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Editors

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Chapter 1

EFFECT OF COAGULATION PROBLEM ON DNA PURITY IN DNA ISOLATION FROM ANTICOAGULATED BLOOD OF FISH AND A SOLUTION PROPOSAL

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1. INTRODUCTION

DNA, due to the genetic material it contains, is frequently used in molecular fisheries studies. In the studies using DNA, however, the high molecular weight DNA molecule should be initially isolated purely as determining the concentration and purity of the isolated DNA is necessary for the eventual molecular techniques. For this purpose, DNA concentration and purity are determined spectrophotometrically by measuring absorbance at 260/280 nm wavelength.

The absorbance ratio at 260/280 nm is mostly used to assess DNA purity, and the range of 1.8 and 2.0 is generally considered as "pure" for DNA. When this ratio is lower than the intended range (≤1.6), it is commonly an indication of contamination with phenol, guanidine or another reagent used during DNA isolation protocol. On the other hand, when the absorbance rate at 260/280 nm is above the ratio of 2, it is interpreted as there is RNA residue in the extract (Matlock, 2015).

The ratio of 260/230 is also considered as criteria to determine DNA purity. The expected 260/230 value for "pure" DNA should range between 2.0 and 2.2. It is stated that if this value is higher or lower than the intended range, it shows that DNA is not considered "pure" (Matlock, 2015).

Blood is commonly used material for DNA isolation in fish as erythrocytes in fish are nucleated like leukocytes and carry genetic material (Strunjak-Perovic et al., 2009). Fish blood, however, tends to coagulate rapidly (Walencik & Witeska, 2007) due to containing many platelets that cause rapid coagulation compared to other vertebrates (Maqbool et al., 2014). This is vital to prevent possible bleeding status in the aquatic environments. During the experimental studies, however, there is a tendency in fish to undergo stress when the blood is taken that causes acceleration of the clotting ratio (Faggio et al., 2014). Therefore, anticoagulants, when using fish blood as a material, should be used for the reliable results in DNA-based molecular studies.

Anticoagulants are the additives to prevent blood clotting (Maqbool et al., 2014). The most applied anticoagulants in fish hematology are heparin and EDTA (Ethylenediamine tetraacetic acid) salts (Walencik and Witeska, 2007). In the diagnostic techniques applied in fish hematology, most of the researchers tend to utilize ready to use microtubes containing anticoagulants to preserve the blood samples. Ready-made microtubes with anticoagulants, however, are mostly produced to utilize in human hematology (Faggio, et al., 2014). Therefore, the concentration of commonly used anticoagulants may sometimes be insufficient as the fish blood tends to clot rapidly. This causes problems in DNA and even obstruct producing high-quality pure DNA. Even if DNA isolation is repeated with the blood samples taken, the intended results cannot always be achieved. This causes loss of time and effort as the trial needs to be re-established and repeated.

This study attempted to find solution of the coagulation problem occurring despite the use of anticoagulated blood in DNA isolation from rainbow trout blood.

2. MATERIAL AND METHODS

Blood samples from 30 rainbow trout were used to perform the current work. Fish samples were anesthetized using clove powder (180 ppm, Arabacı, 2007). Then, 1 ml of blood was taken from the caudal vein of the stunned fish with a 10 ml injector at an angle of 45 degrees and transferred to ready-made EDTA tubes (BD Vacutainer K2 EDTA 1.8 mg / ml). Next, the samples were transferred to the laboratory using transport container with cold chain and their DNAs were isolated within the same day.

Blood samples (0.2 ml) were taken from EDTA tubes and transferred to Eppendorf tubes during DNA isolation. Then, DNAs were extracted using PureLink Genomic DNA Mini Kit (Invitrogen) applying the instructions of manufacturer. Despite the use of EDTA tubes during DNA isolation stage, coagulation formed in some samples. However, the process of isolation was carried out. Absorbance rates of the acquired DNA at 260/280 and 260/230 nm were determined using a NanoDrop 2000 / 2000c (Thermo Scientific).

The rate of 260/280 (DNA purity ratio) in 63% of the samples was within the normal range (1.8 - 2). Although, DNA was obtained in 37% of the samples due to the blood clotting of the samples, the purity rate was higher than 2, which is above the normal range (Fig. 1). In 24% of the samples, 260/230 absorbance rates, which is another DNA purity criterion, should be in the range of 2 - 2.2, while the purity rates were higher than 2.2. This study was conducted according to the absorbance rate of 260/280 and evaluated the results using the peak value given by the nanodrop device. Although the absorbance ratios of 260/230 of some samples were above the value of 2.2, the samples were not considered problematic as the peak values and 260/280 ratios were within the intended range.

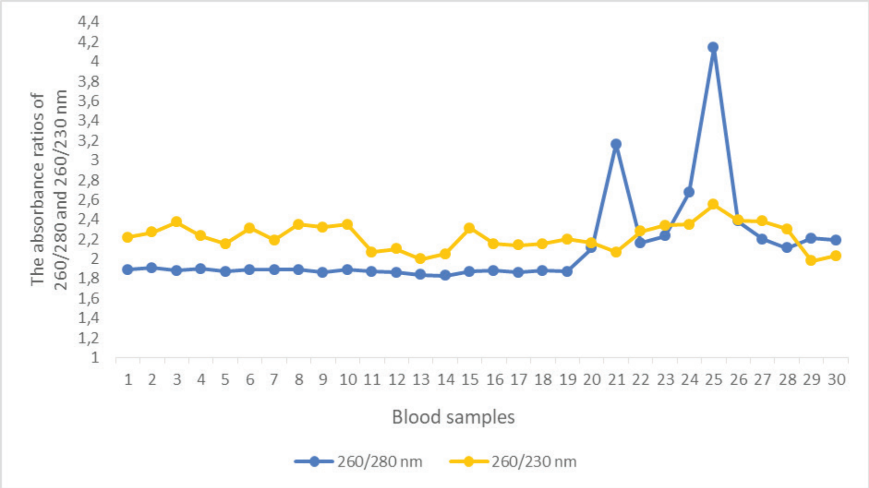

Figure 1. The absorbance ratios of DNA obtained using DNA isolation (260/280 and 260/230 nm).

Considering the absorbance ratios of DNA obtained using DNA isolation at 260/280 nm, the absorbance ratios above 2 shows the RNA residue in DNA extracts. This may cause incorrect connections and formation of unwanted amplicons during the PCR phase (Lee et al., 1997). A preliminary experiment was conducted increasing the amount of RNAse during DNA isolation in order to overcome this problem. The intended results, however, could not be achieved as the RNAse enzyme could not activate due to coagulated structures. Therefore, the clotting problem was attempted to be solved using EDTA (0.1M), which is an anticoagulant. Thus, in the study, it was aimed to dissolve the clot and reduce the absorbance value of 260/280 between 1.8 and 2 that means obtaining the pure DNA. In addition, the improvements in 260/230 absorbance rate were aimed in the current work.

In order to overcome this problem, DNA isolation of clotted blood samples of 11 rainbow trout, which did not provide the intended value, was reconstructed by diluting using EDTA solution. The ratios of blood and EDTA (0.1M) solution were continuously tested against each other to determine the optimum ratio. While the amount of blood was kept constant, EDTA solution was not added to the control group, EDTA solution was added to the other groups as 1, 2, 3, 4 and 5 times, respectively. After EDTA was added to the coagulated blood samples, the samples were vortexed to disintegrate the clots. In the present study, fish blood samples and EDTA amounts (EDTA / BLOOD) were mixed in the ratios of 0/1 (0/200 µl), 1/1 (200/200 µl), 2/1 (400/200 µl), 3/1(600/200 µl), 4/1(800/200 µl) and 5/1(1000/200 µl) respectively by repeating DNA isolation in 11 clotted blood samples.

3. RESULTS

In this study, when DNA isolation with coagulated blood samples was repeated and the EDTA / BLOOD ratio was set to 0: 1, 1: 1 and 2: 1, it was determined that the coagulation increased when lysis buffer was added and the samples solidified, which makes it inconvenient to vortex. Next, the samples were incubated at 56 °C for 30 minutes and then, the samples were transferred into 96% ethanol. Although some of the clotted structures were dissolved, not all the samples achieved to pass through the spin column in the following processes, yet some of the structures even caused blockage of the spin arm. Although DNA was obtained in the subsequent processes, DNA purity range was not found to be intended range (1.8-2.0). Moreover, phenol, which is used in the isolation protocol of DNA, caused contamination with guanidine or other reagents. Although, no significant coagulation was found when EDTA / BLOOD ratio was set to 3: 1 and 4: 1, absorbance values of the obtained DNA at 260/280 and 260/230 nm were not in the required range (1.8-2.0) in five groups. On the other hand, when the EDTA / BLOOD ratio was set to 5: 1, no problem was encountered. When 37% of the samples which does not match with the absorbance rates were re-isolated diluting 5 times with EDTA;

- The average absorbance ratio of 260/280 of the samples was found to be 1.86 ± 0.012.
- Mean absorbance rates of 260/230 were found as 2.16 ± 0.106.

Table 1 shows the absorbance values and concentrations at 260/280 and 260/230 nm of DNA obtained after diluting blood samples 5 times with EDTA.

Table 1. The absorbance values and concentrations at 260/280 and 260/230 nm of DNA obtained after diluting blood samples 5 times with EDTA.

Samples	Nucleic acid concentration (ng/µl)	260/280 nm	260/230 nm
1	648,8 ng/µl	1,85	2,1
2	241,7 ng/µl	1,86	2,2
3	261 ng/µl	1,85	2,26

4	260 ng/µl	1,87	2,24
5	309,8 ng/µl	1,88	2,29
6	73,1 ng/µl	1,86	2,18
7	303,5 ng/µl	1,88	2,33
8	237,1 ng/µl	1,84	2
9	239,3 ng/µl	1,86	2,1
10	330,9ng/µl	1,87	2,07
11	178,9 ng/µl	1,87	2,07

The 260/280 absorbance ratios obtained after diluting 5 times with EDTA with 11 samples with an absorbance ratio of 260/280 greater than 2 were found between 1.8 and 2. Figure 2 shows the absorbance results at 260/280 nm of DNA obtained after diluting and diluting blood samples with EDTA 5 times.

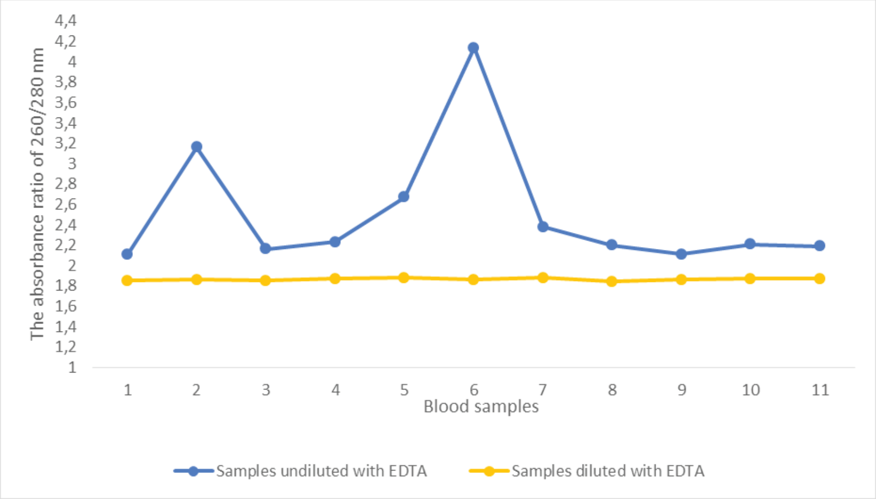

Figure 2. Absorbance values at 260/280 nm of DNA obtained after diluting blood samples 5 times with EDTA and without diluting.

After diluting blood samples with EDTA 5 times, it was observed that the 260/230 absorbance ratios of DNA obtained were substantially normalized. Figure 3 shows the absorbance results at 260/230 nm of DNA obtained after the blood samples were diluted with EDTA 5 times and without diluting.

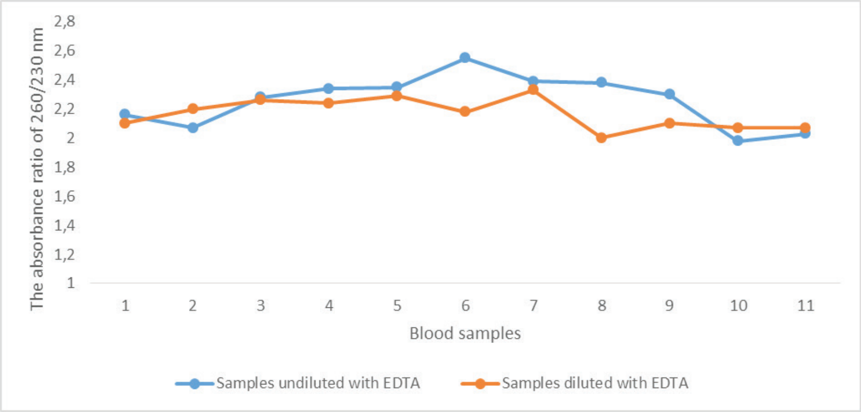

Figure 3. Absorbance values at 260/230 nm of DNA obtained after diluting blood samples 5 times with EDTA and without diluting.

In addition, the results show that the amount of DNA concentration increased after diluting 11 blood samples 5 times with EDTA. Figure 4 shows the concentration amounts of DNA obtained after the blood samples are diluted with EDTA 5 times and without diluting.

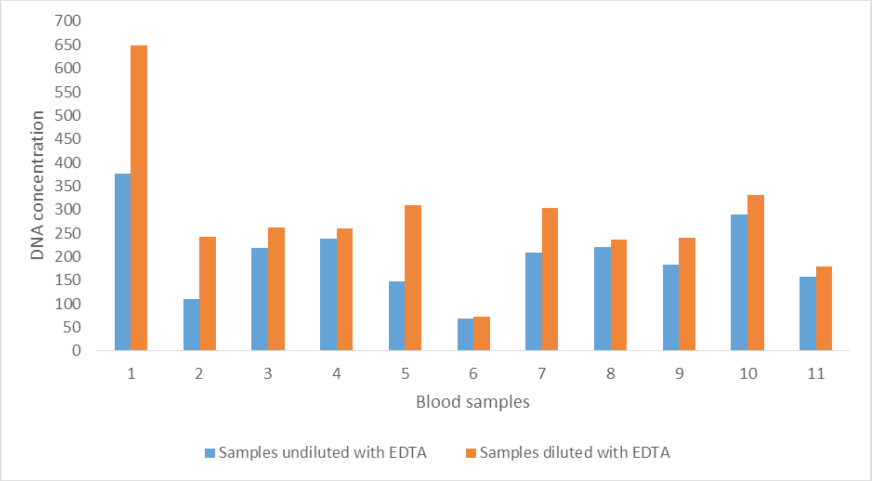

Figure 4. The concentration amounts of DNA obtained after the blood samples are diluted with EDTA 5 times and without diluting.

4. DISCUSSION AND CONCLUSION

The most frequent problem in DNA-based studies is the coagulation of the blood samples. Therefore, most researchers tend to use substances with anticoagulant properties. Although EDTA tubes with anticoagulant properties were utilized in the present study, a coagulation problem was also observed at DNA isolation stage. 63% of the samples of from rainbow trout and blood with anticoagulant were successfully isolated with DNA. 37% of the samples were also isolated, however, the absorbance value was not found in the intended range due to the coagulation. Therefore, this study attempted to find a solution to the coagulation problem observed in DNA isolation from rainbow trout blood.

As it is well known, the concentration and 260/280 absorbance ratio of DNA obtained from the coagulated blood is of lower quality compared to DNA isolated from normal blood (non-coagulated) and does not always provide the intended results (Zhou et al., 2019). To begin with, therefore, the blood clots must be physically dispersed into small pieces before the process of DNA isolation from clotted blood samples. Dispersing blood clots enhances the quality and the concentration of DNA to be obtained. (Xu et al., 2010).

Considering the DNA isolation studies using the coagulated blood, many researchers encountered this problem and attempted to overcome the coagulation problem applying different methods. In some studies, dangerous organic solvents such as phenol, chloroform (Basuni et al., 2000), and ethanol precipitation (Santella, 2006) was applied for isolation of DNA from the clotted blood. Mohammadi et al. (2015), however, stated in their study that the use of toxic organic solvents and destructors such as phenol and chloroform is not only dangerous for the environment, but also threatens the health of laboratory personnel even though these solvents provides a good quality of product in DNA isolation. Another technique to obtain DNA from coagulated blood, suggested by Adkins et al. (2002), is to apply the combination of 100% isopropanol and 70% ethanol.

Ibraheam et al. (2008), in order to disperse the clots, isolated DNA from the coagulated blood by slicing the blood clots with a scalpel and then, added NaCl, EDTA and Tris.cl and and then centrifuged. Xu et al. (2010), however, stated that slicing of clots using a scalpel increases the risk of contamination and requires avoiding sharp objects that could be dangerous to laboratory workers. In another study, it was stated that high-speed agitation would be effective for the dispersion of coagulated blood samples (Lundblom et al., 2011).

Zakaria et al. (2013) used high-frequency ultrasonic vibrations to isolate DNA from coagulated blood samples and they were able to obtain DNA by dispersing the coagulated blood samples. Other studies, on the contrary, claimed that although DNA can be isolated using this method, the ultrasonic vibrations may disrupt the structure of DNA (Zhou et al., 2019). In another study, Mardan - Nik et al. (2019) developed an alternative method for DNA isolation by using metal balls to homogenize and disperse the coagulated blood and then adding phosphate buffered saline (PBS). This method, however, requires additional consumables such as steel or ceramic balls, which are disposable and expensive. Moreover, these metal balls must be cleaned carefully before processing the subsequent sample. However, this process can increase the risk of contamination if the cleaning process is not done properly (Zhou et al., 2019).

Apart from applied methods above, DNA isolation kits were also applied to isolate DNA from coagulated blood samples in other studies. Bank et al. (2013) claimed that they obtained positive results to extract DNA from coagulated blood by applying four commercially available kits. The use of DNA isolation kits, however, can be costly in studies with a large number of samples. Some studies, on the other hand, applied disposable plastic sieves to liquefy blood clots. Solid blood clots were able to be liquefied by passing through a filter screen with a certain centrifugal force using these plastic sieves (Adkins et al., 2002). This method, however, is not suggested by some scholars. Some report shows that not every samples could pass through the sieve. Moreover, the yields and 260/280 ratios of the obtained DNA could be low (Zhou et al., 2019).

The current study repeated DNA isolation using 11 coagulated blood samples by diluting with anticoagulant EDTA 0.1, 2, 3, 4 and 5 times, and the clot was able to be dissolved. It was observed in the current study that when the ratio of blood to EDTA was adjusted to 1/5 with EDTA in clotted blood samples, clotting problem was overcome. In addition, this study achieved to increase DNA concentration, reduced the 260/280 absorbance ratio to the normal limits (1.8-2.0) in all samples, and normalized the 260/230 ratio.

Considering the other studies conducted, researchers applied many different methods to overcome the coagulation problem and somehow achieved their objectives. These methods applied in different studies to isolate DNA, however, had their own advantages and disadvantages. On the other hand, it has been proved that EDTA used in this study has no disadvantage when applied to disperse clotted blood. In contrast to other studies, EDTA did not increase isolation and labour costs, did not cause contamination risk and did not damage DNA structure in the current examination. Furthermore, since EDTA is not a toxic organic solvent such as phenol and chloroform, it has no threat on the health of laboratory personnel.

As a result, it can be seen in the current study that the coagulation problem can be overcome when the blood samples of rainbow trout with coagulation problem were diluted 5/1 (EDTA / BLOOD) with EDTA. Increasing the amount of EDTA solution until achieving to disperse the clotted blood will help the researchers to overcome this problem. Alternatively, adding EDTA to EDTA tubes before use can be a useful procedure to prevent coagulation problem.

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Chapter 2

DETERMINATION OF ENDOPHYTE FUNGAL SPECIES FROM HURMA OLIVES IN KARABURUN PENINSULA, IZMIR

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1. INTRODUCTION

Mediterranean Basin where Turkey is also located in, has worldwide fame in growing olive trees (*Olea europaea*), a drought-tolerant product which has ecological and socio-economic importance (Martins et al., 2016; Malhadas et al., 2017). Olive oil and table olives, obtained from the fruits of these trees, form the basis of the Mediterranean diet (Aydınalp et al., 2004; Mora et al., 2007).

Olive fruit has a high antioxidant effect due to its natural phenolic compounds (Marsilio et al., 2000; Bianchi, 2003). Oleuropein is the main phenolic compound found in the pulp fraction of the olive fruit. Oleuropein is responsible for the bitter taste, a factor that prevents the direct consumption of olive fruit. Thus, oleuropein should be removed from the fruit by the administration of various procedures. Salt is widely used in the processing of table olives and preservation of processed olives. This is especially important for individuals who need to regulate their salt intake (Vinha et al., 2005; Jemai et al., 2009; Ramírez et al., 2014).

Grown in West of Turkey, a type of Erkence olive (*Olea europaea* L.) variety named "Hurma olives" are naturally debittered and produced on the coast of the Aegean region, especially in the Karaburun Peninsula. Hurma olives are known as a suitable option for people who prefer non-salted olive, since it is not a processed olive product and does not contain salt (Aktaş, 2013; Aktaş et al., 2014). Hurma Olive is differentiated from other olives by losing its bitter taste caused by phenolic compounds especially oleuropein during ripening on trees. Thus, when harvested, it can be consumed directly without the need for elimination of the bitter taste (Aktaş et al., 2014; Susamci et al., 2017).

Endophytic fungi are known to live in a part of their life cycle in the plants and do not harm their hosts. So far these organisms have been found in all the plant hosts studied and it was reported that they are colonized in plants living in tropical temperate regions (Sia et al., 2013; Martins et al., 2016). Plant growth hormones can play an important role in assisting their hosts by producing enzymes, and siderophores and are effective against plant diseases and insect pests (Materatski et al., 2019).

For a long time, a fungus named *Phoma oleae* have been reported to be effective in hydrolysis of bitter taste causing phenolic glycoside oleuropein in Hurma olives which only grown in Karaburun Peninsula, Izmir (Panagou, 2006; Aktaş et al., 2014; Susamci et al., 2017). Aktaş

(2013) conducted a study on microbiological characterization of Hurma olives and she has found that the microbial population on the Hurma olives are higher compared to Erkence and Gemlik olives. In the ripening period, the microbial growth in Hurma olives has been interpreted as causing an increase in the enzyme activities which break down the phenolic compounds and causes maturation process called "natural de-bittering" (Aktaş, 2013).

Abacı et al., (2014) identified the fungal isolate from Hurma olives collected from only one garden from the Karaburun Peninsula in Izmir as *Phoma multirostrata* and they stated that this was the first report of *Phoma multirostrata* isolated from *Olea europaea* (Abacı et al., 2014).

The studies on endophytic fungi in olive fruits are very limited (Martins et al., 2016; Malhadas et al., 2017). This study is the first study in the literature to determine the diversity of fungi species that live as endophytes in Hurma olive.

2. MATERIAL AND METHODS

2.1. Sample collection

In our study, samples of tree-ripened Hurma olives growing on the Karaburun Peninsula in Izmir have been collected from Eğlenhoca Village (4), İnecik Village (4), Amberseki Village (3) and Saip Village (3), 20 samples in total were then subjected to endophytic fungi isolation. In addition to this, 12 olive samples were obtained for comperative purposes from Izmir, the non-Hurma Erkence olive variety (5), Gemlik from Manisa (3) and Çelebi (4) from Bursa for total of 12 olive samples were collected and endophytic fungi were isolated.

Olives were collected manually during maturation periods. Hurma olive maturation determined by starting of creasing and colour change to brown. All the samples were collected into sterile jars and kept at + 4 °C until they were analysed.

2.2. Isolation

Surface-sterilization of the olive fruits were made as previously described (Compant et al 2011). For surface sterilization, olive fruits were treated with 70% ethanol (5 min), followed by an immersion in 2.5% sodium hypochlorite solution (5 min). Olive fruits were then rinsed three times with sterile distilled water. For the isolation, the media such as PDA (Potato Dextrose Agar), MEA (Malt Extract Agar) and the MEA containing the flesh of unprocessed olives were used. Surface-sterilized olive fruits were cut with a sterile scalpel, and the samples removed from the inner surface of exocarp with the scraping method were transferred to the designated media. The petri dishes were incubated at 27 °C.

2.3. Phenotypic identification

Morphological studies of the isolate were performed according to Pitt, 2000; Hasenekoğlu, 1991; Barnett and Hunter, 1998; Samson and Pitt, 2000; Boerema et al., 2004; Zheng et al., 2007, Samson et al., 2010, Bensch et al., 2012; Woudenberg et al., 2013 ve Aveskamp, 2014.

After species level identification, specific identification keys are used for each genus.

2.3.1. Identification of *Phoma* genus member

Malt Extract Agar (MEA) and Oatmeal Agar (OA) were used. Cultures were incubated according to Boerema et al., (2004) and Aveskamp, (2014). Eight days after inoculation colony growth was measured. Morphologiacal features were studied after sporulation. During second week the petri dishes were placed in an incubator with a day-night regime of 13 h NUV light and 11 h darkness to stimulate the the pigmentation of the colonies and formation of picnidia (Boerema et al., 2004; Aveskamp, 2014).

2.3.2. Identification of *Penicillium* genus member

Czapek Yeast Agar (CYA 5 ºC, 25 ºC, 37 ºC), Malt Extract Agar (MEA 25 ºC), 25% Glycerol Nitrute Agar (G25N 25 ºC) and Neutral Creatine Sucrose Agar (CSN 25 ºC) were used (Pitt, 2000; Samson and Pitt, 2000; Samson et al., 2010).

2.3.3. Identification of *Rhizopus* genus member

Inoculated Potato Dextrose Agar (PDA) were incubated at 25 and 37 °C (Zheng et al., 2007).

2.3.4. Identification of *Alternaria* genus member

Inoculated Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were incubated at 25 ºC (Samson et al., 2010; Woudenberg et al., 2013).

2.3.5. Identification of *Cladosporium* genus member

Inoculated Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Oatmeal Agar (OA) were incubated at 25 ºC (Bensch et al., 2012).

2.4. Genotypic identification

DNA isolation were made as previously described, with some modification (Liu et al., 2000).

ITS1-5.8-ITS2 region (ITS) of the nuclear ribosomal DNA was amplified with ITS1 and ITS4 universal primers (Samson et al 2014). LR0R and LR7 primers were used to amplify of 28 S RNA (Lin et al., 2015).

Sequencing of the ITS regions were fulfilled in Genmar Laboratory (Izmir, Turkey). After DNA sequencing analyses, identification of the fungi was performed by comparison of other ITS1-5.8-ITS2 region (ITS) sequences in the public database. ITS1-5.8-ITS2 region (ITS) sequences acquired were entered into GenBank database and accession numbers for fungi were obtained.

The determination of the base sequence of the ITS region and 28 S rDNA region amplification in our study was fulfilled in Izmir Institute of Technology Center for Biotechnology and Bioengineering Research Laboratory. Type determination was performed by examining the similarities through the comparison of nucleotide sequences on the web page of Gene Bank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) open to the access of researchers and accession numbers for fungi were obtained.

3. RESULTS

3.1. Isolation

In our study, a total of 26 endophytic fungus isolates were obtained from Hurma olive samples. 6 of the 26 isolates were obtained from the olive samples collected from Eğlenhoca, 8 from Kösedere, 6 from İnecik, 3 from Amberseki and 3 from the villages of Saip (Table 1). In addition, 3 endophytic fungus species were isolated from the other olive samples for comparison purposes.

3.2. Identification of endophytic fungus species isolated from olives by phenotypic and genotypic methods

Endophytic fungus species isolated from Hurma olive samples as a result of phenotypic and genotypic identification were namely *Stemphylium vesicarium* (6), *Stagonosporopsis cucurbitacearum* (9), *Dothiora oleae* (6), *Rhizopus stolonifer* (2), *Penicillium chyrsogenum* (1) and *Bartalinia* sp. (1) (Table 1). Endophytic fungi species isolated from other non-Hurma olive samples were namely *Alternaria alternata* (2) and *Cladosporium ramotenellum* (1) (Table 2).

It was observed that the amount of fungal load determined in Hurma olives was statistically higher compared to that non-Hurma olive variants. ($p = 0.03 < 0.05$).

Table 1. *Endophytic fungus species isolated from Hurma olive samples*

Village	Sample No	Isolate Code	**Species of Fungus Isolated**	**Accession number**	
				ITS	**28S**
Eğlenhoca	E1	H - ST 1	*Stemphylium vesicarium*	**MK681350**	**MK685360**
		H – ST 2	*Stagonosporopsis cucurbitacearum*	**MK713566**	**MK685680**
	E2	H – ST 5	*Stagonosporopsis cucurbitacearum*	**MK713568**	**MK685984**
		H – ST 13	*Dothiora oleae*	**MK713567**	**MK687462**
	E3	H – ST 14	*Dothiora oleae*	**MK713580**	**MK687555**
	E4	H – ST 25	*Penicillium chrysogenum*	**MK713550**	**MK713338**
Kösedere	K1	H – ST 6	*Stagonosporopsis cucurbitacearum*	**MK713570**	**MK687410**
	K2	H – ST 3	*Stemphylium vesicarium*	**MK681349**	**MK685897**
	K3	H – ST 4	*Stemphylium vesicarium*	**MK713546**	**MK685900**
		H – ST 15	*Dothiora oleae*	**MK713581**	**MK688373**
	K4	H – ST 9	*Stagonosporopsis cucurbitacearum*	**MK713572**	**MK687459**
		H – ST 17	*Dothiora oleae*	**MK713582**	**MK688378**
	K5	H –ST 10	*Stagonosporopsis cucurbitacearum*	**MK713573**	**MK696205**
	K6	H – ST 24	*Rhizopus stolonifer*	**MK722192**	**MK704515**
İnecik	İ1	H – ST 7	*Stemphylium vesicarium*	**MK681351**	**MK687409**
		H – ST 18	*Dothiora oleae*	**MK713583**	**MK688393**
	İ2	H – ST 8	*Stemphylium vesicarium*	**MK713496**	**MK687411**
		H – ST 26	*Rhizopus stolonifer*	**MK722197**	**MK705761**
	İ3	H – ST 11	*Stagonosporopsis cucurbitacearum*	**MK713574**	**MK687460**
	İ4	H – ST 12	*Stagonosporopsis cucurbitacearum*	**MK713576**	**MK687461**
Amberseki	A1	H – ST 16	*Stagonosporopsis cucurbitacearum*	**MK713578**	**MK687570**
	A2	H – ST 22	*Stagonosporopsis cucurbitacearum*	**MK713579**	**MK689059**
	A3	H – ST 20	*Bartalinia* sp.	**MK724069**	**MK693698**
Saip	S1	H – ST 21	*Stemphylium vesicarium*	**MK713547**	**MK689360**
	S2	H – ST 19	*Dothiora oleae*	**MK713584**	**MK688554**
	S3	H – ST 23	*Bartalinia* sp.	**MK724070**	**MK689243**

Table 2. *Endophytic fungus species isolated from other non-Hurma olives*

Province	Olive type	Sample no	Isolate code	Species of Fungus Isolated	Accesion Number	
					ITS	28S
Manisa	Gemlik	1	Z - ST 1	*Alternaria alternata*	MK713548	MK713340
		2	Z – ST 2	*Alternaria alternata*	MK674250	MK713341
		3	Z – ST 3	*Cladosporium ramotenellum*	MK722198	MK713374
Izmir	Erkence	1	-	-		
		2	-	-		
		3	-	-		
		4	-	-		
		5	-	-		
Bursa	Çelebi	1	-	-		
		2	-	-		
		3	-	-		
		4	-	-		

As a result of phenotypic and genotypic identification endophytic fungi species isolated from other non-Hurma olive samples; *Alternaria alternata* and *Cladosporium ramotenellum* (Table 2).

As a result of the frequency analysis *Stagonosporopsis cucurbitacearum* (34.6%) was determined to be the most commonly encountered species in Hurma olives. Among the 26 species isolated, this species was the most isolated specie with 9 isolates (34.6%).

4. DISCUSSION

The ripening process of Hurma olives is known to occur only in Izmir / Karaburun Peninsula and only in Erkence olive varieties (Tutar, 2010). A similar olive is found in Greece and is known as "Thrubolea" or "Throuba Thassos". Another type of olive "Dhokar" has been reported by Tunisian researchers (Jemai et al., 2009; Zoidou et al., 2010; Rigane et al., 2011; Aktaş et al., 2014).

Susamci et al., (2017) investigated the development of chemical properties of Erkence and Hurma olives collected from Karaburun Peninsula. In their study, oleuropein content (< 2000 mg kg fresh weight) in Hurma olives have found it to be much lower than in Erkence olives at the beginning of the season (35.000 mg kg fresh weight). These results suggest that the enzymatic oxidation of oleuropein may be responsible for the natural bitter taste in Hurma olives during tree-ripening (Susamci et al., 2017). The main purpose of the table olive processing in local and traditional way is to hydrolyze the phenolic glycoside oleuropein, which gives the natural bitter taste to the fruit (Piscopo et al., 2014). According to a previous study in Greece, elemination of the bitter taste is caused by the presence of *Phoma oleae*, a fungus that grows in the pulp section and hydrolyzes oleuropein. Enzymatic hydrolysis of oleuropein, especially with β-glucosidase, is well known for both bacteria and fungi (Panagou, 2006).

Plant tissues are colonized by a large number of fungi found as epiphytes on the plant surface and as endophytes inside of the tissues. Endophytes provide the plant with the necessary nutrients, indirectly make the plant resistant to pathogens or have beneficial effects on plant growth by directly protecting them with an antagonistic effect on pathogens. Especially in recent years, endophytic fungi have become increasingly important as a biocontrol agent. Endophytic fungi are used to inhibit the growth of other fungi both in the field and during storage (Preto et al., 2017).

In the light of all this information, our aim is to conduct genotyping and phenotyping identification of endophytic fungi isolated from Hurma olives grown in Izmir/Karaburun Peninsula which has been debittered naturally and turn into an edible form. Again for the purpose of comparison, endophytic fungi were isolated from different varieties of non-Hurma Erkence olives from different gardens, Gemlik olives from Manisa and Çelebi olives from Bursa.

Landum et al., (2016) obtained 14 different fungi isolates from uninfected *Olea europaea* cv. *Galega vulgar* olive leaves and infected regions. As a result of ITS region sequence analysis those identified isolates were namely, *Alternaria* sp., *Aspergillus niger*, 2 strains of *Epicoccum nigrum*, *Fusarium* sp., *Anthrinium* sp., and a strain of *Xylariaceae* family that could not be identified at the species level. Again from the uninfected section *Alternaria* sp., *Chaetomium* sp., *Diaporthe* sp., *E. nigrum*, *Fusarium* sp., *Nigrospora oryzae* and an identified endophytic fungi were isolated. Most commonly encountered organisms were *A. nigrum*, *Alternaria* sp., and *Fusarium* sp. And these fungal species were examined for their antagonistic effect on olive anthracnose causing *Colletotrichum acutatum* and as a potential biocontrol agent it is stated that research should continue (Landum et al., 2016; Preto et al., 2017).

In a study, Ferraro et al., (2008) have made the identification of endophytic fungi using Malt Extract Agar (MEA) medium from olive plants grown in two different regions of Sicily. The dominant fungal genus were observed namely *Alternaria*, *Cladosporium*, *Diplodia*, *Phoma*, *Septoria* and *Stemphylium*. It was observed that endophytic fungal species isolated from the olives grown in two regions had similar compositions. Although endophytic fungus studies have been carried out on temperate climate plant species, some studies have shown that tropical plant species host a larger fungal biodiversity. Most of the results so far have shown that tropical endophytes may vary; therefore, this shows the tropical host endophytic fungal species forms a richer ecological community (Ferraro et al., 2008; Sia et al., 2013).

Identified endophytic fungus species isolated from Hurma olive samples by phenotypic and genotypic were *Stemphylium vesicarium*, *Stagonosporopsis cucurbitacearum*, *Dothiora oleae*, *Rhizopus stolonifer*, *Penicillium chyrsogenum* and *Bartalinia* sp. Endophytic fungus species isolated from other non-Hurma olive samples and identified by phenotypic and genotypic methods were *Alternaria alternata* and *Cladosporium ramotenellum*. As in the studies conducted, the fungal population isolated from the olive species of Hurma was higher than the fungal population isolated from other non-Hurma olive species ($p < 0.05$).

Baffi et al., (2012) investigated the fungal diversity of olive fruits *(Olea europaea* L.) and its enzymatic activities determined by biotechnological applications. 7 different genera (*Aspergillus*, *Penicillium*, *Rhizomucor*, *Mucor*, *Rhizopus*, *Lichtheimia* and *Galactomyces*) and 14 different species belong to these genera were identified. *Aspergillus fumigatus*, followed by *Galactomyces geotrichum*, *Penicillium commune* and *Rhizomucor variabilis* var. *regularior* has been the most common species. Almost all strains showed β-glucosidase activity. This enzyme is involved in the degradation of oleuropein, which is responsible for brown color and bitter taste in olive oil, and can be used for the production of bioethanol from lignocellulosic residues (Baffi et al., 2012).

These studies also show the potential of a rich microbiota isolated from olives, suggesting that olives can be used to gain advantages in biotechnology and enzyme production in different sectors. The identified species and their enzymes can be used not only in the olive oil industry, but also in other industries that can use biotechnological processes for biological transformation and the use of agricultural by-products (Baffi et al., 2012).

In their study on endophytic fungal diversity of olive plant *Olea europaea*, Sia et al reported that *Diaporthe*/*Phomopsis* potentially produces a number of biologically active antifungal and antibacterial metabolites, is a rich source of secondary metabolites and is particularly effective on phytopathogenic fungi *Cladosporium sphaerospermum* and *C. cladosporioides* (Sia et al., 2013).

Krohn et al., (1999) reported that herbarulid, an anti-bacterial compoundand ketodivinyllactonic steroid is present in *Pleospora herbarum*, an endophytic fungus of the *Medicago lupulina* plant (Krohn et al., 1999). This suggests that some of our isolated fungal species such as the *Stemphylium vesicarium* an anamorph of *Pleospora herbarum*, may have important anti-microbial properties.

In a study, Abdelfattah et al., (2015) isolated *Colletotrichum*, *Pseudocercospora*, *Fusarium*, *Cladosporium*, *Hypocreales*, *Devriesia*, *Aureobasidium*, *Alternaria*, *Dissoconium*, *Ascomycota*, *Eudarluca*, *Phallus* and *Phomopsis* fungi from an asymptomatic olive fruit. *Aureobasidium* spp., *Cladosposorium* spp. and *Devriesia* spp., have shown to competitive effect against fungal plant pathogens. Based on this information, it is suggested that the activities of endophytic fungal species *Alternaria* and *Cladosporium* that we isolated, should also be evaluated as potential anatagonistic agents (Abdelfattah et al., 2015).

Many studies have proven that endophytes are a potential source of new natural products in modern medicine, agriculture and industry. Numerous new natural products with antimicrobial activity have been isolated from endophytes. Screening of antimicrobial compounds from endophytes is believed to be a promising way to overcome the increasing threat of drug-resistant strains of human and plant pathogens. These outcomes will provide the opportunity to use endophytes as a new resource for antibiotic production (Yu et al., 2010; Malhadas et al., 2017). As a result of the studies so far alkaloids, peptides, steroids, terpenoids, phenols, phenylpropanoids, aliphaticcompounds, polyketides, quinones and flavonoids, as well as volatile organic compounds (VOCs) such as esters, lipids, alcohols, acids, ketones, a wide range of novel antimicrobial compounds produced by endophytic fungi belonging to various structural classes have been reported (Malhadas et al., 2017).

In a study conducted by Malhadas et al., (2017) endophytes (*Alternaria alternata*, *Penicillium canescens* and *Penicillium commune*) attracted a great deal of interest in the development of new drugs against a number of human pathogenic microorganisms. In this study, both *P. canescens* and *P. commune* were found to be the most effective against bacteria, while *A. alternata* showed higher activity against yeasts (Malhadas et al., 2017). According to this information, *Alternaria* and

Penicillium species we isolated should be investigated for the possibility of having antimicrobial potential and their role as producers of bioactive secondary metabolites.

In their new study Abacı et al., (2014) have isolated and identified *Phoma multirostrata* from tree-ripened Hurma olives in Izmir Karaburun Peninsula and published the first report of *Olea europaea* (Oleaceae). ITS and LSU regions were analyzed for molecular identification and their isolates were identified as *Phoma multirostrata* (Abacı et al., 2014). In the studies carried out up this date, it has been reported that *Phoma oleae* is the cause of """natural de-bittering"" process. In our study, we have identified the *Dothiora oleae* species, which is synonymy of *Phoma oleae*.

In a study about the sweet Thassos olives grown on the island of Thassos in Greece showed that the oleuropein responsible for the bitter taste was hydrolyzed to the hydroxy tyrosolase and its derivatives by the enzyme β-glucosidase produced by the fungi during maturation. Again, this situation was also observed in a study about Dhokar olives grown in the southern region of Tunisia. Despite these studies, information on the phenomenon of natural maturation is mostly limited to phenolic alteration, and further studies are needed to identify other chemical alterations occurring in the olive composition during ripening (Aktaş et al., 2014).

A study of sweet Thassos olives grown on the Greek island of Thassos has shown that oleuropein, which is responsible for the bitter taste, is hydrolyzed to the hydroxytyrosol and its derivatives by the β-glucosidase enzyme produced by the fungi during ripening. Again, this was observed in a study of Dhokar olives grown in the southern region of Tunisia. Despite these studies, information on the phenomenon of natural ripening is often limited to phenolic changes and further studies are needed to identify other chemical changes in the olive composition during ripening (Aktaş et al., 2014).

Recently, studies conducted to show the genetic difference of Hurma Olive from other olive varieties draw attention. Doganlar et al. made gene sequencing with other 20 kinds olives grown in Turkey using five genes as Barcodes and observed that 20 different Erkence Olive were of different varieties compared to other olive types. Researchers emphasized that the process that olives should undergo after harvesting, Erkence Olives are completed on the tree thanks to a special fungus and that date Erkence Olives are genetically different from other varieties

(Anonymous, 2017). Cross-talk between endophytic fungi and plants can be solved by a Systems Biology approach such as Boolean modeling used to model and simulate host pathogen interactions (Krouk et al., 2013; Dühring et al., 2015; Guthke et al., 2016; Haliki et al., 2019).

All these studies emphasize the importance of investigating fungal biodiversity in olives and the need for more detailed analysis in this area. At the same time, the studies show the potential of a rich microbiota isolated from olive.

So far, no studies have been conducted on the isolation of endophytic fungi from Hurma olives. For this purpose, we have identified new endophytic species in our study. We think that our study will increase the interest in endophytic fungi and the secondary metabolites produced by them and will highlight many researches about the identification of many undiscovered metabolites.

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Chapter 3

TRADITIONAL USES OF PLANTS IN WOMEN’S HEALTH (WOMEN’S DISEASES, COSMETICS, AESTHETICS AND DIET) IN TURKEY

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INTRODUCTION

As a result of ethnobotanical studies, it has been reported that plants are used for various purposes such as food, spices, dyes, healing, religious beliefs, clothing, shelter, and ornaments (Aumeeruddy, 1996; Kendir and Güvenç, 2010). This information, mostly unwritten and obtained by traditional methods, has been passed down from generation to generation. Traditional folk medicine and treatment methods are still us ed in many rural areas.

Women living in rural areas In Anatolia have undertaken more responsibilities in many jobs than men, from childcare to housework, from crop planting to harvesting, and caring for animals.

As it is known, traditional practices related to health shaped by the customs and traditions, beliefs, and social behaviors of societies are defined as folk medicine (Şar, 2008; Türkdoğan, 1991). Most of the medicines used in this traditional method are of herbal origin. In the production of drugs, plants can be used fresh, as well as many parts such as leaves, flowers, seeds, roots, and shells are used for drying. The most common use of plants is an infusion. However, it is also used in different ways such as decoction, maceration, oil, and powder (Ertuğ 2000; Polat and Satıl, 2012; Bulut and Tuzlaci 2013; Dogan and Ugulu 2013; Akbulut and Özkan, 2014; Ertuğ, 2014; Sargin, 2015; Karaköse et al., 2019; Erşen-Bak and Çifci, 2020; Demir, 2020). It is not as easy for people living in the countryside to access modern medical facilities as in the city people. For this reason, it gives priority to folk medicine for non-vital medical treatments, especially women living in rural areas.

Although there are different applications such as acupuncture, yoga, homeopathy and traditional Chinese medicines in alternative treatment methods, women generally prefer treatment methods that are compatible with their cultural structures (Cutson and Meulemen, 2000; Amanak et al., 2013). Studies have reported that middle-aged and older women primarily resort to herbal therapy for their menopause-related disorders (Mac Lennan et al., 1996; Kang et al., 2002).

A clear list of herbs currently used in more traditional therapy has not been established. It has been stated in different sources that this number may be up to 1000 (Başer, 2001; Koyuncu, 1990). However, no specific grouping has been made between them. While the same plant is used for a single purpose in a region, the same plant can be used for much more purposes in a different region. Special studies on plants commonly used for each disease will be very useful in grouping plants and making alternative solutions more specific.

The aim of the study is to determine the plants used and the usage patterns for especially the gynecological diseases, cosmetic, aesthetic and diet purposes of the women living in the rural areas in Turkey.

MATERIAL AND METHOD

The study was carried out in the 17 cities of Turkey (Table 1, Figure 1). Traditional knowledge was obtained from 2017 to 2019. The data was collected by questionnaire survey and interviews. The interviews were conducted with people living in rural areas in these cities. A total of 340 women were interviewed face to face. The women were asked to show the plants they used, and samples were taken from these plants for identification. During this period, 121 plant taxa used by local women were collected and identified. The book Flora of Turkey was used as the main source for the identification of plants. (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000).

Table 1. Working areas and informant numbers

No	Location	**Questionnaire numbers**
1	Çanakkale	20
2	Manisa	20
3	Kütahya	20
4	Denizli	20
5	Bartın	20
6	Bolu	20
7	Sinop	20
8	Yozgat	20
9	Aksaray	20
10	Mersin	20
11	Ordu	20
12	Kahramanmaraş	20
13	Hatay	20
14	Trabzon	20
15	Rize	20
16	Erzincan	20
17	Ağrı	20
Total		**340**

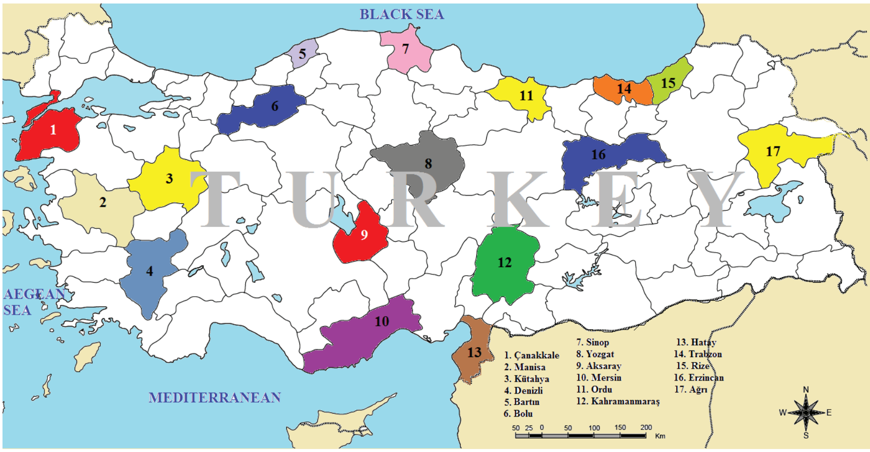

Figure 1. Study areas in Turkey

RESULTS

In the research was identified 119 plants taxa belonging to 47 families that were used by local women (Table 2). Among these are 3 gymnosperms and 116 angiosperms. 3 of the species used are endemic (*Anthemis aciphylla* var. *aciphylla*, *Taraxacum pseudopulchrum, Sideritis arguta*), 21 are culture plants (*Beta vulgaris* var. *altissima, Allium ampeloprasum, Allium cepa*, *Allium sativum*, *Petroselinum crispum*, *Pimpinella anisum*, *Brassica oleracea*, *Raphanus raphanistrum*, *Cannabis sativa*, *Cucurbita moschata*, *Phaseolus vulgaris*, *Juglans regia*, *Ocimum basilicum*, *Morus alba*, *Morus nigra*, *Olea europaea* var. *europaea*, *Sesamum indicum*, *Zea mays*, *Portulaca oleracea*, *Nigella sativa*, *Vitis vinifera*). The plants used for rheumatism pain were also included in the study because they were frequently told by interviewed women. Taxonomic classification was done alphabetically according to first by family and then by scientific names.

The most used families are Asteraceae (20 taxa), Lamiaceae (12 taxa), and Rosaceae (10 taxa).

Commonly used parts of plants for different purposes are leaves (48 taxa), flowers (19 taxa), aerial parts (17 taxa), fruits (15 taxa), seeds (15 taxa), and other parts such as branches, stems, bulbs, branches, roots, stalk, saps, stylus, shoots, latex, cones, wood, resin. In many studies, leaves, flowers, and aerial parts take place as the most used parts of plants in the first three places. These parts of the plants are more preferred because they can be stored for a long time when dried (Sargin, 2015; Rehman et al., 2017; Chaachouay, 2019; Polat, 2019).

The most common traditional preparation method of plants material was infusion (42 taxa), followed by decoction (32 taxa), fresh (29 taxa), oil (16 taxa), mash (11 taxa), cooking (5 taxa), powder (4 taxa), drying (2 taxa), gum (2 taxa), boiled (1 taxa), syrup (1 taxa), warming (1 taxa).

Many of the plants listed in Table 2 are used for similar purposes in traditional medicine. Although some of them are prepared by different methods, they are widely used ethnobotanically in the world. However, the traditional use of some plants was recorded for the first time in this study. The use of fresh leaves of *Cerasus avium* for the treatment of callus on the feet was recorded. The fruit of *Cucurbita moschata* is used externally for infertility treatment after being mashed. The seeds of *Centaurea iberica* are used internally as a breast milk enhancer in the form of decoction (Figure 2-4).

Figure 2. Cerasus avium

Figure 3. Cucurbita moschata

Figure 4. Centaurea iberica

Table 2. Traditional uses of plants in women's health in the study areas

Family	**Botanical name**	**Vernacular name**	**Part use**	**Preparation**	**Traditional knowledge**
Adoxaceae	*Sambucus ebulus* L.	Mürver otu	leaf, fruit	fresh, decoction	Skin diseases, against the allergic effect of *Urtica* spp., against rheumatism pain
Amaranthaceae	*Amaranthus retroflexus* L.	Tilkikuyruğu	leaf	decoction	To lose weight
Amaranthaceae	*Beta vulgaris* var. *altissima* Döll (C)	Şeker pancarı	leaf, seed	boiled, infusion	Boiled leaves are wrapped in rheumatic pain, he seeds are used as tea or mixed with honey or yogurt against fungi.
Amaryllidaceae	*Allium ampeloprasum* L. (C)	Pırasa	leaf	cooking	To lose weight
Amaryllidaceae	*Allium cepa* L. (C)	Soğan	bulb	decoction	To lose weight, breast milk enhancer, for cysts and menstrual irregularities
Amaryllidaceae	*Allium sativum* L. (C)	Sarımsak	bulb	fresh, mash (with olive oil)	Garlic kept in olive oil is placed in the anal area for uterine inflammation, crushed garlic cloves are used to prevent hair loss (for ringworm)
Apiaceae	*Ferula elaeochytris* Korovin	Çağ	leaf, root	infusion, powder	For infertility
Apiaceae	*Foeniculum vulgare* Mill.	Rezene	leaf, fruit	infusion, powder	To lose weight
Apiaceae	*Petroselinum crispum* (Mill.) A.W.Hill (C)	Maydanoz	leaf	fresh, decoction	To lose weight, for menstrual pain
Apiaceae	*Pimpinella anisum* L. (C)	Anason	aerial parts	infusion	To alleviate the effects of menopause, hormone regulator
Aspleniaceae	*Asplenium ceterach* L.	Dalakotu	leaf, shoot	infusion	Against uterine inflammation
Asteraceae	*Achillea millefolium* subsp. *millefolium*	Civanperçemi	aerial parts	infusion	Menstrual regulator, menstrual pain
Asteraceae	*Anthemis aciphylla* var. *aciphylla* (E)	İğne papatyası	flower	decoction	Hair care
Asteraceae	*Anthemis cretica* L.	Dağ papatyası	flower	decoction	Hair care
Asteraceae	*Anthemis cotula* L.	Hozan çiçeği	flower	decoction	Hair care, menstrual pain
Asteraceae	*Anthemis pseudocotula* Boiss.	Acem papatyası	flower	infusion	Hair dye

Table 2. continued

Asteraceae	*Artemisia vulgaris* L.	Kaba yavşan	flowering branches	infusion	Sit baths for menstrual pain
Asteraceae	*Bellis perennis* L.	Koyungözü	flower	decoction, infusion	Menstrual regulator, reducing the effects of menopause
Asteraceae	*Calendula arvensis* L.	Potakal nergisi	flower	decoction, medical oil	Against acne (medical oil), athlete's foot, eczema, psoriasis, gynecological diseases (decoction externally)
Asteraceae	*Centaurea iberica* Trev. ex Sprengel	Deligözdikeni	seed	decoction	Breast milk enhancer
Asteraceae	*Centaurea solstitialis* subsp. *solstitialis*	Çakırdikeni	stem	fresh	Increasing the number of eggs in women
Asteraceae	*Cota tinctoria* var. *pallida* (DC.) U.Özbek & Vural	Boyacı papatyası	flower	decoction	Menstrual pain cutter
Asteraceae	*Lactuca sativa* L.	Marul	leaf	fresh	Breast milk enhancer and breast softener
Asteraceae	*Matricaria chamomilla* var. *chamomilla*	Alman papatyası	flower	decoction	For menstrual irregularities and menstrual pain
Asteraceae	*Helichrysum stoechas* (L.) Moench.	Kudama	flower	infusion	To lose weight
Asteraceae	*Onopordum acanthium* L.	Galagan	seed, leaf	infusion	Skin diseases
Asteraceae	*Pulicaria dysenterica* (L.) Bernh.	Yaraotu	flower	decoction	Hair care and dye
Asteraceae	*Silybum marianum* (L.) Gaertner	Devedikeni	aerial parts	decoction, fresh	For rheumatic pain, psoriasis
Asteraceae	*Solidago virgaurea* L.	Altınbaşak	aerial parts	infusion	For menstrual pain
Asteraceae	*Tanacetum parthenium* (L.) Schultz Bip.	Beyaz papatya	flower sap	fresh	For menstrual irregularities
Asteraceae	*Taraxacum pseudopulchrum* Kirschner & Stèpanek (E)	Afyon çıtlağı	flower sap, latex	fresh	For freckle removal, warts and calluses
Balsaminaceae	*Impatiens noli-tangere* L.	Kına çiçeği	leaf sap	fresh	In hand painting as henna
Betulaceae	*Alnus glutinosa* subsp. *barbata* (C.A.Mey.) Yalt.	Yeykin	leaf	fresh	For cracks and redness on the hands

Table 2. continued

Brassicaceae	*Brassica oleracea* L. (C)	Lahana	leaf	decoction, cooking	Against skin spots, edema remover, for rheumatism pain (externally wrapped in the aching area)
Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	Çobançantası	aerial parts	infusion	In uterine contractions
Brassicaceae	*Raphanus raphanistrum* L. (C)	Turp	seed	infusion, fresh (with honey or yoghurt)	Against fungus
Cannabaceae	*Cannabis sativa* L. (C)	Kenevir	aerial parts	oil	Externally for eczema, psoriasis and fungal diseases
Cannabaceae	*Humulus lupulus* L.	Ömer otu	aerial parts	infusion	To alleviate the effects of menopause, hormone regulator
Cistaceae	*Cistus creticus* L.	Laden	leaf, flower	infusion	For inflammatory skin diseases and fungus
Colchicaceae	*Colchicum speciosum* Steven	Şepart	seed	mash (with garlic)	For rheumatic pain
Cornaceae	*Cornus mas* L.	Kızılcık	fruit, bark	infusion	To reduce hot flashes during menopause
Cornaceae	*Cornus sanguinea* subsp. *australis* (C.A.Mey.) Jáv.	Kansiğdiren	fruit	mash	Skin diseases
Cucurbitaceae	*Cucurbita moschata* Duchesne (C)	Bal kabağı	fruit	mash	Infertility treatment
Cucurbitaceae	*Ecballium elaterium* (L.) A.Rich	Eşek hıyarı	fruit extract	mash	Menstrual remover
Cucurbitaceae	*Momordica charantia* L.	Kudret narı	aerial parts	oil	Externally in eczema treatment
Cupressaceae	*Juniperus drupacea* Labill.	Andız	cone	infusion	Menstrual pain, skin diseases (psoriasis, eczema)
Cupressaceae	*Juniperus oxyc*edrus L.	Ardıç	cone, leaf	oil	Soap made from cones and leaves oil is used externally for acne, eczema, psoriasis and fungal diseases.
Equisetaceae	*Equisetum arvense* L.	Atkuyruğu	leaf	infusion	Menstrual periods regulator
Equisetaceae	*Equisetum telmateia* Ehrh.	Deredoruk	leaf	infusion	For rheumatic pain, eczema
Ericaceae	*Erica arborea* L.	Funda	leaf	infusion	To lose weight
Ericaceae	*Rhododendron ponticum* L.	Kumar	leaf	fresh	For skin rubbing (leaves are warmed)

Table 2. continued

Ericaceae	*Vaccinium myrtillus* L.	Çoban üzümü	leaf	infusion	To revitalize the skin
Euphorbiaceae	*Euphorbia helioscopia* L.	Sarı sütleğen, seherotu	latex	externally fresh	For warts
Euphorbiaceae	*Euphorbia kotschyana* Fenzl	Sütlüce	latex	externally fresh	For warts
Euphorbiaceae	*Euphorbia rigida* M.Bieb.	Sütleğen	latex	externally fresh	For warts
Euphorbiaceae	*Mercurialis annua* L.	Parşen	leaf, flower	decoction	Against uterine swelling (sit bath)
Fabaceae	*Ceratonia siliqua* L.	Keçiboynuzu	fruit	dried fruit, decoction	For menstrual irregularities
Fabaceae	*Glycyrrhiza glabra* L.	Meyan	root extract	decoction	Skin diseases
Fabaceae	*Ononis spinosa* L.	Kayışkıran	root	decoction	Externally as a wound healing
Fabaceae	*Phaseolus vulgaris* L. (C)	Fasulye	seed	mash	For ringworm
Fagaceae	*Castanea sativa* Miller	Kestane	leaf	infusion	Hair care and dye
Fagaceae	*Fagus orientalis* Lipsky	Kayın	wood shaving	externally powder	For skin rubbing
Fagaceae	*Quercus* spp.	Meşe	bark, leaf	decoction, infusion	Externally for the treatment of skin cracks, sitting bath for uterine discharge and pain (infusion of leaves)
Gentianaceae	*Centaurium erythraea* Rafn.	Kırmızı kantaron	flower	oil	For acne and skin blemishes
Geraniaceae	*Geranium collinum* Stephan ex Willd.	Itır çiçeği	aerial parts	decoction	For the treatment of rash skin diseases such as eczema (by adding to bathwater)
Hypericaceae	*Hypericum perforatum* L.	Sarıkantaron	flower	infusion, oil	For skin diseases (external oil), gynecological diseases and menstrual pain (infusion)
Juglandaceae	*Juglans regia* L. (C)	Ceviz	fruit, leaf, seed	decoction	Juice for acne treatment; mash for hair and hands coloring; seed oil as suntan oil in tanning, leaves for hair coloring and care
Lamiaceae	*Ajuga chamaepitys* (L.) Schreb.	Acıgıcı	leaf	decoction	External dressing for skin diseases

Table 2. continued

Lamiaceae	*Astragalus angustifolius* subsp. *pungens* (Willd.) Hayek	Kör geven	root, stem	gum	Breast milk enhancer
Lamiaceae	*Lavandula angustifolia* Mill.	Lavanta	flower, aerial parts	decoction, fresh	Flowers for rheumatism pain, above-ground parts in fresh form for skin problems (compresses)
Lamiaceae	*Melissa officinalis* L.	Oğulotu	leaf	infusion, fresh	For external odor of sweat, menstrual pain, rheumatism pain, mammary gland obstruction (compresses)
Lamiaceae	*Mentha* spp.	Pünk	leaf	infusion	For rheumatic pain
Lamiaceae	*Ocimum basilicum* L. (C)	Fesleğen	leaf	infusion	To soothe the skin
Lamiaceae	*Rosmarinus officinalis* L.	Biberiye	leaf	infusion	Hormone regulator, for the treatment of menstrual irregularity, cyst formation in the uterus
Lamiaceae	*Salvia verticillata* L.	Hart şalbası	leaf	decoction	To alleviate the symptoms of skin diseases, hair care, gynecological diseases, post-menopause
Lamiaceae	*Sideritis arguta* Boiss. & Heldr. (E)	Köy çayı	leaf	infusion	For rheumatic pain
Lamiaceae	*Thymus nummularius* M.Bieb.	Limon kekiği	leaf sap	mash, oil	For uterine inflammation, uterine bleeding (with mash), rheumatism pain (external oil)
Lamiaceae	*Thymus praecox* Opiz	Kaf kekiği	aerial parts	syrup, oil	Syrup, internally for the treatment of menstrual pain, oil, externally for the treatment of eczema
Lamiaceae	*Vitex agnus-castus* L.	Hayıt	bark, seed	infusion	For hormonal regulator and menopause
Lauraceae	*Laurus nobilis* L.	Defne	fruit, leaf	soap in oil, infusion	Oil provides hair growth, relieves rheumatism pain, acne relief, nourishes the scalp, tea obtained from leaves to increase estrogen, eliminate menstrual irregularity
Linaceae	*Linum hypericifolim* Salisb.	Keten	seed	oil	For topical eczema treatment

Table 2. continued

Linaceae	*Linum obtusatum* (Boiss.) Stapf	Akdağ keteni	seed	infusion	To lose weight
Lycopodiaceae	*Lycopodium clavatum* L.	Kurtayağı	aerial parts	decoction	Internally for the treatment of eczema and fungus
Malvaceae	*Malva sylvestris* L.	Ebegümeci	leaf	mash	Externally for the treatment of skin diseases and boils
Melanthiaceae	*Veratrum album* L.	Dokuztepeli	aerial parts sap	fresh	Plant sap for abortion
Moraceae	*Ficus carica* L.	İncir	latex	fresh	Latex, applied externally on the wart
Moraceae	*Morus alba* L. (C)	Akdut	fruit	dried fruit	Dried fruit in paste form externally for eczema treatment
Moraceae	*Morus nigra* L. (C)	Karadut	fruit	fresh	Against cracked lips
Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	Ökaliptus	leaf, flower	oil	Externally for eczema treatment
Myrtaceae	*Myrtus communis* L.	Mersin	leaf, fruit	infusion	For menstrual irregularities
Oleaceae	*Olea europaea* var. *europaea* (C)	Zeytin	leaf	decoction, oil	Leaves to lose weight, soaps made of oil to nourish hair, prevent skin wrinkles
Papaveraceae	*Chelidonium majus* L.	Kırlangıçotu	aerial parts latex	fresh	Latex for removing acne, warts and freckles
Papaveraceae	*Fumaria officinalis* L.	Şahtere, şahdelen	aerial parts	infusion	Internally for the treatment of eczema and fungal diseases
Papaveraceae	*Papaver rhoeas* L.	Gelincik	flower	cooking	Breast milk enhancer if cooked with wheat
Pedaliaceae	*Sesamum indicum* L. (C)	Susam	seed	oil	Externally for acne, freckle spot, scars, eczema and rheumatism pain
Pinaceae	*Picea orientalis* (L.) Peterm.	Ladin	resin	gum	Externally for skin diseases
Plantaginaceae	*Plantago major* L.	Sinirotu	leaf	warming	The heated leaves are compresses for abscess treatment
Poaceae	*Elymus repens* (L.) Gould	Ayrık otu	root, aerial parts	decoction	Externally for eczema treatment, internally for menstrual period pain

Table 2. continued

Poaceae	*Zea mays* L. (C)	Mısır	stilus, seed	decoction, powder	Stylus tea for slimming and reducing menstrual pain, the seed powder is made into a paste with water to make a mask for black spots on the face
Polygonaceae	*Rumex acetosella* L.	Kuzukulağı	leaf	mash	Compresses are applied on acne and boils
Polygonaceae	*Rumex crispus* L.	Labada	leaf	mash, cooking	Mash leaves for burns on the skin, edible leaves to increase breast milk
Portulacaceae	*Portulaca oleracea* L. (C)	Semizotu	aerial parts	fresh, cooking	To lose weight
Primulaceae	*Primula acaulis* (L.) L.	Çuhaçiçeği	flower, parts of plants	mash, oil	Externally for rheumatism pain (mash flowers), externally for the treatment of eczema obtained from the above-ground part
Ranunculaceae	*Nigella sativa* L. (C)	Çörekotu	seed	drying, oil	Drying seeds, uterine cyst preventive, breast milk enhancer, seed oil internally for eczema and fungal diseases, externally for acne
Ranunculaceae	*Ranunculus constantinopolitanus* (DC.) d'Urv.	Kağıthane çiçeği	flower	fresh	For externally rheumatic pain (flowers are crushed)
Rhamnaceae	*Ziziphus jujuba* Mill.	Hünnap	fruit	fresh	For eczema, psoriasis and fungal diseases
Rosaceae	*Alchemilla erythropoda* Juz.	Al şebnemli	leaf	infusion	Internally regulating menstruation, increasing discharge, externally for acne treatment
Rosaceae	*Amygdalus communis* L.	Badem	seed	oil	externally for treating acne, freckle, scars and eczema
Rosaceae	*Cerasus avium* (L.) Moench	Kiraz	fruit stalk, leaf	infusion, fresh	Consumed against menopause, menstrual pain, fresh leaves for the treatment of callus on the feet

Table 2. continued

Rosaceae	*Cydonia oblonga* Mill.	Ayva	leaf	infusion	Internally enhancing breast milk, externally for eczema and acne treatment
Rosaceae	*Potentilla erecta* (L.) Räusch	Kurtpençesi	flower	boiling	Externally to soften the skin, clean skin blemishes
Rosaceae	*Pyrus elaeagnifolia* Pall.	Ahlat	fruit, bark	infusion, fresh	For infertility treatment
Rosaceae	*Rosa canina* L.	Kuşburnu	leaf	decoction	Externally as a tonic against skin wrinkles
Rosaceae	*Rubus canescens* var. *canescens*	Çobankösteği	leaf, root	infusion	Leaves for hair care, roots for menstruation
Rosaceae	*Rubus caucasicus* Focke	Zarif böğürtlen	leaf, root	infusion	Leaves for hair care, roots for menstruation
Rosaceae	*Rubus idaeus* L.	Ahududu	fruit	fresh	To regulate menstruation and reduce excessive menstrual bleeding
Scrophulariaceae	*Verbascum flavidum* (Boiss.) Freyn & Bornm.	Sığırkuyruğu	leaf	decoction	Externally for acne treatment
Urticaceae	*Urtica dioica* L.	Isırgan	leaf, seed	infusion, decoction, fresh	Leaves for hair care (infusion), breast milk enhancer (decoction), rheumatism pain and ear piercing for local anesthesia (freshly externally), seeds mixed with honey for eczema treatment
Vitaceae	*Vitis vinifera* L. (C)	Asma	fruit, leaf	drying, fresh	Dried fruits, fresh leaf salad against nausea in pregnant women

(C) – Cultivation plants, (E) – Endemic plants of Turkey

CONCLUSIONS

Despite advanced medical treatments, the interest in medicinal and aromatic plants remains popular. In this study, 119 plant taxa were identified used by women for various purposes such as aesthetic, feminine ailments, cosmetic and rheumatic pain in Turkey. These plants, which are used in the traditional treatment and using traditional methods, are part of the cultural heritage. Ethnobotanical data are provided directly from individuals and are passed on from generation to generation. Information, many of which are obtained through trial and error, is of medical and pharmacological significance.

Folk medicine has an important place in Anatolian culture. For these people, many of whom live in rural areas, plants are the first source that comes to mind. Therefore, each of the information obtained is worth investigating. Due to the rich flora and culture with Turkey is one of the countries in this regard is quite advantageous.

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Chapter 4

WORLD ORGANIC AGRICULTURAL ACTIVITY, YESTERDAY, TODAY AND TOMORROW[1]

Kurtuluş MERDAN[2]

1 This study 15-16.12. It is the updated and expanded version of the oral statement "The Present State and Future of the World Organic Agriculture Potential" presented at the International Conference On Multidisciplinary Sciences (ICOMUS) between 2018

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1- INTRODUCTION

The rapid increase in the world's population has brought food and housing problems with it. Artificial fertilizer, hormone and pesticides that are used to meet human's needs, spoiled the nature, environment and living being's quality. In order to reach the old quality again, the countries around the world have begun to prefer an agricultural system that does not destroy the nature and is free from the negative effects of chemicals on humans. In this context, a production style that supports sustainable and alternative agriculture has been implemented (Zengin, 2007: 9; Sarikaya, 2007: 110; Demiryurek, 2011; Rehber, 2011; Candemir et al., 2012). Thus, in many countries, the process of shifting from traditional agriculture to organic agriculture has started (Merdan, 2014: 1).

Organic agriculture is a production method which aims to reestablish the natural balance which has disappeared as a result of wrong applications, aims to use production methods that are human and environmentally friendly, recommends to use completely organic pesticides and fertilizers, and adopts the principle of increasing the production quality rather than production increase. It is a form of agricultural production in which all stages from production to consumption are carried out in a controlled and certified manner and there is no use of chemical inputs (Er and Empti, 2008: 13). Organic agriculture is a form of production that aims to increase product quality rather than increase production, to benefit from biological control and to increase the resistance of the plant (Cobanoglu and Isin, 2009: 64). Organic agriculture is an agricultural practice, an agricultural breeding and a production method with its own criteria (Er, 2009: 18). Organic agriculture, which has many definitions similar to each other, aims to produce healthy foods, aims to realize production through natural methods (Atiker, 2004: 1), aims to provide acceptable cost / profit optimization for producers and consumers and to meet the food needs of the society in a reliable way (Merdan and Kaya, 2013: 240) is an alternative production method. This method, whose main purpose is to protect the environment, is also called alternative, biological, natural, ecological, edible or sustainable agriculture (Bayram et al., 2007: 204; Zengin, 2007: 11). The most basic feature that distinguishes organic agriculture from other agricultural techniques is that it has gained a legal framework, control and certification procedures (Tate, 1994; Lampkin, 1996; Demiryurek, 2004).

Organic farming; The ecosystem plays an important role in achieving goals related to biodiversity, sustainable production, climate change, hunger, poverty and water resources. Organic agriculture in the new era; It stands out with the principles of a holistic approach from farm to producer in terms of social equality and fair trade, based on the principles of correct and fair pricing, inclusive and transparent participation, achieving the best in examples, sustainable concepts such as ecology, society, culture and economy in all value chains (Ak, 2004; Merdan, 2014: 6).

At the beginning of the modern age, organic agriculture has been one of the issues that come up frequently. During this period, parallel to the increase in education level, environmental and health awareness also increased. This situation caused an increase in the demand and trade volume for organic products. At this point, states, non-governmental organizations and producers have tried to get more shares from organic agriculture with the regulations they put forward (Deviren and Celik, 2017: 669).

The general distribution of organically produced products in the world countries has been directed towards traditional products. For example, milk and dairy products in Denmark, tea in India, meat and meat products in Argentina, dates in Tunisia, bananas in Africa are among the traditionally produced products (Usal, 2006: 18).

According to the latest findings, organic production is carried out in 1.5% (71.5 million hectares) of the agricultural land in the world. Oceania owns 50% of the world's organic farmland with 36 million hectares of agricultural land. Australia is one of the leading countries with the most organic agricultural land in the world. Argentina comes after Australia with 3.6 million hectares. Liechtenstein is the country that allocates the most land to organic agriculture in its total agricultural land. Following Liechtenstein is Samao (34.5%) and Austria (24.7%) (FIBL and IFOAM, 2020: 20).

The findings of this study show that there is a continuous increase in the fields of organic agriculture in EU countries. In 1999, the number of organic producers which 200 thousand to 2 million in 2013, the year 2018 also reached 2.8 million. It is among the largest government space agencies in the world. The world ‘s largest organic producer is located in Asia (47%). Asia is followed by Africa (28%), Europe (15%) and Latin America (8%). India (1,149,371) ranks first among the countries with the highest number of organic producers. Following India is Uganda (210.352) and Ethiopia (203.602), respectively (FIBL and IFOAM, 2020: 22).

In parallel with all these positive developments in the world, the global market and EU organic food and beverage sales have also exceeded 95 million euros. Although organic food sales are growing at a healthy rate, the demand for organic food continues to be concentrated in the North (America and Europe). Even though the shares of America and Europe are declining, still the majority of global sales are generated by these two regions. The largest global food market belongs to the USA (42%), according to FIBL. This country is followed by the EU (38.5%) and China (8.3%) (FIBL and IFOAM, 2020: 2 2-23).

With this study, the past, present and future of organic farming activities in the world were evaluated. In forming the theoretical and evaluation part of the study, domestic and foreign articles, visual-written media, internet resources, reports and publications prepared by organizations such as FIBL and IFOAM were used. The study utilized the data recorded from the beginning of organic farming until 2018. Based on these findings, organic farming products are made commercially in 186 countries today and organic farming areas are increasing rapidly. As of 2018, the organic farming area in the world has reached 71.5 million hectares, the per capita consumption has reached 12.8 euros, the number of producers has reached 2.8 million and the organic market size has reached 96.7 billion euros. The findings of the study reveal that Australia is the leader in terms of organic farmland, Liechtenstein in terms of the place of organic farmland in total agricultural land, India in terms of producer, USA in terms of organic market size and Switzerland in terms of per capita consumption.

2. HISTORICAL DEVELOPMENT OF ORGANIC AGRICULTURE IN THE WORLD

Negative effects of synthetic agricultural inputs, forgotten with the concern to feed the growing world population, have been seen intensively in developed countries, and this situation has accelerated the search for an alternative system to the traditional farming method. Organic agriculture, which is carried out entirely by using natural resources and accepted as an environment friendly production system, emerged at the beginning of the 19th century, but it reached mass production level towards the end of the 1920s.

In Europe, on the other hand, the term organic farming started to be mentioned as of the 1930s. However, in this period, the growing world population also brought about the concerns about nutrition. At this point, the desire to meet the increasing demand for food made it necessary to use substances such as fertilizers, hormones and antibiotics in agricultural inputs. In the 1950s, the help provided reduced the importance attached to organic agriculture, supported the use of agricultural inputs, and this situation spread rapidly to every unit of production. While organic agriculture was defined as the only sector protecting the environment and natural balance in the 1960s, this feature started to be questioned in the 1970s. The studies, which continued separately until then, made it necessary to gather organic agriculture activities under one roof, to prepare the necessary regulations and to share all developments with its members and farmers in 1972. This situation was moved to different dimensions with the establishment of IFOAM in 1972 and FIBL in 1973. Both organizations publish the developments in organic agriculture in the world and related numerical data regularly every year (Er and Başalma, 2008: 28).

In the 1980s, on the other hand, we faced negativities where the natural balance was destroyed and the environment was irreversibly polluted. In the face of this situation, European countries have turned to alternative products that are environmentally sensitive, do not disturb the natural balance, do not leave toxic effects on humans and living things, and thus a producer- consumer chain has started to form (Merdan, 2014: 46).

Worldwide trade of organic products, began to develop with the 1980s. In this period, concerns caused by issues such as genetically modified products, mad cow disease and dioxin and the issue of sustainability brought a great increase in demand for organic products in the 1990s (Er and Basalma, 2008: 28; Yurudur et al., 2010: 45). Nowadays, as the negative effects of conventional agriculture begin to emerge, this process regarding organic agriculture has brought together producers and consumers who are sensitive to this issue in Europe, America and many northern countries. This process has increased the importance given to organic production and organic agriculture trade (Merdan, 2014: 46).

3. FACTORS AFFECTING THE DEVELOPMENT OF ORGANIC FARMING

Factors affecting the development of organic agriculture can be examined in four main groups (Jones, 2003: 18):

• **Increase in production in organic agriculture:** The interest in organic production is constantly increasing worldwide. The number of organic producers, which was 200 thousand in 1999, reached 2.8 million in 2018 (FIBL and IFOAM, 2020: 22). The increase in the number of organic producers positively affects the development of organic agriculture on a global scale.

• **Increasing consumer demand for organic products:** As the benefits of organic products are known, interest in organic products continues to increase, and this interest is reflected in other product types.

Especially in addition to organic agriculture and food products, organic textiles, health products, ecological hotels and restaurants and related markets are increasing. In terms of sustainable agriculture and development, national and international organizations, governments and producers make great efforts to popularize the organic food market and trade. In line with these statements, per capita consumption for organic products worldwide increased from 10.05 Euros in 2013 to 12.8 Euros in 2018. This finding clearly reveals the increasing demand for organic products.

- **Public interest in terms of sustainability:** The rapid increase in the world population increases the economic activities, and this situation also rapidly deteriorates the social natural environment**.** Organic agriculture offers important opportunities to producers as a sustainable agriculture system that includes a production system in harmony with the environment and adopts a great social responsibility principle.

- **Increasing market share and commercial issues related to the development of organic standards:** Organic global market share, which was 15.2 billion euros in 1999, reached 96.7 billion euros by 2018. As of 2018, the countries with the largest organic market in the world were the USA with 40.6 billion euros, Germany with 10.9 billion euros and France with 9.1 billion euros. The USA alone accounts for 42 percent of the organic food market (FIBL and IFOAM, 2020: 19). Concerns have been raised about cost and capability issues, particularly from developing countries to exporting countries, to meet the certification requirements of organic standards.

4. THE EFFECTIVE FACTORS IN THE ADOPTION OF ORGANIC AGRICULTURE IN THE WORLD

In many European countries and in the USA, the development of organic agriculture takes place with the lead of the farmers. The structure of organic agriculture starts from the producers and develops on the basis of supply and demand from bottom to top. In this case, the factors and motivation sources that play an effective role in farmers' adoption of organic agriculture can vary. In this regard, why farmers prefer organic culture has been a subject for many research. As a result of evaluations made, the factors that are effective in transition from traditional agriculture to organic agriculture can be classified under five categories:

The first factor is related with the deterioration in animal health and the aim to minimize the adversities experienced in the transition regarding classical agriculture such as soil erosion

The second is overcoming economic and financial problems and the ability of the farm to survive in the long term.

The third factor is related with the desire to avoid health problems that may emerge as a result of using certain harmful substances.

The fourth is about the effects of general sociopolitical, religious or environmental factors.

And finally, the fifth factor is associated with the encouragement from organic product sellers, family members, friends and encouragement from the social environment regarding the evaluation and promotion of organic agriculture (Padel, 1994; Padel and Lampkin, 1994; Demiryürek, 2000; Demiryürek, 2004: 64).

5. ORGANIC AGRICULTURE POTENTIAL IN THE WORLD

Organic agriculture activity first began in Europe and the USA, and spread to the whole world in time. The emergence of worrisome situations related to environment and health has been effective in the rapid spread of organic agriculture activity. The increase in the interest in organic agriculture and food products has naturally increased the number of farmer adopting organic agriculture. The continuous increase in this interest has also developed international trade. At this point, some countries which could not find domestic market and demand for organic products, have started to produce and export organic products that are not grown but demanded in Europe.

Statistical data on organic agriculture around the world are constantly changing. This situation is summarized in Table 1.

Table 1. Key İndicators For Organic Agriculture

İndicator	World	**Top Countries**
Countries with organic activities	2013: 170 countries 2018: 186 countries	
Organic agricultural land	(1999: 11 million hectares) 2018: 71.5 million hectares	Australia: 35.7 million hectares Argentina: 3.6 million hectares) China: 3.1 million hectares
Organic share of total agricultural land	2018: 1.5 %	Liechtenstein (%38.5 %) Samoa (34.5 %) Austria (%24.7 %)

Producers	1999: 200.000 2013: 2 Million 2018: 2.8 Million	İndia : 1.149.371 Uganda: 210.352 Ethiopia: 203. 602
Organic market	1999: 15.2 billion euros 2018: 96.7 billion euros	US: 40.6 billion euros Germany : 10.9 billion euros France: 9.1 billion euros
Per capita consumption	2013: 10.05 euros 2018: 12.8 euros	Switzerlan: 312 euros Denmark: 312 euros Sweden 231 euros
Number of countries with organic refulations	2013: 82 countries 2018: 103 countries	
Number of affiliates of IFOAM-Organics İnternational	2018: 779 affiliates from 110 countries	Germany: 79 affiliates İndia: 55 affiliates China: 45 affiliates

Source: FİBL survey 2020, www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The findings summarized in Table 1 reveal that the number of countries with organic agricultural land, agricultural land areas, per capita consumption, number of organic producers and market size have increased. It is seen that the number of countries with organic agriculture certificates, which was 170 in 2013, increased to 186 in 2018, and the organic farming area, which was 11 million hectares in 1999, to 71.5 million hectares in 2018. According to data belonging to year 2016, organic farming lands globally constitute 1% of total agricultural lands; whereas 6.7% of all the agricultural land is utilized as organic farming land in the EU. The share of organic farming land in the total agricultural land is 1.5 % as of 2018. While per capita consumption for organic agriculture was 10.05 euros in 2013, it increased to 12.8 euros in 2018. The number of organic producers, which was 200 thousand in 1999, reached 2 million in 2013 and 2.8 million in 2018. While the organic market size was 15.2 billion Euros in 1999, it increased to 96.7 billion Euros in 2018 (Table 1). The findings of 2018 are in the leading positions of Australia in terms of organic farmland, Liechtenstein in terms of the place of organic farmland in total agricultural land, India in terms of producers, USA in terms of organic market size and Switzerland in terms of per capita consumption reveals that it takes (Table 1).

Table 2. Organic Production Countries, Number of Producers, Export and Import Quantities (2018)

Countries	Number of Producers	Export	Imports
Afghanistan	10	5	-
Albania	52	30	4 (2012)
Australia	1.829	299	161
Argentina	1.366	99 (2016)	-
Brazil	17.508	7	-
Bulgaria (2017)	6.471	6	29
Canada	5.791	-	-
China (2016)	6.308	1.198	66
Czech Republic	4.601	165	303
Denmark (2017)	3.637	80	78
France	41.632	-	545
Finland	5.129	20	63
Germany	31.713	1.208	1.723
Greece	29.594	47	33
India	1.149.371	669 (2012)	-
Italy	69.317	962 (2016)	472
Japan	3.678	-	302
Pakistan	415	37	-
Russia	40	8	-
Turkey	79.563	97	51
Britain	3.544	1	182
Total:	2.796.916	8.846	6.582

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The findings presented in Table 2 give the organic production countries, the number of producers, import and export amounts. Considering the findings obtained, it is seen that the number of organic producers is the highest in India (1,149,371). Respectively after India Turkey (79 563) and Italy (69 317) comes. Table 2 according to most organic product exports performing countries include respectively Germany (1,208), China (1,198) and Italy (962) found that, up to the country's imports of s if Germany (1723), France (545) and Italy (472) is located.

As of 2018, organic agriculture practice is performed in 186 countries with 2.8 million farmers managing 71.5 million hectares of agricultural land. The highest number of organic farmers is seen in India with 1.149,371 farmers, in Uganda with 210,602 farmers, and in Ethiopia 2ith 203,602 farmers. The continents with the largest field of production are Oceania with 35.4 million hectares, which constitutes 50% of the global agriculture fields, and Europe with 15.6 million hectares, which corresponds to 22% of the global organic agriculture fields. These continents are respectively followed by Latin America with 8 million hectares, which is 11% of the global agriculture fields, Asia with 6.5 million hectares, which corresponds to 9% of the global agriculture fields, North America with 3.3 million hectares, which forms 5% of the global agriculture fields, and Africa with 2 million hectares, which constitutes 3% of the global agriculture fields (FIBL VE IFOAM, 2020).

Table 3. Ten Countries with the Largest Organic Farming Land (2018)

Countries	Organic Farm Area (Hectares)
Austria	35.69 million
Argentina	3.63 million
China	3.14 million
Spain	2.25 million
Uruguay	2.15 million
France	2.04 million
United States of America	2.02 million
Italy	1.96 million
Germany	1.52 million

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The country with the largest organic farming area is Austria (35.7 million). Then comes Argentina (3.63 million), China (3.14 million), Spain (2.25 million), Uruguay (2.15 million), France (2.04 million), USA (2.02 million), Italy (1.96 million) and Germany (1.52 million) (Table 3).

According to 2018 data, organic agricultural lands increased by 2.02 million hectares, and the total increase in organic agricultural lands was 2.9%. Organic farmlands show an increase of 1.25 million hectares in Europe, 0.54 million hectares in Asia, 4,000 hectares in Africa, 13000 hectares in Latin America, 0.1 million hectares in North America and 0 1 million hectares (FIBL and IFOAM, 2020: 21).

The introduction of new food and textile products to the world markets has an important effect on the increase of the market network of organic food products. This brings new and refined organic products, the much larger and processed food manufacturers and wholesalers of organic products to market. Apart from organic food and agricultural products, the number of organic textiles, health products, ecological hotels and restaurants are gradually increasing. Governments, Non-Governmental Organizations (NGOs), international organizations and other voluntary organizations make great efforts to develop and disseminate organic agriculture.

6. EVALUATION OF THE GLOBAL ORGANIC AGRICULTURE POTENTIAL IN TERMS OF CONTINENTS

When the world organic farming areas are evaluated in the continental axis, Oceania ranks first. The Oceania continent has 50% of the organic farming area. Europe comes second. The European continent also holds 22% of the organic farming areas.

Table 4. Organic Farming Areas and Shares of the Continents in Organic Farming Area in 2018

Regions	Organic Farming Areas (hectare)	Organic Farming Area Shares of Regions (%)
Oceania	35 9 99 373	50
Europe	15.635.505	22
Latin America	8.008.581	11
Asia	6.537.226	9
North America	3.335.002	5
Africa	2.003.976	3
Toplam	71.519.663	100

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The findings summarized in Table 4 reveal that half of the organic farming areas are owned by the Oceania continent. Oceania is followed by Europe (22%), Latin America (11%), Asia (9%) and North America (5%) (Table 4).

Table 5. Annual and 10-Year Change Rates of the Number of Organic Producers by Continents

Regions	Number of Organic Producers (2017)	Number of Organic Producers (2018)	1 Year Change (Number)	1-Year Change (%)	10- Year Change (Number)	10-Year Change (%)
Africa	806.887	788.858	-18.019	-2,2	+275.890	53,8
Asia	1.231.159	1.317.023	+85.864	7,0	+587.427	80,5
Europe	397.146	418.610	+21.464	5,4	+163.812	64,3

Latin America	460.443	227.609	-232.834	-50,6	-56.756	-20,0
North America	22.966	23.957	+991	4,3	+7.102	42,1
Oceania	26.750	20.859	-5.891	-22,0	+12.393	146,4
Total	2.945.341	2.796.916	-148.425	-5	+989.989	54,8

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The total number of organic producers by continent was 2,945,341 in 2017 and 2,796,916 in 2018. Although the number of organic producers decreased compared to the previous year, an increase of 54.8% was observed in the last 10 years. Between the years 2017-2018 the highest increase in the number of manufacturers was seen in Asia and between the years 2008-2018 it was seen in Oceania. The biggest decrease occurred in Latin America between 2008-2018 (Table 5).

In this section, organic agriculture potential is discussed as the continents of Asia, Africa, Europe, America and Oceania.

6. 1. The Asia Continent

In Asia in 2001 in a total of 420 thousand hectares of organic farmland grew about 15 times in 2018 condensing to run the 6.5 rose million hectares. The resulting value, forms 9% of the total world organic farming. China, with its 3.14 million hectares of organic agricultural land, is the most important country in Asia in recent years towards organic agriculture. The number of organic producers in the Asian continent, which was 100,000 in 2004, has reached 1,317,023 by 2018. During this period, the number of organic producers has increased more than 13 times. In terms of the number of organic producers, India (1,149,371) ranks first. Thailand (58.490) follows.

The Asian continent is considered to be the fourth largest organic market in the world. According to the data of 2018, organic market shares of China are 8.1 billion euros, Japan are 1.4 billion euros and South Korea are 330 million euros .

In the ranking of countries with the largest organic area in Asia, China ranks first with 3,135,000 hectares, India second with 1,938,221 hectares, and Indonesia third with 251,631 hectares (Table 6).

Table 6. Asia: Organic agricultural land, organic share of farmland, producers 2018

Country	Area [ha]	Organic Share [%]	Producers [no.]
Afghanistan	786	0.002%	10
Armenia	694	0.04%	35
Azerbaijan	37.630	0.8%	305
Bangladesh	504	0.01%	9.335
Bhutan	6.632	1.3%	4.354
Cambodia	27.550	0.5%	5.788
China	3.135.000	0.6%	6.308
Georgia	1.452	0.1%	1.075
India	1.938.221	1.1%	1.149.371
Indonesia	251.631	0.4%	18.162
İran (Islamic Republic of)	11.916	0.03%	20
Iraq	63	0.001%	
Israel	6.666	1.2%	349
Japan	10.792	0.2%	3.678
Jordan	1.446	0,1%	23
Kazakhstan	192.134	0.1%	63
Kuwait	22	0.01%	1
Kyrgyzstan	22.118	0.2%	1.107
Lao P.D.R.	*7.668*	*0.3%*	1.342
Lebanon	1.241	0.2%	111
Malaysia	9.576	0.1%	29
Mongolia	636	0.001%	13
Myanmar	12.305	0.1%	48
Nepal	11.851	0.3%	1.622
Oman	43	0.003%	5
Pakistan		0.2%	415
Palestine	4’870	1.6%	1.440
Philippines	218.570	1.8%	12.366
Republic of Korea	24.700	1.4%	15.500
Saudi Arabia	18.631	0.01%	6
Singapore	3	0.4%	
Sri Lanka	77.169	2.8%	1.416
Syrian Arab Republic	19.987	0.1%	2.458
Taiwan	8.759	1.1%	3.556
Tajikistan	8.806	0.2%	953
Hailand	95.066	0.4%	58.490
Timor-Leste	63.882	16.8%	4
United Arab Emirates	4.687	1.2%	95
Uzbekistan	943	0.004%	1
Viet Nam	237.693	2.2%	17.169
Total*	6.537.226	0.4%	1.317.023

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

*Total number includes data for countries with less than three operators.

The findings obtained in Table 5 reveal that the number of organic producers is the highest in India, and the share allocated to organic agriculture within the total agricultural area is in Timorland. According to the data of 2018 in the Asian continent, it was seen that the organic agriculture area reached 6,537,226 hectares and the number of producers reached 1,317,023 (Table 6).

Based on these findings, the demand for organic products continues to increase day by day in the Asian continent and this increase is focused on exports (FIBL and IFOAM, 2020).

6. 2. The African Continent

The African Continent is among the countries where the transition to organic agriculture will be the easiest since it has the lowest input cost. About three per cent of organic farmland of the world is on the African continent (2 million hectares). There are more than one million certified organic farmlands in Africa. Uganda has a large number of organic producers in the largest organic area. Organic products produced include coffee, olive, cocoa, oil, sesame, cotton, fruit and vegetables (www.organic-world.net). Although organic farming activity develops continuously, consumption of the local people remains at a very low level. It is possible to say that organic farming activities of the African continent show a steady increase from 2000 until 2018 (FIBL and IFOAM, 2020).

There are a few problems experienced regarding organic agriculture on the continent of Africa. Deterioration of soil, climate change, poverty and food insecurity have important effects in the emergence of this situation. In 2012, the Second Africa Organic Conference was organized in Zambia so as to eliminate these adversities experienced. With this conference, Africa organic network assumed an institutional structure, and with this structure, important discoveries were made in 2013. The Third East Africa Conference was held in July 2013 in Tanzania. About 200 representatives from 20 countries participated in the conference. When it is considered that East Africa is the leader of the continent in organic agriculture, such conferences can be seen as great opportunities for other African countries and stakeholders from the sub-regional sectors (www.organic-world.net).

6. 3. The European Continent

Globally, Europe has been a pioneer in organic agriculture since the 1990s. Europe has this leadership due to many reasons such as certification in organic products, high consumer demand and the legal process they follow in production. According to 2013 data in Europe, while ecological agriculture is carried out on an area of 11.5 million hectares, in 2018, agriculture is carried out on an area of 15.6 million hectares. The share of organic farming areas in total agricultural lands is 3.1%. Europe has more than 418,000 ecological producers. Turkey (79 563), Italy (69 317) and France (41 632) are the countries with the highest number of organic farmers. (FIBL and IFOAM, 2020). Germany, France and England are among the leading countries in retail sales. The leading countries in terms of consumption per capita, respectively; Switzerland (312 euro), Denmark (312 euro) and Sweden (231 euro).

Tablo 7. Europe and the European Union: Organic Retail Sales 2018: Key Data

	Retail sales [Million €]	Per capita consumption [€]	Growth 2017-2018 [%]	Growth 2009-2018 [%]
European Union	37.412	76.2	7.7%	121%
Europe	40.729	50.5	7.8%	125%

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

According to 2018 data, organic retail sales were 40.729 billion euros in Europe and 37.412 billion euros in the European Union. Per capita consumption is 50.5 Euros, while this rate is 76.2 Euros in the European Union. In Europe, the growth between 2017-2018 was 7.8%, and between 2009-2018, there was a growth above the European Union as 125% (Table 7).

Organic agriculture has shown quite a rapid development in European countries. The reason for this is that there is plenty of financial support and convenience provided to especially organic product producers. The subsidies provided to organic producers, providing adequate information to producers and consumers, product variety, national symbols, legal protection and planning activities are some of these supports and conveniences (Merdan, 2014: 58).

6. 4. The Continent of America

The organic market continues to grow in North America and reached 43.7 billion euros by 2018. The largest organic market in the world is North America. As of 2018, the organic market has grown 3.9 % in Canada and 1.4% in the USA. Consumption per capita was 125 euro in the USA and 84 euro in Canada. Here, the USA and Canada are the most important production locations and markets (FIBL and IFOAM, 2020: 287).

In North America, according to data from the 2018 3 .3 million the hectares of farmland to be organic are processed rack. Of these areas, 2,023,430 hectares are located in the USA, 1,311,572 hectares in Canada, and it develops very rapidly every year. According to the data of 1999, organic agricultural land, which was 0.73 million in North America, increased to 3.4 million in 2018 (FIBL and IFOAM, 2020: 288). The number of organic producers in North America, which was 11,000 in 2004, reached 23,957 in 2018 (Table 8). Based on these findings, organic farmland and the number of producers are constantly increasing in North America.

Table 8. Distribution of Organic Farm Land, Share of Organic Farm Land and Number of Producers in North America According to 2018 Data

Countries	Organic Farming Land (hectares)	Share of Organic Agriculture Land in Total Agricultural Land (%)	Number of Producers
Canada	1.311.572	2	5.791
USA	2.023.430	0.6	18.166
Total	3.335.002	0.8	23.957

Source: Canada Organic Trade Association and United States Department of Agriculture; FiBL survey 2020

Latin America has 8,008,581 hectares of organic farming land and the share of organic farming in the total agricultural land is 1.1 %. In Latin America the countries with the most organic farmland are Argentina (3629968) first, Uruguay (2147083) ranked second and Brazil (1188255) is the third. There are 227,609 organic producers in Latin America and the Caribbean, and producers mostly focus on the production of products such as coffee (248,000 hectares) and cacao (148,000 hectares). Among the leading countries in organic coffee production, Peru is ranked first with 121,000 hectares, Mexico is second with 44,000 hectares, and Nicaragua is third with 24,000 hectares (FIBL AND IFOAM, 2020: 270).

The organic market in the region is developing year by year and the public is more environmentally conscious and has more purchasing power. In addition, there are developments in the manufacturer and procurement process. The standardization and development of organic agriculture is supported by certain collaborators and farmer's markets in the region (IFOAM and FIBL, 2020).

6. 5. The Continent of Oceania

In the whole of the region which consists of Australia, New Zealand, Samoa, Fiji, Papua New Guinea, Vanuatu, Kiribati, New Caledonia, French Polynesia, Solomon Islands, Cook Islands, Niue and Tonga there are 20.860 organic farmers and 35,99 million hectares of organic agricultural land (Table 9). Annual organic consumption per person iss 49 euros.

Tablo 9. Oceania: Organic Agricultural Land, Organic Share Of Total Agricultural Land, and Number Of Producers 2018

Country	Area [ha]	Share of total agr. land [%]	Producer [no.]
Australia	35.687.799	8.8%	1.829
Samoa	97.656	34.5%	2.038
New Zealand	88.871	0.8%	876
Fiji	41.154	9.7%	67
Papua New Guinea	49.573	4.2%	12.742
Vanuatu	25.648	13.7%	47
Cook Islands	24	1.6%	58
French Polynesia	1.512	3.3%	13
Kiribati	1.600	4.7%	900
New Caledonia	94	0.1%	131
Solomon Islands	4.714	4.4%	1.098
Tonga	685	2.1%	1.060
Niue	43	0.9%	1
Total	35.999.373	8.6%	20.860

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

Organic products and uses in Oceania continent are provided on Table 10. According to 2018 data, the permanent areas used for agricultural products are 217,250 hectares. The products that are produced in Oceania densely are coconut, grains, coffee, permanent products, tropical fruits and medical and aromatic plants (Table 10).

Table 10. Oceania: Land Use İn Organic Agriculture 2018

Land use	Crop group	Area [ha]
Arable crops	Arable crops, other	94
	Cereals	41.293
	Fresh vegetables and melons	3.927
	Medicinal and aromatic plants	11.713
	Sugarcane	7
A rable crops total		57.034

Cropland, no details		41.827
Other agricultural land		999.692
Permanent crops	Cocoa	1.935
	Coconut	127.451
	Coffee	24.182
	Fruit	4.567
	Fruit, tropical and subtropical	20.412
	Grapes	7.503
	Medicinal and aromatic plants, permanent	10.650
	Tea/mate, etc.	487
	Permanent crops, other	20.063
Permanent crops total		217.250
Permanent grassland		34.683.571
Total		35.999.373

Source: FiBL survey 2020. www.fibl.org/fileadmin/documents/shop/1663-organic-world-2020.pdf.

The largest organic production in the Oceania continent takes place in Australia and New Zealand. In 2018 total organic market in Australia was 1.2 billion Euros. In the same period, New Zealand's organic market was 155 million Euros. While the organic production of Australia and New Zealand is mostly export-indexed, these products are spices, coconut and tropical fruits (FIBL and IFOAM, 2020).

In Australia, it is authorized by the 'Ministry of Agriculture, Fisheries and Forestry' in accordance with past practices for domestic standards and certification. Organic agriculture sector has become one of the best performing sectors of the economy in the last 5 years. The data obtained in 2018 has shown that, half of the profits from organic corporations are from organic animal husbandry. Although the organic agriculture sector receives limited government support, it continues to grow with donations, sponsorships and research projects and grants (FIBL and IFOAM, 2020).

On the Pacific islands such as Fiji, Papua New Guinea, Vanuatu and Tonga, products with organic certification are produced for export. Australia and New Zealand are seen as international main markets for exported organic products due to their proximity. North America, the European Union and Japan are among other markets. There are deficiencies in the domestic market for certified organic products. At this point, it is necessary to take action according to the 2013-2017 strategic plan, and the Pacific countries and ethical trade community should increase their sources in order to realize their activities.

CONCLUSION AND EVALUATION

In recent years, organic agriculture has spread not only in developed countries but also in developing countries. It can be claimed that this situation is a result of the increase in the importance attached to healthy food consumption and environmental protection among consumers. Depending on these developments, the trade volume of organic agriculture and food products is rapidly expanding especially in West Europe, North America and Oceania.

According to the FIBL report on 2020, the country with the biggest organic farmland was Austria (35.7 million) in the year 2018. Argentina (3.63 million) and China (3.14 million) follow. According to 2018 data, organic agricultural lands increased by 2.02 million hectares, and the total increase in organic agricultural lands was 2.9%. Organic farmland s showed an increase of 1.25 million hectares in Europe, 0.54 million hectares in Asia, 4000 hectares in Africa, 13000 hectares in Latin America, 0.1 million hectares in North America and 0,1 million hectares in Oceania.

Amon the continent with the largest organic production area with 35.4 million hectares, Oceania is the first. Europe follows with 15.6 million hectares. The largest number of organic producers are in India. India is followed by Turkey and Italy respectively.

The USA holds the organic product market with 40.6 billion Euros. The United States is followed by, Germany with 10.9 billion Euros and France with 9.1 billion Euros, respectively. Despite the large market share, organic product consumption in the USA is very low. However, the situation of the markets in France goes parallel with the consumers with a share of 89% consumption of organic products. The countries that export the most organic products are Germany (1.208), China (1.198) and Italy (962), while Germany (1723), France (545) and Italy (472) are among the countries with the highest import.

Recently, double digit growth figures have been observed in most of the important markets. Switzerland and Denmark made the highest expenditure per capita with 312 Euros. While the organic food expenditure per capita in the world countries was 10.05 Euros in 2013, it increased to 12.8 Euros in 2018. Countries with high organic food expenditure per capita can be expressed as countries with high welfare and conscious of organic consumption.

Organic agriculture is practiced on only 1-2% of the global agricultural lands at present. If wasting food and concentrate feed were reduced, a transition to organic agriculture on 60% of available agricultural lands would be possible. At this point, it is necessary to make the use of technology widespread and to increase innovative works. In this context, it is envisaged that with Organic 3.0, a healthy production can be achieved in 2050 in terms of both yield and sustainability.

Climate changes lead to a decrease in the yield in food products and serious economic losses. The importance of climate friendly organic production for the sustainability of food production becomes more evident at this point. Organic production attempts to put forth policies that consider ecological agriculture principles, waste and consumption culture, health conditions, fair distribution, climate changes, future generations, the main reasons of hunger problem, and accessibility to food. Organic agriculture which prioritizes natural entities such as air, water and soil and all living creatures, can be used as an effective tool to feed the world population in the future with the realization of these policies.

One of the most important problems of organic agriculture industry is the problem of trust in organic products. The process from production to consumption is certified, but more efforts should be made to gain the trust of the consumer.

Organic product consumption is directly related with the income status of individuals. As income increases, organic product consumption increases as well. Organic product consumption per capita is 1 dollar in Turkey, while it is 1000 dollars in Germany.

Those engaged in organic agriculture are getting older, and the interest of the youth in production is steadily decreasing. Directing the interest of the youth to the industry and conveying organic agriculture to the future generations, is important for the future of organic agriculture.

To sum up, the world organic agriculture has a great potential, but if this potential is not utilized with correct policies, this situation may create a big problem for the sustainability of the industry.

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