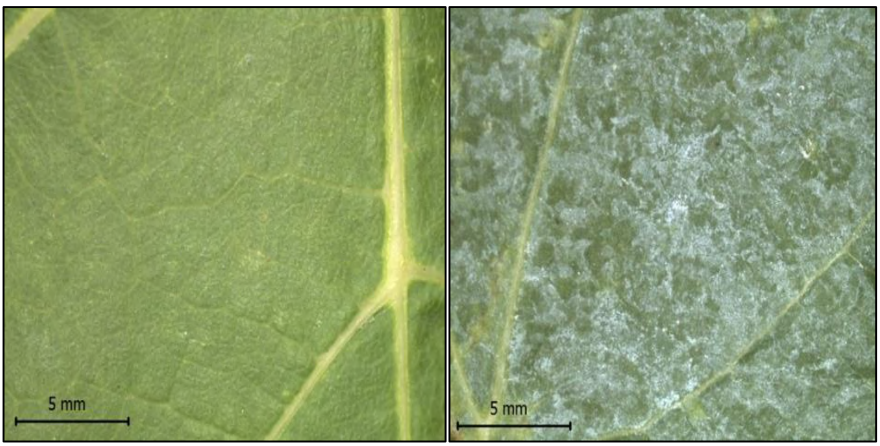

Figure 2. Kaolin particle film treated (right) and control (left) leaf surface under a binocular microscope (Photo: Sevil Cantürk)

Figure 3. Kaolin particle film treated grapevines (Photo: Sevil Cantürk)

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CHAPTER II

DIFFERENCES TO CHEMICAL CONSTITUENT OF AUTOCHTHONOUS GRAPE CULTIVARS IN SIIRT PROVINCE OF SOUTHERN EAST TURKEY

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Introduction

Grape berries are highly complex biochemical units. During development and maturation period, they undergo consecutive modifications in terms of size and composition. By intaking of water, sugars, organic acids, vitamins, and minerals, grape berries, synthesize phenolic compounds and aromatic substances (Kunter et al., 2013).

Grape is defined as a healthy antioxidant fruit with its content. This fruit also helps the growth of bones and teeth with having the mineral substances and providing appropriate pH value for blood (Keskin et al., 2019). Phenolics in grape are primary metabolites absolute for the growth and development of living things such as carbohydrates, protein and fat health by not affecting however affecting physiological and cellular activities secondary metabolites that have positive effects on it (Keskin et al., 2009). The phenolic content of fresh and processed grape products, as well as extract, offers alternative therapies against many diseases affecting human life quality mainly coronary heart diseases and cancer (Gökçen et al. 2017). The presence and proportion of the phenolic compound in grape berries are species and cultivars' characteristics that primarily controlled by genetic, and the amount in the content is formed with depending on the climate and soil effects, maturity and cultural practices in the field of cultivation (Keskin et al., 2018).

Minerals are essential for grapevine health and sustainable for grape production. Mineral compositions of the berries are affected by environmental factors such as soil, temperature, rainfall, sunshine and wind as well as many other growing conditions.

On the other hand, in our country, which has different climatic characteristics, viticulture activities are distributed to different geographical regions. The fact that these regions have different characteristics in terms of climate and soil conditions also constitute essential differences in terms of biochemical compounds that directly affect the quality elements of our grape varieties (Gundesli et al., 2018).

Siirt province, located in the South-Eastern Agricultural Region is one of the highest values of the country in terms of average temperatures and EHS (Effective Heat Summation) during the growing period. This province is suitable for growing of the table, raisin, must, and wine grape cultivars, in point of climatic conditions; and besides, it has the chance to grow all varieties in the extended maturity period, from the earliest to the latest (Keskin et al., 2018).

In this study, mineral and individual phenolic compound contents of four native grape varieties grown in the same vineyard in Siirt province ecology were determined.

Materials and methods

Four native grape cultivars (‘Bağılti’, ‘Besirane’, ‘Emiri’, ‘Tayfi’) with coloured skin which have grown in the Siirt province were used in this study. These cultivars were grown on their roots and trained with traditionally globe-shaped. When total soluble solids (TSS) was 19.0-23.0%, ten vines representing the cultivars were identified, and 3 clusters were harvested from each vine. The clusters were carried in ice containers to the laboratory. Then they stored at - 20 °C until mineral content analyses.

Chemicals

In this study, Phenolics standards were obtained from Sigma-Aldrich (St. Louis, MO, USA) and chemicals with analytical purity were used.

Mineral content analysis

The berries were washed with tap water and wiped. Then they were placed in a paper bag and dried at 65 °C in the oven and then ground by grinding machine (Ika, Germany) (Kacar and Inal 2008; Çavuşoğlu 2018). 200 mg of the ground sample was weighed and placed in an incinerator with 100 ml. Then 2 ml of 67% nitric acid and 8 ml of H2O2 were added. When clear and colourless solution was obtained; the amounts of Phosphorus (P), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Boron (B) and Selenium (Se) were determined in the extracts with external standards of ICP–OES device; while Potassium (K) content was determined with AAS.

Extraction and determination of phenolic compounds

The individual phenolics were determined via HPLC and separation method described by Rodriguez-Delgado et al. (2001). 100 g of the grape whole berry was taken, and 3 g from each sample was transferred to centrifuge tubes. The samples were homogenized then diluted 1:1 with distilled water and centrifuged at 15.000 × g for 15 min. The supernatant was passed through a 0.45 μm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), then injected into the HPLC system (gradient). The chromatographic separation in Agilent 1100 series HPLC took place in DAD detector (Agilent, USA) with 250 mm × 4.6 mm, four μm ODS column (HiChrom, USA). The following solvents in water with a flow rate of 1 ml/min and 20 μl injection volume were used for spectral measurements at 254, and 280 nm: as mobile phase solvent A: methanol-acetic acid-water (10:2:88) and Solvent B: methanol-acetic acid-water (90:2:8)

Statistical analysis

Descriptive statistics for the mineral and phenolic contents were presented as mean and Standard error of the mean. One-way ANOVA was used to compare cultivars. In addition, multidimensional scaling was performed to indicate visual representation of proximities for the characteristics. In multidimensional scaling, ALSCAL algorithm was utilized, and Euclidean distance was computed for distance measurement. Convergence value was fixed as 0.001 for the calculation of stress value. Statistical significance levels were considered as 5%, and SPSS (ver: 13) statistical program was used for all statistical computations.

Results and Discussion

The results for the mineral content of grape cultivars are presented in Table 1. As seen in Table 1, the difference between the varieties was found statistically significant in terms of all mineral substances. Accordingly, when Table 1 is examined in terms of phosphorus; while the highest average belongs to the ‘Bağılti’ variety with 223.450 mg/kg. This was followed by ‘Tayfi’ with 199.180 mg/kg. The lowest average was observed in the ‘Emiri’ with 169.265.

When Table 1 is analyzed in terms of potassium, the highest average was observed in ‘Emiri’ with 2030.78, while the lowest average was observed in ‘Tayfi’with 1460.745. There was no statistically significant difference between ‘Besirane’ and ‘Bağılti’ cultivars.

Table 1. Descriptive statistics and comparison results of minerals

Mineral	Cultivar	Mean	Std. Error	p	Mineral	Cultivar	Mean	Std. Error	p
P	Emiri	169.265 d	2.925	0.001	**Mn**	Emiri	.895 a	.0250	0.001
	Tayfi	199.180 b	4.910			Tayfi	.155 b	.0350	
	Besirane	183.005 c	.145			Besirane	.175 b	.0450	
	Bağılti	223.450 a	1.700			Bağılti	.835 a	.0050	
	Total	193.058	6.095			Total	.464	.1106	
K	Emiri	2030.78 a	40.400	0.001	**Cu**	Emiri	.479 c	.0300	0.001
	Tayfi	1460.745 c	7.325			Tayfi	.157 d	.0185	
	Besirane	1736.985 b	41.555			Besirane	.088 d	.0050	
	Bağılti	1689.575 b	64.245			Bağılti	.757 a	.0235	
	Total	1708.794	63.738			Total	.4205	.0870	
Ca	Emiri	296.125 b	2.265	0.001	**Zn**	Emiri	1.940 a	.0500	0.008
	Tayfi	370.570 a	2.650			Tayfi	1.610 b	.0300	
	Besirane	264.720 c	3.530			Besirane	1.550 b	.1200	
	Bağılti	300.755 b	4.375			Bağılti	1.555 b	.0150	
	Total	296.034	14.090			Total	1.601	.0668	
Mg	Emiri	89.925 ab	4.69500	0.001	**B**	Emiri	.0440 c	.00200	0.001
	Tayfi	96.690 a	1.55000			Tayfi	.0460 c	.00300	
	Besirane	74.175 c	1.76500			Besirane	.0435 c	.00250	
	Bağılti	95.270 ab	1.61000			Bağılti	.0960 a	.00200	
	Total	88.380	2.83895			Total	.0618	.00734	
Fe	Emiri	7.960 a	.18000	0.001	**Se**	Emiri	16.920 b	.1700	0.001
	Tayfi	6.445 b	.27500			Tayfi	15.365 c	.5550	
	Besirane	6.480 b	.36000			Besirane	13.520 d	.2600	
	Bağılti	8.510 a	.04000			Bağılti	19.020 a	.3900	
	Total	6.999	.36889			Total	15.555	.7523	

In terms of calcium, ‘Tayfi’ has the highest average with 370.570 mg/kg, followed by ‘Bağılti’ with 300.755 mg/kg and ‘Emiri’ with 296.125 mg/kg. The lowest average was observed in ‘Besirane’ with 264.720 mg/kg (Table 1).

In terms of magnesium, the highest average was observed in ‘Tayfi’ with 96.69 mg/kg. Although the difference from this variety is not essential, this was followed by the ‘Bağılti’ with 95.207 mg/kg, and the ‘Emiri’ with 89.925 mg/kg and the lowest average was observed in the ‘Besirane’ variety with 74.175 mg/kg.

When examined in terms of iron content, although the difference from the ‘Emiri’ is not statistically significant, while the highest average was observed in the ‘Bağılti’ with 8.510 mg/kg, the difference between the other two varieties was not statistically significant.

The result similar to iron was also obtained in terms of Mangan, and there was no difference between the ‘Emiri’ and the ‘Bağılti’ cultivars, while there was no difference between the ‘Tayfi’ and ‘Besirane’ cultivars. However, these two cultivars were statistically significantly lower than the other cultivars.

When the varieties are examined in terms of copper; while the highest average is 0.757 mg/kg, while it is the ‘Bağılti’ cultivar. This was followed by ‘Emiri’ with 0.479 mg/kg although the difference from ‘Tayfi’ is not significant. The lowest average was observed in ‘Besirane’ with 0.088 mg/kg.

In terms of zinc, the ‘Emiri’ cultivar ranks first with 1.94 mg/kg, while ‘Tayfi’ there was no statistically significant difference between ‘Besirane’ and ‘Bağılti’ cultivars.

When Table 1 is examined in terms of boron; while the highest average was observed in ‘Bağılti’ variety with 0.096 mg/kg, there was no statistically significant difference between the other three cultivars.

Finally, when Table 1 is examined in terms of selenium; while ‘Bağılti’ had the highest average with 19.02 mg/kg, ‘Emiri’ with 16.92 mg/kg and ‘Tayfi’ with 15.365 mg/kg. The lowest average was observed in ‘Besirane’ with 13. 520 mg/kg.

The copper content of grape varieties grown in Mediterranean region (Isparta/Şarkikaraağaç) varied between 0.20-0.33 mg/kg, while Zn content varied between 2.40-4.30 mg/kg (Şamil et al. 2005). Potassium generally exists in high quantities in grape berries. It is known to play an important role in fruit development and wine quality (Martins et al. 2012). Cantürk et al. (2016) determined that potassium content in the seed, flesh and berry skin of ‘Gülüzümü’ (Central Anatolian region) was 205.23, 112.78 and 6.11 mg/100g, respectively. Keskin (2017) determined that P; 252 mg/kg, K; 1145 mg/kg; Ca; 85.90 mg/kg, mg; 140.75 mg/kg, Fe; 5.36 mg/kg, Mn; 0.2 mg/kg, Zn; 0.28 mg/kg and Cu; 0.39 mg/kg of 'Erciş' grape cultivar grown in Van ecology (Southern east region). Keskin et al. (2019) were conducted to investigate 24 grape cultivars from the Mid-Black Sea zone in terms of berry mineral composition. The results indicated that the mineral composition of grapes differs according to cultivars. As a result of their study, 'Hırsız Kesmez' has rich for P, K, Mg; 'Çıtlık' for Ca and B; 'Fenerid' for Fe; 'Siyah Üzüm' for Mn; 'Turşuluk' for Cu; 'Şiredenlik1' for Zn; and 'Kırmızı Üzüm' for Se.

In general, the amount of minerals is lower in arid climatic conditions and dry years. The quantities of minerals are influenced by soil conditions; in addition, some pesticides that used against plant diseases and atmospheric conditions also affect mineral content (Kalkan and Keskin 2018).

Descriptive statistics for the individual phenolic compounds of the cultivars are presented in Table 2.

Table 2. Descriptive statistics and comparison results of phenolics

Phenolics	Cultivar	Mean	Std. Error	p	Phenolics	Cultivar	Mean	Std. Error	p
Protocatechuic acid	Bağılti	2.389	.0160	0.06	**Chlorogenic acid**	Bağılti	8.283	.5040	0.104
	Besirane	1.559	.0080			Besirane	5.031	.6665	
	Emiri	2.650	.0135			Emiri	11.863	.8055	
	Tayifi	1.848	.0295			Tayifi	7.757	.1740	
	Total	2.111	.1629			Total	8.233	.9457	
Vanillic acid	Bağılti	0.120	.0350	0.475	**Syringic acid**	Bağılti	0.161	.0540	0.160
	Besirane	0.105	.0560			Besirane	0.324	.2080	
	Emiri	0.206	.1355			Emiri	0.376	.1390	
	Tayifi	0.171	.0065			Tayifi	1.752	.1220	
	Total	0.150	.0324			Total	0.653	.2475	
Rutin	Bağılti	11.210	.3605	0.080	***q*-coumaric acid**	Bağılti	0.261	.0185	0.261
	Besirane	14.032	.3430			Besirane	0.252	.0085	
	Emiri	7.258	.5995			Emiri	0.375	.1450	
	Tayifi	15.731	.1255			Tayifi	0.555	.0180	
	Total	12.058	1.2210			Total	0.360	.0540	
Gallic acid	Bağılti	4.763	.1600	0.102	***trans*-resveratrol**	Bağılti	1.274	.1005	0.117
	Besirane	3.994	.0475			Besirane	1.544	.0385	
	Emiri	6.747	.4640			Emiri	1.342	.0425	
	Tayifi	2.294	.5620			Tayifi	6.283	.1320	
	Total	4.449	.6207			Total	2.610	.8029	

As seen in Table 2, Protocatechuic acid ranged from 1.559 mg/kg to 2.650 mg/kg. Similarly, for valinic acid, rutin and gallic acids values changed from 0.105 mg/kg to 0.206 mg/kg, 7.258 mg/kg to 15.731 mg/kg and 2.294 mg/kg to 6.747 mg/kg, respectively. The minimum value of Chlorogenic acid was found 5.031 mg/kg in ‘Besirane’ while maximum value was observed as 11.863 in ‘Emiri’.

Syringic acid, *q*-coumaric acid, and *trans*-resveratrol values ranged from 0.161 to 1.752 mg/kg, 0.252 to 0.555 mg/kg, and 1.274 to 6.283 mg/kg, respectively. However, there was no statistically significant difference among the cultivars for all characteristics.

For the cultivars and indivudial phenolics, results of multidimensional scaling are given in Fig 1 and Fig 2, respectively.

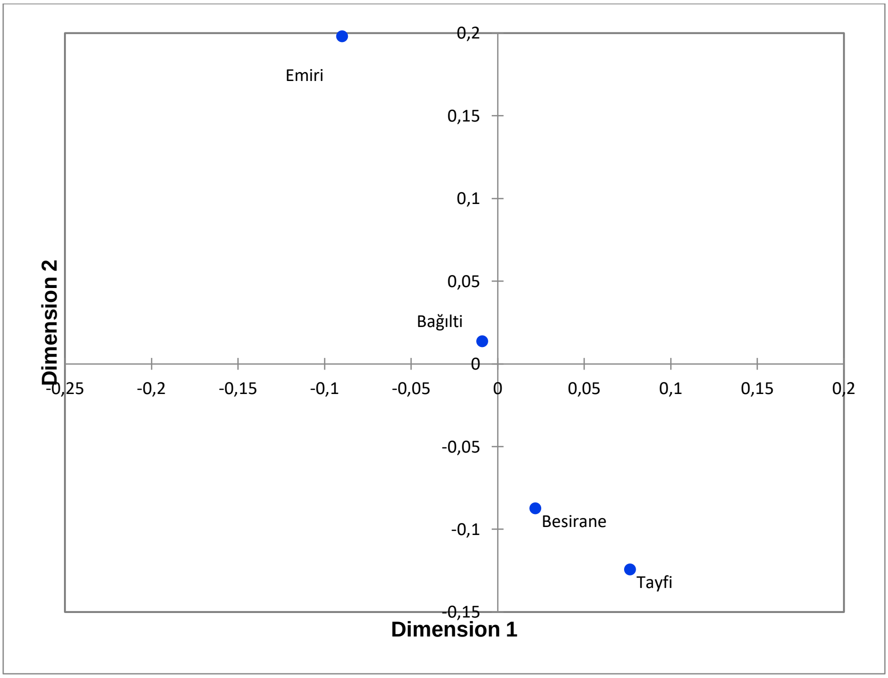

Fig 1. Configuration map of the cultivars

Fig 1, there was considerable similarity between ‘Besirane’ and ‘Tayfi’. Similarly ‘Emiri’ and ‘Bağılti’ were located in the same region.

As shown in Fig 2, *q*-coumaric acid, syringic acid, rutin and *trans*-resveratrol were located in the same region. Similarly, gallic acid, protocatechuic acid, chlorogenic and vanillic acids were grouped in the upper left region of the configuration map. These characteristics were negatively correlated with other characteristics.

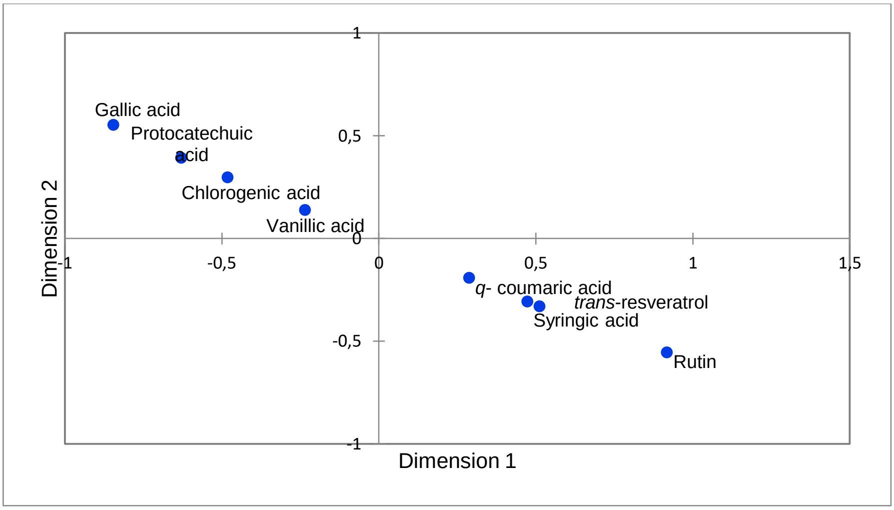

Fig 2. Configuration map of the phenolics

Keskin (2017) was determined gallic acid 1. 37 mg/l; chlorogenic acid 3.18 mg/l; *p*-coumaric acid 0.05 mg/l; vanillic acid 0.33 mg/l and rutin 5.31 mg/l of ancient grape cultivar 'Erciş' in Van region.

Uyak et al. (2020) were conducted to investigate 17 grape cultivars from the Hizan (Bitlis) ecology (Southern east region) in terms of berry individual phenolics. As a result of their study, 'Alaki' has rich for vanillic acid, protocatechuic acid and rutin, 'Siirt Kurutmalık' for *trans*-resveratrol, gallic acid, rutin, 'Kuş Üzümü' for chlorogenic acid, gallic acid, protocatechuic acid and rutin, 'İnek Memesi' for *trans*-resveratrol.

Genetic, agronomic or environmental factors play a crucial role in phenolic composition and concentration (Keskin et al. 2014; Isci et al. 2015; Margaryan et al. 2015; Uyak et al. 2020) reported that. It is well known that the composition of phenols in grapevines depends on variety, species, season and environmental and management factors such as soil conditions, climate and crop load.

Conclusions

Grape is one of the wealthiest fruit species for mineral and phytochemical content. This study determined physical and chemical characteristics as well as phytochemical content of four native grape cultivar grown in Siirt province (Southern east region). It can be considered that these grape cultivars have been partially rich for mineral and phytochemical content. Therefore, the results of this study indicated that Grape cultivars of Siirt have great potential for healthy nutrition, mainly, due to having considerable mineral and phytochemical content.

This study determined differences among the cultivars as well as proximities among the characteristics (individual phenolics). In addition, according to results of this study, using of multidimensional scaling method can be proposed to determine similarity or dissimilarity among the cultivars as well as characteristics.

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CHAPTER III

SOME METHODS USED TO MEASURE THE COLD HARDINESS OF GRAPEVINE DORMANT BUDS

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1. Introduction

Grapevine, which has a long past as much as the existence of humanity, has achieved an important place among horticultural products today (Keskin et al. 2013). Its advantages such as not being very selective in terms of climate and soil requirements, easy routing and evaluated in various ways have made the grapevine one of the most commonly cultivated plants in the world (Keskin et al. 2018). It is known that 79 million tons of grapes are produced in an area of 71.6 million hectares in the worldwide (FAOSTAT 2020). Viticulture in the worldwide is generally carried out in areas extending to latitudes of 4°-59° in the southern hemisphere and 6°-45° latitude in the northern hemisphere (Hai 2016; Jones and Schultz 2016). Economically, however, grape cultivation is carried out in areas with a temperate zone extending between 20°-40° in the southern hemisphere and 11°-52° latitude in the northern hemisphere (Lamar 2011). On the other hand, success in viticulture is directly proportional to the complementary relationship between the climatic factors in the cultivated areas and the genetic structure of the grapevine (Fraga et al. 2016). Genotypes, varieties, and rootstocks, which have genes tolerant to hot and/or cold climates, have easily adapted to suitable climates for their genetic characteristics (Kaya 2020a). Seasonal anomalies caused by global climate change in recent years have adversely been affected viticulture (Kaya 2020b). Especially seasonal anomalies that occur around the world for some years may have a significant effect on the development and yield of the vine, quality of grape, and even the survival of vines (Köse and Kaya 2017; Kaya ve Köse 2017). In fact, it is known that extra-seasonal temperatures have devastating effects on viticulture throughout history as well as today. The "Great Frost", which took place in January 1708-1709 and known as the coldest winter of the last 500 years, has caused great damage to the vineyard regions from Scandinavia to Italy and from Czechoslovakia to the west coast of France (Appleby 1980). After this frost event, the winegrowers developed different methods such as burying the vines in the soil and growing cold-resistant cultivars and burning pruning canes or some substances in the vineyard areas to prevent the grapevines from freezing. (Zabadal et al.

2007). However, in order to prevent or reduce frost damage in such areas, understanding the survival mechanism of the vine and the selection of cold-tolerant cultivars among genetic resources should be the main targets (Evans 2000). In addition, in the selection of varieties that are tolerant to low temperatures and have high adaptability, it is necessary to know well what is effective in frost tolerance of vine tissues, as well as their relationship with the environment during the growing season (Ferguson et al. 2011).

As in most of the deciduous tree species, the hardiness to cold in grapevines shows a complex trait, and this process follows the sequence of acclimation and deacclimation (Rende et al. 2018). In addition, the low temperature hardiness of grapevines; it is directly related to factors such as mainly genetic trait, the falling speed, duration, severity of low temperature, temperatures of dormant period (Khanizadeh et al. 2005). Besides, many factors such as rootstock, altitude and location of the vineyard, pruning method and time of vines, crop load, cultivation type, train systems, fertilization (especially nitrogen), irrigation, disease and pest control level affect their frost hardiness (Kaya and Kose 2018). In other words, the hardiness of the vine to low temperatures has a dynamic trait that differs between cultivars, organs and tissues depending on cultural practices and environmental factors (Hamman 1993). Because of this complex and dynamic trait, it is not possible to give a precise range for the threshold or critical temperatures at which grape cultivars or tissues are damaged by low temperatures. However, considering the rankings of tolerance of vine organs to cold, the trunk is the most resistant, followed by arms, canes, buds and roots, respectively (Paroschy et al. 1980; Ahmedullah 1985; Meiering et al. 1980; Hamman 1993; Wolf and Warren, 2000). These findings have been achieved by researchers for many years using new measurement techniques to detect frost injury in tissues under temperature controlled laboratory conditions or after low temperatures (Kaya and Kose 2020). In this context, many measuring techniques such as tissue browning (visual assessment), electrical conductivity, tetrazolium stain test, differential thermal analysis, kryoscan system, X-ray phase contrast imaging and cryo-scanning electron microscopy have been used to determine the threshold temperatures of grapevine tissues and organs (Badulescu Valle 2003; Kaya 2020a; Karami 2019; Wisniewski et al. 2015; Kovaleski et al. 2019; Kasuga et al. 2020). Frost hardiness in grapevine organs is performed by exposing organ parts to freezing tests in laboratuvar, then frost damage level by using one or more of these techniques together. Devotion to standardized and well defined frost testing protocols as well as estimation tests extremely important for our purpose to exactly determine frost tolerance and compare information from various testing times or methods. In this study, therefore, we review the advantages of the test methods used to guide the selection for usege in vineyard cultivation or research and to guess the frost hardiness of grape buds in the light of literature.

2. Oxidative browning or visual damage scoring method

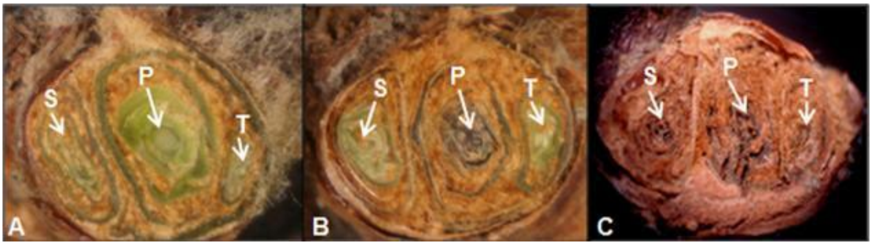
Oxidative browning or visual damage scoring involves examining above ground portions of grapevines in after severe winter-damaged and/or exposure to artificial frost tests in the laboratory (Kaya 2020a). When exposure to low temperatures is applied evenly, this is a simple and direct method to predict the impacts of a similar low temperature exposure under local environmental terms. Test findings are frequently measured by visual assessment of browning of injured cell tissue following an incubation phase (following the 24, 48 or 72-h incubation), so reference to the oxidative browning score (Odneal 1984).

Each node or bud in vine cane has a complex of three primordial or a compound bud (Fennell 2004). The primary bud or "primary primordia" located in the middle of the compound bud are the largest meristematic tissue. The primary bud is located on either side of the cane towards the base of the cane and while some cultivars have a partially fruitful secondary bud, the normally not-fruitful and the bud that is positioned towards the apical part of the cane is the smallest tertiary bud. Recognizing the morphology of the bud structure and making cross longitudinal separable cuts with a single-edged razor blade on each bud, growers or researchers can determine the health status or cold injury of each primordium in the bud. The buds showing vibrant green tissue in complex compound buds are considered viable, while buds showing brown or black tissue are considered dead (Figure 1). In fact, the brown discoloration is caused by the oxidation that occurs as a result of the release of phenolic compounds from injured bud tissues due to the severity of the cold. Additionally, severe cold damage can result in deeper and more pronounced discoloration of damaged or killed tissues, while less severe cold damage can create slightly browned tissues.

How many dormant buds need to be sliced is decided depending on the amount of cold damage the ligament has been subjected to or when the damage occurred. If all of the buds are dead in the first 40-50 bud sample, the chance of find viable dormant buds is very low; so, it may not be necessary to evaluate anymore. Conversely, when the data are quite unstable (damaged and viable from the same vineyard locations and different buds of the same variety), in order to have an understandable thought of the level of injury, amount of buds suggested by the research take about 100 (Moyer et al. 2011). Thus, the percentage damage rate in the vineyard is determined. If the vineyards do not have a uniform structure (different soil, slope, vine size), to determine the potential impact of these variables, it would be better to keep samples from the vineyards or vines. Steps to be applied according to pruning techniques in spring after bud injury is predicted (Table 1).

Table 1. Recommendations according to differences in bud mortality in the vine.

Mortality of bud (%)	Recommended strategy
10-15	When there is damage to this level in the eyes, there is no need to adjust the winter pruning.
20-50	The number of dormat buds should be increased in the winter pruning (+20-30%); i.e. prune to 4 or 5 dormat bud spurs rather than leave more spurs/canes per vine and/or the standard 2-3 bud spurs.
60	The number of buds should be doubled compared to the standard pruning strategy
More than 60	Just establish the bearing structure of the vine or no dormant pruning



Reference: (Sabbatini 2014).

Figure 1. Cross-sections of grape complex buds indicating the location of tertiary (T), secondary (S), and primary (P) buds. A) All three primordial within the bud are alive; B) Primary primordial within the bud is dead, Secondary and tertiary primordial within the bud are alive; C) All three primordial within the bud are dead (Moyer et al. 2011).

3. Electrolyte Leakage Assay (EC)

Tissue damage assessment after artificial frost tests in the laboratory provides useful tools for comparing cold resistance in different grape genotypes, varieties and rootstocks (Jones et al. 1999). The accuracy of cold hardiness assay in dormant buds of grapevine may be determined by using electrolyte leakage test (Reynolds et al. 2014). Following artificial frost tests, the EC has been indicated to be a useful indicator of involution among frost undamaged and damaged bud tissues, and it has been stated for cold hardiness screening of grapevine germplasm (Zhang et al. 2012; Morin et al. 2007; Karimi 2019).

In electrolyte leakage assay, samples are exposed to different test temperatures (e.g. -16, -20, -24 and -28^{o}C in Jan. and Mar., -6, -10, -14 and -18^{o}C in Nov. and Apr.) according to the acclimation and deacclimation stages to determine the frost tolerance responses of the buds. The freezing rate is adjusted to decrease by 2^{o}C h^{-1}, and dormant buds are kept at each frost test temperature for 75 min before being removed from the freezing cabin (Karimi and Ershadi 2015). After sectioning and thawing, buds are incubated in deionized for 24 hours to stabilize the electrolyte diffusion. The ratio of solution volume to bud tissue weight must be low enough to provide a steep diffusion gradient, e.g. 4 ml water per 60 mg bud tissue fresh weight. If the sample containers are small and the ratio is low, shaking of bud specimens is not needed, but sample sizes and larger tubes must swing to ensure steady diffusion (Sutinen et al. 1992). Initial conductivity (EC_1) of samples is read in µmho/cm with a conductivity meter device, preferentially with samples held at a constant temperature or with automatic temperature compensation. After the initial conductivity measurement, the tissue samples of the buds are killed to release the electrolytes remaining in the samples. The samples are then autoclaved at 120°C for 30 min., then the samples are then kept at room temperature to cool. Final conductivity (EC_2) value of samples is measured under the same conditions as EC_1, after 24h incubation.

Frost damage is usually calculated as the relative conductivity, e.a. ratio of the conductivity obtained from the buds to the total conductance after freezing after the bud tissues have been killed. It is assumed that higher frost damage corresponds to higher relative conductivity. Relative electrolyte leakage is determined from EC_1 and EC_2 using the formula:

$$REL = [(EC_1-A_1) / EC_2-A_2)] \times 100$$

where A_1 and A_2 are optional gaps determined before and after heating in the oven. REL ranges from one or a few percent in control samples to about 100% in freeze-killed specimen (Prasil and Zamecnik 1990). Then, the measurement temperatures of the samples are estimated by creating interpolation curves between the REL values read for each sample exposed to different test temperatures and these test temperatures.

4. Tetrazolium Stain Test (TSC)

The TSC is a rapid method for assessing the frost tolerance of dormant vine buds. This method is widely used by scientists to evaluate the potential for cold damage in the buds, to determine the severity of frost damage to the buds, and to determine the problems of resistant or vulnerable grape cultivars after freezing. Indeed, determining the severity of redness of bud tissues exposed to cold after the tetrazolium stain test is a applicable and valid method for determining the amount of damage in both laboratory and field tests of frost hardiness estimation of other woody plants and grapevine (Ershadi et al. 2016). In this method, samples taken under field conditions are exposed to several different temperatures. For example, samples taken during a dormant period are placed in a freezer started at 4ºC in the laboratory, and the freezer chamber is programmed to reduce the temperature gradually by to 0, -3, -6, -9, -12, -15, -18, -21, -24, -27 and 30ºC (drop in 3ºC h^{-1}), then kept for 3h at each temperature (Sarikhani et al. 2014). Bud samples are taken at the end of each freezing temperature treatment and bud survival rates are evaluated using the tetrazolium stain test.

Samples frozen at different temperature segments are removed from the freezer and placed on ice for slow thawing, then 5 mL of a 1% TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) solution is added to each sample tube (Sarikhani et al. 2014). Then, each bud is opened horizontally with a razor blade and surviving or dying buds are investigated by stereomicroscope. Mortality percentage in dormant buds are estimated by dividing the number of dead buds by the total number of buds (Karimi 2019).

5. Differential thermal analysis method

In the DTA method discovered by Quamme et al. (1972), thermocouples were initially used to determine the exotherm of tissues. Thermocouples were inserted into the tissue to provide the connection between the flower primordium of the dormant buds and the thermocouple connection. After silicon grease was applied for maximum heat transfer, the samples were placed in a 2.2 Lt dewar flask, and then the thermocouple cables were taken out of the plastic cover of the dewar flask. DTA tests were performed on samples placed in a temperature-controlled, programmed freezing chamber (Figure 2). On the other hand, Quamme (1978) found that when thermocouples were placed in buds, the physical structure of the tissue was disrupted. In addition, it was very difficult to insert thermocouples into tissue in this method. Then, Nus et al. (1981) and George (1982) developed the precision DTA method using thermistors instead of thermocouple. They designed an environment consisting of a temperature sensor circuit, regulated power supply and freezer unit. After that, Andrews et al. (1983) designed a cooling plate with a thermo-electric module that acts as a kind of thermal that is both sensitive and less expensive. A few years later, Wample et al. (1990) designed a computer-controlled freezer system and a computer-aided data acquisition, storage and evaluation system, automation process of the dta method started by Mills et al. (2006). Finally, when the DTA method was used, it was determined by Kaya and Kose (2020) that sampling or pre-test temperatures had an effect on the bud exotherm values and this method became the standard in frost tolerance estimation (Figure 3).

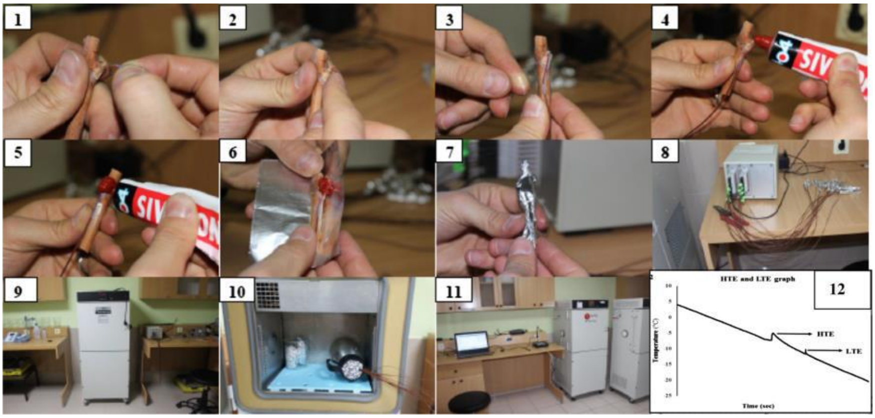


Figure 2. Thermocouples are placed in the intact dormant buds (1, 2) and fixed with elastic band (3). The thermocouple connection is closed using silicon grease to ensure maximum heat transfer occurring in the bud (4, 5). Then, the buds wrap with aluminium foil and inserted in a Dewar flask which was pre-chilled to $-3^{o}Ch^{-1}$ (6, 7, 8, 9, 10, 11). The temperature curve of the high and low temperature exotherm (12) (Kaya and Köse 2017).

In this method, samples are placed in temperature controlled portable coolers that are adjusted to the atmospheric temperature at the time of sampling and the samples are brought to the laboratory by maintaining their temperature during the sampling. Then, the buds kept the laboratory by preserving their temperatures and prepared for the test at these temperatures are tested starting from the temperature value at the time of sampling, so that the buds are not exposed to any temperature changes. For example, samples taken from vineyard conditions at -5°C are stored at -5°C. Samples are prepared for testing at -5°C, and the temperature controlled cabinet is set to 5°C. Then the adjustable controlled cabin temperature is lowered to -5°C, and the samples are immediately transferred to the temperature controlled cabinet. Finally, samples continue to tested until the temperature-controlled cabinet temperature drops to -40°C at cooling rate $4°C\ h^{-1}$ (Kaya and Köse 2017).

Buds whose ambient temperatures are preserved at all stages until the start of the test are placed in modules consisting of 9 wells. At this stage, care is taken to keep the 1-2mm wood tissue under the bud while each bud is cut from the nodes, and then thermal conductive paste is applied to the cut surface of the buds. Four, five or more buds can be placed in each well. In order to increase the heat transfer, foam insulation pads are placed on the samples, and then the covers of the trays are closed and the DTA test is carried out by placing them in the temperature controlled cabinet. For each DTA test, the data obtained from TEMs are sent to the instantaneous computer as the electrical voltage output in mV and the temperatures obtained from the mV peaks are recorded, and then the high and low temperature exotherms of the dormant buds are determined (Kaya and Kose 2020; Mills et al. 2006; Andrews et al. 1983).

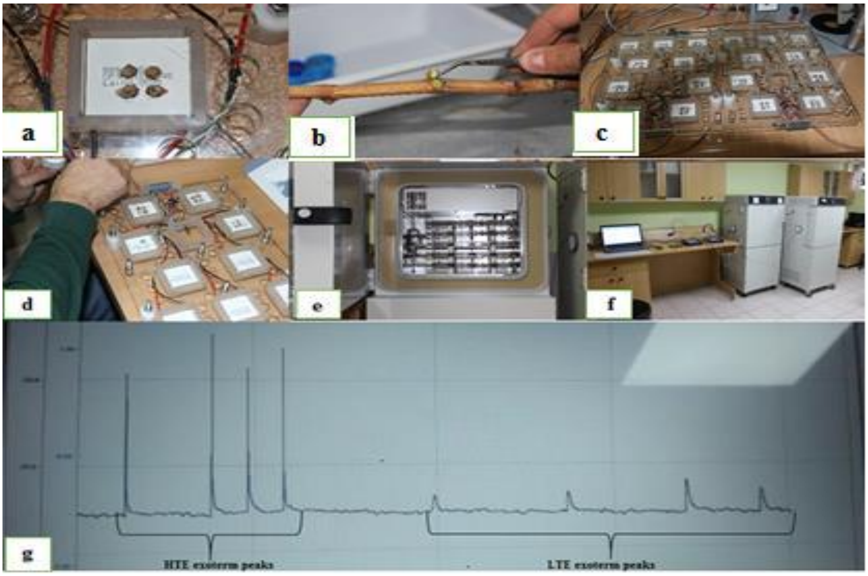


Figure 3. Dormant buds taken from the nodes and placing buds (four grapevine buds) in each TEM **(a)**. Leaving 1-2 mm woody-tissue under the dormant bud **(b)**. 144 dormant buds placed in a total of 36 TEMs for each DTA test **(c)**. The buds are maintained at their temperature during sampling and prepared at a temperature controlled cabinet **(d).** Four trays are placed in one programmable test cabinet (**e**). The temperature curve of the high and low temperature exotherm **(g)** (Kaya and Kose 2020).

6. Kryoscan system

Many authors have used microcomputers for automatic recording, as testing of slow freezing experiments is very time consuming and requires a lot of repetition to obtain reliable results (Andrews at al. 1983; Wample et al. 1990). A special freezing and data acquisition system (Kryoscan) has been developed to overcome the disadvantages of these older instruments previously used, which allows programmed cooling of 16 small wells to temperatures up to -40°C. In this system, bud samples are placed in small copper cavities soldered on a copper block with a diameter of approximately 1 ml, 10 mm. A difference sensor consisting small Peltier module, 2.5 x 4 mm, and 6 elements are situated on the bottom of each well. The freezing event in the bud samples placed in each well causes a temporary increase of its temperature recorded as the temperature difference between the bud and its the well. Into the system, all well sensors are scanned approximately 10 times per second, so that exotherms obtained from buds can be recorded almost simultaneously, but for each sample separately (Badulescu Valle 2003; Badulescu and Ernst 2006). Curves obtained from the buds can be viewed on a computer screen and saved to disc (Figure 4).

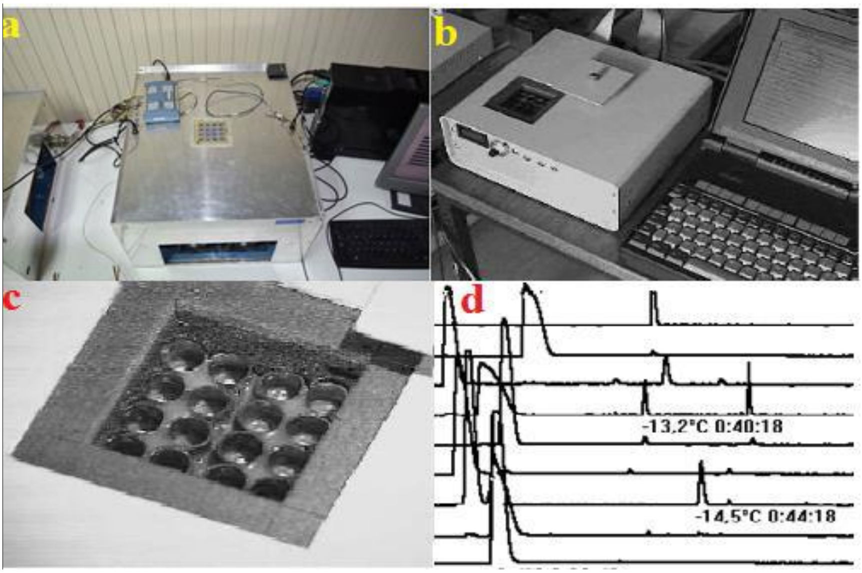


Figure 4. Detailed view of the Kryoscan system (**a**) Kryoscan system (16 wells) with laptop-based controller with freezing block cover removed (**c**), freezing blocks or wells embedded in insulating foam (**b**), high and low temperature exotherms obtained from buds inside the wells (**d**) (Badulescu Valle 2003; Rubio et al. 2019).

7. X-ray phase contrast imaging

When the limits of supercooling are exceeded, it is hypothesized that damage to the vine buds is caused by the intracellular ice formation. The observing the freezing process can help to understand how the damage caused by the freezing event. And also the identifying regions within the bud where supercooling has failed can be the best way to understand how plants control supercooling. Using time-resolved X-ray phase contrast imaging, Kovaleski et al. (2019) aimed to examine frost tolerance-related characteristics of *V. riparia*, *V. amurensis,* and *V. vinifera* bud in real time. They used synchrotron X-ray phase contrast imaging to examine the frost-resistant properties of the buds and time-resolved 2D imaging instruments to visualize freezing of tissues. In this method, bud intracellular freezing temperatures or exotherms (LTE) are detected utilizing thermocouples throughout 2D imaging while the buds are freezed by a N_2 gas cryostream.

In this method, the buds on the cane are attached to a custom-made cylindrical holder by mounting paste, and the holder is attached to a small angle gauge mounted on a Huber 4-circle diffractometer. The monochromatic beam, X-ray energy is expanded to 7mm x 7mm at 15 KeV, imaging is performed at the C-line at the Cornell High Energy Synchrotron Source. The specimen-to-detector interval utilized is optimized to 0.5 m, and the phase setoff occurs when the unperturbed beam mostly commixs by angular deflections in the wave front caused by intensity cahnagins in the bud sample. X-rays are converted to visible light utilizing a rare earth doped GGG (Gd3Ga5O12) crystal plate and imaged utilizing an Andor Neo CMOS camera with a 5x objective lens with a detector pixel size of 6.5 μm (Kovaleski et al. 2019).

The frost in the buds is watched utilizing 2D time-lapse imaging with 2 μm pixel size images and 1 second exposure is used. Imaging of the cooled buds is performed utilizing a N_2 gas Cryo-stream with a fixed coolant speed of about 40°C h^{-1}. A 33-gauge needle thermocouple is inserted into the bud to measure the bud entry temperature during imaging, and the temperature measurements are recorded on an RDXL4SD data logger. LTEs for these buds are determined as temperature deflections from the linear rate of cooling (Kovaleski et al. 2019).

The 2D time lapse X-ray phase contrast utilized in conjunction with a thermocouple can be useful in determining how ice moves within bud tissues. However, in 2D imaging, resolution does not allow ice crystal identification inside the bud, but can evaluated by monitoring the movement of bud tissues that coincide with low LTE values. As a result, in this method has been observed that during the freezing that lasted for a few minutes, the freezing progressed from the middle of the bud to the outer bud scales (Kovaleski et al. 2019).

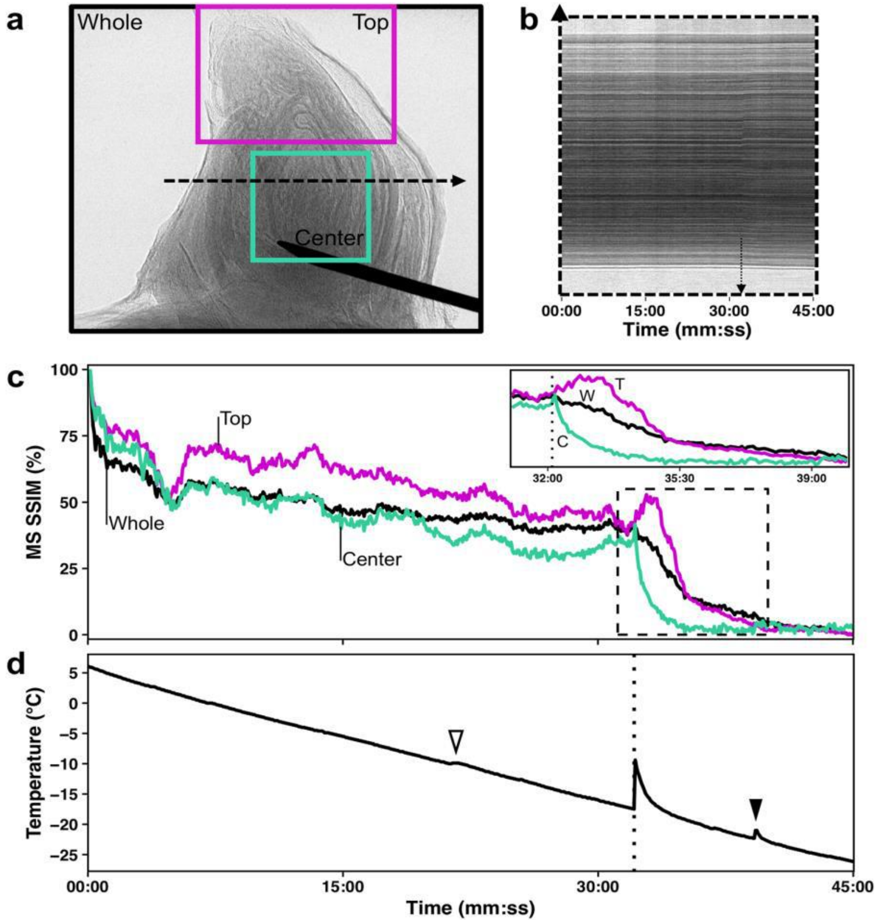


Figure 5. Features of freezing in the bud of *Vitis amurensis*. **(a)** Black (full image); Still image of the bud at the onset of frost; the analyzed areas are magenta and cyan; the dashed line passing throughout the center of the dormant bud marks the pixels utilized to create a kymograph. **(b)** Kymograph originating from the pixel line in the center of the dormant bud; arrows mark the beginning of freezing **(c)** The normalized multiscale structural MS SSIM for the three regions in (a); the dashed box is shown expanded inside, the dotted line indicates the onset of the freeze event. **(d)** Exotherm profile determined by using thermocouple within the dormant bud; the dotted line indicates the onset of the freezing event, the closed arrow-head indicates the secondary bud exotherm, the open arrowhead indicates the slight Delay (Kovaleski et al. 2019).

8. Cryo-scanning electron microscopy (Cryo-SEM)

The cryo-scanning electron microscopy investigations of grape dormant buds under a freezing condition have been provided comprehensive data on freezing behavior that cannot be determined using differential thermal analyses and infrared differential thermal analysis. It also has been used to observe hydrated structures of grape dormant buds, in which water in cells or tissues is kept from conversion to ice by cryo-fixation using very rapid freezing (Kasuga et al. 2020). Thus, with the help of the use of cryo-SEM, detailed observation of the high resolution ice crystal distribution of frozen cell morphology in frozen plant tissues has been obtained. This way, it also has been reached information about the complex structure of the plant freezing behavior at the cellular level and direct visualization of the frost events of cells in most plant tissues has been achieved with the use of cryo-SEM (Figure 6) (Fujikawa et al. 1996; Pearce 1988; Kuroda et al. 1997; Ball et al. 2004).

Kasuga et al. (2020) has used Cryo-SEM to observe for partial dehydration of bud primordial tissue cells of the cold hardiness Yamasachi grape cultivar. Since the use the Cryo-SEM in grape buds was carried out by Kasuga et al. (2020), we tried to explain their methods in the present study. In this method, each dormant bud by a small (1-2mm) of wood tissue is affixed in the hole of a 6 mm diameter samle holder with a little amount of gelatinized starch and moistened with very little MilliQ water.

The dormant bud samples in the sample holders are directly placed in the controllable frost cabinet and the samples are kept at 4 ° C for 1 hour, cooled to -15° C at a rate of 5°C h^{-1}, and then kept for 12h at -15°C. Then, the some frozen bud specimens were cryofixed by dipping directly into cold Freon 22 at -150°C. The remaining bud specimens are cooled in a step-wise manner, i.e. the dormant bud specimens that were kept at -15 °C for 12h are a new cooled to -40°C at a rate of 5°Ch^{-1}, protected at -40°C for 1h, and cryofixed. Cryo-SEM monitoring is performed with the aid of a cryo-scanning electron microscope equipped with a cryo-sample arranging chamber. The cryofixed bud samples are transported to a cold stage in the cryo-sample arranging chamber. The bud specimens are broken with a scalpel blade, engraved at -100° C for 5 minutes, and gold-palladium sprayed coating. The samples were transported to a cold stage in a SEM column. Secondary emission images of samples are obtained, and then the samples are photographed at a voltage of 2.5 kV. Stereomicroscopic pictures of longitudinal sectional surfaces of bud specimens are obtained using a USB digital microscope in a cold chamber of a cryostat (Kasuga et al. 2020).

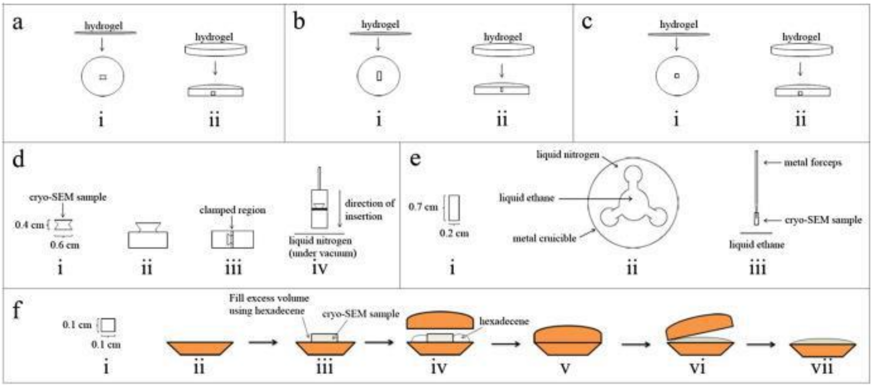


Figure 6. Schematic presentment of how the cryo-scanning electron microscopy specimen was cut from the hydrogels for (a) liquid nitrogen slush, (b) liquid ethane and (c) high pressure freezing where (ii) the IG hydrogels and (i) is the IM. Presentment of the liquid nitrogen slush freezing phases (d): (iv) cross-sectional image of immersing the cryo-SEM sample in liquid nitrogen for aqueous freezing, (iii) aerial image of cryo-SEM sample compressed in sample holder, (ii) Cross section image of the IM cryo-SEM sample held in the sample holder and (i) size of the cryo-SEM samples. Presentment of the LE freezing process (e): (iii) side image of dipping a cryo-scanning electron microscopy specimen into liquid ethane using metal forceps, (ii) aerial image of liquid ethane installation and (i) dimensions of the cryo-scanning electron microscopy specimen. Presentment of the high pressure freezing phases (f): (i) dimensions of the cryo-scanning electron microscopy specimen, (ii) metal sample holder base, (iii) specimen loading with excess volume occupied by hexadecene on specimen carrier, (iv) clamping of sample carrier dome, (v) high pressure freezing of samle inside clamped specimen carrier, (vi) removal of sample carrier dome under liquid nitrogen, and (vii) specimen remaining inside sample carrier ready for analysis by cryo-scanning electron microscopy (Aston et al. 2016).

9. Conclusion

In this study, we reviewed test methods such as as tissue browning, electrical conductivity, tetrazolium stain test, DTA, kryoscan system, and Cryo-SEM used to guide the selection of use in vineyard cultivation or research and to estimate the cold hardiness or frost tolerance of grape dormant buds in the light of the literature. In tissue browning, electrical conductivity, tetrazolium stain tests, samples are exposed to several different temperatures for certain periods of time and the mortality rates or percentage (LT_{50} values) of damaged buds at these temperatures are estimated. In these methods, while the LT_{50} values of the buds are estimated using regression or interpolation curves, the intercellular and intracellular frost temperature limits cannot be determined in the buds. On the other hand, thanks to differential thermal analysis, which has become a standard method for determining both extracellular and intracellular exotherms in supercooled buds, reliable results have been obtained in predicting the cold hardiness of species, cultivars, genotypes and rootstocks. Besides, direct observation of frozen tissue cells by a cryo- SEM and X-ray phase contrast imaging have provided information on the freezing events of primordial cells in buds of grapevine that adapt to sub-freezing temperatures by extraorgan freezing. Although these methods provide information about the frost tolerance of grape buds, it is still unclear which factors estimate the differences in cold hardiness of buds among *Vitis* species. It will therefore be very exciting to investigate what physiological and/or structural factors are involved in the movement of water from the primordium cells of dormant buds at sub-freezing temperatures in future studies, because these investigations may provide useful information for breeding of new freezing-resistant cultivars and the evaluation of grape cold hardiness.

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CHAPTER IV

EXOTHERM TEMPERATURES DETECTED IN SOME FRUIT CROPS TISSUES USING DIFFERENTIAL THERMAL ANALYSIS

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1. Introduction

Since the day humans entered agricultural, it has been recognized that low winter temperatures and late spring frosts are a significant threat to many plant groups. Looking at the date before AD, the Romans considered late spring frosts and low winter temperatures as important factors and carefully selected the species to be cultivated in these regions (Columela 1965). Additionally, about 2000 years ago, they used different methods to protect crops from freezing, as these frosts both damage the plants and cause significant yield losses (Perry 1998). In later years, this situation has led to new research efforts developed to reduce freezing damage, not only by the occuring of new methods for frost injury protection (Rieger 1989) or to delay bud burst (Anderson and Seeley 1993) but also by the works of the physiological and morphological mechanisms involved in freezing injury and its stress (Vyse et al. 2019; Burke et al. 1976). However, although all these efforts have led to the development of new methods such as magnetic resonance imaging, nuclear magnetic resonance, differential thermal analysis, differential scanning calorimeter, low temperature electron microscope, infrared video thermography, tripheny tetrazolium chloride reduction, tissue browning and electrical conductivity for estimating frost tolerance, limited success has still been achieved in protecting plants from frost and in only modest improvements in freezing tolerance (Quamme 1972; Ashworth 1986; Andrews et al. 1983; Wample et al. 1990; Mills et al. 2006; Fennell 2004; Rende et al. 2018; Kaya and Köse 2017; 2019; Sun et al. 2019; Kaya et al. 2018; 2020; Kaya 2020a,b). While considerable efforts have been directed toward understanding how plants response under low temperature conditions using these methods, freezing still accounts for greater losses of vegetables and fruits than any other biological or environmental hazard (Rodrigo 2000).

The response of plants to low temperatures and their frost tolerance has a complex trait (Rende et al. 2018; Ashworth and Wisniewski 1991). The survival process of plants at low winter temperatures varies according to three different stages. These stages follow respectively; pre-hardening or cold acclimation in late fall (Stage-1; 0-10°C), hardening or dormancy in midwinter (Stage-2; < 0 °C;), and loss of hardiness or de-acclimation in early spring (Stage 3; > 0 °C) (Janská et al. 2010). Several several different ways of avoidance and tolerance are involved, including propagation of osmotic regulation by ion transport, extracellular ice, changes in membrane fluidity, and supercooling by nucleation (Sun et al. 2019). These losses of plant tissue hydraulic conductance and minimize cell membrane disruption from intracellular freezing (Gusta and Wisniewski 2013; Neuner et al. 2010; Mayr and Améglio 2016), facilitating bud burst during deacclimation stage or in the spring. A more detailed understanding of these processes is based on observing the frost process in the organs during the exposure of the plant to frost under controlled conditions. Diverse measurement techniques have been developed to understand this complex structure since the end of the 19th century (Wisniewski et al. 2015). Differential thermal analysis (DTA), that includes one of these testing techniques, is widely used to determine threshold temperatures of plant organs such as dormant buds, flowers, parts of flowers, roots, leaves and shoots under laboratory-based frost tests (Wample et al. 1990; Mills et al. 2006; Gao et al. 2014; Kaya and Kose 2019; Kaya et al. 2018;2020; Gale and Moyer, 2017; Fiorino and Mancuso, 2000; Kaya 2020b). Basically, DTA relies on estimation two distinct freezing events, including high temperature exotherm (HTE), which is corresponding to the freezing of water in intracellular region and not cause bud death, and low temperature exotherm (LTE), which corresponds to the freezing of water in the intracellular region and causes bud death (Andrews et al. 1984). In studies conducted on flower buds of many plant groups such as peach, apricot, cherry, grapevine, azalea and plum trees during the resting period, it has been determined that the buds produce two different exothermic temperatures (Durner and Gianfagna 1991; Montano et al. 1987; Salazar-Gutierrez et al. 2014; Graham and Mullin, 1976; Kaya and Kose 2020). On the other hand, many researchers could not identify two different exotherms in the organs of some plant species during bud break or after xylem connections were established in spring (Fiorino and Mancuso 2000; Coleman et al. 1992; Kaya et al. 2018; 2020; Kaya and Kose 2019; Gao et al. 2014; Gale and Moyer 2017). They determined a single-peaked exotherm per sample of plant organs such as pedicel, pistil, petal, receptacle, roots, shoots, stamen and leaves using DTA under temperature controlled laboratory conditions based on freeze assays.

Based on intriguing results developed from the aforementioned researches it can be said that different tissues and organs within the different plant species (both herbaceous and woody) respond in distinctly different ways to sub-zero temperatures. In this study, therefore, we review the literature on how the different tissues of some fruit crops respond to winter temperature and thus, we hope that this response will provide new insights into how the studied species may influence strategies to increase their frost resistance or tolerance.

2. Use of DTA in dormant buds during the acclimation stage

The pattern of freezing in dormant flower buds during the acclimation phase is different than that observed in both deacclimation phase and woody tissues. Buds of some decidious tree species such as grapevine, raspberry, blueberry and several *Prunus spp.* seem to use extraorgan and supercooling freezing (Ashworth and Wisniewski 1991). In those species, it has been found that freezing of the buds starts within the bud the tissues and scales attached to the developing organs of flower buds (Sakai 1979; Ashworth 1982). In other words, water migration from the subtending the bud scales or stem, that is, the supercooled bud tissues to spaces outside, has been observed during deep supercooling of buds (Neuner et al. 2019). In studies, this processs of external formation of freeze dehydration and ice masses has been referred to as extraorgan freezing (Ishikawa and Sakai 1985) and has been operatively related to maintaining a supercooled state within buds (Sakai and Larcher 1987). Indeed, Sakai and Larcher (1987) have classified extra-organ freezing in three types within buds that exhibit freeze dehydration and deep supercooling. Type I buds (e.g. lateral buds of *A. japonicum*), whose can survive at liquid nitrogen temperatures, are considered to be very tolerant of dehydration and become completely dehydrated by freezing (Ishikawa et al. 1997; Neuner et al. 2019). Type II buds that are damaged by frost between -35 and -50ºC can not fully tolerate dehydration frost (e.g. buds of apple) (Pramsohler and Neuner 2013). In Type III buds that remain partially dehydrated, freezable cell water is within the flower bud cells (e.g. many temperate woody species). Cell death in such buds coincides with the moment when temperatures drop below the buds' süper cooling ability (-30 to -25°C), and ice formation occurs in intracellular areas (Quamme 1995). Using DTA, it has been documented that the dormant buds of some deciduous tree species such as grapevine, black and red currants, sweet cherry, blueberry, apricot and rhododendron survive in freezing temperatures by supercooling (Type III; Biermann et al. 1979; Graham and Mullin 1976; Quamme 1974; 1995).

Information about the freezing processes in and around the buds that show Type III freezing can be determined using DTA. DTA studies of peach, grapevine, sweet cherry, blueberry rhododendron, black and red currant dormant flower buds detected two distinct freezing events, including HTE represents the release of heat generated by the water between cells as it moves from liquid to solid and LTE resulting from the freezing of supercooled intracellular water (Quamme 1974;1995; Graham and Mullin 1976; Biermann et al. 1979; Ashworth 1982). Exothermic peaks in the grapevine buds have been presented in detail by the Kaya and Kose (2020) who made the DTA method the most up-to-date. In figure 1, all stages of the DTA method used to determine HTE and LTE values in dormant buds are presented.

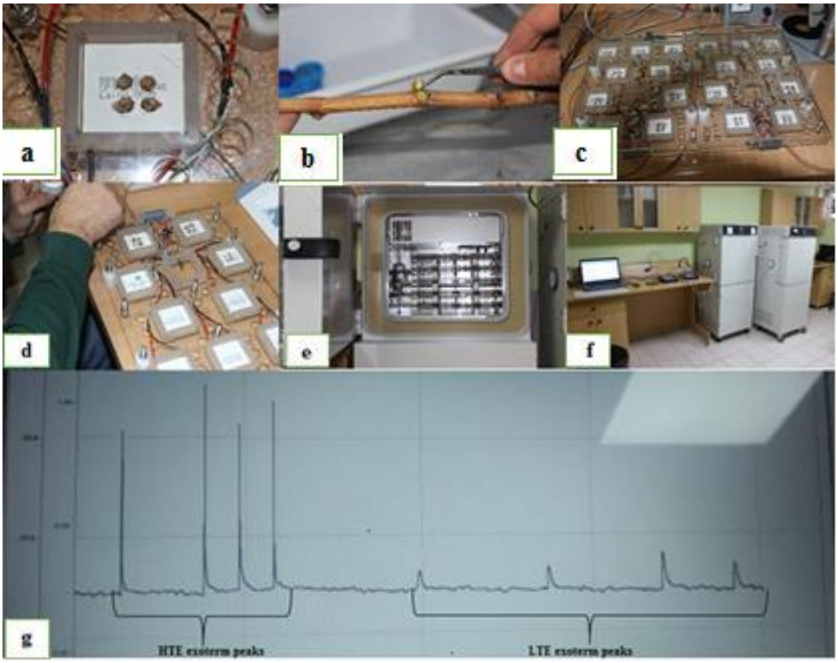

Figure. Dormant buds taken from the nodes and placing buds (four grapevine buds) in each TEM **(a)**. Leaving 1-2 mm woody-tissue under the dormant bud **(b)**. 144 dormant buds placed in a total of 36 TEMs for each DTA test **(c)**. The buds are maintained at their temperature during sampling and prepared in a temperature controlled cabinet **(d).** Four trays are placed in one programmable test cabinet (**e**). The temperature curve of the high and low temperature exotherm **(g)** (Kaya and Kose 2020).

During the dormant season, the HTE values of the vine dormant buds range from -5 to -16°C (Badulescu and Ernst 2006; Andrews et al. 1984). Bud HTE values of 37 species examined by using DTA in previous studies were reported to occur between -0.5 and -3.2ºC (Neuner et al. 2019). For three sweet cherry cultivars that are evaluated the HTEs were determined between -8◦C and -5◦C (Salazar-Gutierrez et al. 2014). Additionally, the HTE values in both *P. padus* and *P. virginiana* occurred between -5.5 to -9ºC during dormancy, as indicated by DTA observations (Kadir and Proebsting 1992). These results are similar to the low temperature range which indicates the occurrence of ice nucleation in trees in nature, i.e., > -3.4ºC (Mayr et al. 2007; Buchner and Neuner

2010; Pramsohler et al. 2012). On the other hand, species, genotypes, cultivars, plant organs and tissues may exhibit different injury at the phenological stage and same temperature, in relation to previous air temperatures, and their cold tolerance to the low temperatures prior to a cold night (Salazar-Gutierrez et al. 2016; Lenz et al. 2013; Sakai and Larcher, 1987). For instance, apple flower buds and bark tissue do not deep supercool, whereas pith cells and stem ray parenchyma cells prevent freezing thanks to its deep supercooling trait (Quamme 1976; Palonen and Buszard 1997). Similarly, it has been reported that pear flower buds do not show supercooling as in apples, whereas shoots avoid freezing by deep supercooling (Quamme 1991; Dongxia et al. 2020). However, it has been determined that LTE values could not be determined using the DTA method in studies conducted on apple and pear buds until today (Quamme 1976; Quamme 1991; Salazar-Gutierrez et al. 2016; Dongxia et al. 2020). Although the failure to detect LTEs in apple and pear buds seems to indicate that the hardness of the of apple flower bud cannot be predicted under artificial frost tests using DTA method, we think that re-analyzes should be made for apple and pear buds using the most up-to-date DTA method.

The extent of supercooling of dormant dormant buds of some *Prunus* species such as apricot, almond, peach and cherry were examined by most researchers using DTA (Andrews and Proebsting 1987; Callan 1990; Kadir and Proebsting 1993; Salazar-Gutierrez et al. 2014). The researchers found that in January, when tolerance to cold was maximum, LTE_{50} values of dormant buds for *Prunus* species were -23.2ºC for apricot, -21.2ºC for sour cherry, -21.6ºC for almod and -21.6ºC for peach (20 Dec.) (Kadir and Proebsting 1993). In general, it is seen in the literature that DTA studies are mostly carried out on grapevine dormant buds. In many studies, it has been revealed that varying bud LTE values depending on the dormant period of the vine occur between -2 and -42ºC (Bordelen et al. 1997; Fennell 2004). It has also been reported that the dormant buds of the cultivars of *Vitis vinifera* can show hardiness temperatures down to -25ºC, while *V. labrusca* × *V. riparia* hybrids (Valiant) can tolerate cold temperatures down to -45.5ºC (Hoover ve Hemstad 2000; Fennell 2004). In addition, it has been determined that the primary buds of *Rubes* spp. (such as red raspberry and blackberry) and *Ribes* spp (such as black and red currant) have supercooling ability. Floral buds of Darrow from among 11 blackberries (*Rubus* subgen. Eubatus) varieties were found to be more tolerant, surviving -33°C in January (in 45% of primordia among primary buds). But, in the work of Hummer et al. (1995) Darrow reported that buds only survived -13°C. At the same time, it has been determined that red currant (*Ribes sativum* [Rchb.] Syme) and black currant (*Ribes nigrum* L.) grown in regions where winter temperatures are very cold are generally very resistant to low temperatures. Warmund et al. (1991) stated that LTE_{50} values for buds of Red Lake cultivar were -22.1, -18.1 and -21.0°C, respectively, whereas for buds of cultivar Danka the corresponding temperatures were -26.5, -23.6 and -26.7°C, respectively in March, January and November.

3. Use of DTA in flower buds during the deacclimation stage

The flower buds of some deciduous species lose their supercooling ability during the deacclimation stage, and HTE and LTE values or two different exotherms can not be obtained from these buds (Rodrigo 2000; Kaya et al. 2018). In previous studies, indeed, a single exotherm was determined in estimating the frost tolerance of flower buds using DTA during and after bud burst. Most researchers have stated that this exotherm obtained from flower buds has the HTE value and agreed that this exotherm has no death point in flowers (Rodrigo 2000; Salazar-Gutierrez et al. 2014; Alhamid et al. 2018). However, Kaya et al. (2020) found a way to use DTA analyses to compare frost tolerance of flower buds of different some fruit crops such as quince, pear, sour cherry, nectarine, wild apricot, plum, peach, apple and sweet cherry during deacclimation (Figure 2). When they subjected the flower buds of those species to DTA tests, they determined a large individual exotherm or a single peak exotherm per sample of flower buds. They also stated that it is difficult to predict where the ice crystals begins in floral tissues when using DTA, but in their study, the exotherm values (cell death point) of floral buds can be achieved by using a thermal conductive paste which provides heat transmission between thermo-electric module and flower samples. The researchers stated that the occurrence of these single exothermic peaks may be due to the presence or absence of xylem in the flower primordia or whether the vessel continuity of the xylem are related to the shoot tissues (Kaya and Kose 2019; Gale and Moyer 2017; Fiorino and Mancuso 2000; Kaya et al. 2018; 2020; Gao et al. 2014). The floral buds of many fruit crops lose their deep supercooling capacity during bud break and bud break and the continuity of xylem vessels coincide in this stage, in this case the ice crystals in the bud tissue spreads immediately via the vascular system (Kuprian et al. 2016; Wisniewski et al. 1997). Therefore, they hypothesized that frost occurs simultaneously in extracellular and intracellular regions of bud tissues due to the xylem vessel connection was established between shoot and bud in most deciduous species during deacclimation stage (Kaya and Kose 2019; Kaya et al. 2018; 2020). On the other hand, this assumption is supported by different researchers with the hypothesis that individual exothermic peaks determined using DTA in different organs of several deciduous species may be the death point. In this context, based on the fact that the only exotherms obtained from flower buds using DTA are the point of death, the response of flowers to low temperatures or their threshold temperatures were discussed in this section.

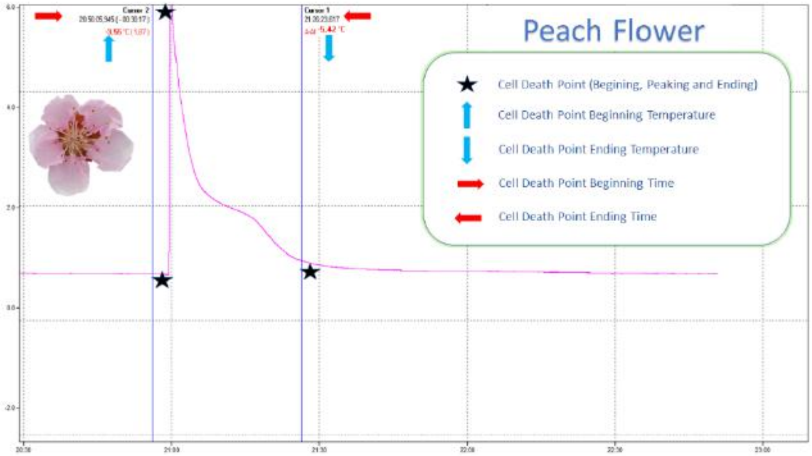

Figure 2. Demonstration of beginning and ending of the cell death point, the temperature of the cell death point, timing of bud death beginning and ending the temperature of the cell death beginning and ending, for flower bud are illustrated (Kaya et al. 2020).

The frost tolerance of buds decreases with the development of floral buds, and also the cold damage of their varies based on different phonological phaese, varieties, genotypes and tissue type (Meng et al. 2007; Kaya and Kose 2019; Szalay et al. 2016; Ashworth et al. 1983; Kaya et al. 2018). Indeed, the cell death point of apricot flower buds sampled at the first white stage was determined between -5.36 (Erzincan Tokaloglu) and -10.65°C (Şalak), whereas cell death point in floral buds of the same cultivars were found to be -4.06 to -8.24°C at full bloom (Kaya et al. 2018). Additionally, the death points of the pedicel, receptacle, petal, stamen and pistil belonging to the sensitive cultivar (Erzincan Tokaloglu) varied between -5.1, -3.3, -10.6, -12.9 and -6.6°C respectively, whereas the death points of these organs belonging to the resistant cultivar (Igdır Salak) varied between -7.8, -5.4, -11.0, -12.0 and -13.3°C, respectively at full bloom stage (Kaya and Kose 2019). In a different study, on the other hand, the flower death point from greatest to least tolerance are as follows: sour cherry (-9.54°C), sweet cherry (-8.61°C), quince (-8.57°C), peach (-7.27°C), apple (-5.44°C), pear (-5.38°C), plum (-3.76°C), nectarine (-2.93°C) and wild apricot (-2.41°C) during first white-pink stage. At full bloom, the flower death point were -2.60°C for nectarine, -3.61°C for plum, -2.17°C for wild apricot, -5.19°C for apple, -6.74°C for peach, -7.93°C for quince, -5.23°C for pear, -8.12°C for sweet cherry and -9.19°C for sour cherry (Kaya et al. 2020). It has been confirmed that these results were compatible with the death point temperature range of flower buds determined by field observations and artificial laboratory test results after spring frosts in different fruit species (Ashworth 1984; Gunes 2006; Rajashekar and Burke 1978; Kaya et al. 2018; Ashworth and Rowse 1982; Dumanoglu et al. 2019).

4. Use of DTA in xylem tissues during the acclimation stage

Studies using DTA have provided evidence that the wood and bark tissues of peach, apple, pear, grapevine and other some deciduous trees exhibit contrasting freezing behavior (Hong et al. 1980; Gale and Moyer 2017; Ashworth et al. 1983; Gao et al. 2014; Quamme et al. 1972;1982; Dongxia et al. 2020). Two distinct freezing events or HTE and LTE values were detected in cold-hardened xylem tissues exposed to low temperatures during the dormant period (Ashworth et al. 1983; George and Burke 1977; Quamme et al. 1972). After the first exotherm, which is not associated with tissue damage and corresponds to freezing of water inside the xylem vessels, a second exotherm, occurring around -40°C, associated with the death of xylem ray parenchyma cells has been identified (Ashworth et al. 1983; Hong et al. 1980). However, changes in the cold resistance of xylem tissues differ depending on the season and give different responses depending on the changes in the extent of deep supercooling (George and Burke 1977). It has been reported that LTE values decrease in the fall during acclimation, reach a minimum level in the midwinter and an increase in the spring during deacclimation (Ashworth et al. 1983).

Quamme (1976) determined that LTE temperatures occurred between -35 and -40 in apple shoots using DTA. Additionally, Dongxia et al. (2020) determined that the shoots of apples at a preconditioning temperature of -7 °C showed LTE at -43ºC. The secondary xylem being most frost tolerant in the shoot, and damage occurs in the shoot of apple spreads and interior radially outwards (Ketchie and Kammereck 1987). Although more than 50% of the xylem tissues are damaged by frost, it is likely for a tree twigs to recover (Warmund et al. 1996). On the other hand, xylem shows deep supercooling in pear shoots and LTE values occur between -33 and -38°C in fully hardened twigs (Quamme 1976). It is also reported that the peak value of the LTE in pear twigs varied between -38 and -41°C in all the pear cultivars (Dongxia et al. 2020). In *Prunus* species, on average, the xylem tissues of the twigs are the most sensitive to frost and the LTE values of these tissues, which show maximum tolerance in the middle of winter, have been determined between -28.7 and -35.3°C (Layne and Gadsby 1995). Apart from these species, xylem tissues of currants (*Ribes* Spp.), grapes (*Vitis* spp.) and blueberry (*Vaccinium* spp.) species are also known to exhibit deep supercooling (Palonen and Buszard 1996). Indeed, it has been determined that blackberry is more sensitive to low temperatures than blackberry and raspberry twigs are dameged at temperatures below -23°C (Ashworth and Wisniewski 1991; Moore and Skirvin 1990). Besides, It has been determined that the LTE values in blueberry shoots occur between -36 and -38ºC temperatures (Dongxia et al. 2020). Additionally, although the branches of *V. vinifera, V. labrusca* and their hybrid varieties vary depending on the periods, their canes can tolerate low temperatures between -8 and -25°C (Fennell 2004). Besides, it was reported by Mills et al. (2006) that injury in the phloem of grapevine started at -14°C and completed in -18°C, whereas cold damage in xylem started at -22°C and completed at -32°C. In a study on grapevine roots, it was determined that phloem was damaged at -6.77 to -10.40ºC, whereas xylem damage was found to be between -12.52 and -22.34ºC in these cultivars and rootstocks (Gao et al. 2014; Kaya and Kose 2018).

Conclusions

This study has given an conspectus of the current state of frost tolerance research in deciduous trees by filtering the lately investigated works on cold hardiness of dormant buds, flower organs and twigs of some both tree fruit and berry crops using DTA. Considering the investigations, it has been observed that the response of fruit crops organ and tissues to subzero temperatures is both complex and varied. The tissues and organs of plants have developed various strategies for dealing with winter temperatures and spring frosts, and when it comes to fruit plants, different tissues and organs in the same plant have been found to react differently. In addition, for the fruit species tested in studies, it was observed that the mechanisms by which cells and tissues respond to low temperature stress are influenced by the unique anatomical and physiological properties of the tissue and organ. Besides, it has been determined that supercooling ability in plant tissues and organs is important in responding to frost tolerance and that the values of HTE and LTE are obtained clear when DTA is used in plants showing supercooling ability. Supercooling capacity were studied in bud and stem tissues of several *Prunus* species (except plum), pear and apple stem tissues, flower buds and stem of raspberry, blueberry and blackberry, and in flower buds, cane and root tissues of currants and grapevines. Therefore, the physical limit of deep supercooling can be a limiting factor to expanding the production of some deciduous tree species such as *Prunus* species and pears. On the other hand, it has been reported that many fruit species lose their supercooling ability during the deacclimation stage. This is explained by the fact that when the xylem continuity is established between the shoot and the flower organs in the flower buds, ice spreads rapidly through the vascular system and this ice nucleates the water within the flower primordial. Thus, it has been hypothesized that a single exotherm was detected in tissues that lost their supercooling character when DTA was used, or that LTE and LTE exotherms coincided. Consequently, it has been suggested that the morphological changes flower bud development of many fruit species leads to the loss of the supercooling capacity and changes in the freezing pattern during spring or deacclimation stage. But, in order to adequately test this hypothesis, more research will be required in the following years using new test methods.

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CHAPTER V

ANTIOXIDANT, PHENOLIC CONTENT and ANTIMICROBIAL PROPERTIES of BILBERIES (*Vaccinium myrtillus* L.)

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1. Introduction

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O^{2-\cdot}$) and hydroxyl radicals ($OH^{\cdot}$) as well as non-free radical species such as hydrogen peroxide (H_2O_2) (Yildirim et al.,2001). These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer‟s disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations (Huang et al., 2005). Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules (Sun et al., 1998). The term ‘antioxidant’ refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors (Khilfi et al., 2006). The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases. Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases (Akinmoladun et al.,2007). *Vaccinium myrtillus* L. is a well- known bilberry shrub belonging to the Ericaceae family. It has been reported to have multiple pharmacological activities from its anthocyanosidic fraction: ophthalmic, vasoprotective, antiinflammatory, wound- healing, antiulcer and antiatherosclerosis. It is to be expected that several activities might be related to a possible antioxidant action from anthocyanosides. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to participate in the pathogenesis of various human diseases and may be involved in the conditions which *Vaccinium myrtillus* L. is used to treat. According to above mentioned it is important and reasonable to get to know and to investigate the antioxidant action and beneficial properties of billberry fruits and leaves grown in Turkey (Escop Monographs, 2003).

In this study, dried fruit and leaves extracts of bilberies (*Vaccinium myrtillus* L.) growed in Turkey have been investigated in two different solvents (methanol and water) antioxidant properties and antimicrobial activity.

1.1 Data on medicinal use

Dry bilberry fruit has been present as single active ingredient in 115 herbal teas on the German market for more than 30 years, traditionally used for unspecific acute diarrhoea, mild inflammation of the oropharyngeal mucosa (Escop Monographs, 2003). Comminuted dry bilberry fruit has been on the Polish market as single active ingredient for more than 30 years and it is also registered as a herbal tea for unspecific acute diarrhoea in Austria. A bilberry methanolic dry extract prepared from fresh fruit (DER 153- 76:1; extraction solvent methanol 70% v/v) containing 36% anthocyanosides, corresponding to 25% anthocyanidins has been on the Italian market for more than 30 years, at least since 1984, being the active substance of three medicinal products, in soft and hard capsules and as a granulate for oral solution (European pharmacopoeia, 2008). Long-standing medicinal use for at least 30 years within the European Community, is therefore demonstrated for the following preparations and indications:
1) *V. myrtillus* L., fructus siccus (dry bilberry fruit), whole or comminuted, as herbal tea for oral use as an adjuvant in unspecific acute diarrhoea. Traditional medicinal use of this preparation is substantiated by extensive bibliography and the presence on the German and Polish market for more than 30 years. The Daily dose in adults and adolescents over 12 years ranges from 15 to 60 g, divided in 3-4 single dose of 5 to 15 g in 250 ml as a 10 minutes decoction. (In Poland it is used also as an infusion (for 10 -20 min under cover: 4 g in 200 ml of boiling water, 2 - 3 times daily) (European pharmacopoeia, 2008). 2) *V. myrtillus* L., fructus siccus (dry bilberry fruit), whole or comminuted, as a decoction for oromocosal use for the topical treatment of mild inflammation of the mucous membranes of the mouth and throat. Traditional medicinal use of this preparation is substantiated by extensive bibliography and the

presence on the German and Polish market for more than 30 years. It is used as a 10% decoction to rinse the mouth several times daily.

1.2 Antioxidant activity

In in vitro studies conducted by Cluzel et al. (1969), it was found that anthocyanins of *V. myrtillus* L. affected the activity of various enzymes of retina in the pig and in the rabbit (inhibiting the activity of phosphoglucomutase, and increasing the activity of lactate dehydrogenase, α-hydroxybutyrate dehydrogenase, 6-phosphogluconate dehydrogenase and α-glycerophosphate dehydrogenase). However, in these studies, the authors used a complex formulation consisting, beyond of an extract of bilberry, of other components, including beta-carotene. Therefore the significant effect of beta-carotene contained in the preparation in large quantities cannot be excluded. It was found, that the extract scavenged superoxide anion and inhibited microsomal lipid peroxidation at all concentrations (25, 50 , 75 and 100 μg/ml) ($p<0.01$) and a 50% inhibition of rate of reaction was observed with a final concentration of 25 μg/ml. The anthocyanoside complex extract was able to inhibit lipid peroxidation (IC_{50} = 50.28 mg/ml) and to scavenge superoxide anion (IC_{50} < 25 mg/ml). The ability to remove hydroxyl radical exerted by this extract was detectable from 50 mg/ml of extract in the reaction mixture (Martin-Aragon et al., 1998). According to Prior et al. (1998), comparison of the antioxidant capacity variety of Vaccinium species have shown high activity of *V. myrtillus* L. Table 1.

Table 1: Antioxidant activity of *V. myrtillus* L. (Prior et al.,1998)

Source	ORAC ROO(μmol/g)	Anthocyanin(mg/100g)	Phenolics(mg/100g)	Ascorbate(mg/100g)
Bilberry	**44.6±2.3**	**299.6±12.9**	**525.0±5.0**	**1.3±0.1**

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44.6±2.3	299.6±12.9	525.0±5.0	1.3±0.1

Direct in vitro influence of the bilberry fruit extracts on the oxidative phosphorylation of isolated rat heart mitochondria was tested by Trumbeckaitė et al. (2013). For testing two types of extracts were used: the hydroethanolic extract (BEE) of the crushed plant material was prepared by maceration with 50% ethanol at room temperature (1:10, v/v), initially for 48 h and thereafter until exhaustion; the aqueous extract (BAE) was prepared using repercolation method (1:10, v/v). The obtained hydroethanolic extract was filtered and concentrated under vacuum (at 50ºC) and then subjected to freeze drying. Freeze dried bilberry powder was packed into a glass jar and dissolved before experiments. The levels of anthocyanidins, measured by use of HPLC, varied in the two extracts (Table 2).

Table 2: Anthocyanidins in the two extracts tested by Trumbeckaitė et al. (2013). Amount of anthocyanidins (ng/ml) in 1 μl) of bilberry fruit extracts

Bilberry extract	**Delphinidin**	**Cyanidin**	**Petunidin**	**Peonidin**	**Malvidin**	**Total**
BAE	0.14±0.1	0.36±0.05	0.22±0.2	0.15±0.3	0.31±0.1	1.18±0.3
BEE	0.18±0.2	0.80±0.1	0.26±0.1	0.19±0.2	0.28±0.3	1.71±0.2

BAE, bilberry aqueous fruit extract; BEE, bilberry ethanolic fruit extract.

When measured the effects of BEs on complex I-dependent substrate pyruvate plus malate oxidation, mitochondrial respiratory rates only in the presence of 5–30 ml/1.5 ml of the BAE extract the mitochondrial state 3 respiration rate decreased from 33% to 61% ($p < 0.05$). Pure anthocyanins, the main components of used extracts, malvidin-3-glucoside, malvidin-3- galactoside, and cyanidin-3-galactoside, had no effect on oxidation of pyruvate plus malate. A statistically significant decrease in H_2O_2 production by mitochondria was found in the presence of bilberry fruit extracts. BAE at concentrations of 1.5 and 15 μl/1.5 ml clearly suppressed this process and caused a 46% and 62% reduction, respectively, in the H_2O_2 generation as compared with that in the absence of BAE. Similar effects (reduction by 50%) were obtained by BEE (15 ml/1.5 ml), whereas lower amounts of BEE (1.5 ml/1.5 ml) suppressed H_2O_2 generation by 16%, that is, less than BAE. The results revealed that the effect of BAE and BEE on mitochondrial function is bivalent: lower concentrations (they correspond to 6–9 mg/l of total anthocyanins) had no effect on mitochondria, whereas at high concentrations (they would correspond to 18–52 mg/l of total anthocyanins), the extracts caused an obvious decrease in the state 3 respiration, but the radical scavenging activity remained increased. The phenolic fraction of *Vaccinium myrtilli* L. significantly decreased generation of reactive oxygen and nitrogen species (RONS), of oxidizing lipids, proteins and DNA. Antiradical activity was evaluated spectrophotometrically as the ability of the tested substances to reduce 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The extract has shown scavenging activity; 50% inhibition was achieved at 3.99 ± 0.14 μg/ml (IC_{50}). In the same experimental condition, IC_{50} of the synthetic analogue of vitamin E, trolox, was 2.15 ± 0.06 μg/ml (8.57 ± 0.25 μmol/l) (Valentova et al., 2007). Estimation of the antioxidant activity of the BE was also investigated in the xanthine/XOD superoxide generating system. The BE scavenged the superoxide radical and its activity was equivalent to 108 ± 7.2 units of SOD per mg of extract. In the same system, trolox had an activity equivalent to 16.4 ± 0.19 units of SOD/mg (Valentova et al., 2007). Ogawa et al. (2011) studied the lipid peroxidation and free radical scavenging activity of a BE (containing more than 25% anthocyanosides – no futher detail) in murine stomach tissue homogenates. BE in the concentration dependent range significantly induced decrease of activity of the lipid peroxide levels and revealed strong scavenging activity against superoxide and hydroxyl radicals. Table 3; Table 4)

Table 3 Superoxide anion radical scavenging activity of the bilberry extract and its main anthocyanidins (delphinidin, cyanidin and malvidin) (Ogawa et al.2011).

Compound	IC50			
	µg/mL		µM	
Bilberry extract	1.2.	(1.0-1.5)		
Delphinidin	1.2.	(0.9-1.6.)	3.5.	(2.5-4.7)
Cyanidin	31.8.	(21.8-50.9)	98.4.	67.5-157.8)
Malvidin	1.0.	(0.7-1.4)	2.8.	(2.0-3.8)
Trolox	130.8.	(113.4-154.0)	522.4.	(453.1-615.1)

Table 4 Hydroxyl radical scavenging activity of the bilberry extract and its main anthocyanidins (delphinidin, cyaniding and malvidin) (Ogawa et al.2011).

Compound	IC_{50}			
	µg/mL		µM	
Bilberry extract	116.	(80-192)		
Delphinidin	237.	(203-205)	0.7.	(0.6-0.9)
Cyanidin	>323	(>1.0.)		
Malvidin	>367	(>1.0)		
Trolox	325.	(275-400)	1.3.	(1.1-1.6)

IC_{50}, 50% inhibitory concentration. The parentheses show 95% confidence limits.The antioxidant activity of phenolics (at concentrations of 1.4, 4.2, and 8.4 µg of purified extracts/ml of liposome sample) such as anthocyanins, ellagitannins, and proanthocyanidins from bilberry.

1.3 Antimicrobial activity

It has long been known that several phenolic substances such as flavonoids, phenolic acids, tannins and lignans have antimicrobial activity (Heinomen, 2007). It is believed that it is the flavonoid anthocyanins component in *V. myrtillus* that exerts such an effect. The mechanism of antimicrobial activity may include antiadhesion activity, destruction of the cytoplasmic phospholipid bilayer of the cell wall in microbes, damage of the outer membrane with disintegration of the liposaccharide (LPS) layer by phenolics, tannins complexation of metal ions and inhibition of plasma coagulation by bacteria. Another mechanism is the inhibition of antibacterial multidrug resistance (MDR) and impairment of the efflux pump activity in bacteria (Heinomen, 2007). Rauha et al. (2000) evaluated the antimicrobial activity of a number of plants, including bilberry. To the in vitro studies, an aqueous solution of the dry extract prepared from the dry plant material (acetone/methanol 70% V/V – no further detail) was used to determine the diameter of the inhibition zones in the agar cultures of bacteria. Clear antimicrobial effect has been found for the BE (500 μg samples) against the Micrococcus luteus (inhibition zone (i.z.) of sample= 3 - 4 mm > i.z. of methanol and slight antimicrobial activity against Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli and Candida albicans (i. z. of sample = 1 - 3 mm) > i.z. of methanol. The antimicrobial activity of many plants, including *V. myrtillus* L. extract (acetone-water 70:30 V/V; elution with MeOH) prepared from fresh frozen berries was screened against the human pathogenic microbial strains on agar plates to estimate their growth and adherence of the bacterial cells to a berry material. The BE (1 mg/ml) revealed the death of the culture of *Helicobacter pylori*, very strong inhibition of growth of Bacillus cereus and strong inhibition of growth of *Clostridium perfringens* and *Staphylococcus aureus* (Nohynelc et al., 2006). Activity of bilberry against Gram positive and Gram negative intestinal pathogens was examined in in vitro cultures of Salmonella, Staphylococcus, Listeria and Lactobacillus bacteria. The BEs (water/ethyl acetate/methanol) prepared from fresh frozen berries containing phenolic acids and fractions eluted with methanol (ellagitannins and anthocyanins) were tested. BE (2 mg/ml) inhibited the growth of Staphylococcus aureus for 12 and 24 hours (5 x 101 - 5 x 102) and Salmonella enterica Typhimurium (10- 5 x 101). BE fractions (10 mg/ml) exhibited stronger inhibition against Staphylococcus aureus (>5 x 104) for 12 and 24 hours compared with control. Stronger inhibition of growth was also seen against Salmonella enterica Typhimurium (5 x 102 - 5 x 103) compared with control. Influence of various preparations of BE prepared from fresh berries, on trophozoites of Giardia duodenalis viability and spontaneous excystation of Cryptosporidium parvum oocysts was examined in in vitro experiments by Anthony et al. (2011).

The water soluble extracts of bilberry containing polyphenols (167 µg/ml of gallic acid equivalents) killed 90.4 + 2.8% of Giardia duodenalis trophozoites. Increase of the spontaneous excystation of Cryptosporidium parvum oocysts observed after administration of the BE (equivalent to 213 µg/ml of the gallic acid). Because anthocyanins represent more than 70% of the polyphenols, it is believed that they are responsible for antiprotozoan activity of bilberry (Anthony et al. ,2011).

2. Material and Method

Aerial parts from *Vaccinum myrtillus* L. (Bilberry) were randomly collected from Artvin, Turkey. Plant materials was harvested at the flowering period in March and April 2018. The taxonomic identification of plant materials was confirmed by a plant taxonomist. Collected plant materials were dried in the shade, then separated from the stem of the plant.

2.1 Preparation of extracts and their solutions : Extraction was followed by filtration through Whatman No 1 filter paper and evaporation of the filtrate to dryness at 30^{O}C in the Büchi V-700 rotary vacuum evaporator. The dry residue was mixed with 150 ml of metanol in a screwcapped Erlenmeyer flask and placed on a Nüve SL 350 shaker (Nüve, Ankara, Turkey) to obtain an metanol extract. Extraction was repeared until the solvent became colourless; 200 ml of metanol was used in total. The combined extracts were filtered through Whatman No 1 filter paper and evaporated to dryness at 40^{O}C in the Büche V- 700 rotary vacuum evaporator. The residue obtained after filtration was left in a dark place at distilled water. This extract was filtered and the filtrate was freezedried in a Labconco 117 freeze-dryer at 5 m Hg and -50^{O}C. The dried samples of all the extracts were stored under nitrogen at 4^{O}C until use. For antioxidant activity measurements, dried extract solutions were prepared by dissolving 20 mg of dried exctract in 20 ml of solvent. Although the same solvent were used for all the assays, concentrations differed from assay to assay as described below. There was no detectable effect of the solvents on any meaured activity, as established by control experiments in which solvents containing no extract were used in the assays (Demir and Bicim, 2019). In all cases, three independent experiments, each with duplicate measurements, were performed. The results shown are the means of these measurements.

2.2 The 2,2-diphenyl-1-picrylhydrayl (DPPH) Free Radical Scavenging Assay: The antioxidant activity of the plants was evaluated by the 2,2-diphenyl-picrylhydrayl (DDPH) free radical scavenging assay, previously implemented . Briefly, 10 µL of plants were diluted to a final volume 150 µL with methanol (HPLC-grade). Then, 4 mg of DPPH were diluted in 100 mL of methanol to obtain a working solution with an absorbance at 515 nm. Diluted plant s was mixed with 2.85 mL of DPPH and incubated 24 h at room temperature in the dark. Finally, absorbance at 515 nm was measured in a visible light spectrophotometer. Methanol (HPLC-grade) was used as a blank, and trolox(6-hydroxy-2,5, 7,8-tetramethylchroman-2-carboxylic acid, Sigma) was used for the standard curve. Antioxidant activity was expressed as μg of trolox equivalents per 1 mL of extracts (μg trolox equiv./g DE). All determinations were performed in triplicate (Scherer and Godoy, 2009).

2.3 Total Phenolic Content : Total phenolic compound contents were determined by the Folin Ciocalteau method. The extract samples (0.5 ml; 1; 10 diluted) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 20-200μg/ml (R^2=0.987) which is a common reference compound. Total phenolic contents can be calculated from the formula: T = CV/M **,**Where, T=Total Phenolic concentration C= Concentration of gallic acid from caliberation curve (μg/ml) V= Volume of extract (ml) M= Wt. of extracts of plants (Asadi et al.,2010).

2.4 Antimicrobial Activity : Disk diffusion susceptibility test was applied to determine the antimicrobial activity of plant extracts. From these extracts, 50 μL of these were impregnated to antibiotic disks in aseptic conditions using a micropipette with a diameter of 6 mm (Schleicher & Schül, Nr 2668, Germany). In our study, Mueller Hinton Agar (OXOID) was used to determine the antimicrobial activity of bacteria and yeast as medium. Plaques in which bacteria were inoculated were incubated for 24 hours at 35 ° C and plates inoculated with yeasts were incubated for 3 days at 30 ° C. When the time was over, the diameters of the inhibition zones formed around the disks were measured. The antimicrobial activity experiments against all the test microorganisms were repeated three times.

3. Results

Natural products have always been a prefered choice of all as it plays a great role in discovering new medicines. There are many organic compounds which are capable of acting as antioxidants. Many natural substances with an antioxidant effect can protect particularly unsaturated fatty acids against oxidative damage, the process being a crucial step in the development of coronary heart diseases such as stroke and heart attack. The antioxidant compounds from food participate in the removal of reactive oxygen speice that is why a balanced diet rich in natural phenols and other antioxidants is required for the prevention of some lifestyle diseases. Furthermore, natural polyphenols have neuroprotective ability and maintain normal cognitive function in the process of brain ageing (Demir, 2018). Antioxidant activity (DPPH) results were given in Table 1. Standard curve is shown in Figure 1.

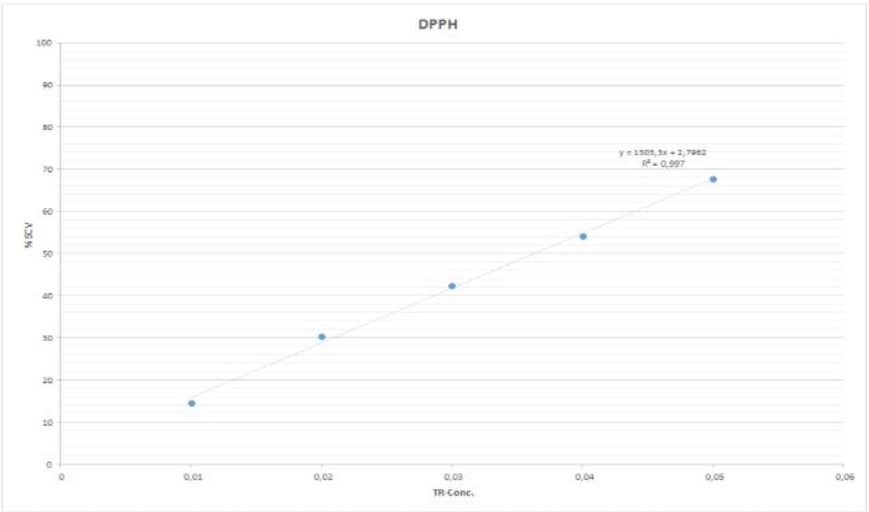

Fig 1. Trolox standard graphic for antioxidant activity(DPPH)Gallic acid standard curve was used to calculate total phenol contents(Fig 2) and total phenolic content of the samples defined as mMGAE/g(Table 1).

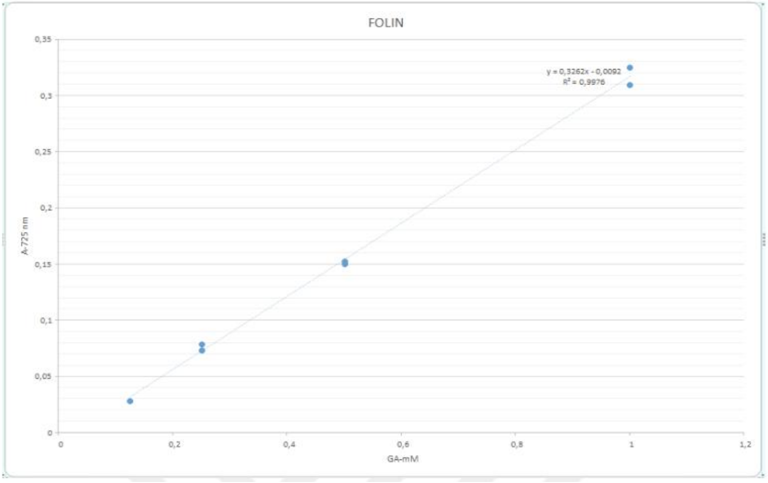

Fig 2. Standard graphic prepared with gallic acid for total phenol content

Table 5 Antioxidan activity(DPPH)μMTE/g, phenolic compound(Folin) mMGAE/g of plant extracts

Plant Extracts	Antioxidan activity(DPPH)μMTE/g	Phenolic compound(Folin) mMGAE/g
Vaccinum Myrtillus leaves(ETOH)	60.85	1.037
Vaccinum Myrtillus fruits (ETOH)	85.21	0.081
Vaccinum Myrtillus leaves (H_2O)	949.49	0.673
Vaccinum Myrtillus fruits (H_2O)	151.98	0.099

4. Discussion

In the present study, the antioxidan activity and phenolic compound of *Vaccinum myrtillus* L. extracts were investigated. Many reports had described the chemical constituents of different extracts of *V. myrtillus* L. including antioxidan activity and phenolic compounds. In the present study, antioxidan activity of water extract from *V. myrtillus* L. leaves revealed 949 μMTE/g. *V. myrtillus* L. leaves had the highest antioxidan activity(949 μMTE/g) followed by water extract from *V. myrtillus* fruits(151.98 μMTE/g), while the lowest value was observed in methanol extract from V. *myrtillus*(60.85 μMTE/g) followed leaves followed by methanol extract from V. myrtillus fruits(85.21 μMTE/g). The phenolic compound of *V. myrtillus* varied from 0.081 to 1.037 mMGAE/g. The highest phenolic compound was determined by methanol extract from V. myrtillus leaves (1.037 mMGAE/g), and the lowest phenolic compound was found by methanol extract *V. myrtillus* fruits (0.081 mMGAE/g).

Phenolic compounds such as flavonoids, phenolic acids and tannins are widely distributed in plants and have gained much attention due to their antioxidant activities and free radical scavenging abilities, which potentially have benefit for human health (Aidi et al., 2020). Results obtained in the present study revealed that level of these phenolic compounds in the various extracts of the *V. myrtillus* leaves and fruits were considerebly higher in methanol extract than that in other aqueous, and this could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds. Morever, the phenolic content of *V. myrtillus* leaves observed in this study corroborated with the findings of Gardeli *et al* (2008) and Nassar *et al*. (2010) on different fractions of this plant. In italy, Giovanelli and Buratti (2009), collected *V.*

*myrtillus f*ruits and made total polyphenols, DPPH analyses. They reported that the *V. myrtillus* had a high antioxidant capacity. In addition to this, they found that the fruit of *V. myrtillus.* Patriot showed the highest activity among the all cultivars. It can be said that the similar results were determined from our study for *V. myrtillus*. According to results of Burdulis *et al.* (2009) , study, *V. myrtillus* had high radical scavenging and antioxidant activity. In other study showed that *V. myrtillus* had high total polyphenols 11.539-20.742 mg GAE/g dry sample. The antioxidant activities found with DPPH, expressed as trolox equivalent antioxidant capacity ranged from 0.143 to 0.297 mmol TEAC/g dry sample. According to the study of Tumbas(2010) phenolic compound in bilberry (*V. myrtillus* L.)observed 494.31 μg/g dry sample. ROS play a role in signal transduction; whereas excessive ROS production lead to oxidative stress which has been involved in the pathophysiology of many cardiovascular diseases such as endothelial dysfunction, atherosclerosis and hypertension (Frombaum etal., 2012). Among all the extracts, water extract from *V. myrtillus* leaves was shown to possess significant radical scavenging activity against DPPH, evidencing its ability of having extracted a considerable amount of polyphenols and flavonoids with specific structure with many hydroxyl groups. According to many reports, there is a highly positive correlation between polyphenols, flavonoids and antioxidant activities in many plant species, and this is mainly due to their redox properties, which allowa them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Govindon et al.,2013). Furthermore, they scavenge free radicals and have a metal chelating potential. DPPH radical scavenging methods are common spectrophotometric procedures for determining antioxidant capacities of components, and they are based on the ability of DPPH radical to decolorize in the presence of antioxidants by accepting an elecron or hydrogen donated by an antioxidant compound.Thus, the strong scavenging capacity of *V. myrtillus* extract as possibly due to the hydrogen/electron donating ability of the polyphenolic compounds present in these extracts, which made them good antioxidants acting as free radical inhibitors or scavengers. Our results is in agreement with that obtained by Hayder et al., who studied the effect of extraction solvent on DPPH scavenging activity of myrtle leaf extract from Tunisia (Hayder et al., 2008). Myrtle extracts showed potent antioxidant activity mainly due to their richness in phenolic compounds. Therefore, these polyphenols should be considered to contain conjugated ring structures and hydroxyl groups that have the potential fo function as antioxidant in vitro by scavenging free radicals involved in this oxidative processes.

Phenolic compounds are the most abundant and potent antioxidants in plants, there is also a numbe of non-phenolic compounds that contribute to the overall antioxidant activity of plant extracts. The antioxidant activity of phenolic compounds critically depends on the number and position of phenolic hydroxyls in the aromatic ring moieties. Generally, monophenols are less effective than catecholic phenols, and phenolic aglicons have higher antioxidant activity than their respective glycosides (Çelik, 2006). Although the leaves and fruits of V. myrtillus are commonly used in traditional medicine as antihypertensive therapy, the pharmacological evidences of their activity are lacking. Our data reveal that aqueous extract showed a significant antioxidant activity than methanol extract, and this is most possibly due to its high total contents of phenolic compounds. In general, bluberry fruits have a high antioxidant content. Its fresh and dried fruits are sold in many markets. In addition, leaves of bluberries can be found in markets as tea. According to the results of some previous researches, fruits of blueberries prevent memory lossing and aging since they includes antiageing features. Anthocyanins, phenolic compounds and flavonoids have the ability to neutralize free radiacals. Blueberries contain high amounts of phenolic compounds and have a high antioxidant activity (Pereira et al., 2012). In the present study, our data highlight the good antioxidant proprieties of different extracts from *V. myrtillus* L.This antioxidant potential is probably attributed to the presence of polyphenolic compounds which may have many benefits in treating exidative stress related diseases. These results lay the ground work for further studies on the molecular mechanisms underlying the biologycal profile of the extracts and isolation and purification of more active principles in each extract as well as clarification of their mode of action.

Tablo 6 Result of antimicrobial activity

Mikroorganims	Leaf H2O extract	Fruit H2O extract	Leaf ETOH extract	Fruit ETOH extract
Escherichia coli ATCC 10536	7 mm	0 mm	7 mm	10 mm
Staphylococcus aureus ATCC 6538	7 mm	0 mm	0 mm	9 mm
Pseudomonas aeruginosa ATCC 15442	0 mm	0 mm	0 mm	7 mm
Bacillus subtilis ATCC 6633	8 mm	0 mm	6 mm	10 mm
Candida albicans ATCC 10231	0 mm	0 mm	0 mm	0 mm
Aspergillus niger ATCC 16404	0 mm	0 mm	0 mm	0 mm

In our study, extracts of leaves and fruits of blueberries prepared with two different solvents, indicated maximum antimicrobial activity on *Escherichia coli* (10 mm) and *Bacillus subtilis* (10 mm). Fruit and leaf extracts were found to be more effective in gram- positive bacteria. This may be since gram-negative bacteria has a multi-layered structure which consists of a lipopolysaccharide layer on the outermost wall of the cell.

This structure ensures gram-negative bacteria to be more resistant. In the antimicrobial activity of the plant's ethyl alcohol extracts on the test organisms, fruit extracts showed antimicrobial activity against microorganisms in the range of 7-10 mm and leaf extracts between 6-7 mm. Fruit ethyl alcohol extract indicated antimicrobial activity against *Escherichia coli*, *Bacillus subtilis* (10 mm), *Staphylococcus aureus* (9 mm), *Pseudomonas aeruginosa* (7 mm). No antimicrobial activity was detected on *Candida albicans* and *Aspergillus niger*. An antimicrobial activity was observed in leaf ethyl alcohol extract against *Escherichia coli* (7 mm), *Bacillus subtilis* (6 mm) while no inhibition zone was formed in *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger.*

In this study, the antifungal properties of fruit and leaf extracts are weaker than those of antibacterial properties. It can be asserted that this is due to sterols in the eukaryotic cell membrane. While antimicrobial agents must bind to sterols in the cell membrane to inhibit eukaryotic fungal cells, such binding is not necessary for prokaryotic bacterial cells that do not carry sterols (Erdogrul, 2002). In leaf extracts prepared with water, *Bacillus subtilis* (8 mm), *Staphylococcus aureus* and *Escherichia coli* (7 mm) showed antimicrobial activity but *Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* did not form an inhibition zone. Antimicrobial activity was not observed in fruit extracts. Ethanol extract of dried fruit and leaves of blueberries was found to be very effective against gram positive bacteria *Escherichia coli* which causes diseases such as urinary tract infections, meningitis, peritonitis and even showed better activity than ampicillin used as standard drug. In a study, it was observed that the natural species of blueberries, dried fruits and dry leaves showed more antimicrobial activity than wet fruits and fresh leaves. Among the extracts examined, the activity of the extract of blueberry dried fruit in ethanol was found to be highest. In a study by Burdulis et al. (2009), it was found that the antimicrobial activity of the blueberry fruit grown in Lithuania showed the highest activity among the test microorganisms against one of the gram positive bacteria, Escherichia coli. Today, continuous exposure of people to substances with toxic activity, increase in diseases such as nutrition-related cardiovascular diseases, cancer, and lack of reach to sufficient and quality food increases the significance of quality nutrition. Striving to produce foods with high nutritional value and long shelf life has also increased the importance of the quality of the produced foods and the ingredients used. Due to the devastating effects of synthetic antimicrobials on the body, the search for natural preservatives that can replace synthetic substances continues swiftly. Blueberry extracts with high antimicrobial activity should be determined through these types of studies and continuity of the studies should be ensured for industrial application by examining their protective effects on food systems.

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CHAPTER VI

ENVIRONMENTAL SUSTAINABILITY IN LIVESTOCK FARMS

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1. Introduction

According to the United Nations' latest estimates, the world population is expected to reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion in 2100 (UN 2017). The rapidly increasing population in recent decades brings with it an increase in basic vital needs. It is observed that industry, intensive agriculture, and livestock production have shown rapid development in order to meet the especially food needs.

Animal-sources foods constitute a significant part of the protein that a person must take daily for an adequate, balanced and healthy diet. Products such as meat, milk, eggs, honey, wool, leather can be produced in different parts of the world in different demand and production systems. Meeting the needs of the growing population and the demand for food for the specified reasons played an active role in the development of the livestock animals, which are intensely produced in the unit area, have become widespread. As animal-source production continues to grow, releasing large quantities of greenhouses gases and waste in environment.

The transition to intensive production in the livestock sector has increased the amount of waste generated as a result of production in parallel with the production. Also, as a result of intensification in production, the raw material input in the farms, the emissions that occur during the production period within the livestock farms and the effects of wastes, as well as the final desired product, increase the environmental impacts and greenhouse gas emissions.

Competition has increased in the use of limited resources such as land, water, and energy in livestock farms, and emissions released during all production stages have severe impacts on air, water, and soil ecosystems (De Vries and De Boer 2010). The feed has an essential place in livestock production. Global warming caused by greenhouse gas emissions caused by the use of water and fuel in feed production and some crops in feed content has the highest environmental impact rate. Manure, water consumption, electricity consumption, fossil fuel, diesel fuel consumption, dead animal, emissions from feed consumption, solid and liquid wastes, dust, and particulate matter business structure that occur outside the livestock barns during the livestock production process. These emissions cause global warming, eutrophication, acidification, energy use, ozone depletion potential effect, terrestrial toxicity, biotic and abiotic degradation by livestock barns (González-García et al. 2014, Leinonen et al. 2014, Cesari et al. 2017).

Global warming, the most important of these environmental problems, poses the threat of our world's future and the destruction of resources, directly or indirectly. Global warming; is because greenhouse gas emissions (mainly carbon dioxide, methane, and dinitrogen monoxide), which are mostly the result of human activities, cause an increase in temperature on the world. The concept of carbon footprint has emerged to determine the effects of emissions from anthropogenic activities on global warming. Carbon footprint; is the measurement of greenhouse gas emissions from anthropogenic activities in terms of carbon dioxide equivalence. Tier 1-2-3 methods have been developed by the International Climate Change Panel (IPCC) to measure the emission amounts of greenhouse gases (IPCC 2006a).

In addition to environmental effects such as global warming, the use of natural resources also has an impact on ecological sustainability. The use of water in agriculture is essential in terms of both the sustainability of the resource and the evaluation of its interaction with the environment. Increasing water consumption with the increase in production in agriculture and the pollution of water resources created by the wastes afterwards pose a threat to existing water resources. The concept of water footprint has emerged to determine the consumption of water resources and the effects of pollution. The water footprint is the result of activities performed or a unit of product. It is a benchmark for determining the total amount of water consumed for its production or the water pollution resulting from these processes.

The cumulative impact that these environmental problems will cause over time will face the danger of the destruction of resources and irreparable damage to the environment in the future. It is crucial to carry out ecological sustainability assessment to ensure the minimum pollution of the environment and the protection of resources and the continuity of production.

2. Pollutant Sources in Livestock Farms

The livestock sector is a significant polluting source with its environmental impacts on the water ecosystem, soil ecosystem, and atmosphere in all areas, from local production to global scale production.

There are many factors necessary for livestock production, especially in intensive livestock farms. Activities such as feed, mechanization, electricity and water, heating and cooling systems, and ventilation systems constitute inputs in the production process. The products, by-products, and wastes required as a result of production constitute the production process's output. Every activity to be carried out during the life cycle of chicken meat production and the greenhouse gas emissions from the resulting products and wastes will harm global warming (Da Silva et al. 2014, González-García 2014, Leinonen 2014).

Animals, feed, manure, litter material, heating systems carbon, nitrogen, phosphorus, volatile organic particles and microorganisms found in livestock farms are potential sources of an environmental footprint (Xin et al. 2011).

In livestock farms is the most significant emissions source, production of feed. Generally, feed are obtained from crops such as barley, wheat, corn, soybean meal, soybean and contain various vitamins and minerals. Water used during feed production, diesel fuel consumed in machine use, manure, fertilizer and pesticide uses constitute the emission sources. The use of water and space in the feed production process represents the most critical factors in resource consumption. Diesel fuel consumed in the mechanization used for harvesting, the use of natural and chemical fertilizers in plant development, and the use of pesticides in the removal of unwanted weeds and pests increase the emissions of CO_2, CH_4, CO, NO_x and volatile organic compounds (VOC) to the atmosphere.

Anaerobic fermentation and manure are the most important sources of CH_4 emissions in the livectock farms during the production period. The formation, storage, and application of manure on the farm, NH_3, N_2O, and small amounts of CH_4 are the direct sources of gas emissions (Leinonen et al. 2014). Poultry is a non-ruminant animal with a simply built stomach in which a small amount of microbial fermentation occurs (Dunkley et al. 2015). Ruminants, emit CH_4 and N_2O gas emissions with anaerobic fermentation to the environment. Although poultry has fewer effects than ruminant animals because they have a simple stomach digestive system, they are considered a source of greenhouse gas emissions. Vergé et al. (2009) stated that only manure is the source of CH_4 in the poultry sector because enteric fermentation in the digestive systems of poultry is not as significant as the amount of fermentation that occurs in ruminant animals.

In livestocks farms, various tools, equipment, and systems are used to create a better indoor environment for animal such as feed distribution systems, manure belts, automatic feeders and drinkers, ventilation, mechanic manure scraper and lighting systems operated in. It is used is realized with electricity consumption, and as a result, CO_2 emissions are released.

3. Global Warming and Climate Change

Short wave radiation coming from the sun to the earth's surface warms the earth by transforming from light into heat. The Earth's surface reflects some of this radiation into space as long-wave infrared radiation. Most of these long-wave infrared radiation returns to space, while some are absorbed and trapped in the atmosphere by various gases such as water vapor (H_2O), carbon dioxide (CO_2) and methane (CH_4). Thus, the earth's surface and atmosphere become hotter than it should be, and the warmed air remains in the earth's atmosphere. This event is called the greenhouse effect, and the gases that warm up the world are called greenhouse gases (Korkmaz 2007).

With the industrial revolution that began in the 18th century, industrialization movements gained accelerating with each passing century. With the introduction of coal in steam engines in the Industrial Revolution and later the use of petroleum and other derived fuels, the most important greenhouse gas, CO_2, has increased excessively. Together with the developing industry, activities such as population growth, increasing fossil fuel use, developing technology, decreasing forests, and intensive agriculture have increased. As a result of this, increasing greenhouse gas emissions in the atmosphere the greenhouse effect and the temperature of the world. This situation triggers global warming, which is a severe problem for our world (Köse 2018).

Global warming; are the emissions of important greenhouse gases, firstly carbon dioxide, to the atmosphere, especially as a result of the burning of fossil fuels such as coal, oil, and gasoline as a result of human-made activities and the reduction of large-scale forest areas (Houghton 2005). In another definition, global warming; at the beginning of the 20th century, especially since the end of the 1970s, it has been stated as the long-term increase in fossil fuel consumption emissions from the Industrial Revolution in the world (Anonymous 2019a).

Global warming is the primary cause of climate change due to emissions of greenhouse gases (CH_4 and N_2O, especially CO_2) to the atmosphere. The greenhouse gases that cause global warming are produced the burning of fossil fuels (energy), industry and manufacturing (chemical processes and cement production), transportation, land-use change, solid waste management and storage, agricultural production and management (livestock breeding, manure management, stubble burning, rice production) activities (Şahin and the Avcıoğlu 2016). The uncontrolled increase of greenhouse gases after the industrial revolution has caused the world's average temperature to rise by 1˚C. The most significant effects of global warming on climate change are; world warming, melting glaciers, rising sea levels, changing rainfall patterns, extraordinary weather events significant increases in the frequency and intensity of the ecological structure, and changes due to these changes (Bayraç 2010).

Climate change changes in the normal state or variability of the climate for decades or longer (MGM 2019). An international breakthrough in 1992 to limit and reduce greenhouse gas emissions in the fight against climate change was achieved with the United Nations Framework Convention on Climate Change (UNFCC). The Kyoto Protocol was discussed in 1997 in Kyoto, Japan. However, since it must be signed by countries responsible for at least 55% of worldwide emissions, this rate was only reached and entered into force in 2005 when Russia signed the protocol (Anonymous 1998).

Since the Kyoto Protocol will expire in 2020, the UNFCC 21th Conference of the Parties signed the Paris Agreement, which sets the framework for climate change since 2020. The Paris Agreement, which is expected to come into force in 2020, sets a more limiting goal of allowing the global average temperature to rise by a maximum of 2˚C and reducing greenhouse gas emissions by staying constant at 1.5˚C (Anonymous 2019b).

Livestock breeding directly contributes to global warming potential with enteric fermentation, manure and indirectly feed production. Greenhouse gas emissions from small ruminants are mainly associated with methane (CH_4) from both enteric fermentation and manure management, whereas with for nitrous oxide (N_2O) it appears to be only related to manure management (Opio et al., 2013). In anthropogenic activities, the systems producing the highest amount of CH_4 are beef and dairy cattle farming activities. Therefore, CH_4 emission produced in cattle systems and its impact on global climate change is a worldwide concern (Petrovic et al., 2015).

Animal production constitutes 14.5% of the global warming potential, and 8% of the emission from this sector originates from poultry farming and cattle breeding (Gerber et al., 2013; Kılıç et al., 2018). Poultry production is an important source of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) emissions. NH_3 emission rates are higher in poultry houses compared to other livestock farms. Therefore, they are considered as NH_3 emission source. Since NH_3 is not greenhouse gas, it is not discussed in this study.

4. Environmental Sustainability

In today's world, the population is increasing day by day, and with the increasing living standards and needs, a consumer society brings with it. People's have needs, especially for food, health, transportation, communication, clothing, etc. as well as many vital needs. Animal proteins constitute an essential part of the amount of protein that a healthy person should take daily. Industrialization and vertical expansion have become common to meet excess demand. To meet the increase in demand for livestock products, the increase in the amount of production by intensive breeding causes the resulting a large amount of waste. The resulting waste and gas emissions have cumulative effects on the environment over time, destroying the environment and causing severe damage. Ecological sustainability is an important issue to focus on to avoid irreversible damage to the environment over time and to ensure that resources are not destroyed and protected for the next generation (Hellstrand 2013).

Sustainability as a word means to provide continuity and productivity of existing resources for the next generation while meeting today's needs. The report 'Our Common Future,' published by the United Nations Commission on Environment and Development (WCED) in 1987, described sustainable development with sustainability. In this report, it is stated that sustainable development can be achieved by meeting the needs of today's people without compromising humanity's ability to meet the needs of future generations. A holistic, sustainable development can be achieved through economic, social, and ecological sustainability, and the foundation of this is the provision of ecological sustainability because ensuring ecological sustainability will play an active role in ensuring economic and social sustainability along with creating the infrastructure for sustainable development (UN 1987).

Ecological sustainability ensures that the ecological environment and the resources in nature are used cleanly and permanently, both by meeting the needs of today and protecting at least today's level for the next generation. The amount of intensive waste generated by the high production potential in intensive enterprises constitutes a problem in ensuring the continuity of production in the farms and its effects in the natural environment. The effects of the wastes on the environment must be evaluated and taken precautions before they become irreparable. Environmental sustainability assessment; waste mitigation, evaluation, or to be brought under control, ensure the conservation of resources use and cleanly, and for companies and institutions in terms of ensuring continuity of production have also become important (Toprak 2006, Akgül 2010).

Meat production has the most environmental impact and livestock farms have the greatest impacts (Djekic & Tomasevic, 2016). Water and land usage, methane, nitrous oxide and carbon dioxide emissions, acidification and eutrophication potential, manure are among the most important environmental problems derived from livestock farms (Waveard et al., 2007; Reckmann et al., 2012). The most important indicators for environmental sustainability in livestock farms are greenhouse gas inventory, carbon footprint and water footprint.

5. Greenhouse Gases and Greenhouse Gas Inventory

The rapid industrialization process after the Industrial Revolution has led to a rapid increase in the greenhouse gas emission intensity in the atmosphere. Beacuse urbanization was increased with the migration of the population from rural areas to cities, and begining of intensive production in the agricultural sector. Greenhouse gas emissions into the atmosphere increase as a result of activities such as the growth of production potential in intensive livestock operations and the increased use of manure, fertilizers, pesticides, and chemical drugs used in cultivated areas. These factors show that the agricultural sector is a severe source of greenhouse gas emissions and has severe effects on global warming (Korkmaz 2007). Gerber et al. (2013); stated that 14.5% of the global warming potential of human origin was caused by animal production, and 8% of it was from chicken meat and egg production.

Carbon dioxide (CO_2), methane (CH_4), dinitrogen oxide (N_2O), hydro fluoride carbons (HFCs), perfluorocarbons (PFCs) and sulfur hexafluoride (SF_6) are mentioned in the Kyoto Protocol as greenhouse gases and cause global warming. The seventh greenhouse gas added in the second alignment process to the Kyoto Protocol, which expires in 2020, is NF_3 (nitrogen trifluoride). According to emission factors determined by the Intergovernmental Panel on Climate Change (IPPC), CH_4 and N_2O gases are expressed in kgCO_2 equivalence. Table 1 states their equivalents in terms of greenhouse gases and CO_2. According to Table 1, CO_2 is less dangerous, and SF_6 is a hazardous greenhouse gas. However, the greenhouse gas most commonly found in the atmosphere is CO_2. It is, therefore, considered the most dangerous greenhouse gas. Sulfur hexafluoride is not yet a danger because it is not a greenhouse gas dense in the atmosphere (IPCC 2006b).

Considering the IPCC's 100-year time period, N_2O and CH_4 are the greenhouse gases that are the highest in the atmosphere after CO_2. The IPCC states that their global warming potential in terms of CO_2 equivalence is equivalent to 1 million tons of CH_4 gas, 25 million tons of CO_2, and 1 million tons of N_2O gas with 298 million tons of CO_2. These data show that the capacity of N_2O gas to absorb heat in the atmosphere is higher than that of CH_4 gas (IPCC 2006b).

Table 1: Greenhouse gases and CO_2 equivalents (IPCC 2007)

Greenhouse gases	CO_2 equivalence
Carbon dioxide (CO_2)	1
Methane (CH_4)	25
Dinitrogen oxide (N_2O)	298
Hydro fluoride carbons (HFCs)	124-14800
Perfluorocarbons (PFCs)	7390-12200
Sulfur hexafluoride (SF_6)	22800

According to the United Nations Food and Agriculture Organization (FAO), 18% of the total greenhouse gas is released as a result of agricultural activities, and 14.5% of this value comes from livestock farms.

Turkey Statistical Institute (TUIK) 's total greenhouse gas emissions, according to the latest published data showing a 135.4% increase from 2016 to 1990, 496.1 million tonnes (Mt) of CO_2 was stated to be equivalent. The highest share was generated from energy-related emissions with 72.8%, while industrial operations and product use accounted for 12.6%, agricultural activities accounted for 11.4%, and waste generated by 3.3% (TÜİK 2018).

While emissions from agricultural activities were 42.4 million tons in 1990, they increased by 33.25% in 2016 and reached 56.5 million tons. 55% of CH_4 emissions and 77.6% of N_2O emissions have emerged from agricultural activities (Table 2) (TÜİK 2018).

Table 2: Percentages by CH_4 and N_2O emission sources

Greenhouse gas sources	CH_4 (%)	N_2O (%)
Agricultural activities	55,54%	77,60%
Energy	18,63%	12,10%
Industrial processes and product use	0,03%	3,80%
Waste	25,80%	6,50%
Total (thousand tons)	2 188,7	107,3

6. Carbon Footprint and Carbon Management

After the Industrial Revolution, climatic characteristics began to change with the increase of greenhouse gases that caused warming as the world entered a rapid development process. The increase in the use of fossil fuels by industrialization, the deforestation created by the opening of new areas to meet the increasing needs, and the release of greenhouse gases from different industry areas into the atmosphere are among the most significant sources of global warming (Bekiroglu 2011). The concept of carbon footprint has emerged to demonstrate the emissions of greenhouse gases that cause global warming in terms of CO_2 equivalence throughout the processes of the formation of products, enterprises, and services.

Carbon footprint is a measure of the damage caused to the environment by human activities in terms of the number of greenhouse gases produced, measured in units of carbon dioxide (Çınar 2013, Kılıç ve Amet 2017). Wiedmann and Minx (2008) described the carbon footprint as the total measurement of CO_2 emissions, either directly or indirectly, in each process during the life cycle of an activity or a product. In other words, of the total amount of greenhouse gases released by institutions or individuals resulting from activities such as transportation, heating, electricity consumption is expressed in terms of equivalence of carbon dioxide. Since carbon dioxide is the most concentrated greenhouse gas in the atmosphere, the carbon footprint is calculated in CO_2 equivalence (Wiedmann and Minx 2008).

Carbon footprint; primary (direct) footprint and secondary (indirect) footprint can be examined in two groups according to their scope. The first footprint; is a measure of direct carbon dioxide emissions from burning fossil fuels, including household energy consumption and transportation. The secondary footprint is a measure of all indirect carbon dioxide emissions associated with their degradation during the life cycle of products or activities due to their manufacture, operation, and use. Carbon is the most critical component of the ecological footprint. Turkey's ecological footprint is formed by the impact of carbon footprint with a ratio of 46% (Ozsoy 2015).

There are some international standards in determining carbon emissions related to global warming and carbon footprint. The most current and widely used of these standards are ISO 14067:2018, GHG protocol, and PAS 2070. These standards guide in determining the sources of the output of greenhouse gas emissions, in calculating data, in creating process maps, in assessing the precision of measurements, and in helping to make a holistic greenhouse gas assessment. Various devices and mechanisms have been developed that measure the necessary greenhouse gas concentrations in calculating the carbon footprint. A wide range of computational methods has been developed for a detailed calculation of the results of measured concentrations, from simple approaches to advanced life cycle assessment and input-output based methods.

International Panel on Climate Change has developed Tier 1-2-3 approaches for calculating greenhouse gas emissions measurements:

• Tier 1 method is a method of calculation using existing national and international statistics and default emission factors.

• Tier 2 method is a calculation method using national energy statistics and emission factors specific to regions.

• The Tier 3 method is a complex and detailed modeling method that requires more data.

As a result of the studies, scientists have stated that the effects of the agricultural sector on global warming are undeniable. Livestock farms are significant producer resources affecting global warming. Environmental footprints are an essential method for determining environmental impacts such as global warming and water use caused by waste and gas emissions during the life cycle of food products in livestock farms. As a result of carbon footprint calculations, food consumption with 52% accounted for the highest value resulting in carbon emissions and, therefore, greenhouse gases, while sources used for food products with 17% accounted for (Anonymous 2012a). Livestock production results in more significant greenhouse gas emissions than other food products (De Vries and de Boer 2010, Kebreab et al. 2016). There are studies on carbon footprint in certain areas in Turkey, and there are not enough studies for agricultural production.

Henriksson et al. (2011) determined the greenhouse gas emissions resulting from milk production with their carbon footprint in a study they conducted. They reported that the most important parameters affecting the carbon footprint of milk are feed dry matter intake, enteric CH_4 emission, N content in dry matter in feed, N-fertilizer rate and diesel used rate. It is concluded that the carbon footprint of milk production is on average 1.13 kg $CO2_e$ / kg milk.

In a study conducted in the UK, Leinonen et al. (2014) identified the environmental impacts of two different breeding systems (battery cage and enriched cage systems) used to improve animal welfare within the broyler and layer hen houses. They stated that the low-density system in broiler production has 2% more effect on global warming potential due to the need for heating compared to the standard closed system. 3% reduction occurred when the heat exchanger is added. It stated that enriched cage systems have 8% less energy usage and 3% lower global warming potential effect in laying hens production than battery cage systems. The impact of acidification and eutrophication caused by using different methods are tiny differences.

In a study conducted, Dunkley et al. (2015) examined broiler, pullet, and breeding farms. They discussed the greenhouse gases (CO_2, N_2O, and CH_4) that emerged under mechanical (combustion, transportation, and electricity) and non-mechanical (manure) emissions in these farms. They determined the carbon footprint by calculating the total CO_2 equivalence. The greenhouse gas emissions in the four farms studied were between 917 tons CO_2/year and 176 tons CO_2 /year.

Shepherd et al. (2015) conducted a study in three different commercial egg production system (conventional cage system, enriched cage systems and perched alternative cage system). As a result of the observations, the highest amount of NH_3 emission was measured with 0.112 gr/hen/day value in the perched alternative cage system. In contrast, CH_4 emissions were similar and small values (0.07-0.08 gr/hen/day) in each order. It was determined that NH_3 emissions from manure depots account for 60-70% of the emissions within the farm.

In a study conducted in Tunisia, Ibidhi et al. (2017) calculated water, soil, and carbon footprint for the use of natural resources and greenhouse gas emissions for one kg of meat production in sheep and poultry farms in different systems. According to the study, they determined the carbon footprint for the production of one kg of chicken meat equivalent to 3 kg of CO_2 and stated that the carbon footprint of chicken meat is smaller than the carbon footprint of sheep.

7.Water Footprint

¾ of the Earth is covered with water. The saltwater in the oceans accounts for 97.5% of the total water, while only 0.5% of the remaining 2.5% is potable water. More than 90% of fresh water is found in poles or underground sources. Increasing global warming and ongoing climate change are increasing the pressure on water resources. Increasing demand for water consumption as industrialization increases, and greenhouse gas emissions increase dramatically, triggering climate change, resulting in rapid reduction, contamination, and extinction of water resources (Anonim 2012b, Turan 2017).

While there is an essential place for water use in every sector, for agricultural activities is much more important. As part of global water scarcity and concerns about food security, the water footprint is emerging as an essential sustainability indicator for the agriculture and food sectors (Ridoutt et al. 2010). The concept of water footprint was developed in 2002 by Arjen Hoekstra while working at the UNESCO-IHE Research Institute. Arjen Hoekstra, a professor in the Department of Water Management, University of Twente, co-founded the Interdisciplinary Research Area Water Footprint Network (WFN) in 2008, which studies the relationships between water management, consumption, and trade.

There are many definitions related to the water footprint. Water footprint, in general, is a term used to measure the amount of water consumed in the performance of products or services, or the extent of pollution caused by water. Hoekstra (2003) and Gerbens-Leenes et al. (2013) stated that the water footprint is an indicator of water use related to consumed products and that the water footprint coverage is determined by evaluating water consumption and water pollution. By another definition, the water footprint is a measure of clean water use or used polluted waters used by humans (Anonymous 2019c). In other words, a water footprint is the total amount of clean water resources used for the production of goods and services consumed by the individual or society, or used by the manufacturer for the production of goods and services (Anonymous 2014).

The water footprint addresses the use of water, both directly and indirectly. The water footprint can be examined under three headings according to the utilization types and quality of water. These; green water footprint, blue water footprint, and grey water footprint:

• Green water footprint; is the amount of rainwater used throughout the life cycle of a product or service. Since rainfall affects green water supply and demand, climate change and variability should be considered when evaluating the green water requirement of a region (Anonymous 2014).

• Blue water footprint; it measures the amount of water used from the surface and underground freshwater sources of water consumption required for the production of a product or service (Anonymous 2014).

• Gray water footprint; it is an indicator of pollution in freshwater resources. It is the amount of freshwater required for the disposal or reduction of pollutants in polluted water due to contamination of pollutants directly or indirectly to water resources as a result of production activities (Anonymous 2014).

Agricultural production accounts for 92% of the global clean water footprint. Approximately one-third of the total water footprint of agricultural production is water footprint resulting from animal production (Hoekstra and Mekonnen 2012). The need for water in livestock production is due to the need for water for the production of their feed, rather than the amount used for drinking water and cleaning of animals. ISO has developed an international standard for the identification and evaluation of the water footprint. The standard, titled ISO 14046, defines the principles, requirements, and guidelines for assessing and reporting clean water footprint at local, regional, and global levels (ISO 2014).

Turkey, which is among the countries with water restrictions, is also considered an agricultural country with its animal and crop production. This situation makes the existence of water an even more strategic issue for Turkey. Thus, water footprint assessment and the determination of water consumption and pollution will prioritize and guide the taking of the right measures for the protection of water resources for the future. However, not enough studies about water footprint have been done in Turkey yet. Foreign literature includes some studies on water footprints in livestock production.

Ridoutt et al. (2011) calculated carbon footprints and water footprints in six different beef farms in South Australia. When the processes from the cradle to the farm gate were examined, they found that the farms had a carbon footprint of 10.1-12.7 kg CO_2 equivalence/kg per living weight and a water footprint of 3.3-221 L H_2O equivalence/kg.

By Mekonnen and Hoekstra (2012) in a study by considering different production systems in different countries (China, India, the Netherlands, and the United States) different livestock products (beef, mutton, goat meat, pork, chicken, eggs, milk, butter, powdered milk, cheese, and leather) green, blue and grey water footprints were calculated. When considering the weighted averages of selected countries, the lowest water footprint was obtained from chicken meat production with 4.325 m^3/ton, while the highest water footprint was derived from beef production with 15.415 m^3/ton.

A study by Gerbens-Leenes et al. (2013) examined the water footprint on poultry, pig, and cattle farms. They stated that the process in which the water footprint occurs most is feed production and that this process depends on feed conversion rate, feed composition, and feed type. They found that the water footprint generated by poultry, in general, has a smaller value than the production of pork and beef. They stated that most water footprints were from cattle production.

In a study conducted in Germany, Krauß et al. (2015) examined water uses for feed production, drinking water, cleaning, and settlement frequency in four different broiler fattening systems to measure water efficiency. 90-93% of total water use in all fattening systems is due to feed production. They stated that water use for breeding chickens accounted for 7-10%, and drinking water and cleaning water accounted for less than 1%. Water efficiency values for all fattening systems were determined to consist of 0.5 kg carcass weight per m^3 of water input, 2.8 MJ energy, and 57 g protein.

8. Conclusion

In this study, widespread environmental impacts and indicators used in determining the ecological sustainability of livestock farms are explained. Using these indicators, the ecological sustainability of farms can be calculated. In order to use the indicators specified in the study, the data recording systems of the farms should be very good. Sustainability assessment in livestock farms is vital in terms of sustainable food production and understanding the strengths and weaknesses of resource use in livestock systems (Atanga et al., 2013).

It should be worked and developed methods on reducing the global warming potential, reducing the use of natural resources and increase natural source efficiency using in throughout the life cycle of the production chain in livestock farms. Methane conversion rate can be reduced by changes in dietary composition or feed management to reduce enteric fermentations. N_2O and CH_4 emissions from manure should be reduced using some manure improvements in manure management systems. Some strategies provide energy conversion should be developed, and studies should be carried out on emission reduction systems.

In the world, 29% of the water footprint caused by the agricultural sector consists of livestock production (Mekonnen and Hoekstra, 2012). Methods should be investigated to reduce water use or provide more effective water usage in production or feed production on livestock farms. Policymaker should form some policies and legal regulations related to water usage in livestock farms.

It is important for farms to protect themselves within the framework of legal regulations, to reduce their impact on the environment and to calculate their environmental sustainability for a cleaner production. Livestock producers should be encouraged to implement cleaner production applications such as the recycling of waste, energy-saving consumption, development of wastewater quality improvement technologies, and reaching zero waste in livestock farms.

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CHAPTER VII

ORGANIC AGRICULTURE AND MARKETING OF ORGANIC PRODUCTS IN ÇANKIRI PROVINCE/TURKEY

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1. Introduction

We need nutrition in order to provide our life energy. We have met this need with ongoing agricultural activities since the beginning of human history. Global population increasing is one of the most important reasons why the importance of agriculture has been increasing in our life. This change in agriculture has shown a great acceleration with the development of technology and industry. From 1700s years to today's world, the agricultural production has been increased to a certain point with using of chemical manure. Excessive and unconscious chemical using for this purpose caused many negative effects such as environmental pollution, losing quality and flavor of foods and on the health of human and other livings. Due to the negative effect of this agricultural production method called conventional or modern agriculture, it has been started to look for an alternative agriculture production method. Producers and consumers, from all around the world, especially in developed countries, have begun to be organized and prefer to produce and consume healthy agricultural products while maintaining the ecological balance.

In order to feed world population increasing and maintain the ecological balance “Integrated Struggle works” has been started at the end of researching for alternative production way. Owing to the Ministry of Agriculture and Rural Affairs attended the development together with the world in 1990s years and offers success with an increase in product basis. Integrated Struggle Studies; It was the most important factor in the transition to “Good Agricultural Practices” and “Organic Agriculture”.

2. Organic agriculture

Organic agriculture maintains the ecological balance protecting soil against the use of chemicals and keeps disease and pests under control with biologically. In organic agriculture, there is no use of chemical manures or drugs to increase productivity. In addition, it is advised to use green manure and organic manure or implement crop rotation and soil conservation. Every stage of production until reaching to the last consumer is kept under control, and all these certificated production ways mean organic agriculture [1]. The organic agriculture definition was approved by International Federation of Organic Agriculture Movements (IFOAM) in 2008 is this: “Organic Agriculture is a production system that sustains the health of soils, ecosystems, and people. It relies on ecological processes, biodiversity, and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation, and science to benefit the shared environment and promote fair relationships and good quality of life for all involved.” [2]

General aims of organic agriculture;

- To maintain balance in nature,
- To maintain live in nature keeping diseases and pests under control,
- To ensure the continuity of lives in nature by controlling diseases and pests,
- To ensure productive production using natural sources and energy in optimum condition,
- To maintain natural sources for the next generations,
- To protect nature, humans and animals against chemicals,
- To maintain soil fertility for long-term under natural/organic conditions,
- To prevent soil and genetic resource erosion,
- To protect water sources and quality,

- To use renewable energy sources and save energy,
- To protect farmers and staffs who work in farms,
- To enhance the level of incomings for farmers in little farms and maintain the cycle of production,
- To support the economy,
- To obtain healthy and quality production.

Basic principles of organic agriculture

1. Not to use of genetically modified seeds,
2. Not to use of chemical fertilizer polluting soil,
3. In the struggling with pests and diseases; not to use chemicals that are permanent, harmful to nature and not breaking down,
4. To make the certification and labeling of the product. Although organic agriculture brings with the costs of certification and labelling, it saves chemical input costs, does not require investment costs since external input is not required. It can create a self-sufficient production system, increase product quality and quantity and has a demand on a national and international basis. As it is a form of production, it has begun to be implemented as a form of agricultural production that is accepted and applied all over the world, as it has the potential to export and contribute to rural and national economic development [3].

2.1. Organic agriculture in the world

Organic agriculture was first begun in the European Union (EU) and the United States of America (USA), then it appeared in other countries. Due to increasing worries about the environment and health, organic agriculture takes enormous demand from all around the world. It was observed an increase in the number of farmers who interested in organic agriculture depending on the attention. This demand has enhanced the potential in international trade. Although there is no domestic market and demand for organic products in their own countries, some countries have started to produce and export organic products that are not grown and demanded in Europe [4].

Table 1: Chronological development of organic agriculture in the world [1]

1972	The International Federation of Organic Agriculture Movements (IFOAM) was established
1973	Organic Agriculture Research Institute (FIBL) was established
1985	The first legal arrangement was made in France of Europe
1991	2092/91 EU Council Regulation entered into force
1999	Livestock was also included in the EU Council Regulation
1992	Legislation has been published for countries to export organic products to the EU
2000	Japan Ministry of Agriculture has published the JAS Standard for organic herbal products
2001	NOP Standard has been published by the U.S. Department of Agriculture
2009	Council Regulation on Organic Production and Labeling 834/2007 came into force in the European Union

In the world, organic agriculture activities had begun in 1972 with the foundation of The International Federation of Organic Agriculture Movements (IFOAM) although it began after late of World War 2. In the beginning, organic agriculture was begun to protect farmlands; then this movement led to protecting the rights of organic farmers and supporting to produce healthy and natural agriproducts. As the first institution, the IFOAM defined and transcribed the rules on organic production worldwide [5, 13]. In 1998 the directory of the rules developed as basic principles was modified as the IFOAM Basic Standards and came into force after being accepted by the general assembly. The IFOAM is also cooperating closely with international organizations such as the EU, the United Nations Agro-Food Organization (FAO), the World Trade Organization (WTO), and the International Association for Nature Conservation (IUCN). As the most authorized institution, the IFOAM enriches the regulation with the additions annually.

In particular, for the last 5 and 6 years, organic agriculture has shown fast development worldwide. In the present, in 150 countries and at 32.3 million hectares farmland and 1.2 million agriculture businesses in the world have implemented organic agriculture. The countries having the largest organic farmlands in the world are listed as Australia (11.8 million hectares), Argentina (3.1 million hectares), China (2.3 million hectares) and the USA (1.6 million hectares), respectively. These countries are being followed by Italy, Brazil, Spain, Germany, Uruguay, England and others. When compared to conventional production areas, the proportionally highest organic production area is in Europe [3].

In the world, there are essential factors in the development of organic food products marketing. Organic food products are export-based, but also support the development of domestic marketing. Significant food producers and wholesalers bring new and processed organic products to this market. Aquaculture is also another branch that developed in many countries all around the world. In addition to organic agriculture and food products, ecologic hotels and restaurants, organic textile, health products, and related products spread worldwide. Civil Society Organizations (NGOs) and other voluntary organizations are making great efforts to promote organic agriculture, promote the organic food market and trade [4]. In a study conducted by The Organic Farming Research Foundation (OFRF) in the USA, it was determined that 83% of the organic farming enterprises in the USA are family businesses [5]. Table 2 shows the number of organic farming areas and producers in the world.

Table 2: Organic farming areas and the number of producers in the world [3]

Continental	**Organic Farming Areas (million hectares) and Percentage**		**The Number of Producers**
Africa	0.9	3	530.000
Asia	2.9	9	230.000
Europa	7.8	24	200.000
Latin America	6.4	20	220.000
North America	2.2	7	12.064
Oceania	12.1	37	7.222

There are many reasons why organic products are significantly chosen by consumers that the first and important one is to concern about health. A survey conducted that in Germany and England states that the priority level of choosing food in mentioned countries, 70% and 46% is health respectively. All over the world, the rapidly growing organic agriculture is usually traditional products of the country, for example, tea in India, milk and dairy products in Denmark, meat in Argentina and its products, bananas in Central America and Africa, palm in Tunisia, olive oil, dried and hard-shelled fruits in Turkey are the first organic products. Briefly; current knowledge and high adaptability have enabled an easier transition to organic agriculture [5].

In the organic agriculture marketing of the world, North America (48%) and the West Europa (48%) are the pioneer countries. Between 1999 and 2009 years, the value of the market reached from 15.2 Billion $ to 55.0 Billion $ (267% rate of increase). 5% percentage of the total market of food and beverage composed of organic products in 2009. At the same year, 5% of total food and beverage market consisted of organic product sales in the world [4]. In Figure 1, it was presented the logos of the organic products in the world.

Figure 1: The logos of the organic products in the world [6]

2.2. Organic agriculture in Turkey

Organic agriculture movements in Turkey, not by farmers like ones in the world; it was launched through representatives of European organic farming companies. This situation is the result of agricultural products not grown in the EU and exported by Turkey into the EU For instance, in the Aegean region, organic agriculture was introduced and assisted through the representatives of European organic farming companies. Depending on the demand coming from Europe, the projects of organic agriculture and the varieties of organic agriculture had been carried out in Turkey since the middle of 1980s [4].

According to the statistical data provided by FIBL, Organic agriculture is carried out with 65 thousand producers in 1.9% of the total agricultural land of 461 thousand hectares [12].

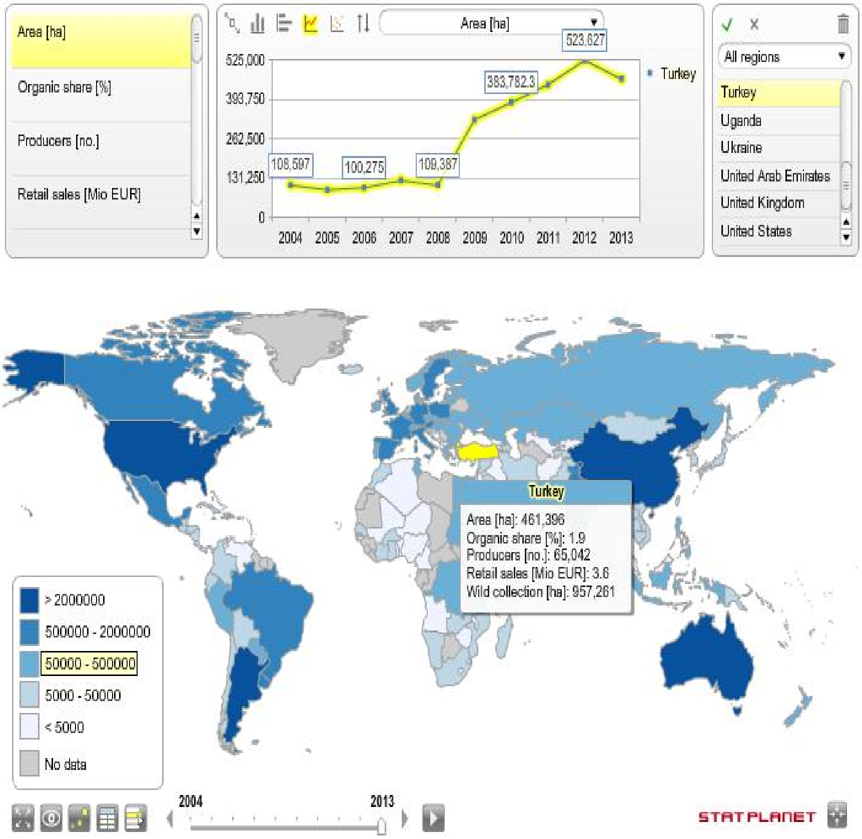

Figure 2: Organic agriculture lands of Turkey [12]

In 2008, 69 different organic agriculture and food products were exported to 34 countries from Turkey. When the product groups are analyzed, the most foreign currency is obtained from organic nuts, figs, apricots, grapes, strawberries, olives, cotton and fruit juices. More than 40% of our total organic product exports are made to Germany. Figure 2 shows that the organic field in Turkey and manufacturer distributions. EU countries such as France, Netherlands, the U.K., Italy, Denmark, Austria, Sweden and Portugal are leading in our organic exports [4].

2.3. Organic agriculture in Çankırı province

Organic Apple; production is made with 656 farmers in the Central District, Eldivan, Ilgaz, Korgun, Yapraklı and Şabanözü Districts, and collection is made for processing rather than direct consumption.

Organic Melon; between 2012 and 2014 years it was implemented in an area of 200 decares with 15 farmer families in the Village of Hasakça of the Central District. In 2004 the amount of production reached 400 tones.

Organic Honey; in the central district Eldivan, Ilgaz and Şabanözü Districts the production is made with totally 37 beekeepers and in 2900 beehives. In 2014 the production reached to approximately 58 tones.

Organic Grape; between 2012 and 2015 it was implemented in an area of 9000 decares with 53 farmer families in the Village of Alaçatı of the Central District. Organic products were started to be harvested from the table grapes, which are in the transition process, in 2015.

Organic Strawberry; in the central district, Yapraklı, Ilgaz and Eldivan Districts, the production of organic strawberry are made with totally 10 farmer family and in 10 decares of area.

Organic Almond Cultivation; in the village of Bakırlı of the Şabanözü District it was built on 45 acres, and it organically started to produce in 2015 [6]. Table 3 shows the organic agriculture production of Çankırı for the last 5 years.

Table 3: Organic agriculture production in Çankırı province in the last 5 years [14]

Turkey	Çankırı Province	Years
120.152.079	402.505	2015
119.237.661	446.915	2016
135.885.136	374.042	2017
159.142.178	405.339	2018
195.831.757	556.362	2019

3. Organic agriculture legislation

In order to ensure organic agriculture movement progress properly in Turkey, it was established the Ecological Agriculture Organization (ETO) in İzmir province. In 2004, in order to make a contribution to improving the organic agriculture movement in Turkey “the Association of Organic Product Manufacturers and Industrialists” was established [7]. According to the EU EEC No 2092/91 and on the 24th of June 1991 issued by the EU for this purpose in Turkey, the first legal regulations on organic agriculture were issued on 24th of December 1994 and 22145 dated "Regulation of Plant and Animal Products to be produced with Ecological Methods" to become and the powers were given to the Ministry of Agriculture and Rural Affairs [15]. In 2003, the Alternative Agricultural Production Techniques Department was established within the General Directorate of Agricultural Production Development of the Ministry of Agriculture and Rural Affairs (MARA). As a result of the studies carried out "Organic Agriculture Law" came into force on the 3rd of December 2004. The basis of the legal regulations regarding organic agriculture was established with the entry into force of the "Regulation on Principles and Implementation of Organic Agriculture" published in the Official Newspaper dated 18 August 2010 and numbered 27676, with the leadership of the relevant units of MARA and with the participation of non-governmental organizations engaged in organic agriculture. Regarding organic agriculture; there is an advisory committee consisting of members from various general directorates of TKB and related public institutions, universities, NGOs and company representatives on organic agriculture. At the end of the study conducted by the advisory committee, A "National Strategy" and an "Action Plan" related to organic agriculture were created. In 2013 with the restructuring of the Ministry of Agriculture and Rural Affairs, to the Ministry of Food, Agriculture and Livestock, General Directorate of Plant Production, Good Agricultural Practices and Organic Agriculture Department, it conducts studies on the financial support of organic enterprises and producers (organic agriculture support, appropriate business and investment credits) for the organization and development of farmer and personnel training, extension, research activities related to organic agriculture.

MARA informs its staffs about the current national and international regulations and standards. At the same time, MARA is responsible for providing international standards and criteria to the control and certification companies to increase the export of our products [16]. The national database on organic agriculture has been provided by the MARA through the control and certification companies for recent years. Export figures are kept by both the Undersecretaries of Foreign Trade and the Associations of Aegean Exporters. This situation, as mentioned before, results in the inconsistency of organic production and export data in some years. Therefore, in collaboration with all institutions related to organic agriculture, MARA started to create a new organic agriculture information system that includes all marketing processes from the production to export of organic products [4].

3.1. The Law of organic agriculture

The "Organic Agriculture Law" numbered 5262, which came into force on 03.12.2004 and consisted of 5 parts. This Law covers the fulfilment of the control and certification services related to the execution of organic agriculture activities and the audit procedures and principles of the Ministry and the powers, duties and responsibilities. It has been issued to determine the procedures and principles for taking necessary measures to improve the production of organic products and inputs to provide reliable, quality products to the consumer. In the second and third sections of the Law, the provisions regarding the Entrepreneurs and Authorized Institutions have been determined. According to this;

1. Products that are not certified by the Association of Control and Certification (ACC) cannot be sold under the name of organic product.
2. Authorized Institutions and their controller/certifications must have the work permit from the Ministry of Agriculture and Rural Affairs.
3. In certain periods with the framework of regulation, it is compulsory to present report, knowledge and documents to the Ministry of Agriculture and Rural Affairs.
4. Enterprises are obliged to work and comply with the legislation under the control of the authorized institutions.
5. Enterprises are obliged to present the necessary documentation to the authorities during the inspection.
6. Organic product labels and logos are used only for organic products. Labels and logos cannot be false, misleading, erroneous impression according to the structure and content of the product.
7. Organic products and inputs that do not carry the necessary certificates cannot be exported or imported.

8. The organization, enterprise, entrepreneur etc. within the scope of the Law control authority has been given to the Ministry of Food, Agriculture and Livestock (MFAL).

In section 4, where criminal proceedings take place, administrative fines ranging from 10 to 50 thousand Turkish Liras (TL) are introduced to those who produce, market, sell, import and export organic products and inputs under the Law [8].

3.2 The Regulation about the foundations and implement of organic agriculture

"Regulation on Principles and Practices of Organic Agriculture" numbered 27676, which entered into force on 18.08.2010, has been prepared for the purpose of determining the principles and procedures for the protection of ecological balance, conducting organic agricultural activities, regulation, development and dissemination of organic agricultural production and marketing. Producing of every kind of animal and vegetable products according to the procedures of organic agriculture, collecting of every kind of food, yeast, forestry products to the procedures of organic agriculture, processing-packaging-labeling certification-storage-transport-controlling and punishment procedure all processes are included by the regulation. The General Rules of Organic Agriculture in article 5 of the Regulation states that,

1. Registration of enterprises should be done to the database within 45 days.
2. Conventional production tools should not be used before disinfecting them.
3. All activities during organic production can be recorded and watched step by step.
4. GMO seeds, fertilizers, etc. products should not be used.
5. Production should not be done without disturbing the ecological balance.
6. It is to ensure using animal and vegetable wastes as renewable sources in production.
7. It is to prevent the damages coming from conventional agriculture.
8. Controls of the measures taken should be checked by the authorized institution.

In the Second Part of the Regulation; Crop Production, Mushroom and Yeast Production by Organic Agriculture Method; Animal Production with Organic Agriculture Method; The conditions to be carried by the enterprises that will operate in relation to the Organic Agriculture Method and Aquaculture, the products to be evaluated within the scope of the organic product, and the production details are included.

Processing and packaging of organic products;

1. It should be composing updated procedures to introduce products systematically.
2. It should be taken hygienic measures to prevent contamination in the production area.
3. It should not be used GMO products and inputs.
4. It should be taken records about harvest days and time information etc.
5. It should be provided with appropriate storage conditions in order to prevent contaminations.
6. Organic product quality should not deteriorate while packaging.
7. It should be preferred to use biological, mechanical and physical methods.
8. It is banned to use ionic radiation in the packaging step.

Labelling of Organic Products,

1. The transition period should be taken into account the minimum 1 year transition period for the products.
2. In transition products, it should be found the transition period expression and used “Logo of Organic Product”.
3. It should be stated who the product belongs to and that it is produced in accordance with this regulation.
4. On the product, it should be found the name of authorized institution, logo, number of code and the certification number of the product.
5. It should be used the logo of organic product in domestic production and marketing.
6. The label and packaging of non-organic products should not be similar to organic product ones.
7. Although the words "organic, ecological and biological" are considered equivalent and used, "bio, bio, eco, eco, org" prefixes should not be used.

Marketing of Organic Products,

1. It can be marketed the products as organic products that have the certification of organic product.

2. The reduction of the number of products sold must be notified to the authorized organization within 7 days, and stock tracking must be done by the authorized organization.
3. Organic products should be sold in such a way that they do not mix with conventional products.
4. If the organic product is sold by hand changing and is not processed, it does not need to be recertified again.
5. In retail sales, the invoice and packing slip must be given to the seller together with the product along with the certified copy of the existing product certificate by the operator and must be provided when necessary. It is given in Figure 3 are used in organic agriculture logo in Turkey.

Figure 3: The Logo of organic agriculture in Turkey [4]


Yetkilendirilmiş Kuruluş Logosu

ÜRÜN SERTİFİKASI

Sertifika No
TR001OT35-01001

Müteşebbis İsmi : İzmir Gıda Tarım ve Hayvancılık İl Müdürlüğü
Proje İsmi : Birgi ve Çevre Köyleri Organik Meyvecilik Projesi
İletişim Bilgileri : Üniversite Cd. No: 47 B. Blk. Z. Kat PK: 35100 Bornova/İZMİR
Tel : 232 435 10 02-05 Dahili : 1201 e-posta : organiktarım35@gmail.com

Ekli Listede yer alan 100 üreticiye ait listede belirtilen arazıler içindir.

İşletmenin Ana Faaliyet Konusu
Organik Bitkisel Üretim (Meyvecilik)
Ürünler: İncir, Kestane, Ceviz, Zeytin

Ürün Bilgileri
PartiNo: -
FaturaTarihiveNumarası: ../../20..-000000001
AlıcıFirma: Muhtelif
ÜrünKaynağı: 350000TR
SözleşmeNo: 350000TR000B000

İş bu belge Yetkilendirilmiş Kuruluş Tarafından yukarıda bahsi geçen Müteşebbisin, Ürünler başlığında yer alan ürünleri TR 5262 sayılı Organik Tarım Kanunu kapsamında çıkarılan mevzuata uygun olarak "organik" veya "geçiş ürünü" niteliğinde ürettiğini gösteren Belgedir.

Ürünler	Niteliği	Süreci	Üretim Miktarı	Geçerlilik Tarihi
Ürün1	Renk, Şekil, Boy vb.	Organik	...... Kg/Yıl	...... Kg/Yıl
Ürün2	Renk, Şekil, Boy vb.	Geçiş1	...... Kg/Ay	...... Kg/Ay
Ürün3	Renk, Şekil, Boy vb.	Geçiş2	...... Kg/Hafta	...... Kg/Hafta

Geçerlilik Süresi: .../.../20.. Tarihine kadar
Kontrol Tarihleri: .../.../20.. .../.../20.. .../.../20.. .../.../20..
Veriliş Yeri/Tarihi: 31 Aralık 2012

Akreditasyon Kuruluşu

Tarım Gıda Hayvancılık Bakanlığınca yetkilendirilmiş Kontrol ve Sertifikasyon Kuruluşunun Adı - Ticari Ünvanı - Adresi

Yetkili Kişinin Adı-Soyadı-Ünvanı-İmzası

Figure 4: The Logo of organic agriculture in Turkey [4]

The processing of control and/or certification system:

The properties of organic production are to control every stage of production and certificate the products. In Figure 4 it is seen an example of a production certificate. According to the provisions, there are two basic factors to secure the product that is control and certification. The control and certification processes can be done by the same institution or by the separate institution.

1. For Enterprises, it is compulsory to present to the certification and/or control authorized institutions the necessary documents about the organic productions.
2. The information and documents are kept in records by authorized institutions.
3. The authorized institution controls the enterprises once a year (as announced or unannounced).
4. Organic agriculture entrepreneur certificate and product certificate are given by the control and certification institution.

In other parts of the regulation there are Provisions regarding the Authorized Institutions and Entrepreneurs, Supervision, Criminal Practices, Organic Agriculture Committee and Organic Agriculture National Steering Committee [4].

3.3. The Operation of organic agriculture law

It can be said that there is the accordance between our country and the EU about the legislation. Operations of organic agriculture control and certification are conducted by the independent-authorized institutions appointed by the MARA. There are still 17 control and certification institutions working in our country [9]. Private control and certification institutions can arrange organic product certificate at the result of their researching and examination. However, these private control and certification institutions have to take the necessary permissions from the MARA.

4. The Market of organic products in Turkey

The organic agriculture model implemented in our country is carried out by the organic producers through the mentioned companies by contract. Producers reassure the companies that not to use chemical fertilizer or chemical substances and produce in ecologically friendly and natural conditions. Control and certification processes are carried out by the Ministry of Agriculture and Rural Affairs and the control and certification institutions (CCI), which are accredited by the European Union (EU), independent of production and marketing companies [4]. In the early of 1990 while the numbers of products produced in Turkey were only 8, in 2008 the number reached 247 products. At Table 4 it is showed the given financial support in Turkish Lira (TL) and encouragement to ensure the development and trade in organic production in Turkey. State support is given to farmers engaged in organic agriculture in Turkey that includes low-interest loans, direct income support, and supports for the protection of agricultural land for environmental purposes.

Table 4: Loans to organic agriculture by years (2004-2011) [4]

	Organic Agriculture		Organic Fertilizer		
Years	**Number of Producers**	**Sum (TL)**	**Number of Producers**	**Sum (TL)**	**Total duty (TL)**
2004	113	3.180.000	3	233.000	3.413.000
2005	263	6.157.000	10	912.000	7.069.000
2006	524	14.629.000	21	1.236.000	15.865.000
2007	651	18.008.000	30	1.553.000	19.561.000
2008	715	19.933.000	45	3.329.000	23.262.000
2009	468	15.411.000	16	1.100.000	16.511.000
2010	1359	59.529.000	19	5.494.000	65.023.000
2011	1274	27.370.000	-	-	27.370.000
TOTAL	**5367**	**164.217.000**	**144**	**13.857.000**	**178.074.000**

As a result of working on organic agriculture, there is a rapid increase in production and export in Turkey. In comparison with other organic product markets all around the world, Turkey has two significant advantages. The first one, the quality of our organic products and the other one, is the variety of our organic products. Most of our organic products are exported to other countries. The most important export countries are the EU countries (80% ratio) and the USA (15%). Germany is the first one in the EU countries. After Germany, significant countries we export that are France, Netherlands, England, Denmark and Switzerland. In addition, North Europe countries, Canada and Japan also are the market taken attention. In 2006 it was exported total 10.374 tones organic products and earned 28 million the USD [7]. There are a few studies and resources about the situation of organic products in the domestic market. Since the late 1990s, there have been promotional attempts in large supermarkets aimed at improving domestic demand. Still, these attempts have not been successful due to insufficient consumer awareness, the limited number of products and high prices.

There is little potential in the domestic market that composed of people who are educated, middle aged-group, high-income level and sensitive to health risk [1]. There are two different delivery ways to consumers for organic products.

4.1. The Markets

Produced by entrepreneurs; unprocessed and unpackaged organic products are collected through producers associations or intermediaries and delivered to enterprises that will process the organic product. In these authorized facilities, organic products are certificated and labelled; then there are delivered to the markets. The inventories of these products entered on a postpaid basis are recorded by certification institutions. The authorized controllers of both the certification institutions and the MARA control the label and the certification of the products if it is necessary, they have the products analyzed. The organic products definitely should be put on shelves separately from conventional products to prevent contamination risk.

In addition to the basic label information, the organic agriculture logo or label, the manufacturer's information, and the information of the certificate issuer must be included on the label of the products called organic products. Organic product sellers have to present the organic product certification to the customer who requested to see. Owing to packaging and processing the organic products increased durability, markets as it provides to reach the desired product variety by the mass of consumers who want to consume healthy and durable organic products, at any time and in a fresh way; it has been the reason for the preference for the consumer [17].

4.2. The Ecologic (organic) markets

Especially in big cities, organic food markets started opening due to increasing consumer demand. Due to the problems arising from the provisions of the Organic Agriculture Law and the Law on Organic Products Wholesalers and the marketing of the organic products sold with conventional products are problems [10]. Producers prepare products harvested in the garden for the market before the market opening. Products are sold in open-air benches, and complaints from the consumers are met with the solution coming from the producer directly. In such markets, producers and consumers come together and get communication about the products directly. This communication is significant for the trustworthy between two sides [11]. Organic products are accepted to these markets by invoice, delivery note, producer certificate or certificate of receipt. In addition, products are weighed both at the market entrance and at the exit. In this way, both the quantities submitted are checked and the quantities sold by the producer that week are reported to the relevant certification institutions. It is provided some information such as where produced, who produced, etc. about the products through the certification that submitted on the bench on the market. In addition, there are also options for changing the product, and it is determined upper and lower price limits for the fair price. With these markets also aims social benefits such as creating ecological life awareness and sensitivity in consumers and breaking the image that organic products are expensive luxury [17].

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CHAPTER VIII

PREPARING THE NEW GENERATIONS TO REAL LIFE BY TEACHING HOW TO FISH

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Introduction

Many individuals today think that the pandemic poses a threat not only to our health, and lives, but also to the social and economic order we are in [1] and they may not wrong in this regard. The IMF says that the decline in the economy as the worst since the Great Depression of the 1930s [2]. The UN's Framework for the Immediate Socio-Economic Response to the COVID 19 Crisis declares that: "Without urgent socio-eco-nomic responses, global suffering will escalate, jeopardizing lives and livelihoods for years to come."[3] Under these circumstances, today's people are worried and stressed about self-sufficiency [1, 4, 5] as well as their life with the emergence of possible economic problems [2] and collective panic over [6-9] time, even if they manage to protect themselves from the virus.

While the self-sufficiency, which has been on the agenda especially since the 1970s, was the area of interest of a minority before the pandemic, today this concept is a problem for the masses [1, 3]. In this case, it seems that the number of individuals who are hungry for education about self-sustainability and even survivability will increase, as they feel insufficient to meet their basic needs and their loved ones. But will this demand be met satisfactorily?

Unfortunately, there is no comprehensive curriculum in formal education institutions where individuals are taught knowledge and skills to meet basic needs and survive in extreme conditions. Although some courses that provide education such as first aid and preparation for natural disasters, these are insufficient and most of them are within the scope of elective courses. Outdoor activities such as camping, scouting, and bushcraft organized by school clubs are only preferred by the individuals who are interested in these subjects. Currently, private institutions, organizations, and businesses provide satisfactory services for individuals with a special interest in gaining both self-sufficiency and survival knowledge and skills. What should be, is to fulfill this need by official educational institutions.

In this study, the two popular movements, “Permaculture” and “Survivalism” will be discussed with the hope that it will shed light on the work to be done in the field of self-sufficiency education in official institutions.

Forgotten Knowledge and Skills

Nowadays, when it comes to any disaster signal, the main reason behind the exaggeration of the food and goods stock; looting, theft, and the increase in the images of people crushing each other in the markets in the race to obtain basic needs is that individuals cannot produce these resources themselves and think that they should have them ready-made [6]. Moreover, these scenes are seen not only in underdeveloped or developing countries but also in what we call world giants, which are regarded as the cradle of civilization. The Wall Street Journal reported that the NYPD had seen a 75% increase in burglaries of businesses from March 12 to March 31 [7]. In the UK, the Union of Shop, Distributive and Allied Workers (USDAW) declared that shop workers have been assaulted, threatened, or abused at least once a week since the start of the crisis, while it is just once every two weeks in 2019 [8]. According to IMF chief economist Gita Gopinath "If the crisis is badly managed and [the response] is viewed as having been insufficient to help people, you could end up with social unrest," told Reuters [9].

It should be known that self-sufficiency is no longer a hobby of a certain segment, and it is on the way to be an area of interest of large masses [1]. But there is a problem: where do we get the sound knowledge and skills on these issues? The reason for this question is that individuals were raised to meet all their basic needs such as water, food, shelter, and clothing until the Industrial Revolution has forgotten the importance of this knowledge and skills over time and not passed on from generation to generation as they can buy these needs with money. While we are currently buying our needs from the markets, we need governments for the supply of them and most of the services [10].

Governments, on the other hand, are obliged to always remain incapable of meeting these needs [11] and remain dependent on foreign sources, unless the collective consciousness of each citizen, accustomed to consumption, not production [12] change. Moreover, they cannot form communities of powerful individuals, each of whom has the knowledge and equipment to meet their own needs. It is imperative that the new generations, especially city-dwellers, can empathize with the world and nature we live in, develop community awareness, and be a part of production to produce longer-term solutions.

The Problem of Sufficiency of Self-Sufficiency Education

We can list 3 factors that are necessary for an individual to survive healthily: clean water, food, and air. The chance of survival of the individual decreases in the absence of shelter, clothing suitable for climatic conditions, and a safe environment [13]. Providing these basic needs is the basic right of every individual. Even if governments try to provide these basic needs to the citizens or parents to their children, they miss a big point: the acquisition of necessary knowledge and skills to meet these basic needs. Since the Industrial Revolution, individuals perceive the knowledge and skill of obtaining basic needs as determining the supplier and providing them with money.

Since the 19th century, the concept of freedom has also changed in time. Today, there is a misconception that individuals who can buy all their needs, not the ones who can meet them as much as possible without needing any external resources, are freer [14]. Today, even though giant steps have been taken in the name of freedom of thought and human rights with laws and practices, we depend on each other and the resources of the governments to meet our basic needs more than ever, therefore less free.

While the main purpose of the curriculum organized by authorized institutions in primary, secondary, high schools and even universities today is to prepare individuals for real life, what is really meant is to reduce the difference between what they learned in school and the conditions they encounter in working life [15, 16]. What is meant by preparing individuals for "real life" is not to provide them with survival knowledge and skills. Today, how many of the individuals who have received education until the first adulthood have the knowledge and skills to meet their basic needs and survive in extreme conditions? Although rural individuals may have the knowledge and skills to obtain one or more of the basic needs listed, city-dwellers do not have such a chance except what they learn with personal curiosity.

In this context, The United Nations Decade of Education for Sustainable Development (2005-2014) encourages to mobilize the educational resources of the world to help create a more sustainable future by stating that: “Many paths to sustainability (e.g. sustainable agriculture and forestry, research and technology transfer, finance, sustainable production and consumption) exist, and are mentioned in the 40 chapters of Agenda 21, the official document of the 1992 Earth Summit. Education is one of these paths. Education alone cannot achieve a more sustainable future; however, without education and learning for sustainable development, we will not be able to reach that goal” [17, 18].

It was on the agenda of sensitive individuals, academic and non-governmental institutions and organizations before the pandemic that individuals in the city should be actively involved in meeting their needs and should be encouraged to be a part of not only consumption but also production [17, 18]. In today's conditions, this issue seems to be on the agenda of the masses. Today, the educational hunger for self-sufficiency and survival knowledge and skills is unfortunately not covered by the curricula of formal education institutions. Private institutions and organizations, relevant communities come into play in this field. Communities that give services in cities and metropolises educate city-dwellers to sow, harvest, utilize even the smallest areas such as balconies and terraces [19-21], and even survive [22]. Besides, there are lots of businesses serving in touristic regions or rural areas help individuals who want to learn these skills by sharing their knowledge and skills within the scope of ecotourism or farm tourism [23, 24].

However, movements and studies related to creating self-sufficient closed or semi-closed systems are only possible when the environmental conditions are ideal or close to ideal. When it comes to extreme climatic and social conditions, a survival dimension is added to the issue of self-sufficiency. In extraordinary situations, which are caused by human or natural ways, such as earthquakes, hurricanes, nuclear attacks, economic collapse, pandemic, war, famine survival skills, and knowledge come into question. The question to be asked today is how many of the individuals who have graduated from university or even have post-graduate education, have good jobs, and live above the social standard are prepared for the extreme conditions mentioned [25, 26]? Even the medical world has been desperate when it comes to the pandemic, it's terrifying to think about the rest [27, 28].

Numerous courses are on the agenda to prepare individuals for extreme conditions with theoretical and practical knowledge in all weather conditions and disasters. Individuals attend courses for both self-sufficiency and survival to look to the future more confidently since knowing that you are prepared for such conditions gives individuals confidence and relief in some way [29]. Furthermore, the possibility of an individual who is equipped with the necessary knowledge and skills to acquire basic needs to commit to someone else's property and life also decreases [30].

A movement that stands out for self-sufficiency: Permaculture

The reason why Permaculture is prominent in studies conducted on self-sufficiency and of course being discussed in this study is that it includes many movements and studies that emerged before and after it because of its holistic approach. Dating back the 1970s, Permaculture is a system based on creating long-term sustainable, economically viable, and ecologically healthy systems or farms where families and communities obtain enough products for themselves.

One of the most important features that distinguish this system from others is the concept of "design", which is made by considering the relationships of each element such as buildings, animals, plants, energy systems, and multiple benefits (Fig. 1). The purpose of permaculture is not to go back to the old times when human beings were slaves of the land, but to use energy the most efficiently by combining ancestral knowledge and technology. In this way, it is aimed to design a system that can be controlled with almost small touches once installed. The promotion of community awareness, the use of local species and resources, intensive horticulture, the promotion of polyculture agriculture, biodiversity, and energy generation through environmentally friendly systems are among the main topics [31].

Due to its multidisciplinary approach, Permaculture was criticized by the academic community in the first years of its emergence. However, it comes back to the agenda in the scientific sense over time as a result of its noticeable achievements in developing environmentally friendly simple, and applicable technologies, obtaining high efficiency without harming nature and saving energy. Permaculturists, on the other hand, far from the clumsiness of the academic bureaucracy; they develop, improve and share their work in many different disciplines such as organic agriculture, ecological architecture, zoology, and energy systems day by day. While economical feasibility and practical applicability of the technologies is an important criterion, bulky systems, scarce raw material, requiring expensive and complicated technology, and high footprint are not preferred when designing a Permaculture system [31]. Today there are no restrictions or rules for sharing existing Permaculture knowledge. Individuals who have received this education, can organize a course and share their experiences for the fee they determine whenever they feel competent and ready.

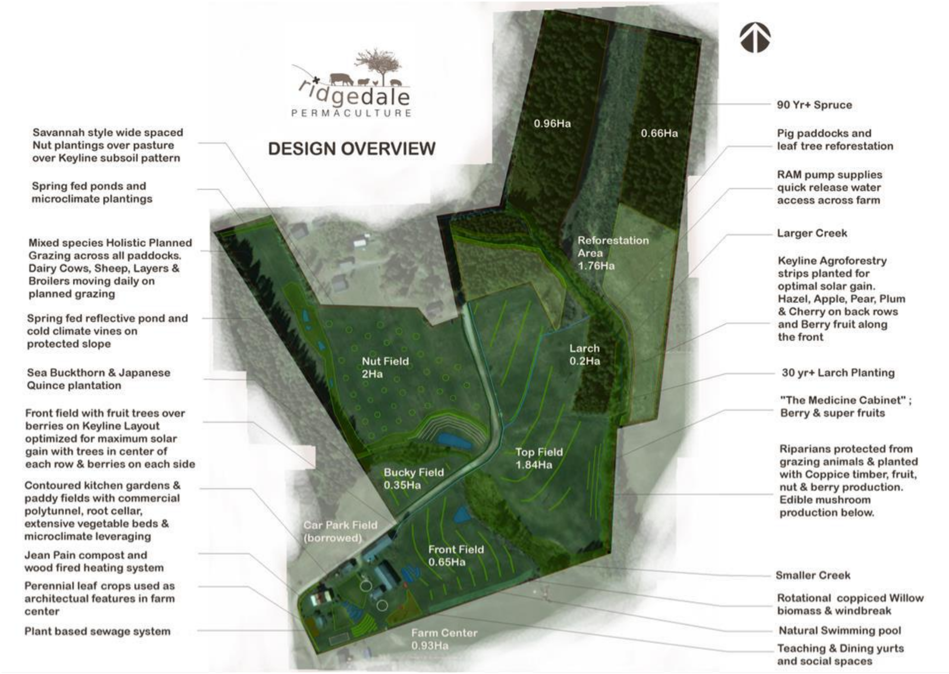

Fig 1. Design overwiew of Rigdale Permaculture Farm [32].

Although the ethicality of charging an astronomical price for education is debated due to the principle of generous sharing of the knowledge, there is no sanction regarding the lower and upper limit of the fee.

A movement that stands out for survival: Survivalism

Like Permaculture, Survivalism has been discussed in this study since it has a wide-ranging perspective, has been studied almost since the 1930s, and of course, its popularity is increasing day by day. It should also be noted that the Survivalism movement includes subgroups with very different tendencies shaped around specific doomsday scenarios and religious beliefs [33-36]. The part of this movement that concerns this study is the content of the theoretical and practical training provided for individuals to meet their basic needs to survive without harming nature and others.

Survivalism, a trend that could be called Radical by many in the past, is among the issues that are again on the agenda in today's world. Survivalism is a movement of individuals or groups who actively prepare for emergencies, including possible disruptions in the social or political order, on scales from local, national, and international. Some Survivalist groups share their experiences and knowledge by opening survival schools that offer education in different specialties [33, 37, 38].

The Bushcraft movement specializes in living in nature, whether voluntarily or compulsively. During this training, it is aimed to teach the knowledge and skills of providing comfort and survival in nature with completely primitive /natural techniques and practices by making use of materials and opportunities in nature as much as possible. Course titles include fire lighting, making edged tools, hunting, foraging in the wilderness, plant and tree identification, shelter building, navigation, carving, basketry, bow making, cordage, and bark work, bone and hide work, blacksmithing, primitive technology, tailoring, camp craft, water and hygiene, overseas expeditions, fire lightning, wildlife awareness and predator safety, emergency signals, weather conditions are predicted (Fig.2) [39, 40].

On the other hand, the Prepping movement ise contains many elements of bushcraft and survival. However, it more focuses on food storage for emergencies, prepared luggage and escape plans, protective clothing, knowledge about independent self-sufficiency, and the handling of weapons [37, 38]. In addition to natural disaster scenarios such as avalanche, flood, tornado, a tsunami, a hurricane, a forest fire, an earthquake, a volcanic eruption, Survavilists also organize courses for unexpected situations such as mountain climbing accident, being stranded in a desert, being capsized in a boat or ship due to the weather getting lost while hiking in woods. Extreme conditions such as a pandemic, economic crises, nuclear accidents, and terrorist attacks, war, famine, which could be predictable or emergent, are certainly the conditions that the Survivalists are preparing for. In addition to the mentioned skills, the curriculum of the courses include skills such as hiding and self-defense [37, 38]. Apart from the courses, there is an incredible demand for "go-bags", which are defined as "things you can use in an emergency to ensure your survival for 72 hours”.

Fig 2. Bushcraft/Survival Courses run in the Brecon Beacons South Wales [41].

Currently, over 100,000 emergency and survival kits are sold on Amazon.com. There are a wide variety of options from simple bags with knives and dust masks to luxury models with tents and shovels. Meanwhile, survivalists are conducting research and studies to produce more vital medical needs such as insulin [42-44].

Conclusion

The curriculum of today's official educational institutions, which claim to prepare new generations for life, is incomplete and insufficient in terms of knowledge and skills that enable individuals to meet their basic needs and survive. Today, individuals who want to receive education in self-sufficiency and survival generally try to acquire this knowledge and skills by attending courses organized by independent institutions and organizations.

Paying a certain fee for education is one of the factors that creates limitations in sharing Permaculture and Survival knowledge. Although it is seen as another disadvantage that there is no inspection and sanction regarding the adequacy and accuracy of this information, it is an advantage in terms of the rapid dissemination and sharing. What should be, is to fulfill this need by official educational institutions. Citizens should not have to pay to acquire this basic knowledge and skills which helps them to survive. Furthermore, the subjects that have no academic validity yet should be studied and developed by scientists. Also, it should be known that learning this information should not be arbitrary, but essential. It has to be seen that the work of self-efficacy and survival is worth more than just weekend activities attended by enthusiasts or adventurers to enjoy.

Convincing students of the necessity of the information in the curriculum is one of the main challenges facing most educators today. The student cannot come to the desired readiness to learn unless he is convinced that the information that is claimed to be learned will be useful in real life. If not convinced, the student usually studies only for exams and after a while, this information is forgotten as it is deemed unnecessary. To want to remember this information permanently, students must be convinced that they will use it in real life.

The topics mentioned in both permaculture and survivalist movements contain an accumulation of knowledge and skills that will not be overlooked and have been developed over the years by both experts and individuals and independent groups who are interested in the subject. All of the studies on sustainability and survival, certainly are based on theoretical knowledge and practical applications in applied sciences such as mathematics, physics, chemistry, biology, geography. If we really want to prepare individuals for real life, what is necessary is to compile this resource, to reorganize the theoretical and practical training of applied sciences in the current curriculum, taking into account the survival and self-sufficiency instincts. When individuals know that the information they will learn will save their lives and their loved ones in the future, they will be more motivated to learn and the education will be more permanent.

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<u>CHAPTER IX</u>

WORK-RELATED HEALTH STATUS OF THE HARVESTING AND TRANSPORT WORKERS IN FORESTRY

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1. Introduction

Forest operations differ greatly from industrial works in terms of ergonomics. Mechanization of forest operations has increased productivity since it has accelerated some phases of operations. Although there has been no physical development in human body, there have been lots of technological developments especially in this century. Working and living conditions for forest workers are generally poor in Turkey. Physically heavy work, inadequate working methods, tools and equipment cause occupational accidents, diseases and unnecessary fatigue. Forestry in Turkey appears to be one of the most hazardous occupations with frequent and severe accidents and many diseases. The improvement of safety, health, well-being and efficiency is a basic condition for prosperity, and ergonomics is a very important tool for this. The forestry workers working in wood extraction continuously encounter various health problems due to the works they perform, the difficulty of different climate and land conditions.

Appearance of worker health, which is a social and economic problem, falls on the end of 18th century. Due to the fact that poor working conditions started to threaten the health of the employees and disease and accident rates increased, labor protection has gained importance. Worker health is a field aiming to maximize the physical, mental and social aspects of the employees in each job, to maintain this maximization, to prevent the factors that will spoil this order, to employ workers in the works that are suitable to their physiological and psychological abilities, and thus to adapt the work to the person and the person to the work. The object here is to provide maximum health capacity to the employees, to protect them from the negative aspects of the working conditions, and to provide the optimal harmony between the work and the worker (Dertli, 1999; Shemwetta et al., 2002).

Forestry works are the works performed under biological and physical conditions in order to meet different demands of people in forestlands. These works can be gathered around main points such as wood harvesting, forestation, plantation, road repair, and forest protection. Forestry works are included in heavy works due to high energy consumption, static workload density, frequent repetition of the movements such as lifting and carrying heavy loads, leaning, kneeling and standing, vulnerability to climatic factors such as extreme heat, high humidity, wind, snow and rain, negative technological effects such as noise, vibration, gas, dust, and chemicals, health disorders they create in the main systems of people such as central nervous system, skeletal system, cardiovascular system, and accident risks for all the organs of the body. These features of forestry works require the forestry workers to be strong, skilled and trained, thus the forestry has a different quality than the labor in many other business lines. Performing forestry activities timely and in accordance with the technique, and providing high productivity are primarily based on the employment of forestry workers who received a good education and whose living and working conditions are arranged (Engür, 2006).

Production works in forestry are composed of the processes such as cutting and toppling trees, cleaning the branches, girdling, classifying, wood extraction, loading, transporting, stacking and carrying the woods to the final storage. The peasants taking a production work from the forest administration perform these processes in periods of time that may take 1-5 months with the periods changing year by year. While on the one hand, they continue their working under hard land conditions; they perform cutting, dragging and carrying processes despite the harsh winter conditions (winter cutting) on the other hand. Compared to other sectors, forestry workers who are quoted in unit price in forestry generally work continuously and without resting in order to do a lot of work and earn more money by eating on the run and pushing their limits, and thus undesired situations such as fatigue and insomnia appear (Menemencioğlu, 2012; Çığ, 2013). Forestry works generally performed in steep and rough areas far away from the main residential areas under various climate, vegetation and soil conditions increase the health problems of the forestry workers and thus the productivity. Moreover, working environments for forest-working are insufficient either technically or socially and are high-risk areas. For example, the risk increases considering the fact that the works performed manually and with chainsaw in the cutting process require physical power and energy. Therefore, in order to perform forestry works effectively, it is important to have experience and practical thinking as well as a healthy forest worker (Astrand et al., 2003). In this scope, while the wageworkers depending on an employer are evaluated within Social Security Institution (SSI), social security of the self-employed (forestry workers) in the status of those who “make a production of goods and services on his/her own behalf without employing any employees” is provided by the Social Security Organization for Artisans and the Self-employed within the Law No. 2926, Law on Social Insurance for the Self-employed in the Agricultural sector (Tunay and Emir, 2015).

The aim of this study is to reveal all the changes by performing all the physical examinations of the forest workers who take charge in production and transport operations in forestry such as measurement-logging, cutting with chainsaw, dragging with tractor and transportation with trucks within certain periods in the hospital environment, to detect the disorders occurring due to the work effect, to make comparative evaluation between the obtained results and the work performed by the forest workers, and to propose solutions.

2. Material and Method

The study material is 12 forest workers (forest villagers) who take charge in different lines of work such as measurement-logging, cutting with chainsaw, dragging with tractor and transportation with trucks in the production and transport operations in forestry in West Black Sea region. The study was conducted in 5-year periods (2011-2012 / 2015-2016 production years) within the forest workers attending the study to detect the health conditions. They were taking charge in different lines of work such as measurement-logging, cutting with chainsaw, dragging with tractor and transportation with trucks (3 workers in each line of work) in production and transport operation in forestry was compared and the differences were detected and detailed information on their general health status was obtained.

In order to obtain general health status of workers, first, a questionnaire form was arranged in order to reveal the awareness for introductory information, social condition, personal habits and then to detect disorders their general examinations and necessary tests were made in a general health center.

Necessary tests were divided into four sections as laboratory tests, audiometric, radiological and cardiologic examinations. Within laboratory tests, biochemical (biochemical, hemogram, hematology and hormone tests, urinalysis and microbiological tests (sedimentation, serological tests), pure tone audiometry test for detecting the hearing loss were applied, for radiological tests bilateral wrist-ankle joint radiography, pelvic radiography, waters (sinus) radiography, lung radiography; for cardiologic tests echocardiography, effort and Holter tests were applied.

3. Findings and Discussion

In work area, chainsaw operator performs the processes of cutting, toppling and classifying the trees; the measurement-logging worker performs the processes of cleaning the branches, bark peeling, and classifying; the tractor operator performs the processes of wood extraction and stacking the products; and remote transportation (transportation with trucks) operator manages the processes such as loading and carrying the products to the final storage.

Age and experience distributions of the forestry workers taking charge in different lines of work are given below (Fig 1). Accordingly, it is seen that the individuals with high average age (61) work in measurement-logging work that requires less energy and mobility than the other work segments, which is followed by the work segments such as cutting with chainsaw, remote transportation and dragging with tractor. Moreover, it is understood that the forestry workers taking charge in these work segments are experienced from their early ages (<20).

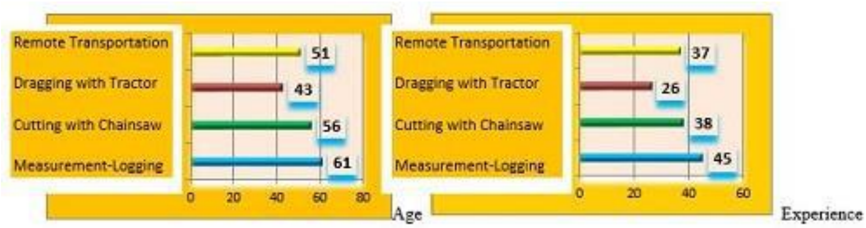

Fig. 1 Age and experience distribution of the forest workers taking charge in different parts of work (years).

Distribution of body mass index (BMI) of the forestry workers which is calculated by dividing the body weight (kg) and length into its square in meters is below (Fig 2). Tractor and truck operators are seen to be overweight.

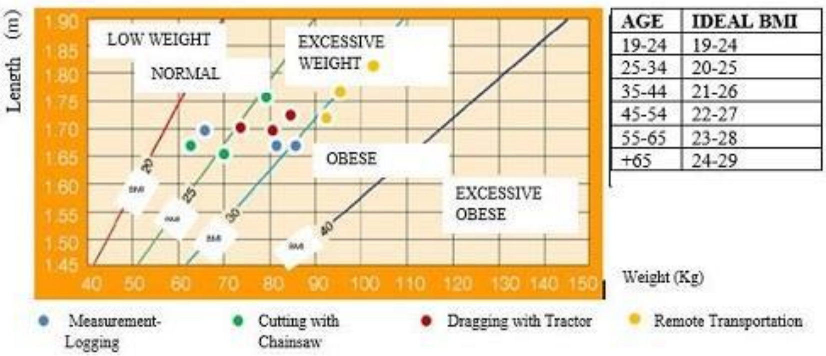

Fig. 2 Body Mass Index distribution of the forest workers in different parts of work.

Annual production (working) amounts of each forestry worker taking charge in different parts of work, whose general health conditions are thought to be associated with the evaluation of the changes are given below (Table 1).

Table 1: The last 5-year production (working) amounts of each forest workers (m^3).

	Measurement-Logging			Cutting with Chainsaw			Dragging with Tractor			Remote Transportation		
Production Years	*1st worker*	*2nd worker*	*3rd worker*	*1st worker*	*2nd worker*	*3rd worker*	*1st worker*	*2nd worker*	*3rd worker*	*1st worker*	*2nd worker*	*3rd worker*
2011-2012	342	75	198	341	342	294	421	98	103	258	418	201
2012-2013	-	339	676	463	361	341	192	107	146	170	201	58
2013-2014	289	161	289	161	289	229	161	161	161	156	161	114
2014-2015	312	422	360	422	312	310	422	124	422	376	422	252
2015-2016	385	140	385	140	385	294	170	140	140	361	478	250
TOTAL	1328	1137	1908	1527	1689	1468	1366	630	972	1321	1680	875

According to the questionnaire form, all of the forestry workers are primary school graduates but generally they are experienced in terms of work security, yet they do not want to go to the doctor except for routine disorders. In terms of personal habits It is seen that especially the forestry workers in chainsaw and measurement-logging segment have a high level of drinking tea (>20 tea cups) and smoking because of stress factor in the working environment. Moreover, it is detected that previous complaints of the forestry workers still continue.

While the patient was evaluated, patient history was examined through his/her anamnesis which was combined with the physical examination and necessary examinations were made, thereby true diagnosis could be provided (Fig 3).

Fig. 3 Blood sampling for laboratory tests and physical examination.

According to examination, while general views of the chainsaw (56), tractor (43) and remote transportation (51) operators are good and their body is quite youthful, the forestry workers working in measurement-logging (61) segmentare observed deformities related age.

Besides, it is understood that CK-MB value which is available in cardiovascular muscles and gives an idea in the diagnosis of the damage in heart (myocardial infarction) and other muscles was high in two of every three patients working in measurement-logging and in one of every three patients working in other work segments (Table 2).

Among the potential causes, excessive smoking and alcohol consumption, age (40 and above in males), high blood pressure, fatness, ongoing stress and genetic factors as well as high muscle breakdown due to work-related difficult exercise conditions are regarded as effective factors.

Table 2: CK-MB paid attention in the diagnosis of myocardial infarction (Ref. Range: 0.3-4.0 ng/ml).

	Measurement-Logging		*Cutting with Chainsaw*		*Dragging with Tractor*		*Remote Transportation*	
	25.10.11	*25.03.16*	*25.10.11*	*25.03.16*	*25.10.11*	*25.03.16*	*25.10.11*	*25.03.16*
1st worker	3.7	3.4	3.6	6.2	1.2	4.3	0.9	1.2
2nd worker	3.2	5.4	1.7	3.1	1.2	1.7	2.1	3.3
3rd worker	5.2	7.3	3.4	3.6	0.9	1.6	5.4	5.5

Within reference values   Out of reference values

It is understood that cholesterol level (ref. range 140-200 mg/dl) and triglyceride levels (ref. range 50-160 mg/dl) were high in two of every three patients working in measurement-logging segment and in one of every three patients dragging with tractor and remote transportation segments.

Moreover, low monocyte level (ref. range 0.15/0.70 10^3/µl), the role of which is to terminate the infected organisms, attracted the attention in all forestry workers taking charge in all lines of work. Acordingly diseases such as flu, cough, chill, fever, thamuria as well as malaria, tuberculosis, and anemia is thought to be caused by the physical conditions of the working area, together with seasonal changes.

Through performing "pure tone audiometry" which determines the minimum intensity of sound (threshold of hearing) that both ears can hear in different frequencies (high and low pitch), hearing levels were measured as dB and air and bone conduction hearing levels were determined and hearing loss was revealed (Table 3).

Table 3: Hearing sound level changes (dB) of each forest worker in a 5-year Period.

		Measurement-Logging				*Cutting with Chainsaw*				*Dragging with Tractor*				*Remote Transportation*			
		25.10.11		*25.03.16*		*25.10.11*		*25.03.16*		*25.10.11*		*25.03.16*		*25.10.11*		*25.03.16*	
		left	*right*	*left*	*right*	*left*	*right*	*left*	*right*	*left*	*right*	*left*	*right*	*left*	*right*	*left*	*right*
1	*Air*	13	15	22	18	12	13	25	25	10	7	20	17	7	5	15	12
	Bone	12	10	18	12	10	10	20	20	5	2	10	8	2	2	8	10
2	*Air*	17	18	20	17	13	10	13	12	12	13	13	12	7	10	13	12
	Bone	12	15	18	12	5	5	10	8	7	10	12	10	2	5	10	10
3	*Air*	13	12	22	18	30	23	30	25	10	12	15	13	10	12	13	12
	Bone	12	10	15	12	25	22	28	25	2	5	7	10	5	7	7	10

When long exposure to the noise resulting from chainsaws and presbycusis (age-related hearing loss) were evaluated together for the forestry workers (61) with a high average of age and working in measurement-logging segment, hearing loss levels were evaluated, as a result of sensorineural (neural) decreases occurring in high frequencies (tone), while "mild hearing loss" was observed in the forestry workers cutting with chainsaw; "very mild hearing loss" was observed in the forestry workers performing measurement-logging (Rubak et al., 2008). When feature of the work is considered for the causes of this innate or acquired loss, long exposure to the noise resulting from chainsaws was an effective cause.

Hearing levels were normal (threshold average below 15 dB) in the forestry workers taking charge in dragging with tractor and remote transportation (Fig 4). It is understood that the workers did not pay attention to the condition as the loss was not that much and did not disturb them much.

Fig. 4 Pure tone audiometry test.

With PA lung graphy, relationship of trachea with the middle line and the diameter thereof, main bronchial patency and potential mass following the same, cardiothoracic rate, heart configuration and potential size differences, calibration of aorta and pulmonary arteries, enlargement of both hila, mass and lymphadenopathy developments, control of mediastinal contours and potential mass-related enlargements, lung parenchymal areas, pleural thickening and especially calcifications and waters (sinus) graphy, maxillary and other paranasal sinus mucosal inflammatory (various microbial agents) pathologies and septum (a wall dividing the nose into two equal parts inside) and nasal concha (narrow and curled shelf of bone providing nasal secretions) were evaluated (Fig. 5).

According to this evaluation, opacity in the form of mucosal thickening was observed in the bilateral maxillary sinuses in at least one of the forestry workers taking charge in different lines of work, and sinusitis secondary to upper respiratory tract infections was diagnosed. Moreover, bilateral hilar fullness was observed and chronic bronchitis was diagnosed in at least one worker in the chainsaw operators. Diagnosis of sinusitis and bronchitis in all of the workers in the previous examinations was associated as the period (autumn) when the examinations were made and the seasonal changes. In addition, as a result of vertebral fatigue and strain of the ligaments occurring due to standing for a long time and chainsaw weight, scoliosis (curvature of the spine) was monitored in one of every three workers working in chainsaw and measurement-logging segment. Cardiothoracic rate giving an idea in the diagnosis of cardiac insufficiency in one of the workers taking charge in measurement-logging segment increased and aortic arch was prominently observed, and the other evaluations were studied at normal levels.

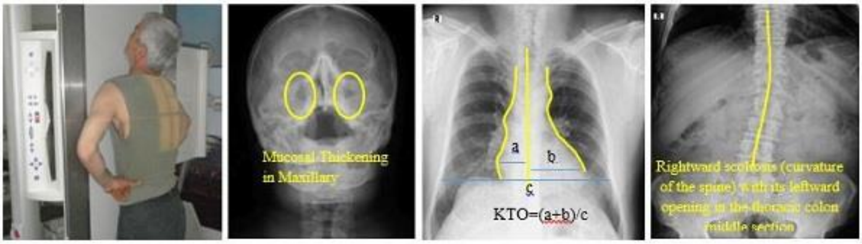

Fig. 5 Waters (sinus) and PA lung graphy and their interpretation.

To see the lumbar and hip disorders and the pathological conditions occurring in the wrist and ankle joints, first orthopedic examinations of the forestry workers were made, and then pelvic radiography, bilateral wrist and ankle radiography were taken, joint MRI was also performed due to the suspicion based on clinical laboratory data, and advanced imaging examinations were applied.

Accordingly, herniated disk disorder was monitored, in a respective order, in two of every three workers working in chainsaw and measurement-logging segment, in one of every three workers dragging with tractor and in all of the forestry workers working in remote transportation segment (Fig 6). Looking at the causes of this condition, in addition to a lot of factors such as lifting heavy, frequently performing sudden and repetitive movements, strains occurring as a result of the fact that the work requires physical strength, and standing for a long time; disk displacement due to the age-related loss of protective liquid content between the disks for the forestry workers with high average of age and working in measurement-logging segment, weight of the cutting with chainsaw for the forestry workers cutting with chainsaw, frequent repetition of the sudden movements for the forestry workers working in the segment of dragging with tractor and also weight for the forestry workers working in remote transportation segment were evaluated as effective factors.

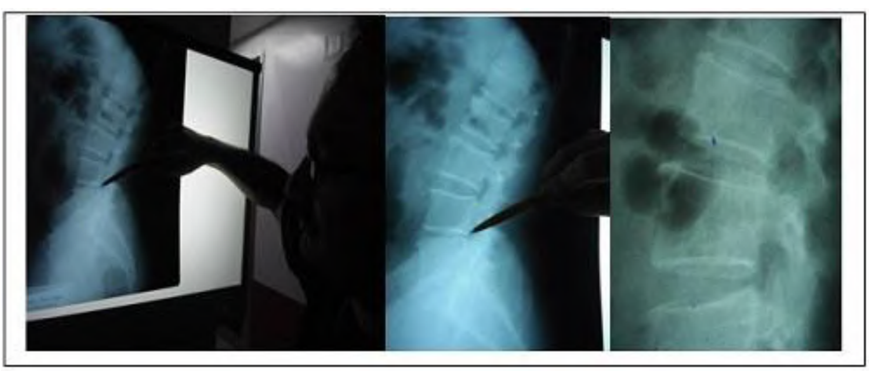

Fig. 6 Radiological evaluation of the spine

As result of the complaints occurring based on age, structure and the environment in the forestry workers working in measurement-logging segment in time; while mild height loss was monitored in T10, T11, T12 (10, 11 and 12th dorsal vertebra) and L1 (1st lumbar vertebra) vertebra corpus anterior, spur formation (calcification) was monitored in vertebral corpus corners in T12, L1, L2, L3, L4, L5 vertebrae. Moreover, L5-S1 intervertebral disk height was also reduced. While mild height loss was monitored in T10, T11, L1, L2, L3, L4 vertebra corpus anterior in the forestry workers cutting with chainsaw, spur formation was monitored in vertebral corpus corners in L1, L2, L3, L5 vertebrae. Besides, grade I anterolisthesis (slight slipping of upper vertebral body forward onto the vertebra below) was seen in L5 vertebra. In the forestry workers working in remote transportation segment, however, bulging disc (protrusion of the disc outside the space it normally occupies between the vertebrae) in L2-L3, L3-L4 intervertebral disk levels, mild disk herniation (herniated disk) in L4-L5, L5-S1 intervertebral disk levels and degenerative changes were monitored. Furthermore, decrease in T10, T11 vertebra anterior and L4-L5 intervertebral disk height and straightening of lumbar lordosis (lumbar lordosis is straightened as a result of the spasm of thick muscles around the vertebra due to lumbar pain) were seen (Fig 7a). First, quality physiotherapy was advised with the use of a corset, and surgical intervention is found appropriate unless success cannot be achieved. It is understood from the previous examinations that the same forestry workers had complaints of lumbar disorders and due to the low pressure intensity in the initial stage necessary treatment was not applied and it showed progression in time.

As a result of bilateral wrist and ankle radiograph and joint MRIs; spur formation (cystic calcification areas and various damages in some anatomic regions) was monitored in a level of Achilles tendon joint in both calcanei (heel bone) in two of every three workers working in measurement-logging segment and in one of every three workers cutting with chainsaw, respectively, due to standing for a long time, and due to being above the ideal weight for those working in measurement-logging segment. Joint spaces were normal in the forestry workers working in the other lines of work and no pathologic opacity was detected in the visible soft tissues. In the wrist radiograph/MRIs applied, however, no fracture or lesion was detected in the bones examined, and the result was evaluated as normal wrist radiograph (Fig 7b).

However, hand nerve compression (carpal tunnel syndrome) which manifests itself as hypokinesia during wrist movements, tingling and severe pain, loss of strength in the hands, electrification in fingers, pain spreading to the shoulder and elbow and increasing aches was detected in all of the workers working in cutting with chainsaw and measurement-logging segment that require excessive use of the wrist, application of surgical treatment that would require local anesthesia in which it is aimed to relieve the nerve cells by relaxing the median nerve was found appropriate.

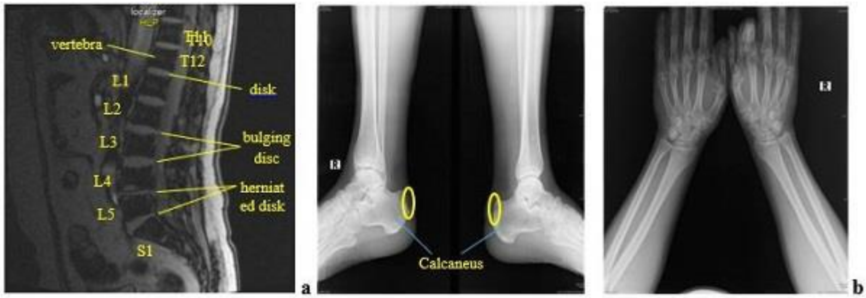

Fig. 7 (a) Lumbar spine MRI (of the remote transportation operator), (b) bilateral ankle and wrist radiograph (of the forest worker working in measurement-logging segment).

Finally, electrocardiography (ECG), through which cardiac electrical activity is measured, was performed in order to reveal the cardiovascular system related problems, application of the Holter test in which the heart beats are recorded throughout the entire 24 hours of the day to detect the short-term palpitations which are not seen during the examination but seen within the day and all the cardiac rhythm disorders such as feeling of fainting, and the effort tests aiming to detect transient ischemic symptoms that may appear in the myocard and revealing the disorders not seen in ECG performed during normal resting were provided (Fig. 8).

Fig. 8 Application of ECG, holter and effort tests.

According to the Holter test results, ventricular extra-systole was detected in at least 15 forestry workers taking charge in measurement-logging segment. Rare supraventricular premature beats were detected in the forestry workers working in the other parts of work. According to the effort test results, however, coronary vessels of the forestry worker (taking charge in dragging with tractor) whose effort tests applied in 2011 and 2016 were positive from the ischemic aspect (2 mm ST depression in d2-3, avf and V4-5-6 derivations) were examined with coronary angiography, a diagnostic tool showing the structure of coronary vessels along with the first symptoms, and medical treatment was decided without any other process after coronary angiography. For the same forestry worker, the cardiologic test results performed in 2016 were described as suspicious positive effort test (due to genetic reasons and working environment (stress)) after the evaluation thereof with the previous tests and continuation of the medical treatment was found appropriate. Besides, the new effort test was evaluated as positive (>2 mm ST depression in d2-3, avf and V4-5-6 derivations) from the ischemic aspect in the forestry worker taking charge in measurement-logging segment and having negative effort test result in the previous tests, and thus a stent (steel case) was applied to the vascular occlusion (Fig. 9).

Fig. 9 ST Segment depression of the forest worker working in measurement-logging that was revealed by effort test.

4. Conclusions

According to the results of the study, forest workers experienced in terms of occupational accidents and working in the segments of measurement-logging (61), chainsaw (56), tractor (43) and remote transportation (51) failed to show the same success in terms of worker health; although their previous complaints still continue, they do not need to go to the doctor except for the epidemics (upper respiratory tract infections, chill, fever, thamuria, etc.) increasing in the seasonal shifts and the stress factor is effective in the working environment. Likewise, it is understood that in addition to work-related difficult exercise and working environment conditions, excessive cigarette consumption, age, weight, ongoing stress, nutritional conditions and genetic factors, etc. are the factors affecting the health conditions.

When the hearing loss levels are evaluated, in the forestry workers who perform the processes of cutting with chainsaw and are exposed to the noise from the chainsaws for a long time, mild hearing loss was evaluated while in the forestry workers (61) with a high average of age and working in measurement-logging segment, long exposure to the noise resulting from chainsaws and presbycusis (age-related hearing loss) were evaluated together.

In line of orthopedic examinations, as a result of vertebral fatigue and strain of the ligaments occurring due to standing for a long time and chainsaw weight, scoliosis (curvature of the spine) was monitored in one of every three workers working in chainsaw and measurement-logging segment. Moreover, herniated disk disorder was monitored, in a respective order, in two of every three workers working in chainsaw and measurement-logging segment, in one of every three workers dragging with tractor and in all of the forestry workers working in remote transportation segment. Looking at the causes of this condition, in addition to a lot of factors such as lifting heavy, frequently performing sudden and repetitive movements, strains occurring as a result of the fact that the work requires physical strength, and standing for a long time; disk displacement due to the age-related loss of protective liquid content between the disks for the forestry workers with high average of age and working in measurement-logging segment, weight of the cutting with chainsaw for the forestry workers cutting with chainsaw, frequent repetition of the sudden movements for the forestry workers working in the segment of dragging with tractor and also weight for the forestry workers working in remote transportation segment were evaluated as effective factors.

According to the cardiologic examination results, ventricular extra-systole was detected in at least 15 forestry workers taking charge in measurement-logging segment. Rare supraventricular premature beats were detected in the forestry workers working in the other lines of work. Medical and life changing treatment of the forestry worker who is working in the segment of dragging with tractor and whose effort tests were positive (due to genetic reasons and working environment (stress)) from the ischemic aspect in the previous examination was made. Besides, the new effort test was evaluated as positive (due to age and weight and working environment) (>2 mm ST depression in d2-3, avf and V4-5-6 derivations) from the ischemic aspect in a forestry worker taking charge in measurement-logging segment and having negative effort test result in the previous tests, and thus a stent (steel case) was applied to the vascular occlusion.

While the wage workers depending on an employer are evaluated within Social Security Institution (SSI) in the Occupational Health and Safety Law No. 6331, social security of the self-employed in the status of those who “make a production of goods and services on his/her own behalf without employing any employees” is provided by the Social Security Organization for Artisans and the Self-employed within the Law No. 2926, Law on Social Insurance for the Self-employed in the

Agricultural sector. Due to the fact that seasonal quality of working in the sector is dominant and the employment statuses are transitive with each other, a lot of problems preventing the establishment and management of the insurance appear, and social security of the employees working in the forestry sector cannot be provided completely. In order to provide the social security of the employees working in this sector completely with the regulations to be made, underwriting opportunities for the forestry workers covering the periods they work for the General Directorate of Forestry and even the opportunity of integrating these premiums with the optional insurance application should be provided. Above all, those who are structurally convenient should be employed and those who are employed should be followed with periodic controls. It is quite important to establish mobile service systems that will provide the fastest and the most effective access to provide health services. Moreover, within the framework of the protocols to be made between Ministry of Forestry and Water Affairs and Ministry of Health in order to enforce the Law No. 6331 in the production works, opportunities of performing routine health examinations of the forestry workers in certain periods should be evaluated. This will provide the peasants to approach the circumstance more consciously and will lay the way open for professional forestry workers.

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