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Chapter 1

EFFECTS OF IRON FORMS ON IRON USE EFFICIENCY AND LEAF SPAD METER READINGS OF RICE CULTIVARS

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Introduction

Iron is an essential element for various cellular functions of the plants including chlorophyll synthesis, photosynthesis, respiration, DNA synthesis, protein synthesis and nitrate reduction into ammonia (Ishimaru et al., 2006; Kumar et al., 2013). It is also the primary component of cellular redox system like hemoprotein including cytochrome, catalase, peroxidase, Fe-S (ferredoxin), aconitase, super oxide dismutase enzymes (Marschner, 1995). Fe^{+2} and Fe^{+3} redox couple increase enzymatic redox reactions and thus play a great role in plant growth and development (Gill and Tuteja, 2010).

In oxidized soils, iron generally exists in ferric (Fe^{+3}) forms and bound as oxides and hydroxides. Besides, plant iron uptake is primarily realized in Fe^{+2} form (Lindsay and Schwab, 1982). Therefore, iron should be reduced to Fe^{+2} form to be available for plants. Otherwise, for Fe to be up taken and used by the plants, Fe^{+3} form should be transported by chelating agents of the roots. Therefore, there are two mechanisms developed against negativity in growing environment (Romheld and Marschner, 1986; Rogers and Guerinot, 2002; Epstein and Bloom, 2005). The first one of these mechanisms is reduction of rhizosphere pH following the release of protons (H^{+}) by the plant roots. Low pH levels in rhizosphere may reduce iron into soluble forms or reduce Fe^{+3} ion into Fe^{+2} form. Then, reduced form of iron is transported along the cell membrane with the aid of specific Fe^{+2} transport system of the plant. This type of mechanism is primarily encountered in dicotyledonous plants and herbaceous monocotyledons (Epstein and Bloom, 2005). The second mechanism is the release of phytosiderophores by the plant roots inducing iron uptake of the plants. These phytosiderophores form a complex with Fe^{+3} without reducing Fe^{+3} ion into Fe^{+2} ion and this Fe^{+3}-siderophore complex is then transported along the cell plasm membranes (Epstein and Bloom, 2005). Takagi et al. (1984) indicated that release of chelating compounds or siderophores is not specific to dicotyledonous plants, but specific to grass or herbaceous plants. Chelating compounds are characterized as amino acids non-existing in protein structure, mugineic acid and avenic acids.

Dos Santos et al. (2017) investigated iron absorption and transport in rice plants. Palmer and Guerinot (2009); Kobayashi and Nishizawa (2012); Bashir et al. (2013) indicated that in terms of iron absorption and transport, rice plants exhibited combined characteristics of Strategy-I and Strategy-II plants. As can be inferred from Figure 1, in Strategy-I plants, rice plants release H^{+} ions into rhizosphere ambient and dissolve insoluble form of Fe^{+3} and also reduce Fe^{+3} into Fe^{+2} and take it inside the cell with the aid of ferric reductase oxidase enzyme. On the other hand, in Strategy-II plants, rice plants release phytosiderophores from the roots and turn insoluble form of Fe^{+3} into soluble forms and then take it inside the cells. Therefore, previous researchers indicated that rice plants behaved like both the Strategy-I and the Strategy-II plants in terms of iron absorption and transport and these two strategies emerged in a combined fashion in rice plants.

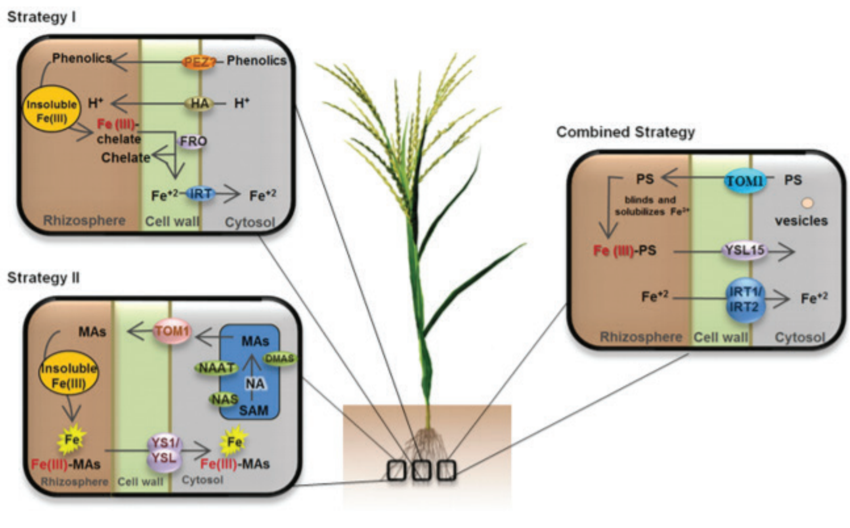

Figure 1. *Iron absorption and transport in rice plants (Palmer and Guerinot, 2009; Kobayashi and Nishizawa, 2012; Bashir et al., 2013)*

If the growing media is limy and media pH is over 7.2, then Fe-EDDHA should be applied. Stability of iron chelates in root growth media relies on media pH level or lime content and proper chelates should be selected for chelated to have positive contributions to plant iron nutrition. If the media pH or lime content of the media is not suitable for stability of the chelates, then it is impossible to increase iron level of soil solution. Fe-EDDHA is the most stable iron chelate especially in limy environments. Fe-EDDHA was reported to be more stable than Fe-DTPA and Fe-EDTA. The Fe-EDTA could be applied or remain stable when the media pH is 5.5 and 6.0. The Fe-EDTA chelate is not stable at high pH or limy environments. In such environments, calcium replaces the iron and plants could not get sufficient iron. Iron sulphate ($FeSO_4 \cdot 7H_2O$) is an inorganic iron salt. Iron sulphate solubility in soil or plant growth media relies on media pH and plant iron uptakes from this iron compounds are lower as compared to chelated iron compounds (Loué 1986; Lucena, 2006).

Chlorophyll meter (or SPAD meter) is a simple portable diagnosis device measuring greenness or relative chlorophyll content of the leaves (Inada, 1985, Kariya et al., 1982). During the last ten years, various optical techniques have been developed for instant and non-destructive measurement of chlorophyll content (Markwell et al., 1995, Richardson et al., 2002). These methods rely on absorbance or reflection of certain wavelengths of the light by the firm leaves of the plants and will in turn replaced the conventional chemical extraction methods. However, there is still a need for such chemical extraction methods as to serve bases for comparisons in assessment of performance of optical methods. On the other hand, although SPAD meter could be used to indicate relative chlorophyll content of the leaves, it could not indicate absolute chlorophyll content or concentrations of the leaves (Richardson et al., 2002).

Maruyama et al. (2005) indicated that despite the increasing Fe concentration of young rice leaves, SPAD meter readings decreased. Researchers indicated that rice plants accumulated Fe in old leaves; iron was efficiently distributed in young barley leaves; SPAD meter values of the second leaf of the rice plants continuously remained high.

Rong-li et al. (2012) indicated that chlorosis was encountered in young leaves of the rice plants 4 days later 0.1 mmol L^{-1} Fe-EDTA treatments. SPAD meter readings taken on 3rd day of the treatments were significantly lower in control plants as compared to iron-treated plants. After the 3rd day, SPAD values continuously decreased in control plants, but a decrease was not seen in iron-treated plants. Despite decreasing SPAD meter values under iron deficiency, in that study, iron deficiency did not influence shoot and root dry matter productions.

In present study, effects of different iron forms on iron use efficiency and SPAD meter readings of rice plants were investigated.

Material and Method

In present experiments, 5 different rice cultivars (Biga incisi, Osmancık-97, Hamzadere, Ronaldo and Edirne) were used.

Seed sterilization was achieved through keeping rice seeds in 5.0 % (v/v) sodium hypochlorite solution for 15 minutes. Seeds were then washed through deionized water and germinated in moist cloth bags. Germinated seeds were transferred to perlite-filled containers (40x25x5 cm dimensions) and grown there for 10 days to get rice seedlings. Seedlings of 5 different rice cultivars were transplanted into plastic pots filled with 1 kg of quartz sand as to have 10 seedlings in each pot.

Rice cultivars were supplied with $FeSO_4.7H_2O$, $FeSO_4.7H_2O$+EDTA, Fe (III) EDTA forms as to include 0 and 45 µM Fe. Totally 1460 mL nutrient solution was applied to plants until the end of the experiments. The pH of plant nutrient solution was adjusted to 5.5 with the use of diluted HCl or KOH solutions. Composition of plant nutrient solution (without iron) is provided below:

500 µM NH_4NO_3; 60 µM $NH_4H_2PO_4$; 230 µM K_2SO_4; 210 µM $CaCl_2$; 160 µM $MgSO_4 \cdot 7H_2O$; 2.5 µM $MnCl_2$; 0.75 µM $(NH_4)_6Mo_7O_{24}$; 3.2 µM H_3BO_3; 0.1 µM $CuSO_4$;

2.0 µM $ZnSO_4 \cdot 7H_2O$

Experiments were conducted in 5×4 factorial design with 3 replications and experiments lasted for 50 days.

At the end of the experiments, rice shoots were harvested. Harvested shoots were dried in an oven at 65 ºC and weighed. Then, rice shoots were ground in a grinder with stainless steel blades and made ready for analyses.

Total iron content of shoot samples was determined with the use of an atomic absorption spectrophotometer (AAS) device in accordance with Kacar and İnal (2008).

Iron use efficiency (%) of rice cultivars for different iron forms was calculated with the use of the following equation:

Iron use efficiency for different iron forms, % = $[(A-B) / C] \times 100$

where;

A = Shoot iron uptake of 45 µM Fe-applied cultivars, µg/pot

B = Shoot iron uptake of control (Fe 0) plants, µg/pot

C = Total iron quantity applied to the cultivars, µg/pot

Plant tolerance index to different iron forms was calculated with the use of the following equation:

Plant tolerance index to iron form = $(K / L) \times (M / N)$

where;

K = Shoot dry matter quantity of the cultivar in control treatment, g

L = Shoot dry matter quantity as the average of all cultivars in control treatment, g

M = Shoot dry matter quantity of iron form-applied cultivars, g

N = Shoot dry matter quantity as the average of all iron form-applied cultivars, g

Following the plantation of rice seedlings, control and iron-containing nutrient solutions were applied to plants. At the end of 40th day, SPAD meter readings were taken from the mid-point of fully developed leaves with a portable SPAD meter device (Konica Minolta SPAD-502 Plus).

Statistical analyses were conducted with the use of SPSS 17.0 software in accordance with 5×4 factorial design.

Results and Discussion

Effects of different iron forms on shoot dry matter content of rice cultivars and plant tolerance index values for different iron forms

Variance analysis results for the effects of 3 different iron forms on shoot dry matter quantities of rice cultivars are provided in Table 1.

Table 1. *Variance analysis results for the effects of 3 different iron forms on shoot dry matter quantities of rice cultivars*

Sources of Variation

Parameter	Iron Treatments		Cultivars		Iron treatment × cultivar interactions		Error	
	DF	**MS**	**DF**	**MS**	**DF**	**MS**	**DF**	**MS**
Shoot dry matter quantity, g/pot	3	1.55**	4	0.58**	12	0.14**	40	0.008

$^{**}p<0.01$; DF: Degree of Freedom; MS: Mean Squares

The effects of 3 different iron form treatments on shoot dry matter quantities of rice cultivars are provided in Table 2.

Table *2. Effects of 3 different iron form treatments on shoot dry matter quantities of rice cultivars*

Rice Cultivars	Shoot dry matter quantity, g DM / pot				
	Fe 0 (Control)	$FeSO_4{\cdot}7H_2O$ 45 µM Fe	$FeSO_4{\cdot}7H_2O$+EDTA 45 µM Fe	Fe III-EDTA 45 µM Fe	Average
Biga incisi	1.49h	2.55b	2.39bc	2.46b	2.22**A**
Osmancık-97	1.68fg	1.79ef	2.21d	2.17d	1.96**B**
Hamzadere	1.46h	1.57gh	2.16d	1.92e	1.78**C**
Ronaldo	1.47h	2.19d	2.15d	1.82ef	1.91**B**
Edirne	1.74f	2.29cd	2.43bc	2.74a	2.30**A**
Average	1.57**C**	2.08**B**	2.27**A**	2.22**A**	

There is no significant difference at 0.05 between means shown with the same letters

As can be inferred from Table 1, iron forms, cultivars and iron form × cultivar interactions had significant effects on shoot dry matter quantities of rice cultivars at $p<0.01$ level.

As compared to the control, iron treatments in different forms significantly increased shoot dry matter quantities of rice cultivars grown in sand media. As compared to the control, dry matter quantity increased by 44.58% with $FeSO_4 \cdot 7H_2O$+EDTA, 41.40% with FeIII-EDTA and 32.48% with $FeSO_4 \cdot 7H_2O$ treatments. Effects of EDTA-chelated Fe^{+2} or Fe^{+3} valance iron forms had similar effects on dry matter quantity; however, effects of chelating were greater as compared ton on-chelated $FeSO_4 \cdot 7H_2O$.

In terms of shoot dry matter quantities, rice cultivars were found to be significant different. The greatest dry matter quantity was obtained from Edirne cultivar and the lowest from Hamzadere cultivar. Based on average dry matter quantities, rice cultivars were ordered as Biga incisi ~ Edirne > Osmancık-97 > Ronaldo > Hamzadere (Table 2).

It was observed that effects of three different iron forms on dry matter quantities largely relied on rice cultivars (Figure 2).

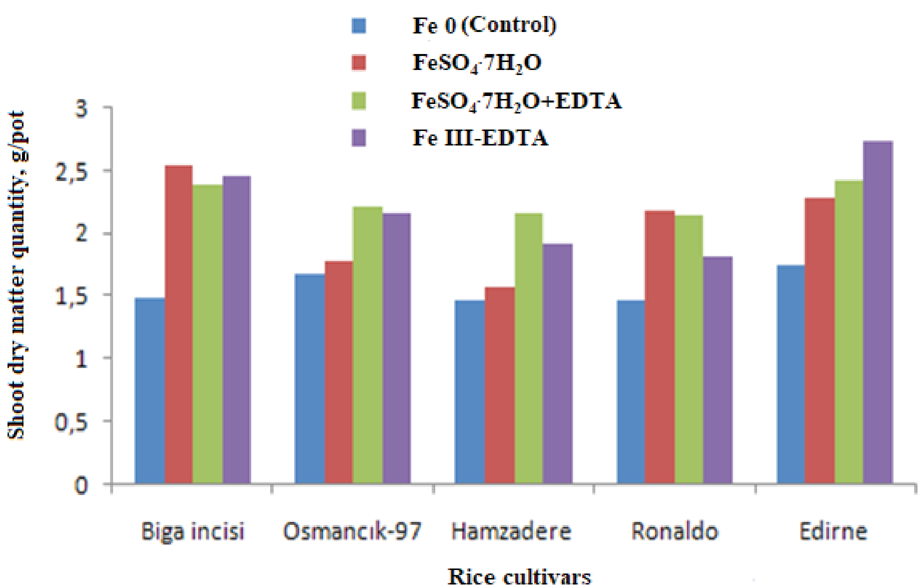

Figure 2. *Effects of three different iron forms on shoot dry matter quantities of rice cultivars*

Rate of increase achieved in shoot dry matter quantities with different iron sources as compared to control varied with the cultivars. Based on increase provided in shoot dry matter quantities, iron sources were ordered as $FeSO_4 \cdot 7H_2O$ > FeIII-EDTA > $FeSO_4 \cdot 7H_2O$+EDTA in Biga incisi cultivar; as $FeSO_4 \cdot 7H_2O$+EDTA ~ FeIII-EDTA > $FeSO_4 \cdot 7H_2O$ in Osmancık-97 cultivar; $FeSO_4 \cdot 7H_2O$+EDTA > FeIII-EDTA > $FeSO_4 \cdot 7H_2O$ in Hamzadere cultivar and as FeIII EDTA > $FeSO_4 \cdot 7H_2O$+EDTA > $FeSO_4 \cdot 7H_2O$ in Edirne cultivar. Present findings revealed that investigated rice cultivars were able to use ferrous (Fe^{+2}) and ferric (Fe^{+3}) iron.

In iron sulphate ($FeSO_4 \cdot 7H_2O$) treatments, the greatest rate of increase in shoot dry matter quantity (71.14%) was observed in Biga incisi cultivar and the lowest rate of increase (6.54%) was observed in Osmancık-97 cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Ronaldo > Edirne > Hamzadere ~ Osmancık-97.

In $FeSO_4 \cdot 7H_2O$+EDTA form treatment, the greatest rate of increase in shoot dry matter quantity (60.40%) was observed in Biga incisi cultivar and the lowest rate of increase (31.54 %) was observed in Osmancık-97 cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Hamzadere ~ Ronaldo > Edirne > Osmancık-97.

In FeIII-EDTA form treatment, as compared to the control, the greatest rate of increase in shoot dry matter quantity (65.10 %) was observed in Biga incisi cultivar and the lowest rate of increase (23.80 %) was observed in Ronaldo cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Edirne > Hamzadere ~ Osmancık-97 > Ronaldo.

In present study, in terms of shoot dry matter quantity, Biga incisi cultivar yielded the greatest response to 3 different iron forms.

Tolerance index values of rice cultivars to different iron forms as compared to the control are provided in Table 3. As can be inferred from Table 3, the rice cultivars best tolerating $FeSO_4 \cdot 7H_2O$ were identified as Edirne and Biga incisi cultivar; the cultivars best tolerating $FeSO_4 \cdot 7H_2O$+EDTA were identified as Edirne, Osmancık-97 and Biga incisi cultivars; the cultivars best tolerating Fe-III EDTA were identified as Edirne, Biga incisi and Osmancık-97 cultivars. On the other hand, the cultivar least tolerating $FeSO_4 \cdot 7H_2O$ was identified as Hamzadere cultivar; the cultivars least tolerating $FeSO_4 \cdot 7H_2O$+EDTA and Fe-III EDTA were identified as Hamzadere and Ronaldo cultivars.

In terms of tolerance index, iron forms were ordered as $FeSO_4 \cdot 7H_2O$ > Fe-III EDTA > $FeSO_4 \cdot 7H_2O$+EDTA in Biga incisi cultivar; as $FeSO_4 \cdot 7H_2O$+EDTA ~ Fe-III EDTA > $FeSO_4 \cdot 7H_2O$ in Osmancık-97 cultivar; as $FeSO_4 \cdot 7H_2O$+EDTA > Fe-III EDTA > $FeSO_4 \cdot 7H_2O$ in Hamzadere cultivar; as $FeSO_4 \cdot 7H_2O$ > $FeSO_4 \cdot 7H_2O$+EDTA > Fe-III EDTA in Ronaldo cultivar; as Fe-III EDTA > $FeSO_4 \cdot 7H_2O$ > $FeSO_4 \cdot 7H_2O$+EDTA in Edirne cultivar.

Table 3. *Tolerance index values of rice cultivars to iron forms as compared to control (Fe0)*

Rice cultivars	Iron tolerance index of rice cultivars		
	$FeSO_4.7H_2O$	$FeSO_4.7H_2O$+EDTA	Fe-III EDTA
Biga incisi	1.16	1.00	1.05
Osmancık-97	0.92	1.04	1.04
Hamzadere	0.70	0.88	0.80
Ronaldo	0.98	0.88	0.76
Edirne	1.22	1.18	1.36

Fageria et al. (2008) classified rice cultivars based on tolerance index values to irons source or iron dose as: tolerant for index value of > 1.0; moderately tolerant for index values of between 0.5-1.0; sensitive for index values of < 0.5. Based on this classification, Edirne and Biga incisi cultivars were highly tolerant to $FeSO_4 \cdot 7H_2O$; Edirne, Osmancık-97 and Biga incisi cultivars were tolerant to $FeSO_4 \cdot 7H_2O$+EDTA; Edirne, Biga incisi and Osmancık-97 cultivars were tolerant to Fe-III EDTA.

Shoot iron ratio coming from iron fertilizers

Variance analysis results for the effects of iron forms on shoot iron ratio coming from iron fertilizers are provided in Table 4.

Table 4. *Variance analysis results for the effects of iron forms on shoot iron ratio coming from iron fertilizers*

Sources of Variation							
Cultivar		**Iron Form**		**Cultivar × Iron Form Interaction**		**Error**	
DF	MS	DF	MS	DF	MS	DF	MS
4	303.32	2	407.27	8	65.96	30	147.85

DF: Degree of Freedom; MS: Mean Squares

The effects of iron forms on shoot iron ratio coming from iron fertilizers in rice cultivars are provided in Table 5.

Table 5. *Effects of iron forms on shoot iron ratio coming from iron fertilizers*

Rice Cultivars	Shoot Fe ratio coming from different iron sources, %			
	$FeSO_4 \cdot 7H_2O$ 45 µM Fe	$FeSO_4.7H_2O$+EDTA 45 µM Fe	Fe III-EDTA 45 µM Fe	Average
Biga incisi	70.8	81.5	73.6	75.3
Osmancık-97	59.2	75.3	64.4	66.3
Hamzadere	56.5	65.0	67.6	63.0
Ronaldo	75.0	84.3	67.9	75.7
Edirne	68.4	75.6	77.6	73.9
Average	66.0	76.3	70.2	

Shoot iron ratios coming from iron fertilizers were not significantly influenced by rice cultivars, iron forms and cultivar × iron form interactions. The greatest ratio was obtained from $FeSO_4 \cdot 7H_2O$+EDTA and the lowest ratio was obtained from $FeSO_4 \cdot 7H_2O$ treatments. Despite insignificant differences in shoot iron ratios of the rice cultivars coming from the iron fertilizers, the greatest ratio was observed in Ronaldo and the lowest ratio was observed in Hamzadere cultivar.

Iron fertilizer use efficiency

Results of variance analysis for the effects of iron forms on iron fertilizer use efficiency of the rice cultivars are provided in Table 6.

Table 6. *Results of variance analysis for the effects of iron forms on iron fertilizer use efficiency of the rice cultivars*

Sources of Variation							
Cultivars		**Iron Form**		**Cultivar × Iron Form Interaction**		**Error**	
DF	MS	DF	MS	DF	MS	DF	MS
4	85.81	2	251.54**	8	17.32	30	36.81

***p<0.01; DF: Degree of Freedom; MS: Mean Squares*

The effects of iron forms on iron fertilizer use efficiency of rice cultivars are provided in Table 7.

Table 7. *Effects of iron forms on iron fertilizer use efficiency of rice cultivars*

Rice Cultivars	Iron use efficiency, %			
	$FeSO_4 \cdot 7H_2O$ 45 µM Fe	$FeSO_4 \cdot 7H_2O$ + EDTA 45 µM Fe	Fe III-EDTA 45 µM Fe	Average
Biga incisi	8.3	16.0	10.8	11.7
Osmancık-97	7.0	16.5	8.0	10.5
Hamzadere	4.6	7.4	8.4	6.8
Ronaldo	9.8	17.9	7.9	11.87
Edirne	10.1	21.4	14.8	15.43
Average	8.01**B**	15.89**A**	10.01**B**	

There is no significant difference at 0.05 between means shown with the same letters

As can be inferred from Table 6, iron forms had significant effects on iron fertilizer use efficiency at $p < 0.01$ level, but the effects of rice cultivars and cultivar × iron form interactions on iron use efficiency were not found to be significant.

In terms of iron use efficiency, iron forms were found to be significantly different. Based on iron use efficiencies, iron forms were ordered as $FeSO_4 \cdot 7H_2O$+EDTA > Fe III-EDTA ~ $FeSO_4 \cdot 7H_2O$. When the $FeSO_4 \cdot 7H_2O$+EDTA and Fe III-EDTA iron forms were compared, it was observed that rice cultivars were able to use +2 and +3 valance iron. Rice cultivars were able to better use +2 valance $FeSO_4 \cdot 7H_2O$+EDTA as compared to Fe III-EDTA. Considering the general averages, it was observed that iron use efficiency was identified as 8.01% for $FeSO_4 \cdot 7H_2O$; 15.89% for $FeSO_4 \cdot 7H_2O$ + EDTA and 10.01% for Fe III-EDTA.

In terms of iron use efficiency, although the differences between the cultivars were not found to be significant, the greatest iron use efficiency was observed in Edirne rice cultivar.

Besides Fe^{+3}/phytosiderophore complex, rice plants could also take +2 valance iron from the rhizosphere (Ishimaru et al., 2006). As a result of low solubility of oxide ferric form under aerobic conditions, iron turns into the most limiting nutrient for plant growth and development, then it is absorbed as Fe^{+3}/phytosiderophore (Zuo and Zhang, 2011; Samaranayake et al., 2012).

Fe $(OH)_3$ form of iron is dissolved by phytosiderophores of muguneic acid (MA) family as to form Fe (III)-MA complex. The Fe (III)-MA complex is then transported from plasma membrane of root cells through a transporter of Yellow Strip (YS) family (Inoue et al., 2009). The YS Fe transporter was the first isolated from maize and called as YS1 (Curie et al., 2001).

In cells of rice plants, iron could move in Fe-citrate, Fe^{+2}-nicotinamine (NA) and Fe^{+3} - 2'-deoximugineic acid (DMA) forms (Koike et al., 2004). These complexes could easily be taken by specific receptors allowing passage of iron from one cellular component of another (Bashir et al., 2006).

Effects of different iron forms on leaf SPAD meter readings of rice cultivars

The results of variance analysis for the effects of different iron forms on leaf SPAD meter readings of rice cultivars are provided in Table 8.

Table 8. *Results of variance analysis for the effects of different iron forms on leaf SPAD meter readings of rice cultivars*

Sources of variation							
Fe form		**Cultivar**		**Iron form × cultivar interaction**		**Error**	
DF	MS	DF	MS	DF	MS	DF	MS
3	2308.8**	4	25.30**	12	11.500**	40	2.460

$**p<0.01$; $*p<0.05$; DF: Degree of Freedom; MS: Mean Squares

The effects of different iron forms on leaf SPAD meter readings of rice cultivars are provided in Table 9.

Table 9. *Effects of different iron forms on leaf SPAD meter readings of rice cultivars*

Rice cultivars	Leaf SPAD meter readings Fe 0 (Control)	$FeSO_4 \cdot 7H_2O$ 45 µM Fe	$FeSO_4 \cdot 7H_2O$ + EDTA 45 µM Fe	Fe III-EDTA 45 µM Fe	Average
Biga incisi	9.9f	38.55a	35.28bc	37.60a	30.58**C**
Osmancık-97	15.58e	37.75ab	39.75a	38.67a	32.95**B**
Hamzadere	10.47f	38.80a	38.82a	38.17a	31.57**C**
Ronaldo	18.50d	39.12a	38.98a	40.48a	34.27**A**
Edirne	13.40e	34.77c	37.85ab	39.78a	31.44**C**
Average	13.57**C**	37.80**B**	38.14**AB**	39.13**A**	

There is no significant difference at 0.05 between means shown with the same letters

As can be inferred from Table 8, iron forms, cultivars and iron form × cultivar interactions had significant effects on leaf SPAD meter readings of rice cultivars at $p<0.01$ level.

As compared to the control treatment, different iron forms significantly increased leaf SPAD meter readings. Again, as compared to the control treatment, 178.55% increase was achieved in SPAD readings with $FeSO_4.7H_2O$ treatments; 181.06% with $FeSO_4{\cdot}7H_2O$ + EDTA treatments; 188.35% with Fe III-EDTA treatments.

In terms of SPAD-increasing effects, iron forms were ordered as Fe-III EDTA > $FeSO_4{\cdot}7H_2O$+EDTA > $FeSO_4{\cdot}7H_2O$. EDTA (Ethylene diamine tetra acetic acid) chelating of iron element improved the efficiency of iron in chlorophyll synthesis. In terms of leaf SPAD meter readings, rice cultivars were ordered as Ronaldo > Osmancık-97 > Hamzadere > Edirne > Biga incisi.

Effects of different iron forms on leaf SPAD meter readings varied with the rice cultivars (Figure 3).

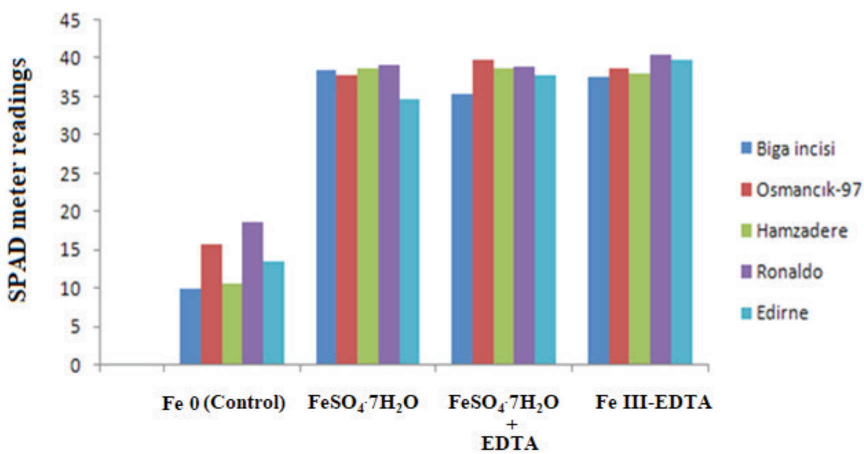

Figure 3. *Effects of different iron forms on SPAD meter readings of rice cultivars*

The SPAD meter readings of the rice cultivars were lower under Fe deficiency conditions (Fe0) than under different iron forms. In terms of SPAD readings under iron-deficit conditions, present cultivars were ordered as Ronaldo > Osmancık-97 > Edirne > Hamzadere > Biga incisi.

In $FeSO_4{\cdot}7H_2O$ treatments, SPAD meter readings of Edirne cultivar were significantly different from the SPAD meter readings of the other 4 cultivars; but Biga incisi, Osmancık-97, Hamzadere and Ronaldo cultivars had similar and close SPAD meter readings. In this iron form, the greatest SPAD meter reading was observed in Ronaldo cultivar and the lowest reading value was observed in Edirne cultivar.

In $FeSO_4{\cdot}7H_2O$+EDTA treatments, SPAD meter readings of Biga incisi, Osmancık-97, Hamzadere and Ronaldo cultivars were different, but Edirne and Biga incisi cultivars had similar values. In terms of SPAD meter readings in this iron form, Osmancık-97, Hamzadere, Ronaldo and Edirne cultivars had also similar values. In this iron form, the greatest SPAD meter reading was observed in Osmancık-97 cultivar and lowest value was observed in Biga incisi cultivar.

In Fe-III EDTA treatments, SPAD meter readings of all cultivars were similar and close to each other. Maruyama et al. (2005), despite increasing Fe concentrations in Fe concentrations of young leaves, reported decreasing SPAD meter readings. Researchers indicated that rice plants accumulated Fe in old leaves; iron was efficiently distributed in young barley leaves; SPAD meter values of the second leaf of the rice plants continuously remained high.

Rong-li et al. (2012) indicated that chlorosis was encountered in young leaves of the rice plants 4 days later 0.1 mmol L^{-1} Fe-EDTA treatments. SPAD meter readings taken on 3rd day of the treatments were significantly lower in control plants as compared to iron-treated plants. After the 3rd day, SPAD values continuously decreased in control plants, but a decrease was not seen in iron-treated plants. Despite decreasing SPAD meter values under iron deficiency, in that study, iron deficiency did not influence shoot and root dry matter productions.

Conclusion

As compared to the control, dry matter quantity increased by 44.58% with $FeSO_4{\cdot}7H_2O$+EDTA, 41.40% with FeIII-EDTA and 32.48% with $FeSO_4{\cdot}7H_2O$ treatments. Effects of EDTA-chelated Fe^{+2} or Fe^{+3} valance iron forms had similar effects on dry matter quantity; however, effects of chelating was greater as compared to non-chelated $FeSO_4{\cdot}7H_2O$. Based on average dry matter quantities, rice cultivars were ordered as Biga incisi ~ Edirne > Osmancık-97 > Ronaldo > Hamzadere.

In iron sulphate ($FeSO_4{\cdot}7H_2O$) treatments, as compared to the control, the greatest rate of increase in shoot dry matter quantity (71.14%) was observed in Biga incisi cultivar and the lowest rate of increase (6.54%) was observed in Osmancık-97 cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Ronaldo > Edirne > Hamzadere ~ Osmancık-97.

In $FeSO_4.7H_2O$+EDTA form treatment, as compared to the control, the greatest rate of increase in shoot dry matter quantity (60.40%) was observed in Biga incisi cultivar and the lowest rate of increase (31.54%) was observed in Osmancık-97 cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Hamzadere ~ Ronaldo > Edirne > Osmancık-97.

In FeIII-EDTA treatments, as compared to the control, the greatest rate of increase in shoot dry matter quantity (65.10%) was observed in Biga incisi cultivar and the lowest rate of increase (23.80%) was observed in Ronaldo cultivar. In terms of rate of increase in shoot dry matter quantities, with this iron treatment, rice cultivars were ordered as Biga incisi > Edirne > Hamzadere ~ Osmancık-97 > Ronaldo. In present study, Biga incisi cultivar had the greatest shoot dry matter quantity in all 3 iron forms.

According to present assessments, Edirne and Biga incisi cultivars were highly tolerant to $FeSO_4{\cdot}7H_2O$; Edirne, Osmancık-97 and Biga incisi cultivars were tolerant to $FeSO_4{\cdot}7H_2O$+EDTA; Edirne, Biga incisi and Osmancık-97 cultivars were tolerant to Fe-III EDTA.

Considering the general averages for iron ratios coming from iron fertilizer, the greatest ratio was obtained from $FeSO_4{\cdot}7H_2O$ + EDTA and the lowest ratio was obtained from $FeSO_4{\cdot}7H_2O$ treatments. Despite insignificant differences in shoot iron ratios of the rice cultivars coming from the iron fertilizers, the greatest ratio was observed in Ronaldo and the lowest ratio was observed in Hamzadere cultivar.

In terms of iron use efficiency, iron forms were found to be significantly different. Based on iron use efficiencies, iron forms were ordered as $FeSO_4{\cdot}7H_2O$+EDTA > Fe III-EDTA ~ $FeSO_4{\cdot}7H_2O$. When the $FeSO_4{\cdot}7H_2O$+EDTA and Fe III-EDTA iron forms were compared, it was observed that rice cultivars were able to use +2 and +3 valance iron. Rice cultivars were able to better use +2 valance $FeSO_4{\cdot}7H_2O$ + EDTA as compared to Fe III-EDTA. Considering the general averages, it was observed that iron use efficiency was identified as 8.01% for $FeSO_4{\cdot}7H_2O$; 15.89% for $FeSO_4{\cdot}7H_2O$ + EDTA and 10.01% for Fe III-EDTA.

As compared to the control treatment, different iron forms significantly increased leaf SPAD meter readings. Again, as compared to the control treatment, 178.55% increase was achieved in SPAD readings with $FeSO_4{\cdot}7H_2O$ treatments; 181.06% with $FeSO_4{\cdot}7H_2O$ + EDTA treatments; 188.35% with Fe III-EDTA treatments.

In terms of SPAD-increasing effects, iron forms were ordered as Fe-III EDTA > $FeSO_4{\cdot}7H_2O$+EDTA > $FeSO_4{\cdot}7H_2O$. EDTA (Ethylene diamine tetra acetic acid) chelating of iron element improved the efficiency of iron in chlorophyll synthesis. Effects of different iron form on leaf SPAD meter readings varied with the cultivar.

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Chapter 2

A STUDY ON THE DETERMINATION OF SOME PHYSICAL AND PHYSIOLOGICAL PROPERTIES OF NETTLE SEEDS (*URTICA DIOICA* L.)

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1. INTRODUCTION

Plants included in the *Urticaceae* family, which has 48 genera and 1050 species in the world, are evaluated by many sectors (Güneş et al., 2019). These plants, which can be propagated by seed or cuttings, generally show a natural spread (Baytop, 1963). Turkey has 5 types although there is the most common type of *U. dioica* (Arslan et al.,2015; Deveci et al., 2016). Nettle is a herbaceous perennial plant with a herbaceous structure found in large clusters. Nettle plants that can grow to 40-150 cm in length which in Turkey also known by names "*dızlağan, çızlağan,cızgan, dalagan, cınçar, ağdalak, ısırgı*" (Baytop,1999). Contains many chemical compounds in its burning hair (Ayan et al., 2006). It is stated that this property is due to formic acid, histamine, serotonin and choline. In addition, it has been determined that the leaves of the plant are rich in lecithin, carotenoids, flavonoids, sterols, tannins and vitamins and minerals (Taylor, 2005). Also contained 2.5% dry matter and 18% fats, the seed was found to be approximately 8-10% in the fixed oil (Ayan et al., 2006). Nettle has been evaluated in the textile industry in Europe in the 19th century due to the advantages of its fiber properties (Vogl & Hartl, 2003). It is used extensively as a dye, in cosmetic products such as supplementary food supplements, shampoo, and in the pharmaceutical industry. there are studies showing that nettle is analgesic and pain reliever (Yongna et al., 2005), antimicrobial (Gülçin et al., 2004), antibacterial (Aksu & Kaya, 2004), antidiabetic (Farzami et al., 2003), cardiovascular (Testai et al., 2002), it has diuretic (Tahri et al., 2000) and antirheumatic (Riehemann et al., 1999) effects. Although it is not a soil choice in general, it has been determined that nettle, which loves slightly alkaline (Lieres, 1995) soils, can grow in mild climate conditions. It is recommended for producers as an alternation plant for the utilization of soils under the threat of erosion, empty and bare lands.

2. MATERIAL AND METHOD

This study was carried out in the laboratories of Ege University Faculty of Agriculture Agricultural Machinery and Technologies Engineering and Bingöl University Faculty of Agriculture Biosystem Engineering departments in 2019-2020. The seeds were randomly sampled according to the randomized plot design and some physical (shape-size, surface area, average arithmetic-geometric diameter, sphericity and weight of a thousand grain) and physiological (germination rate, time, shoot-root length) belonging to these seeds. The values obtained were evaluated according to the basic statistical parameters (minimum-average-maximum-standard deviation). (Dumanoğlu, 2020(b)).

Each seed has its own basic characteristics (length (mm), width (mm) and thickness (mm). The characteristics of the seeds vary depending on the climate differences in which the plants are grown, soil properties (pH, salinity, drought, etc.) and genotype differences (Dumanoğlu, 2020(a)). The geometric (long-medium-short) and shape (round-oval-long) characteristics of the seeds are classified according to the basic parameters determined based on the researches (Yağcıoğlu, 2015) (Table 1). In addition, by using these basic properties, other data such as arithmetic and geometric diameter (mm), sphericity of the seeds can be determined with the help of the equations stated below (Alayunt, 2000; Kara, 2012; Mohsenin, 1970). By using all these values, it is ensured that the seeds are left to the seed bed with the least loss. With the selection of the appropriate planter and planting machine, a successful sowing process can be realized, and in the product processing stage, the seeds are classified and separated and the packaging processes are completed.

In this study; 100 seeds were selected from among the nettle seeds (*Urtica dioica* L.) randomly sampled and the basic characteristics of the seeds were determined with a stereo microscope (Nexius Zoom brand) with its own software (Image Focus 4.0 v2.4) (Dumanoğlu & Geren, 2020). In addition, 1000 seed counts of the seeds were made in triplicate and the weighing process was completed with Radwag AS 220.R2 analytical balance (to 0.0001 g precision) (ISTA, 2007; Dumanoğlu & Mokhtarzadeh, 2020).

Table 1. Classification of seeds according to their geometric and shape features

Seeds according to their geometric features	**Grain width / Grain length (b/a) (mm)**	**Seeds according to shape characteristics**	**Length (a), Width (b), Thickness (c) (mm)**
Long	0,6	Round	$a \approx b \approx c$
Middle	0,6 – 0,7	Oval	$a/3 < b \approx c$
Short	> 0,7	Long	$c < b < a/3$

$$D: (L + W)/2 \tag{1}$$

D: Average arithmetic diameter of the seed (mm)

L: Seed length value (mm)

W: Seed width value (mm)

$$Do: (L * D^2)^1/3 \tag{2}$$

D_0: Average geometric diameter of the seed (mm)

L: Seed length value (mm)

D: Average arithmetic diameter of the seed (mm)

$$\Phi : Do / L \qquad (3)$$

Φ : Sphericity Value of the Seed

Do : Average geometric diameter of the (mm)

L : Seed length value (mm)

Depending on the time and storage conditions of the seeds, there may be changes in their germination abilities (Ceylan, 1997). For this reason, it is necessary to take samples and germinate from existing seeds before each production period. Thus, the producer can make an estimate of the yield to be obtained during the production period. Of course, the production conditions are one of the factors that directly affect the yield, but one of the most important production inputs, the high germination ability and the health of the seed is among the requirements of high quality and standard production. In this study, nettle seeds (*Urtica dioica* L.) were germinated in glass plates under controlled conditions in laboratory conditions at a temperature of approximately 20-30 ° C and 70% humidity in four repetitions in accordance with ISTA (2007) rules. The germination time (day) and rate (%) of the seeds were determined. In addition, 100 plants were randomly sampled, with 25 plants in each variety, and their shoot-root lengths (cm) were measured.

3. RESULTS AND DISCUSSION

According to the physical characteristics of the nettle seeds (*Urtica dioica* L.), the average length of the seeds is 1.462 mm; It was measured with a microscope that it has a width of 1.088 mm and a surface area of 1.204 mm^2. According to the data obtained here, it was calculated that the seeds had an average arithmetic diameter of 1.274 m, a geometric diameter of 0.579 mm and a sphericity value of 0.396. (Table 2). Depending on these values, it was determined that the stinging nettle seeds examined in the study had a short and oval seed structure.

Table 2. Some physical properties of nettle seeds

Seed Features	Min.	Max.	Aveg.	Stdv
Length (mm)	1.231	1.670	1.461	0.086
Width (mm)	0.965	1.264	1.088	0.069
Surface area (mm^2)	0.968	1.525	1.204	0.114
Sphericity of the seed	0.310	0.533	0.396	0.051
Average geometric diameter of the seed (mm)	0.398	0.827	0.579	0.086
Average arithmetic diameter of the seed (mm)	1.108	1.422	1.274	0.059
Thousand grain weight (g)	2.510	2.600	2.560	0.047

In this study, a thousand grain weight of nettle seeds was determined as 2.600g, while Ayan et al. (2006) stated the weight of thousand grains as 0.14 g in their research (Table 2). When we examine the physiological properties of nettle seeds (*Urtica dioica* L.); It was determined that the seeds had a 77.0% germination percentage, germinated within 5.5 days and the average shoot length was 1.61 cm and the average root length was 3.01 cm (Table 3).

Table 3. Some physiological characteristics of nettle seed

Seed Features	Nettle (*Urtica dioica*)
Germination Percentage (%)	77.0
Average Germination Time (day)	5.457
Average shoot length (cm)	1.61
Average root length (cm)	3.01

In this study, some physical and physiological properties of nettle seeds (*Urtica dioica* L.), which are used extensively by many sectors, were determined. It is aimed that the results obtained will constitute the infrastructure for the improvement studies to be carried out and increase the production possibilities with mechanization.

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Chapter 3

IMPORTANT SPECIES OF TURKEY, MUSSELS (FAMILY: MYTILIDAE): CAPTURE, AQUACULTURE AND TRADE

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INTRODUCTION

Mussels are a member of Bivalvia classes of mollusk that has been produced for many years. It is consumed with pleasure by many countries of the world, especially in the Far East and European countries. Over the years, due to the high consumption, aquaculture has replaced capturing. In recent years, the share of world mussel aquaculture in total mussel production is more than 95%. The number of facilities that mussel farming has been increasing in recent years in Turkey and mussels have taken their place among the important cultivated species. Turkey mussel aquaculture constitutes 1.12% the total aquaculture in 2019. The mussels of exports in Turkey are expected to increment with the increase of the sustainable capture and aquaculture of mussel.

1.Mussel Production Over the World

A considerable amount of food need has been provided from fisheries resources. The most important species belong to fish, crustacean, macro algae and mollusks. Mollusks compose 11% of the world fisheries production. Mussel is one of the most demanded species which can be cultured. Mussel aquaculture accounts for 2% of the world cultivation amount. Mussel cultivation and fishery have been performed in the world and Turkey for many years. World mussel aquaculture includes much higher data compared to mussel fishery. The total mussel production of the world in 2018 was 2196796 tons and 2112638 tons of this amount belonged to aquaculture (Figure 1). The world mussel culture value in 2018 was approximately 4.5 billion dollars (FAO, 2018).

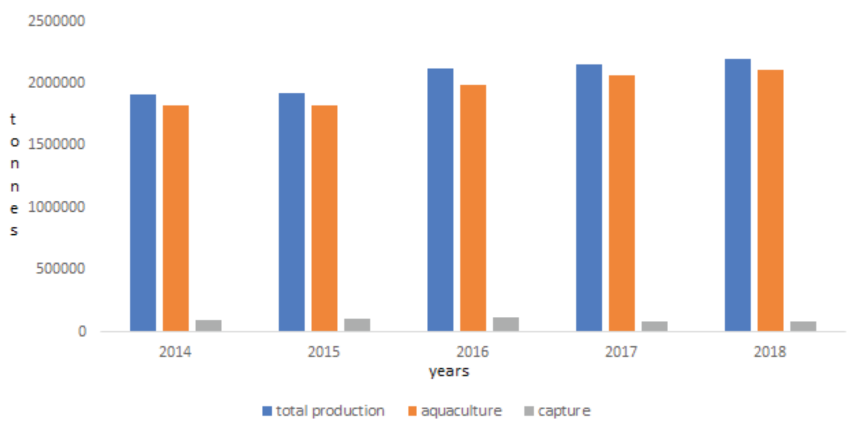

Figure 1. World Mussel Production (t) (FAO, 2018)

In addition to being a very delicious food, mussel is rich in nutrient content, making it one of the most preferred seafood by the world countries. Khan and Liu (2019) reported that daily mollusks consumption strengthens the immune system and protects against many diseases. High protein content and richness in vitamins and minerals (Anacleto et al., 2016; Khan and Liu 2019), high antiviral activity (Dupuy et al., 2004; Luk'yanov et al.,2007; Roch et al. 2004; 2008), rich omega-3 and omega-6 fatty acids composition (Cherifi et al., 2018; Dernekbasi et al., 2015) of the mussel meat were reported in previous studies.

World mussel aquaculture accounts for 95% of world mussel production. The three countries with the most cultivation are China, Chile, and Spain (Table 1). Forty-four countries carried out mussel aquaculture in 2018 and Turkey took the 32nd place.

Table 1. Countries with the Most Mussel Culture in the World and Their Cultivation Amounts (t) (FAO, 2018)

Countries	2014	202 2015	2016	2017	2018
China	769.919	809.415	862.829	927.609	903.361
Chili	240.821	211.356	302.344	341.427	368.916
Spain	769.919	809.415	862.829	927.609	903.361
New Zealand	240.821	211.356	302.344	341.427	368.916
Italy	220.449	225.308	215.855	241.785	283.801
France	97.483	76.811	94.037	99.716	86.176
North Korea	63.700	63.700	63.700	63.700	62.035
Netherland	57.633	56.802	57.149	57.148	57.148
Thailand	51.463	53.536	54.642	76.161	49.500
Indonesia	0	0	0	0	38.120

Asia is the leading continent in mussel aquaculture with 1.1 million tons and it is followed by Europe (532000 t) and America (411000) respectively. The most cultivated species are given in Table 2. While the Mediterranean mussel is at the fourth place among the cultivated species, it is at the top in Turkey in terms of production and consumption amounts.

Table 2. The Five Most Captured Mussel Species in the World (t) (FAO, 2018)

Species	**Scientific Name**	**2018**
Chilean Mussel	Mytilus chilensis	365 595
Blue Mussel	Mytilus edulis	159 466
Green Mussel	Perna viridis	124 184
Mediterranean Mussel	Mytilus galloprovincialis	103 011
New Zealand Mussel	Perna canaliculus	86 176

European countries especially put an emphasis on mussel culture. The amount of mussel aquaculture in the European Union (EU) is almost 513000 t. The most cultivated mussel species in the EU are blue mussel (*Mytilus edulis*) and Mediterranean mussel (*Mytilus galloprovincialis*) (Figure 2).

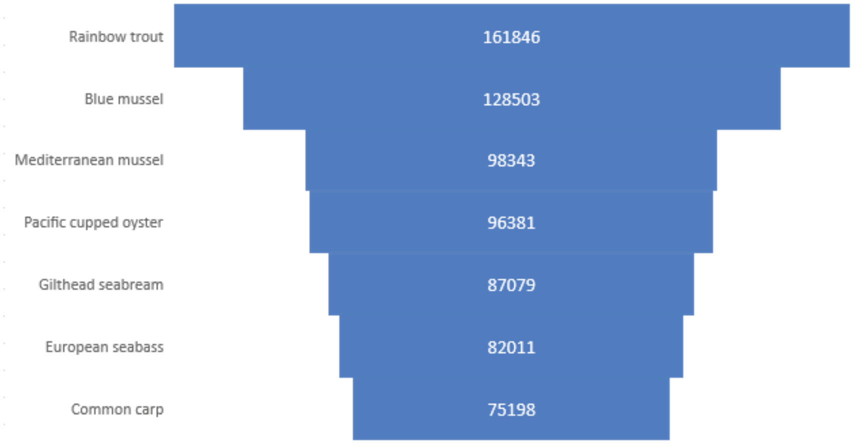

Figure 2. The Most Cultured Aquatic Species in the EU (t) (FAO, 2018).

Less attention is paid to mussel fisheries than that of cultivation over the world. The amount of capture-based mussel production accounts for 4% (84158 t) of the total mussel production. Denmark is the leading country in mussel capture (Table 3). A decrease trend occurred year by year during the last twenty years and mussel production reduced almost 70%. The main reason of this reduction is due to starting and strengthening mussel cultivation instead of mussel capture especially in Italy and Thailand. Denmark has also directed the mussel industry to aquaculture and reduced mussel capture almost 70% which had a capacity of over 100000t in the beginning of 2000. Overfishing is also problematic for aquaculture because of reducing mussel spats which are needed for cultivation. This situation increased the demand on hatcheries and the importance of mussel production from egg to rootstock individual is getting bigger. However, production cost in hatcheries is expensive. Therefore, an acceptable amount of rootstock individual capture from the sea during a suitable period of the year is a crucial point. Furthermore, decreasing stocks of filter feeder mussels creates an environmental problem. Bivalve mollusks are one of the most significant species in the coastal ecosystem and they generally dominate the macro benthos (Dame, 1996). Similarity in habitat preference of *Crassostrea gigas* with native blue mussel (*M. edulis*) and Mediterranean mussel (*M. galloprovincialis*) was reported in many studies (Angles d'Auriac et al., 2017; Dolmer et al., 2014; Özcan Gökçek et al., 2020).

Export amounts of mussels have been increasing year by year over the world. In the last decade, a 35% of increase occurred and 360000 t of export corresponding to 970 million dollars was noticed in 2018. Import values also increased almost 25% during the last decade. Global import amount and value in 2018 were reported to be 315000 t and 875 million dollars, respectively. The most exported and important products were mussels of the genus *Mytilus* spp., live, fresh or chilled. The main importing countries were France, Netherlands and Italy. On the export side, Chile, Spain and Netherland dominated the market in 2018 (FAO,2018). Increasing demand to bivalve consumption which resulted in higher prices has been negatively affected due to covid-19 pandemic. Especially cessation of economic and logistic activities in Chile, which is the leading country, caused a sharp decrease in mussel sales.

Table 3. Mussel Fisheries Amounts of Top Countries in the World (t) (FAO, 2018)

Countries	2014	202 2015	2016	2017	2018
Denmark	41.363	45.300	42.909	40.644	39.382
Chili	4.616	8.010	11.263	11.602	10.227
USA	12.393	14.456	15.031	9.170	7.943
North Korea	6.467	6.076	9..334	6.748	6.863
Indonesia	4.024	6.701	5.214	3.387	6.512
Brazilian	5.329	4.810	4.810	4.810	4.810
Mexico	1.896	2.196	2.970	2.438	3.326
Peruvian	5.866	4.476	3.682	2.688	1.324
England	570	979	1.617	687	948
Turkey	204	240	78	536	604

2. Mussel Culture and Capture in Turkey

Total mussel production of Turkey was 5338 t in 2019. While capture based production accounted for 22%, cultivation composed 78% of the total production. Turkish mussel fisheries showed fluctuations recently (Figure 3). Mussel fisheries have been performed along the surrounding waters of Turkey except Mediterranean Sea in the last years. However, in 2019, mussel fishery was done only in the Black Sea (TUİK, 2019).

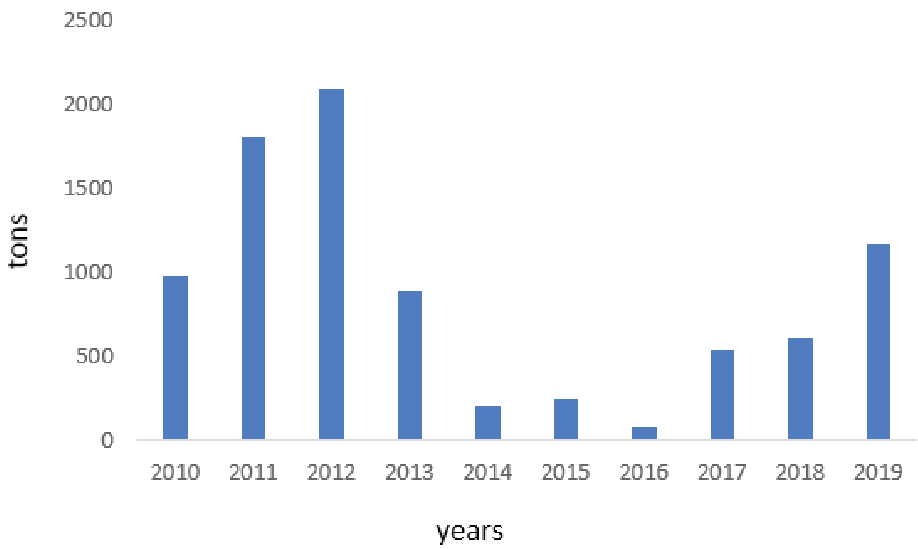

Figure 3. Catch Amount of Turkish Mussel Fisheries (TUİK,2019)

Mussel capture in Turkey is generally conducted illegally. Mussels have been collected from the areas where they may be risky for human health. Those areas are generally pillaring of bridges and ports, straits with intense marine traffic where are heavily polluted and contain heavy metals. İstanbul, Çanakkale, İzmir and Bursa are the provinces where unregistered mussel fishery is performed the most. Capture in those cities is conducted by divers and dredge gears hauled by small boats. Collected mussels are illegally distributed to all parts of Turkey via a huge network. The center of this network is İstanbul. The volume of illegal mussel fishery is estimated to be high when the collected amounts of mussel form cities such as İzmir, Çanakkale and Bursa are considered. While the price of black mussel was 1.42 TL/kg in 2018, it raised to 3.82 TL/kg in 2019 according to TÜİK (2019).

Although the illegal mussel fishing is lacking in the official statistics, Turkey is the 3th in Europe and 10th over the world in mussel fishery. Because mussel fishery increased 94% in 2019 compared to previous year, Turkey seems to save its position within the first ten countries. Furthermore, illegal mussel fishery is the major income source for many people in Turkey. By the entry of law number 7191 "Law on Amendments to Fisheries Law" into force in 2019, it is planned to prevent illegal mussel fishery in Turkey (Official Newspaper, 2019).

Mussel cultivation has gained importance recently in our country. The number of breeding enterprises is gradually increasing. Traceability is one of the most important advantages of mussel cultivation facilities. Samples taken from the mussel farms are periodically examined by the district directorates of agriculture ministry. Thus, it is checked whether there is a toxic substance in mussels and whether there is any situation that may affect human health. These inspections resulted in increased confidence to cultivated mussels. Mussel cultivation started in 1993 in our country, and research and development studies have been carried out for many years (Aral, 1999; Hindioğlu et.al., 2001; Lök, 2001; Yıldırım, 2004; Yıldız et.al. 2006).

There are 12 enterprises engaged in mussel farming in Turkey which are registered and approved by Republic of Turkey Ministry of Agriculture and Forestry (TOB, 2019). Five of these enterprises are in the Marmara region, 5 in the Aegean region and 2 in the Central Anatolia region. Except for the ones in the Central Anatolia region, all the enterprises carry out mussel farming by the rope production method. In these enterprises, mussels are harvested and packaged at certain times and they offer these products for sale with its own label and certificate of origin. These facilities also create micro habitats for other living things and contribute to ecology by breeding mussels with ropes (Serdar and Yıldırım, 2018). However, mussels are an important bivalve type used in the IMTA (Integrated Multi-Trophic Systems) system to reduce the organic load in the water column, such as fish feed (Giangrande et al., 2020; Irisarri et al., 2013; Jiang and Fang, 2021).

Two of the 13 important bivalve species on the coasts of our country are *M. galloprovincialis* (Mediterranean mussel-black mussel) and *Modiolus barbatus* (bearded horse mussel). The only bivalve species produced by breeding is the black mussel (Serdar and Yıldırım, 2020).

The amount of mussel aquaculture in 2019 in our country is 4168 tons and has increased by 360% compared to 2018 (Figure 4). Turkey was at the 32nd place in the world mussel culture ranking and has a huge potential to be at higher places with the aquaculture amount of 2019 and the expected amounts during the following years.

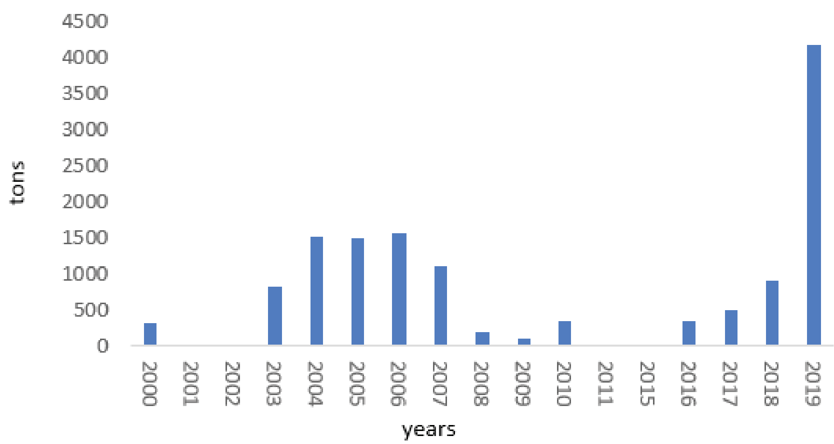

Figure 4. Mussel Aquaculture Amounts of Turkey (t) (TUİK,2019)

3. Mussel Trade and Marketing in Turkey

Today, the most widely used species in Turkey is the Mediterranean mussel. It is generally consumed fresh or processed most as the stuffed mussel. Stuffed mussel is one of the most important street flavors in Turkey. Popularity of stuffed mussel increased in the recent years by the effect of internet and growing online trade. Stuffed mussels, which were previously offered for sale at street stands, are recently produced and sold in markets which are registered to and approved by the Republic of Turkey Ministry of Agriculture and Forestry. Under the counter production is targeted to avoid by this regulation. Mussels are also used in various dishes in restaurants and hotels. Mussels which are very popular in many countries are a financially rewarding resource. Mussels are the livelihood of many people in our country. Families in İzmir and İstanbul get the biggest share from the mussel market in Turkey. Mussel marketing process is given in Figure 5.

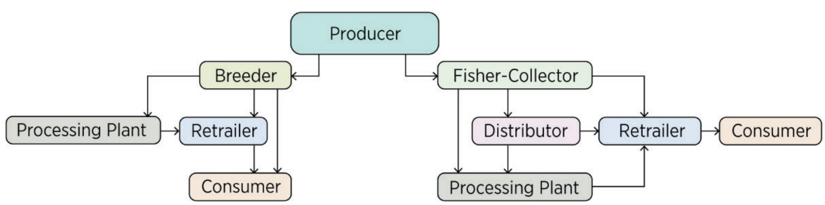

Figure 5. *Mussel marketing process in Turkey*

Each channel in the ranking of mussel marketing is the income source for many families. Collectors and distributors can be the same person, as well as manufacturers and sellers are likely to be the same person. While some of the processed mussels reach the consumer through the Retailer, some of them are marketed abroad by export. The mussel export is approximately 45 tons in 2019 (Figure 6). There are 14 companies that export mussels in 2020. Five of these companies are in Istanbul, 4 in Izmir, 2 in Çanakkale and others in Hatay, Adana and Denizli (EİB, 2020).

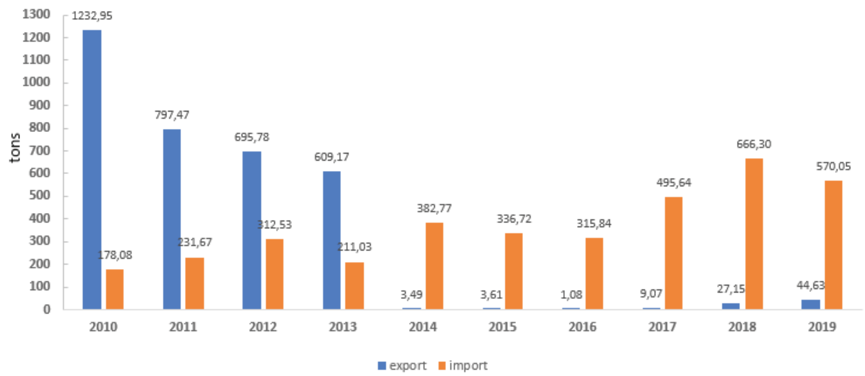

Figure 6. Export and Import Amounts of Mussels in Turkey (Foreign Trade Statistics, 2020)

While the amount of mussel export was over 1000 t in 2010, a considerable decline occurred in the recent years. The greatest decline was observed between 2013 and 2014. Similarly, it is seen that the decrease in mussel fishing was reflected in exports. Recently, there has been an increase in mussel exports due to mussel farming. Mussels import in Turkey has been increased during the years in general.

Approximately 65% of mussel exports in 2019 were processed or canned mussel products, and 25% were frozen mussel products of the genus *Mytilus*. Similarly, almost 75% of mussel imports were processed or canned mussel products, and 15% was composed of frozen mussel products of the genus *Mytilus* (Foreign Trade Statistics, 2019). The most exported country is the Turkish Republic of Northern Cyprus (Table 4). Germany, Spain and Austria are the export countries in 2019 according to TÜİK indicating the entrance to European Market.

Table 4. Countries of Mussel Export (Foreign Trade Statistics, 2019)

Export	2017	2018	2019
	kg	kg	kg
KKTC	5894	12700	14909
Kuwait	1500	3860	6745
UAE	-	-	6407
Spain	-	-	3654
Iraq		270	3600
Georgia	1614	8321	2418
Germany	-	-	2100
Azerbaijan	-	-	1762
USA	60	500	1023
Sweden	-	-	1020
Qatar		1000	720
Austria	-	-	100
Tunis	-	-	60
Turkmenistan		500	60
Saudi Arabia	-	-	50

The country with the biggest amounts of import is Chile (Table 5). Products imported from Chile are processed or canned mussels and frozen mussels of genus *Mytilus*. Frozen mussels of the genus *Mytilus* were imported from China and only live/fresh/cold *Mytilus* mussels were imported from Greece.

Table 5. Countries of Mussel Import (Foreign Trade Statistics, 2019)

İmport	2017	2018	2019
	kg	kg	kg
Chile	483.500	504.682	523.451
China	-	-	24.000
Greece	10000	161000	22.600
Norway	-	616	-
Spain	2138	-	-

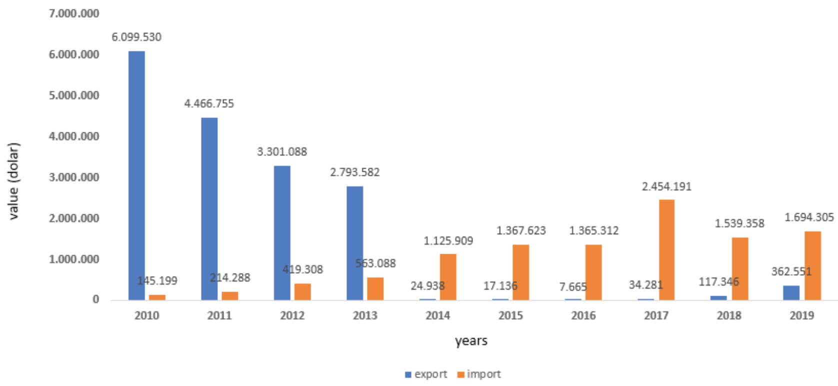

Figure 7. Value of Mussel Export and İmport in Turkey (Foreign Trade Statistics, 2020)

Value obtained from intensive mussel export in the early 2010s, decreased considerably towards the end of the 2010s with the decrease in exports. In addition, mussel import has increased recently in Turkey to meet the demand for mussels. Approximately 1.7 million dollars' worth of mussels were imported in 2019 (Figure 7).

CONCLUSION

Turkey is one of the countries with high rates of mussel consumption, but statistical dimension of the consumption is not known exactly. The most important reason for this case is the illegal mussel fishery. Collected mussels are transported in sacks and can be easily transferred to production facilities. This situation significantly increases informality.

Article 11 of the "Law on The Amendments of The Fishery Law" contains the penalties to be imposed on those who act contrary to the prohibitions, restrictions and obligations in the regulations under the heading "administrative sanctions". Accordingly, amendments have been made in Article 36 of the Fisheries Law No. 1380 and these amendments are closely related to unregistered fishing in the fishery sector (Official Newspaper, 2019). With the amendment, administrative fines have been imposed on those who perform fishing activities with purse seiners, trawlers, beam trawls with illegal and unlicensed boats, those who dive and collect aquatic organisms such as sea cucumber or mussel illegally, and those who catch with light in the Marmara, Istanbul and Çanakkale Straits and the Black Sea. Publication of this law in the Official Newspaper on November 22, 2019, highly affected the unregistered mussel fishing. Serious sanctions have been imposed on those who continue fishing despite the law. It is aimed to decrease illegal fishing by this implementation.

In addition to these sanctions, it should be possible to correct the wrong fishing practices with the guiding regulations in accordance with the law. There may be ways to contribute in the long run rather than short term enforcements. Some examples of this approach are as follows:

- Facilitating certain mandatory conditions for licensing unregistered boats and guiding by public institutions
- The necessity of mussel purification process after the fishery to be conducted by the state
- Allowing fishery only in areas and at certain sizes determined by public institutions
- Helping fishers to establish cooperatives

Therefore, preventing unregistered hunting will result in contribution to the economy of the country and positive employment.

Demand for mussels cannot be met due to decrease in fishery and thus imported amount increases. The acceleration of mussel cultivation in recent years is important for the development of the aquaculture industry. Because mussel meat includes high protein content and mussel is a suitable bivalve type for culture. It consists of protein up to 60% of its dry weight. Its fat ratio and cholesterol concentrations are low. Mussel meat also contains A, B1, Niacin, B2, C, D and E vitamins and iron and calcium elements (Ayvaz, 2018). Technological and scientific developments in the aquaculture sector also provide basis for the cultivation of new species. it is expected that mussel cultivation will increase during the following years under these considerations.

After 2013, Turkey's mussel exports decreased sharply due to the reduction in aquaculture and fishery. Since 2016, there has been a revival once again. This increase is related to aquaculture and fishery production. The mussels export may increase at an increasing rate in the following years and contribute to the economy of the country. Mussels must comply with European standards for export procedure. The export of mussels to Europe in 2019 can be considered as an important development point considering the mussel consumption of Europe. Despite high mussel production, European countries have not been able to meet the demand in recent years and have turned towards imports. It is seen that the export of mussels can increase by growing mussels in accordance with the desired standards. Mussel is a seafood with high economic value. It is a product which creates higher incomes than other exported goods from Turkey (Table 6).

Table 6. Prices of Some Export Products in 2020 (Dollar/kg) (Foreign Trade Statistics, 2020)

Product	Price (Mean ± S.D.)
Octopus, prepared or preserved	20.39 ± 1.95
Shrimps, prep. or pres., not in airtight containers	11.96 ±3.79
Mussels, prep. or pres., not in airtight containers	11.14 ±3.34
Crayfish, prepared or preserved	10.08±2,76
Salmon, prepared or preserved	9.63±5.68
Mackerel, prepared or preserved	6.86±0.43
Turkey, Other prep. or pres. meat, meat offal or blood, not in airtight containers	6.55±2.89
Anchovies, prepared or preserved	5.99±3.12
Euthynnus other than tuna, skipjack prep. or pres.	4.58±0.89
Sheep, Other prep. or pres. meat, meat offal or blood, not in airtight containers	1.86±0.56

It is possible to meet the high demand in the domestic market and export to foreign countries with the production. Employment can be created by the development of mussel aquaculture as well as contributing to the country's economy. Pawiro (2010) stated that the development of the bivalve industry in developing countries will depend on growth prospects, establishing reliable monitoring and inspection programs, and implementing sustainable farming.

Our country has made very important progress on these issues. Thus, the increase in exports will continue with the existence of aquaculture facilities producing mussels that we can consume safely.

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Chapter 4

EFFECT OF PREPARATORY CUTTING IN NATURAL REGENERATION OF ANATOLIAN BLACK PINE

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1. INTRODUCTION

Natural regeneration is one of the most important forestry practices to transmit present genetic diversity from generation to generation by naturally, and conversion of degraded forest to productive forest. The practice is getting importance to establish resistance forest to a-biotic and biotic damages. Climate change is one of the most important potential a-biotic damages. And its effect on forest area and forestry practices cannot be foreseen by foresters. Tíscar and Linares (2011) reported that it has many uncertainties and normally unable to predict the direction and magnitude of the change at the small scale needed by forest managers. However, effect of climate change can be managed and to be minimize by improvement of forestry practices. Black pine [*Pinus nigra* Arnold.] could be accepted as an indicator species to monitor climate change effect because of its large natural and artificial distribution in different ecological conditions. The species has wide natural spread around the world at 40° east longitude in Turkey, west longitude 5° in Spain and Morocco, 35° north latitude in Morocco and Cyprus island, between latitude 35° north in Crimea and 48° north in Austria (Figure 1) by five sub species as (Gaussen et al., 1964; Critchfield and Little, 1966; Vidakovic, 1974; Nikolic and Tucik, 1983; Alptekin, 1986; Yaltirik, 1993; Lauranson-Broyer and Lebreton, 1995; Isajev et al., 2004; Farjon, 2010; Caudullo et al., 2017):

Pinus nigra Arnold. subsp. *nigra* (Austrian pine): Austrian pine is native in southeast European continental climates with some summer rainfall of Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Macedonia, Montenegro, Romania, Serbia and Slovenia generally between 200 and 1200 m.

Pinus nigra Arnold. subsp. *larico* (Corsican pine): Corsican pine grows naturally between 950 and 1800 m asl. at Corsica of France and Sicily and Calabria of Italy by several protected stands. The taxa classified as least concern is widespread, although locally subject to logging or habitat loss.

Pinus nigra Arnold. subsp. *dalmatica* (Dalmatian black pine): Dalmatian black pine is mainly native on three islands of Croatia at between 400 and 700 m elevation and smaller 300 km². it has been classified as endangered because of many a-biotic and biotic damages, and limited natural distribution.

Pinus nigra Arnold. subsp. *salzmannii* (Pyrenean pine): Pyrenean pine is native to Algeria, France, Morocco and Spain at elevations of 400-1500 m. It has large natural and plantation forest in Spain, while the North Africa populations are small and isolated, and probably genetically distinct.

Pinus nigra Arnold. subsp. *pallasiana* (Lamb.) Holmboe (Anatolian black pine): Anatolian black pine is a native forest tree species in Cyprus, Russia, Turkey, and Ukraine at between 100 and 1900 m asl. The species mainly occurs in Turkey, where large stands remain in the Pontic and Taurus Mountains; in the Taurus it forms pure stands, or is mixed with *Cedrus libani*, *Pinus brutia* and *Abies* taxa in mainly occupies north-facing mountain slopes and ravines (Figure 2).

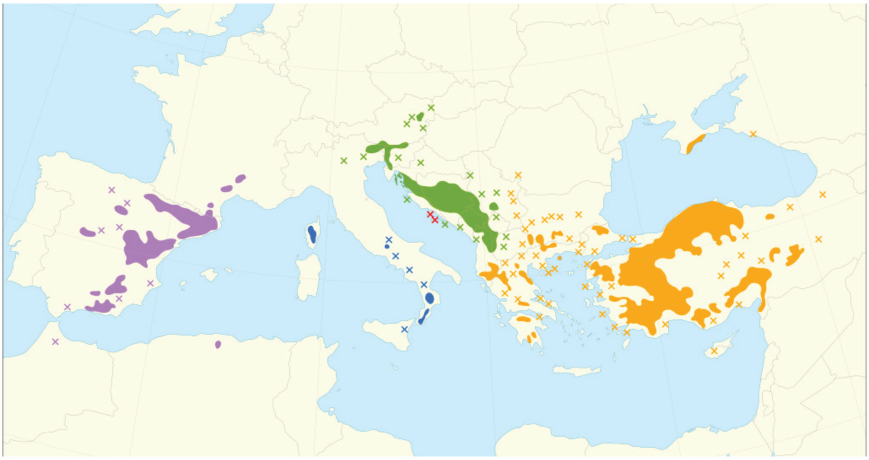

Figure 1. *Natural distribution of Pinus nigra (EUFORGEN, 2009)*

Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe], by its five varieties *Pinus nigra* subsp. *pallasiana* var. *pallasiana*, *P. n* subsp. *p.* var. *şeneriana*, *P. n* subsp. *p.*var. *yaltirikiana*, *P. n* subsp. *p.*var. *columnaris-pendula* var. *nova*, *P. n* subsp. *p.*var. *pyramidata* (Gaussen et al., 1964; Alptekin, 1986; Yaltirik, 1993; Ansin and Ozkan, 1993), is one of the most important commercial and ecological tree species used afforestation practices widely (Ayan et al., 2017) by 30-35 m height and up to 1 m diameter (Ansin and Ozkan, 1993), which has 2.84 million ha productive and totally 4.36 million ha natural distribution in 22.74 million ha in Turkish forestry according to the latest forest inventory (OGM, 2020, Table 1) (Figure 2). Anatolian black pine occupies hot and dry area, also resistance to winter colds.

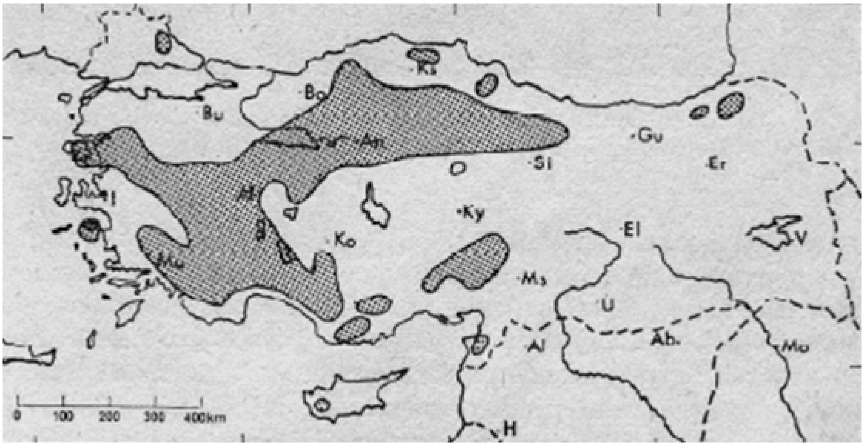

Figure 2. *Natural distribution of Pinus nigra in Turkey*

Table 1. *Area of Turkish forestry and Anatolian black pine*

	Productive		Degraded		Total
	Area*	**%**	**Area***	**%**	**Area***
Turkey	13.08	57.5	9.66	42.5	22.74
Anatolian black pine	2.84	65.1	1.52	34.9	4.36

**; million ha*

Anatolian black pine is resistance to shadow, drought and frost. It is also closest to step because of its silvicultural characteristics (Saatcioglu, 1976; Yaltirik, 1993; Ansin and Ozkan, 1993). These characteristics show importance of the species for climate change and potential for degraded forest area, and restoration practices in forestry.

Anatolian black pine can be considered as a key species in modification of silvicultural treatments because of its large natural distribution in different ecological conditions. Effect of preparatory cutting in natural regeneration of the species is discussed by a case study from southern part of Turkey of Anatolian Black pine.

2. REGENERATION METHODS

Regeneration is defined as the renewal of a forest crop by natural or artificial means. Regeneration methods are divided into two groups as natural regeneration defined as the renewal of a forest crop by self-sown seeds or by coppice or root suckers, and artificial regeneration (i.e., afforestation, reforestation, restoration) or natural regeneration supplemented by artificial regeneration. However, it is known that there could be many environmental and biological factors such as species, age of the tree, soil conditions, climate, crown, other externals effective on decision and its practices (Saatcioglu, 1976; Ata, 1995; Odabasi et al., 2007). For instance, physiographic conditions, physical soil properties, organic matter, climate, and outer soil status factors are effective on success of regeneration efforts in the species (Yazgan and Ozel, 2013).

3. NATURAL REGENERATION

Natural regeneration is the process by which juvenile plants and coppice that have established naturally replace plants which have died or have been killed, or harvested. While it changes for stand structure, species and other factors, shelter wood method of cutting, which included preparatory, seed, light and removal cuttings, is suggested for natural regeneration in productive forests of the species by 80-120 seed tree/ha, and clear cutting method for degraded forest area in Anatolian black pine (Saatcioglu, 1976; Ata, 1995; Odabasi et al., 2007).

3.1. Preparatory cuttings

Preparatory cutting is first step of shelter wood method. It could be applied one or more than one times based on stand structure and other factors. Purposes of the preparatory cutting are equal canopy, increasing of seed crop, improvement of edaphic characteristics and increasing of resistance of wind effect to stand (Saatcioglu, 1976; Ata, 1995; Odabasi et al., 2007). For the purpose, preparatory cutting (25°25’58” N latitude, 41°98’305” E longitude, 1520 m, north-east aspect, 35% slope) and un-preparatory cutting (25°31’38” N latitude, 41°98’000” E longitude, 1400 m, north-east aspect, 35% slope) also called as control lands were sampled from a restricted natural regeneration area applied practices by local forestry three years ago to minimize effect of other factors from southern part of Turkey of Anatolian black pine which the species was dominant (Figure 3).

Fifty trees chosen randomly were sampled from the lands at end of growing period of 2020. Diameter at base (D_0), Diameter at breast height ($D_{1.30}$), and tree height (H) were measured together with number of two-year cones (C_N) at the trees of the lands.

Preparatory cutting and un-preparatory cutting lands were compared for the characteristics by one-way analysis of variance. Growth characteristics and cone numbers were correlated by Pearson phenotypic correlation analysis using SPSS statistical package program.

Figure 3. *Un-preparatory cutting (left) and Preparatory cutting (right) lands*

3.1.1. Growth characteristics and cone number

Averages and ranges of the characteristics were given for the lands in Table 2. Large differences within treatment showed importance of genetic structure of seed tree and its selection. For instance, diameters at based were between 32.5 cm-52.0 cm in preparatory cutting land, and ranged from 29 cm to 52 cm in un-preparatory cutting land (Table 2). The differences among individuals within treatment showed great variation for cone production such as between 20 and 300 in preparatory cutting land (Table 2). There could be many factors effective in the variation such as age and crown diameter. However, the characteristics showed similar performances for the lands except of tree height according to results of analysis of variance (Table 3). Averages of mature cone numbers were 119 and 87 in natural stands of Anatolian black pine (Cercioglu, 2018). It was emphasized that year is very important in cone production in the species (Cercioglu, 2018). It is also well accordance with good seed year of the specie which is one time in each two-three years (Ata, 1995; Odabasi et al., 2007). Beside, it is known that altitude and aspect are also effective factors in cone production in the species (Saatcioglu, 1976; Ata, 1995). The results emphasized local forestry practices in natural regeneration.

Table 2. *Averages and ranges in preparatory and un-preparatory cutting lands*

	Preparatory cutting		Un-preparatory cutting	
	average	**range**	**average**	**range**
D_0 (cm)	43.5	32.5-52.0	42.9	29.0-52.0
$D_{1.30}$ (cm)	36.1	27.5-49.0	36.8	26.0-50.0
H (m)	13.5	9.5-21.0	15.0	9.5-25.5
C_N	96.0	20.0-300.0	107.2	12.0-312.0

Table 3. *Results of analysis of variance for the characteristics and lands*

Characteristics	Source of variation	Sum of Squares	df	Mean Square	F	P value
D_0	Between Groups	6.76	1	6.76	.279	.598
	Within Groups	2372.99	98	24.21		
	Total	2379.75	99			
$D_{1.30}$	Between Groups	13.32	1	13.32	.569	.452
	Within Groups	2294.87	98	23.42		
	Total	2308.19	99			
H	Between Groups	61.62	1	61.62	11.32	.001
	Within Groups	533.41	98	5.44		
	Total	595.03	99			
C_N	Between Groups	2872.96	1	2872.96	.674	.414
	Within Groups	417531.04	98	4260.52		
	Total	420404.00	99			

Coefficient of variation (Cv) was higher in preparatory cutting land (71.4%) than un-preparatory cutting land (57.36%) for cone production (Figure 4). It emphasized method practices, and importance of balancing of trees performance in future process for higher genetic diversity in reproductions and sustainable forestry. Large differences were found among populations and within population, and also between years for reproductive characteristics included strobili, seed and cone productions in natural stands of Anatolian black pine (Cercioglu, 2018). Large differences among populations and within population for reproductive characteristics were also found in different forest tree species (Cercioglu, 2013; Keles, 2015; Yazici and Bilir, 2017).

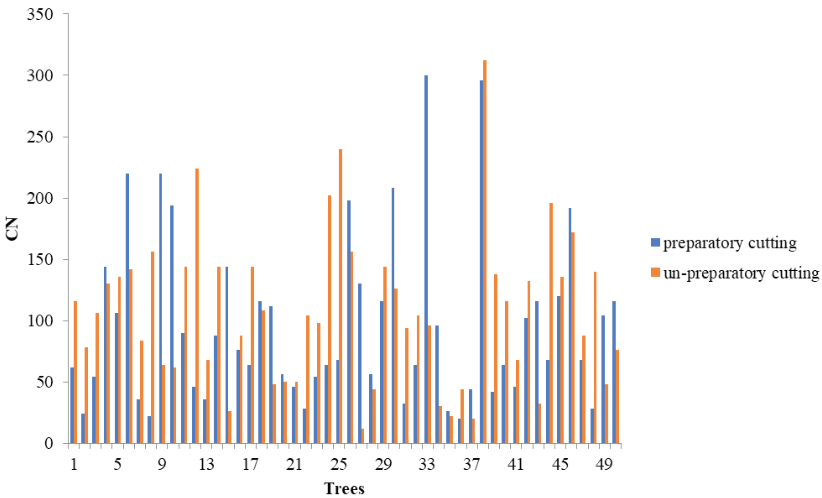

Figure 4. *Cone numbers in un-preparatory and preparatory lands*

These results show importance of period, repetition and degree of preparatory cutting, and period of next step in natural regeneration. For instance, in present practice, canopy is decreased to 0.6-0.7 after preparatory cutting. Preparatory cutting serve many purposes in practices. However, the study focused mainly on cone production. Success of preparatory cutting and next practices can be measured by different parameters.

Generally, number of natural reproduction per square meter is used for succession criterion of natural regeneration in traditional forestry (Baker, 1934; Saatcioglu, 1976; Guner, 2001; Pariona et al., 2003; Odabasi et al., 2007; Yazgan and Ozel, 2013). Guner (2001) suggested local natural regeneration practices in the species. However, Guney (2014) studied on evaluation of natural regeneration success by number of natural reproductions together with morphological characteristics of the reproductions. It was also suggested that soil treatment with machine is more successful in semi arid regions (Guney, 2014). Seedling height, root collar diameter, age and terminal shoot height values were examined to estimate success of natural regeneration practices in the species (Ertugrul and Bilir, 2020). Beside number of advance sapling could be taken into consideration in regeneration area (Figure 5) also suggested by Genc (1994) to competition from the same or other plant species may prevent successful seedling recruitment. It is known that number of natural reproduction is an important criterion to estimate success of natural regeneration. However, number of natural reproduction at biological independence, and survival of the reproductions could be also used based on climate change. For instance, natural reproductions reach 50 and 80 cm height at 9 and 13 years, while they reach generally average 80 cm height at 10 years in Anatolian black pine (Guzel, 2013).

Figure 5. *Advance regenerations in the area*

Growth characteristics were not significant (p>0.05) effective on cone production in both lands based on results of correlation analysis. Generally, positive and significant (p<0.05) relations were reported between growth and reproductive characteristics in natural stands of Anatolian black pine (Cercioglu, 2018). However, the relations were changed for characteristics and populations/altitude and year in natural populations. Negative relations were found between growth and strobili production in natural populations of *Pinus sylvestris* by Nikkanen and Velling (1987). Age was an important factor on seed production in natural populations of *Pinus sylvestris* (Boydak, 1977) and *P. brutia* (Eler, 1990). Present study was carried out in a limited area and by one year data. However, there could be many factors in these relations. The results showed new studies in different populations of the species.

4. CONCLUSIONS

A few years of preparatory cutting seems not sufficient for its practical purposes. However, effect of preparatory cutting to next practices and edaphic characteristics should be examined. Present study was carried out in a limited area of natural regeneration. New studies should be organized for large area and different treatments such as different canopy. Present regeneration practices should be improved based on climate change for sustainable forestry. Utilization possibilities of advanced sapling should be studied for success of regeneration practices. Effect of preparatory cutting on germination, number of filled seeds, growth performances and number of reproduction should be also investigated. Present study was carried out on pure stand of the species. Effect of preparatory cutting in mixed stand could be also examined by different species.

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Chapter 5

THE ECOLOGICAL SIGNIFICANCE OF NON-WOOD PRODUCTS IN OUR COUNTRY

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1.INTRODUCTION

One Benefiting from forest ecosystems is not limited to obtaining wood raw materials; this is only one part of the benefits. Non-Wood Forest Products (NWFP) is gaining commercial, cultural, and ecological or biological significance. NWFP are shrubs, bushes, and all sorts of plants and their parts that emerge in forest habitats and are cultivated or gathered for commercial and non-commercial uses in forest ecosystems. These products are also called "secondary products", "special forest products", "non-traditional products" [1].

Farm species, game animals and wild animal resources, water production basins, and animal feed resources are all considered non-wood forest products (NWFP) [2]. The demand for NWFP, which is described as "all kinds of herbal or animal products grown in forests and openings that people and other creatures profit from by meeting their own needs or earning income through their trade," has risen in recent years on both domestic and international markets. Furthermore, these products are widely applied in the home economy, especially among forest villagers [3]. The versatility of of the resulting value (economic, social, cultural, ecological), as well as the broad variety of beneficiaries and areas of use, make non-wood products significant. Indeed, these goods are attracting interest in Turkey today, especially in rural areas, in terms of income balance, manufacturing, ecological tourism, and other factors. In certain areas, NWFP will provide rural areas with a higher level of income than traditional forestry and wood raw material production. After understanding the importance of the multifaceted benefits provided by NWFP, progress has been made on the need to attach sufficient importance to these products in the management of forest resources. As a result, it is anticipated that non-wood goods would contribute more at the national or regional level. A large portion of NWFP is consumed by local people for their own needs, particularly in developing countries; the remainder is mainly sold in cities at low prices through intermediaries or exported to other countries. Since most exports are in the form of raw materials or semi-finished goods, the revenues generated are low and far below the potential revenues. On the other hand, developed countries that import these products (mainly Germany and the Netherlands), after processing, sell the products they produce at high prices in the domestic market or provide large foreign currency revenues to their countries through re-export [4]. People's preference for sustainable and organic goods has been growing in recent years. Our country has a very rich accumulation in terms of non-wood forest products, and the number of people who use these services is growing day by day. If these herbal products harvested from our forests are assessed from an ecological standpoint, it is clear that when they are collected and evaluated correctly and well, they will create serious gains for both the people living in the region and the economy of the country. It is necessary to consider that non-wood products are sold as processed rather than raw sales by establishing a stock exchange in our country.

The aim of this study is to examine the importance of non-wood products in our country, which are significant for environmental and human health, as well as the current situation in our country.

2. BIOLOGICAL DIVERSITY AND ECONOMIC IMPORTANCE OF NON-MODEL PRODUCTS

Non-wood forest products (NWFP), also known as forest subsidiary products, make up the majority in the biological diversity of our country's woods, and cover 27.2 percent of the country's 21.2 million hectares [5]. Turkey, which is one of the most important countries in the world in terms of non-wood products due to its geographical structure, has a significant floristic wealth. Because of their economic importance, non-wood goods, which have been used by the public as folk medicine and spices since ancient times, are also important for the food, medicine, and cosmetics industries. The topographical structure, climate and soil differences have made the forests of Turkey quite rich in terms of plant diversity. Especially the richness of relic and endemic plants increases the importance of Turkey's forests in terms of biological diversity [6. Turkey, which has around 9,000 plant species, 3,000 of which are endemic, and rich fauna resources, is among the countries rich in biodiversity among the temperate zone countries. Because of the abundant ecological diversity and the preservation of natural or semi-natural forest status in most of the country's areas, the country's forests are of global significance [7].

Biodiversity is defined as "the diversity of living organisms, the ecological ecosystems (terrestrial, marine, and aquatic) in which those organisms live, and the ecological processes provided by that organism environments" in the Biological Diversity Convention, which was agreed at the "United Nations Environment and Development Summit" held in Rio de Janeiro in 1992 and to which our country is a member. This includes intra-species and ecosystem diversity. In our country, there is a wide range of economically significant families. Species diversity is one of the hierarchical categories of biological diversity, and forest ecosystems in areas falling under forest regime are very important in terms of species diversity and endemic species. In the forest ecosystem, non-wood products play a significant role. It especially contributes to the protection of the ecological balance of nature [8].

The distribution of NWFP in our country's forests varies depending on the areas. In this sense, our nature and forests offer rich resources to humanity, considering the region, region, country and even globally, but this resource cannot be utilized sufficiently. Despite this, the plants collected or harvested or the products obtained from them are collected by the forest villagers, and an income is obtained by selling after personal needs are met. This utilization has become an important source of income for certain regions and products (pine nuts, bay leaves, etc.). Pesticides and artificial fertilizers are not applied in the production of non-wood products. It has a significant benefit over other agricultural products in this regard. Furthermore, the amount of labor required for the production of these products is less than the agricultural products. Since these materials are harvested from their natural habitat, they do not require practices such as seed planting, hoeing, or irrigation that are used in agricultural production.

3. NON-WOODEN PRODUCT APPLICATIONS

Plants that fall into the category of non-wood products and products derived from these plants have a wide range of applications. With the advancement of technologies, the range of applications has broadened. Plants usually have several use zones, which can vary depending on the location. The following are few examples of non-wood herbal product applications: while walnut, pistachio pine and carob are consumed raw with fruit or seeds, some plants such as kenger, hibiscus and beef tongue are consumed raw as well as cooked and cooked. Many different plants in different regions of our country are used as food [9].

A)Appplication as medical (medicine):

One of the areas where the usage areas of plants are the widest is their medicinal use. The use of the oil of St. John's Wort (Hypericum perforatum) plant as a wound healing, licorice root (Glycrrhiza glabra) and its roots as a bronchitis, breast softener and expectorant, and the use of the fruits, seeds and flowers of the Rosehip (Rosa spp) plant in upper respiratory tract infections. are the facts of folk medicine known for many years [9].

B) Cosmetics and Perfumery Applications:

In his book called Essential Oils Used in Aromatherapy and Aromatherapy, the role of natural substances in new cell formation and cell metabolism cannot be denied, that aromatic waters (hydrolates) as well as essential oil mixtures are used in skin, nail and hair care, *Ocimum basilicum* (basil), *Salvia sclarea* (Musk sage), Thymus (linalol type), Rosmarinus officinalis (Bird tongue) Eucalyptus olivies, Matricaria recutita (*May chamomile*) and Lavantula angustifolia (Lavender) are frequently applied in aromatherapeutic skin care. Essential oils of some plants such as lavender and mint are used in perfume production [9].

C) Usage as a Natural Dyestuff:

Fabrics were colored with natural dyes derived from plants during times when synthetic dyes were not widely available or inexpensive. This tradition, which survives in Anatolia, although in a minor form, is a significant part of our history. Some of the plants applied for this purpose are; Smoke tree, Dyer's sumac (*Cotinus coggyria* Scop.) Buckwheat (*Rhamnus petiolaris*), Castor oil plant (*Ricinus communis* L), Yellow chamomile (*Anthemis tinctoria* L.), Thuja oak (*Quercus infectoria* Olivier). There are many plants used for this purpose. It is possible to count many types; examples of plants used in parks and gardens are *Aesculus hipocastanum*, Platanus spp., Juniperus spp. In addition, especially geofit species such as *Fritilleria imperialis* L., *Fritilleria persica* L., Anemone blanda Schott et Kotschy are used as decorative and ornamental plants [9].

D) Application in Spice Industry:

The organs of some plants such as leaves, fruits, seeds, shells, and cones are used to give flavor to foods and dishes after various processes. Thyme, mint, bay leaf, almond, fenugreek, saffron, and sumac are some of the most well-known spices [9].

E) Application in Different Industrial Fields:

Aside from the uses listed above, non-wood materials are applied in a variety of industries. Various examples are given in the publication named "Diagnosis and Promotion Guide of Some Important Forest Secondary Products in Our Country". For example, it is stated that Çöven (*Gypsophila* L.) is widely applied in the food industry in the production of halva and ice cream in the soap and detergent industry due to its bleaching properties and also its good foaming properties. The oil of laurel (*Laurus nobilis* L.) is applied in the pharmaceutical and cosmetic industries, especially in the manufacture of soap, as well as in the sweetening of soups, cakes, confectionery, sausages, and meats. He said that for castor oil (*Ricinus communis* L.) it is widely used especially in the manufacture of aircraft engine oils due to its viscosity remaining stable against large and sudden temperature changes [9].

4. RESEARCH ON NON-WOODEN PRODUCTS AND ITS ECOLOGICAL EXAMINATION

The socioeconomic aspects of non-wood products in the world and in Turkey were investigated in a report, which first looked at which products stand out in terms of NWFP in various countries, which products acquire value in terms of NWFP trade, and information about NWFP trade from around the world. In our country, the Aegean Region has been determined as a work area and three products, thyme, laurel and pine nuts, which have significant export values in this region, were purchased and the trade groups of these products were discussed. A survey was conducted with four relevant groups identified as producer/collector, intermediary/trader, exporter and forestry organization, and recommendations were made on the relations between these groups, their problems, suggestions for solutions, and the determination of policies and management style in this regard [10].

According to another study, the most common non-wood forest products grown in Turkey between 1990 and 2009 were shrub, laurel, thyme, pistachio pine cones, sage, spruce, myrtle, rosemary, carob and chestnut. The most exported products are hazelnuts, cotton linters, basic chromium sulphate, cumin, pomegranate, thyme, chestnut, laurel, strawberry and anise, while the highest import is walnut, synthetic organic materials used in debagat, kiwi, almond, hard kernels suitable for chipping, henna, basic chromium sulphate, mimosa, preparations and kebrako have also been revealed [11]. In the study, plant taxa used as non-wood forest products in various industrial areas such as chemistry, paint and cosmetics, mainly food, medicine and pharmacy in Isparta Sütçüler region were investigated. A total of 74 non-wood herbal forest products belonging to 62 genera of 38 families were evaluated. The scientific names, families, parts of non-wood forest products, local areas of use, determination of endemic ones, and their contribution to the local community and economy have all been documented in a database [12].

In a study, theses, research master plans, priorities, science and technology policies, publications in electronic databases, which emerged as a result of research methods, were examined in terms of research priorities and the place they gave to multi-faceted benefits. As a result of the examination, it has been determined that this issue is rarely addressed in the postgraduate studies prepared in Turkey, the same situation can be found in the projects supported by Scientific And Technological Research Council Of Turkey (TÜBİTAK), and the subject is increasingly included in the Forestry Research Master Plans, and multi-directional utilization is among the principles of the Turkish National Forestry Program [13]. As a result of human activities, ecosystems, species and genetic diversity are being destroyed much faster than the natural rate. The rate of decline in diversity is believed to be 1000 to 10,000 times higher than in previous periods. This dramatic decrease in biological diversity jeopardizes the ecological, economic, spiritual and cultural gains that we still derive from the earth's living resources. Our natural resources and biodiversity are in an unfortunate process of degradation, decay and extinction. Our natural resources and biodiversity are process of degradation, decay and extinction at an alarming rate. The main reasons for this process are rapid population growth and the lack of education; all of which leads to the irrational and unconscious use of our natural resources.

5. CONCLUSIONS

In our country, there are many types that can be expressed as NWFP. Furthermore, the number of consumers who profit from these goods in both rural and urban areas is high to be underestimated. Despite the fact that our country is very rich in terms of NWFP and there are many people who need these products, efficient studies have not been available yet. As a result, the necessary revenue is not available. This condition arises from a variety of reasons. The distribution of the collected products to the market and the point of contact with the customer are the most significant of these.

General Directorate Of Forestry's work on NWFP is generally not sufficient. Many institutional and scientific studies on NWFPs have been conducted in our country in recent years. Since more urban people are included in studies and rural people are not, this term is regarded as medicinal plants or medicinal plants in culture, and its relationship with forests is ignored. Even though the idea is unknown conceptually, it is believed that public service advertisements and social media posts can attract people's attention in order to inform that the products in question grow in forest areas and have a place in forestry discipline. Furthermore, organizing conferences at certain times would raise awareness of local NWFP types. In Turkey, the rich non-wood forest product potential in the degraded forest areas of approximately 10 million hectares should be preserved in the balance of the ecosystem, priority should be given to the village legal entities in its evaluation and technical support should be provided by the relevant government institutions in this regard. The conservation of biological diversity has a very important role as one of the goals of organic agriculture as well as being of vital importance for the future of human history.

Non-wood products must be protected and collected without damaging the natural environment in our country, which has a diverse flora. These are critical and effective applications for the continuity of plants. On the other hand, it will be contributed to the ecological protection by increasing educational activities, especially by preventing the unconscious collection of non-wood products collected from nature.

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Chapter 6

EFFECTS OF TOXIC IRON TREATMENTS ON SHOOT GROWTH, IRON TOXICITY TOLERANCE INDEX, THE DEGREE OF TOXICITY SYPMTOMS AND CONTENT OF TOTAL CHLOROPHYLL OF SOME RICE CULTIVARS

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1. Introduction

Iron toxicity is encountered in rice plants at Fe^{+2} concentrations of between 10-1000 mg L^{-1} (Kirk, 2004). Aquatic species exhibit various resistance mechanism against iron toxicity including; Fe^{+2} oxidation in rhizosphere, inhibition of Fe^{+2} uptake by the roots, accumulate excess Fe^{+2} ion in apoplast and vacuoles, detoxification of reactive oxygen species through the enzymes. Besides, the primary defense mechanism of some plants is widely accepted as the release of oxygen (O_2) from the roots to rhizosphere. Aerenchyma of rice plant transports O_2 from the atmosphere to the roots under inundated conditions (Armstrong et al., 1991; Butterbach Bahl et al., 2000). The O_2 leached into the rhizosphere results in precipitation of ferric oxides and ferric hydroxides-like reddish Fe^{+3} compounds on plant root surfaces (Begg et al., 1994; Kirk, 2004). These reddish color oxidized regions are mostly encountered around the active roots (Atulba et al., 2015). Since root biomass development may vary significantly based on development stage of the rice plants, roots may release O_2 at different levels, thus fluctuate soil redox conditions.

It is estimated in rice fields that distinctive black zones were related to reduced regions and red zones were related to oxidized regions. Dark greyish color relies on formation of iron sulphate (Paul & Clark, 1996); reddish colors rely on ferric oxide and ferric hydroxide formation (Flessa & Fischer, 1992; Kirk, 2004). Therefore, emergence of specific colors in soil profile of rice fields may vary throughout the growing season. Analysis of these colors may indicate spatial dynamics of redox conditions (Schmidt et al., 2010).

Iron toxicity is encountered when a toxic Fe concentration is accumulated in rice leaves (Sahrawat, 2004). Fe levels of ambient solution generating an iron toxicity may vary between 10-500 mg Fe L^{-1} or over 500 mg Fe L^{-1}. Such a large range for iron toxicity may resulted from source and form of Fe supplemented to nutrient solution, cultivar Fe tolerance, concentration of the other nutrients, temperature and solar radiation (Marschner & Çakmak, 1989; Bode et al., 1995). Iron toxicity generates an important problem in rice farming performed under inundated soil conditions (Genon et al., 1994; Sahrawat, 2004). Nutritional disorders in rice plants, so called as “Akagare Type-I” in Japan, “Akiochi” in Korea and “Bronzing” in Sri Lanka, are attributed to Fe toxicity (Ponnamperuma et al., 1955; Tanado & Yoshida, 1978).

Previous researchers explained ferrous (Fe^{+2}) iron-induced toxicity symptoms in rice plants (Tanaka et al., 1966; Howeler, 1973). Toxicity symptoms included initiation of brown spots on old leaves, then formation of reddish-brown color spreading from the tips along the edges to the base of these leaves. Due to iron toxicity, decreases were encountered in number of tillers, stem height (Olaleye et al., 2001), plant fresh and dry weights and spikelet sterility (de Dorlodot et al., 2005). Iron toxicity symptoms in rice plant roots are encountered as formation of ferric (Fe^{+3}) precipitates on outer surfaces (Green & Etherington, 1977). Roots are not sufficient, short and have dark brown color under iron toxicity (Sahrawat, 2004). Such symptoms are mostly related to "Fenton reactions" leading production of toxic hydroxyl radicals (OH^-) and the other reactive oxygen species (H_2O_2, O_2^-) (Bode et al., 1995; Thongbai & Goodman, 2000; Estevez et al., 2001).

Rice plants have a greater tendency of iron uptake than the other plants. Besides the effects of high inner-cell Fe concentrations on plant growth and development, high Fe^{+2} concentrations in rhizosphere have antagonistic effects on uptake of several basic nutrients (Fageria et al., 2008). Therefore, iron toxicity is frequently characterized as "multi-nutrient disorder".

Rice roots exhibit three primary functions against iron toxicity. These functions include oxidation of rhizosphere iron to keep iron concentration of growth media at low levels. In this process, molecular oxygen is transferred from the shoots to roots through air chambers and aerenchyma, then passed into growth media. Such a case results in greater oxidation of rhizosphere than the soil.

In rice cultivars resistant to iron toxicity, less iron is absorbed or less iron is transported from the roots to leaves. Both processes are so called as "physiological avoidance". Iron-resistant rice cultivars accumulate less iron in photosynthetic leaves and keeps superior photosynthetic potential in case of iron existence hold in leaves (Audebert & Sahrawat, 2000). Since plant growth is dependent on dry matter production of the leaves (Jiang et al., 2004), iron toxicity-tolerant rice cultivars are expected to have greater weight of shoot biomass than the sensitive plants. Iron-tolerant rice cultivars may have high Fe levels without serious damages on plant tissues.

The content of chlorophyll pigments in plants is also considered an indicator of their response to the habitat, weather and anthropogenic conditions (Selzer & Busso, 2016). It can also be a reliable indicator of the vitality of plants and their resistance to stressful thermal and humidity conditions (Golińska, 2007). Modern indexing methods enable quick and easy measurement of the current content of chlorophyll. For this reason, research on the chlorophyll content is becoming more and more common and it can be done easily even in a field (Gáborčik, 2003). The measurement of the chlorophyll content may be an important indicator of the plant's life processes, which may affect the yield of biomass (Zielewicz et al., 2020).

This study was conducted to determine the effects of nutrient solution treatments containing toxic iron levels on shoot growth and iron toxicity index values, total chlorophyll content of fresh leaves, and the degree of toxicity symptoms of some rice cultivars.

2. Material and Method

Seeds of rice cultivars used in greenhouse experiments were supplied from Black Sea Agricultural Research Institute. These cultivars included: Biga incisi, Osmancık-97, Hamzadere, Ronaldo and Edirne rice cultivars.

2.1. Experiments

Seed sterilization was achieved through keeping rice seeds in 5.0% (v/v) sodium hypochlorite solution for 15 minutes. Seeds were then washed through deionized water and germinated in moist cloth bags. Germinated seeds were transferred to perlite-filled containers (40x25x5 cm dimensions) and grown there for 10 days to get rice seedlings. Rice seedlings were transplanted into plastic pots (12x12 cm) filled with 1 kg of quartz sand as to have 10 seedlings in each pot.

Rice cultivars were subjected to four different treatments in the form of iron sulphate ($FeSO_4 \cdot 7H_2O$) as of: I) 0 µM Fe (control), II) 45 µM Fe (sufficient Fe), III) 3.50 mM Fe (toxic Fe), IV) 3.50 mM Fe + % 10 bentonite (soda-activated bentonite).

Nutrient solutions were applied to 5 different rice cultivars at equal quantities as to have 3 cm water head over the sand in experimental pots. Nutrient solution pH was adjusted at 5.5 with the use of dilute HCl or KOH solution. Experiments lasted for 50 days.

As specified by Zhang et al. (1998), following non-iron containing nutrient solutions containing essential nutrients were used:

500 µM NH_4NO_3; 60 µM $NH_4H_2PO_4$; 230 µM K_2SO_4; 210 µM $CaCl_2$; 160 µM $MgSO_4 \cdot 7H_2O$;

2.5 µM $MnCl_2$; 0.75 µM $(NH_4)_6Mo_7O_{24}$; 3.2 µM H_3BO_3; 0.1 µM $CuSO_4$; 2.0 µM $ZnSO_4 \cdot 7H_2O$

At the end of the experiments, above-ground parts of rice plants were harvested and determined with a precise balance. Rice cultivars were separated into above-ground parts and dried in an oven at 65 ºC. Then, shoot dry weights were determined.

Visual assessment scale for iron toxicity at 2-5 leaf stage of rice plants and corresponding symptoms are provided in Table 1.

Table 1. *Visual assessment scale for iron toxicity in rice plant (IRRI, 2002)*

Severity of toxicity	Symptoms encountered in plant
1	Development and tillering are normal
2	Development and tillering are normal; formation of reddish-brown spots or orange color on tips of old leaves
3	Development and tillering are normal; old leaves are in reddish-brown, purple or orange or yellow color
5	Development and tillering are delayed; several leaves loose normal green color
7	Development and tillering are terminated, majority of the leaves loose normal green color or die
9	Almost all plants die

The tolerance rate to iron toxicity = (A / B) × 100

Where;

A = Shoot dry weight at toxic iron (3.5 mM Fe) concentration, g pot^{-1}

B = Shoot dry weight at sufficient iron (45 µM Fe) concentration, g pot^{-1}

As compared to sufficient iron level (45 µM Fe), tolerance index of rice cultivars to high iron doses in bentonite-supplemented and non-supplemented sand media was calculated with the use of the following equation (Fageria et al., 2008):

Plant tolerance index to toxic iron dose = (A / B) × (C / D)

Where;

A = Shoot dry weight of a cultivar at sufficient iron concentration (45 µM Fe), g

B = Average shoot dry weight of all cultivars at sufficient iron concentration (45 µM Fe), g

C = Shoot dry weight of a cultivar at toxic iron concentration (3.50 mM Fe), g

D = Average shoot dry weight of all cultivars at toxic iron concentration (3.50 mM Fe), g

Total chlorophyll (chlorophyll a+b) content was measured using the method of Arnon (1949).

2.2. Statistical Analyses

Experimental data were subjected to analysis of variance in accordance with 5×4 factorial experimental design with the use of SPSS 17.0 software. The *comparison* of *means* was *performed* through the *tests* of *Duncan* using *SPSS* 17.0 software

3. Results and Discussion

3.1. Shoot dry weight

Variance analysis results for the effects of toxic iron levels on shoot dry weight of rice cultivars grown in sand media are provided in Table 2.

Table 2. *Variance analysis results for the effects of toxic iron levels on shoot dry weight of some rice cultivars*

Sources of variation							
Iron sulphate dose		*Cultivar*		*Iron sulphate dose x cultivar interaction*		*Error*	
DF	MS	DF	MS	DF	MS	DF	MS
3	13.24**	4	0.434**	12	0.093	40	0.065

$^{**}p<0.01$; $^{*}p<0.05$ DF: Degrees of Freedom; MS: Mean Square

The effects of toxic iron levels on shoot dry weight of rice cultivars are provided in Table 3.

Table 3. *The effects of toxic iron levels on shoot dry weight of some rice cultivars*

Rice cultivars	Shoot dry weight, g pot $^{-1}$				
	Fe 0 (Control)	45 µM Fe	3.50 mM Fe	3.50 mM Fe + 10% Bentonite	Average
Biga incisi	1.49	3.91	1.72	2.51	2.41A
Osmancık-97	1.68	3.26	1.48	2.25	2.17BC
Hamzadere	1.46	3.21	1.37	1.86	1.98C
Ronaldo	1.47	3.38	1.11	2.09	2.02C
Edirne	1.74	3.60	1.39	2.57	2.33AB
Average	1.57C	3.48A	1.42C	2.26B	

**Means indicated with the same letters are not significantly different at 5% level.*

As can be inferred from Table 2 and 3, effects of iron dose and cultivars on shoot dry weight were found to be significant at $p < 0.01$ level. In terms of average shoot dry weight, rice cultivars were ordered as Biga

İncisi ~ Edirne > Osmancık-97 ~ Ronaldo ~ Hamzadere. As compared to the control, sufficient iron level (45 µM Fe) applied in iron sulphate heptahydrate form significantly increased shoot dry weight. On the other hand, toxic iron level (3.50 mM Fe) applied in iron sulphate form did not significantly influence shoot dry weight as compared to the control (Fe0); but significantly reduced shoot dry weight as compared to sufficient iron level. Also at toxic iron level, soda-activated bentonite supplementation into growth media significantly increased shoot dry weight as compared to the control (Fe0). As compared to sufficient iron level, toxic iron treatments reduced shoot dry weight by 59.19%. Average tolerance ratio of rice cultivars to toxic iron level was calculated as 40.80%. Tolerance ratio of the rice cultivars to toxic iron level was identified as 45.98% in Biga incisi, 45.39% in Osmancık-97, 42.67% in Hamzadere, 32.84% in Ronaldo and 38.61% in Edirne cultivar. As compared to sufficient iron dose, soda-activated bentonite supplementation (10%) into sand media reduced shoot dry weight by 35.05% and tolerance ratio of the cultivars to toxic iron level increased to 64.94%. Present findings revealed that soda-activated bentonite supplementation into sand media increased tolerance ratio of rice cultivars to toxic iron level. Effects of cultivar × iron dose interactions on shoot dry weight were not found to be significant. In terms of shoot dry weight in sand media without bentonite supplementation at toxic iron level, rice cultivars were ordered as; Biga İncisi > Osmancık-97 > Edirne ~ Hamzadere > Ronaldo. In non-supplemented sand media, the lowest shoot dry weight was observed in Ronaldo cultivar because of toxic effects of high iron dose and the greatest value was observed in Biga incisi cultivar. In terms of shoot dry weight in bentonite-supplemented sand media at toxic iron level (3.50 mM Fe), present cultivars were ordered as Edirne ~ Biga incisi > Osmancık-97 > Ronaldo > Hamzadere.

Samaranayake et al. (2012) used two different rice cultivars to investigate the effects of excessive iron ions (Fe^{+2}) on growth and iron content of rice plants. Researchers exerted iron stress on 21-day old plants through adding 250 mg Fe^{+2} L^{-1} in the form of $FeSO_4$ into nutrient solution and plants were harvested 14 days after application of Fe^{+2} stress. Researchers identified Bw 272-6b cultivar as sensitive to Fe^{+2} stress and Bw 267-3 cultivar as resistant to iron stress. Ferrous (Fe^{+2}) iron stress negatively influenced shoot dry weight of resistant and sensitive rice cultivars. Besides, a significant decrease was observed in shoot dry weight of sensitive Bw 272-6b rice cultivar. Relative decrease in shoot dry weight was 10 times greater in Bw 272-6b cultivar as compared to Bw 267-3 cultivar. These findings proved that Bw 272-6b rice cultivar was more sensitive to Fe^{+2} stress as compared to Bw 267-3 cultivar. Besides, in both rice cultivars, root dry weights were not significantly influenced by Fe^{+2}

stress. Since there were not significant differences in root dry weights under stress and control conditions, relative decrease in root dry weights were not calculated. Under control conditions, root iron contents of both cultivars did not change significantly. Such a case revealed that in case of moderate iron content of growing media, the cultivars differentiating in resistance to Fe^{+2} ion had similar nutrient absorption. Under stress conditions, root iron contents of both cultivars were significantly greater than the control treatments. Independently from the resistance against Fe^{+2} stress, significantly increasing Fe^{+2} ion absorption was observed in both cultivars with increasing iron content of growing media. Besides, root iron content was significantly lower in iron stress-resistant cultivar than in sensitive cultivar. Such a case indicated that Fe^{+2} stress-resistant cultivar was able to exclude excessive Fe^{+2}; on the other hand, sensitive cultivar had a low power of exclusion as previously indicated by Peng & Yamauchi (1993). Shoot iron contents of both cultivars were greater under stress conditions than under control conditions. Besides, shoot iron contents of both cultivars under stress conditions were not found to be significantly different from each other. Despite insignificant differences in shoot iron contents of both rice cultivars under stress conditions, iron toxicity-dependent symptoms of two rice cultivars were significantly different; while 7 point was assigned to symptoms encountered in sensitive rice cultivar, 1 point was assigned to symptoms encountered in resistant rice cultivar. There might be two possible reasons of these scoring. Firstly, shoot system of the resistant cultivar might have a mechanism able to separate irons into tissues without generating cellular damage; sensitive cultivar may not have such a mechanism. It was reported that different organs of rice plants had different iron distribution levels and highly sensitive cultivars did not have an iron barrier (Audebert et al., 2006). Secondly, leaf symptoms might have been related to a chemical signal transmitted through the root system. The signal transmitted by the roots of sensitive cultivars with significantly greater iron weight in root systems may be stronger than the signal transmitted by the roots of resistant cultivars with low iron weight in root systems. Further research is needed for better comprehension of such an important phenomenon.

3.2. Tolerance index values of some rice varieties to iron toxicity and the degree of toxicity symptoms

Tolerance index values of some rice varieties to iron toxicity were calculated in accordance with Fageria et al. (2008) and provided in Table 4 together with iron toxicity severity levels (IRRI, 2002) at toxic iron dose.

Table 4. *Tolerance index values of some rice varieties to iron toxicity and the degree of toxicity symptoms*

Rice cultivars	Bentonite supplemented sand media	Unsupplemented sand media	
	Tolerance index value to toxic iron dose	Tolerance index value to toxic iron dose	Iron toxicity severity level (IRRI, 2002)
Biga incisi	1.24	1.36	3
Osmancık-97	0.93	0.97	5
Hamzadere	0.75	0.88	7
Ronaldo	0.89	0.75	9
Edirne	1.17	1.01	7

According to Fageria et al. (2008), rice cultivars are classified as: tolerant for tolerance index values of >1.0; moderately tolerant for tolerance index values of between 0.5-1.0; sensitive for tolerance index values of <0.5. Based on this classification, Biga incisi was identified as the most tolerant cultivar to toxic iron dose (3.50 mM Fe). On the other hand, Ronaldo rice cultivar was identified as moderately tolerant to toxic iron dose. In terms of tolerance index values to toxic iron dose in non-bentonite-supplemented sand media, rice cultivars were ordered as; Biga incisi > Edirne > Osmancık-97 > Hamzadere > Ronaldo. In non-supplemented sand media, Ronaldo cultivar was identified as the most sensitive cultivar to toxic iron dose or iron toxicity. In bentonite-supplemented sand media, tolerance index value to toxic iron dose increased only in Edirne cultivar. In terms of tolerance index values to toxic iron dose (3.50 mM Fe) in bentonite-supplemented (10%) sand media, present cultivars were ordered as; Biga incisi > Edirne > Osmancık-97 > Ronaldo > Hamzadere. These findings revealed that Biga incisi cultivar was more resistant to iron toxicity. Observation-based iron toxicity level of Biga incisi rice cultivar (3) was better. On the other hand, observation-based iron toxicity level of Ronaldo cultivar, the least resistant cultivar, was identified as 9. Observation-based results were parallel to the calculated iron toxicity index values.

Iron toxicity in rice leads to the accumulation of the element in plant tissues accompanied with ethylene biosynthesis in shoots, drastic reduction of root growth and loses of yield (Becker & Asch, 2005). Iron toxicity symptoms are various among rice cultivars and the most common ones are spots on the inter-veins and purplish brown of the leaves followed by drying. Roots become scanty, coarse, short and blunted (Peng & Yamauchi, 1993).

Bronzing scale is a classical parameter used in assessment of sensitivity of rice cultivars to iron toxicity. It was reported that ferrous iron toxicity interrupted growth and development of rice plant (Nyamangyoku & Bertin, 2013). Number of tillers, shoot height, fresh and dry weights are also significantly influenced by iron toxicity (Ponnamperuma et al., 1955; Olaleye et al., 2001; de Dorlodot et al., 2005). Leaf bronzing level is recommended as an efficient measure to assess the severity of iron toxicity (Ota, 1968). Bronzing symptoms primarily rely on an antagonism between Mg and Fe in formation of secondary oxidative stress or porphyrin group in chlorophyll (Becana et al., 1998). Rice genotypes exhibit large differences in tolerance to ferrous iron stress (Ponnamperuma et al., 1955; Howeler, 1973; Bode et al., 1995; Luo et al., 1997). Such differences in iron toxicity tolerance of genotypes are mainly attributed to the differences in genotypes and Fe^{+2} oxidation power in rhizosphere (Green & Etherington, 1977; Ottow et al., 1982; Bienfait, 1989).

3.3. Effects of toxic iron levels on total chlorophyll content of fresh leaves of some rice cultivars

Variance analysis results for the effects of toxic iron levels on total chlorophyll content of fresh leaves are provided in Table 5.

Table 5. *Variance analysis results for the effects of toxic iron levels on total chlorophyll content of fresh leaves*

Sources of variation								
Parameter	*Iron sulphate dose*		*Cultivar*		*Iron sulphate dose x cultivar interaction*		*Error*	
	DF	MS	DF	MS	DF	MS	DF	MS
Total chlorophyll	3	11.55**	4	0.86**	12	0.723**	40	0.055

***p<0.01; *p<0.05; DF: Degree of freedom, MS: Mean Square*

Effects of toxic iron levels on total chlorophyll content of fresh leaves of some rice cultivars are provided in Table 6.

Table 6. *Effects of toxic iron levels on total chlorophyll content of fresh leaves of some rice cultivars*

	Total chlorophyll content (mg / g fresh leaf)				
Rice cultivars	Fe 0 (Control)	45 µM Fe	3.50 mM Fe	3.50 mM Fe + % 10 Bentonite	Average
Biga incisi	1.92hij	2.31gh	2.31gh	3.27cd	2.45C
Osmancık-97	1.86ij	2.75ef	1.82ij	4.54a	2.74B
Hamzadere	1.71j	2.57fg	2.76ef	3.74b	2.70B
Ronaldo	1.28k	3.56bc	3.24cd	3.74b	2.96A
Edirne	0.97k	2.82ef	2.21ghi	3.06de	2.27C
Average	1.55D	2.80B	2.47C	3.67A	

**Means indicated with the same letters are not significantly different at 5% level.*

As can be inferred from Table 5, effects of iron dose, cultivar and cultivar x iron dose interactions on total chlorophyll content of fresh leaves were found to be significant at $p<0.01$ level.

As compared to the control, sufficient iron level (45 µM Fe) significantly increased total chlorophyll content of fresh leaves. On the other hand, toxic iron level (3.50 mM Fe) significantly reduced total chlorophyll content of fresh leaves as compared to the sufficient iron level. Toxic iron levels in bentonite-supplemented sand media (3.50 mM Fe + 10% Bentonite) significantly increased total chlorophyll content of fresh leaves as compared to the other iron level (Fe0, Fe-sufficient, Fe-Toxic). Such a case revealed that bentonite increased total chlorophyll content, reduced iron uptake and prevented iron toxicity.

Along with the aforementioned changes related to the oxidative stress, total chlorophyll, soluble protein and carbohydrate contents declined and amino acid content increased under toxic iron levels (250 and 500 mg L^{-1}). The results indicate that iron toxicity induced greater oxidative stress in rice plants and supplemental potassium was ineffective in preventing iron accumulation in shoots and consequently did not ameliorate plant growth under iron toxic levels (Mehraban et al., 2008).

The Fe^{+2} excess causes free radical production that impairs cellular structure irreversibly and damages membranes, DNA and proteins (Arora et al., 2002; de Dorlodot et al., 2005). Increase in the amount of H_2O_2 and phenolica and decrease of chlorophyll and soluble protein content by oxidative stress have been reported by several researchers as Vichnevetskaia & Roy (1999), Schutzendubel & Polle (2002), Blokhina et al. (2003).

In terms of general averages on total chlorophyll contents, rice cultivars were ordered as Ronaldo > Osmancık-97 > Hamzadere > Biga incisi > Edirne (Table 2). According to these findings, the greatest total chlorophyll content of fresh leaves was observed in Ronaldo cultivar and the lowest in Edirne cultivar (Table 6).

Effects of increasing iron concentrations on total chlorophyll content of fresh leaves varied with the cultivars (Figure 1).

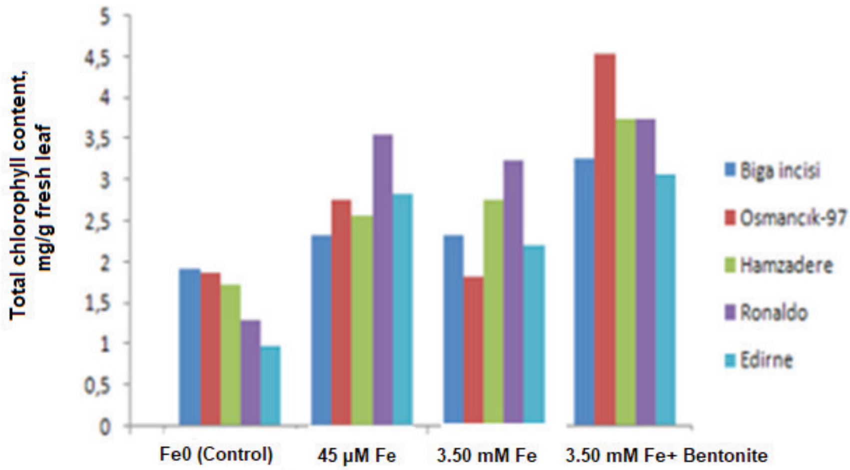

Figure 1. *Effects of increasing iron concentrations on total chlorophyll content of fresh leaves of some rice cultivars*

As can be inferred from Figure 1, under iron deficiency (Fe0) conditions, the greatest total chlorophyll content of fresh leaves was observed in Biga incisi and Osmancık-97 cultivars and the lowest in Edirne cultivar.

Under sufficient iron levels (45 µM Fe), the greatest total chlorophyll content of fresh leaves was observed in Ronaldo cultivar and the lowest in Biga incisi cultivar. Under toxic iron levels (3.50 mM Fe), the greatest total chlorophyll content of fresh leaves was observed in Ronaldo cultivar and the lowest in Osmancık-97 cultivar. Under toxic iron levels of bentonite-supplemented sand media (3.50 mM Fe + 10% Bentonite), the greatest total chlorophyll content of fresh leaves was observed in Osmancık-97 cultivar and the lowest in Edirne cultivar.

As compared to sufficient iron level, effects of toxic iron levels on total chlorophyll content of fresh leaves varied with the cultivars. As compared to sufficient iron level, the change in total chlorophyll content of fresh leaves with toxic iron treatments was calculated as 0.0% in Biga incisi; -33.8% in Osmancık-97; 7.39% in Hamzadere; -9.98% in Ronaldo and -21.6% in Edirne cultivar. Accordingly, toxic iron levels reduced total chlorophyll content of fresh leaves in Osmancık-97, Ronaldo and Edirne cultivars and increased total chlorophyll content of fresh leaves in Hamzadere cultivar as compared to sufficient iron levels.

4. Conclusions

As compared to sufficient iron level, toxic iron treatments reduced shoot dry weight by 59.19%. Average tolerance ratio of rice cultivars to toxic iron level was calculated as 40.80%. Tolerance ratio of the rice cultivars to toxic iron level was identified as 45.98% in Biga incisi, 45.39% in Osmancık-97, 42.67% in Hamzadere, 32.84% in Ronaldo and 38.61% in Edirne cultivar. As compared to sufficient iron dose, soda-activated bentonite supplementation (10%) into sand media reduced shoot dry weight by 35.05% and tolerance ratio of the cultivars to toxic iron level increased to 64.94%. Present findings revealed that soda-activated bentonite supplementation into sand media increased tolerance ratio of rice cultivars to toxic iron level. Biga incisi was identified as the most tolerant cultivar to toxic iron dose (3.50 mM Fe). On the other hand, Ronaldo rice cultivar was identified as moderately tolerant to toxic iron dose. In terms of tolerance index values to toxic iron dose in non-bentonite-supplemented sand media, rice cultivars were ordered as; Biga incisi > Edirne > Osmancık-97 > Hamzadere > Ronaldo. In non-bentonite-supplemented sand media, Ronaldo cultivar was identified as the most sensitive cultivar to toxic iron dose or iron toxicity. In bentonite-supplemented sand media, tolerance index value to toxic iron dose increased only in Edirne cultivar. In terms of tolerance index values to toxic iron dose (3.50 mM Fe) in bentonite-supplemented (10%) sand media, the greatest value was observed in Biga incisi and the lowest value in Hamzadere cultivar. In terms of tolerance index values to toxic iron dose in bentonite-supplemented sand media, present cultivars were ordered as; Biga incisi > Edirne > Osmancık-97 > Ronaldo > Hamzadere. These findings revealed that Biga incisi cultivar was more resistant to iron toxicity. Observation-based iron toxicity level of Biga incisi rice cultivar (3) was better. On the other hand, observation-based iron toxicity level of Ronaldo cultivar, the least resistant cultivar, was identified as 9. As compared to the control, sufficient iron level (45 µM Fe) significantly increased total chlorophyll content of fresh leaves. On the other hand, toxic iron level (3.50 mM Fe) significantly reduced total chlorophyll content of fresh leaves as compared to the sufficient iron level.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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