JOURNAL OF AGRICULTURAL PRODUCTION

Year: 2021 | Volume: 2 | Issue: 2 | e-ISSN 2757-6620 | agriprojournal.com

Journal of Agricultural Production

Volume: 2 Issue: 2 Year: 2021

e-ISSN: 2757-6620

agriprojournal@gmail.com
www.agriprojournal.com

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JOURNAL OF AGRICULTURAL PRODUCTION
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RESEARCH ARTICLE

Abundance of Culturable Heterotrophic Marine Bacteria in *Ulva lactuca* Associated with Farmed Seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum*

Albaris B. Tahiluddin[12*] • Tadzmahal I. Alawi[1] • Nurul Syakila A. Hassan[1] • Sitti Nurhaliza A. Jaji[1] • Ertugrul Terzi[3]

[1]Mindanao State University, Tawi-Tawi College of Technology and Oceanography, College of Fisheries, Tawi-Tawi/Philippines
[2]Kastamonu University, Institute of Science, Department of Aquaculture, Kastamonu/Turkey
[3]Kastamonu University, Faculty of Fisheries, Department of Aquaculture, Kastamonu/Turkey

ARTICLE INFO

Article History:
Received: 30.09.2021
Accepted: 09.11.2021
Available Online: 03.12.2021

Keywords:
Seaweed
Heterotrophic marine bacteria
Eucheuma denticulatum
Kappaphycus
Ulva lactuca

ABSTRACT

Ulva lactuca is one of the macro-epiphytes of farmed seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum* in Tawi-Tawi, southern Philippines, especially during the summer season, which affects the growth and health of farmed seaweeds. In this study, the abundance of culturable heterotrophic marine bacteria from *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum* was investigated in the seaweed farms of Tongsibalo, Sibutu, Tawi-Tawi, southern Philippines, using serial dilution procedure. Results revealed that the average bacterial counts obtained from *U. lactuca* associated with *Kappaphycus alvarezii, K. striatus*, and *E. denticulatum* were 2.48 x 10^{10} CFU g^{-1}, 1.14 x 10^{12} CFU g^{-1}, and 1.32 x 10^{11} CFU g^{-1}, respectively. In addition, agar-digesting bacteria were observed from *U. lactuca* samples associated with *K. alvarezii* and *K. striatus* manifested by the depression and liquefaction of the marine agar after 2-3 days which were suspected as pathogenic bacteria causing ice-ice disease. Therefore, *U. lactuca* may serve as a vector for these potential pathogens to farmed seaweeds.

Please cite this paper as follows:

Tahiluddin, A. B., Alawi, T. I., Hassan, N. S. A., Jaji, S. N. A., & Terzi, E. (2021). Abundance of Culturable Heterotrophic Marine Bacteria in *Ulva lactuca* Associated with Farmed Seaweeds *Kappaphycus* spp. and *Eucheuma denticulatum*. *Journal of Agricultural Production*, 2(2), 44-47. https://doi.org/10.29329/agripro.2021.360.1

Introduction

In the marine environment, most colonizers on the surface of macroalgae are bacteria (Armstrong et al., 2000). Macroalgae can supply food (organic matter) and oxygen that are beneficial to associated bacteria. Seaweed-associated marine bacteria play an important role in the morphogenesis and growth of seaweeds, both direct and indirect ways (Singh & Reddy, 2014). The most abundant bacterial communities on the surface of seaweeds belong to Proteobacteria and Firmicutes (Albakosh et al., 2016). Marine bacterial communities associated with seaweeds provide a vast array of benefits such as producing plant growth-promoting substances, bioactive compounds, quorum sensing signaling molecules, and other effective molecules which are responsible for the seaweeds' normal development, morphology, and growth (Singh & Reddy, 2014).

However, some pathogenic bacteria in seaweeds can lead to the development of the disease (Largo et al., 1995a; Tahiluddin & Terzi, 2021a; Tahiluddin et al., 2021). *Cythophaga-Flavobacrterium* complex and *Vibrio-Aeromonas* complex are some of the identified aetiological agents of ice-ice disease in *Kappaphycus*, especially when the seaweeds are stressed due to low or high temperature, salinity, and light intensity which can lead to the whitening of the thalli (Largo et al., 1995b; Tahiluddin & Terzi, 2021a; Tahiluddin & Terzi, 2021b). *Vibrio* species entered the tissue of seaweeds by pumping the carraginase enzyme causing the thalli to become pale and white and resulted in making the tissue soft and easily

* Corresponding author
E-mail address: albarist20@gmail.com

broken, which is called medullary seam (Yulianto & Mira, 2009). Some bacteria have been associated with decaying microalgal blooms linking to degradation processes in microalgae (Teeling et al., 2012). Largo (2002) reviewed different seaweed diseases and indicated that seaweed diseases are generally caused by microorganisms coupled with unfavorable environmental conditions.

Green alga *Ulva lactuca* is more abundant in polluted sites due to high nutrient contents (López-Gappa et al., 1990). In Tawi-Tawi, southern Philippines, *U. lactuca* is one of the macro-epiphytes, which hinders seaweed production during "green tides" outbreak, especially in the summer season as a consequence of inorganic nutrient disposal after inorganic nutrient enrichment of *Kappaphycus* (Tahiluddin, 2018). Since heterotrophic marine bacteria are usually attached to seaweeds in general, epiphytic green alga *U. lactuca* attached to farmed seaweeds *Kappaphycus* and *Eucheuma* species may contain pathogenic bacteria. It might be possible that these bacteria may contaminate and infect the farmed seaweeds that could induce ice-ice disease, a disease that threatens seaweed industry in the Philippines. Thus, this study investigated the abundance of culturable heterotrophic marine bacteria in green alga *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum*.

Materials and Methods

Study Site

The study was carried out at the seaweed farms of Tongsibalo, Sibutu, Tawi-Tawi, southern Philippines (Figure 1). Sample analysis was done at the Microbiology Laboratory, College of Fisheries (COF), Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, southern Philippines (5.1042° N, 119.8121° E) from March 22, 2019 to April 1, 2019.

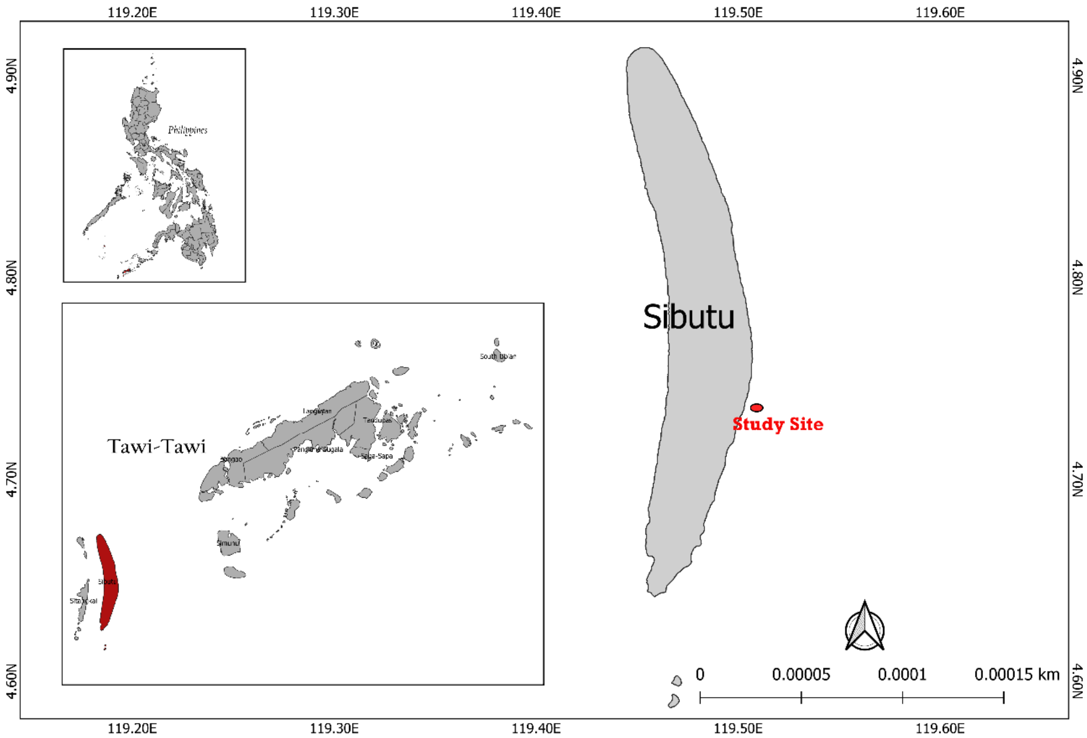

Figure 1. Map of the study area showing the study site (Source: QGIS version 3.18)

Sample Collection

Ulva lactuca samples were collected individually in three different farmed seaweeds such as *E. denticulatum*, *K. alvarezii*, and *K. striatus*. Nearly 5 g of samples were placed in different sterile containers with filtered 20 ml seawater. All samples were collected in triplicate. These were chilled inside the styrofoam using seawater ice and were immediately stored in the freezer in the study site.

Transporting of Samples

Frozen samples inside the styrofoam were transported via motor launch to Chinese Pier, Bongao, then from Chinese Pier to Microbiology Laboratory, COF, MSU-TCTO, Sanga-Sanga, Bongo, Tawi-Tawi, and was immediately stored in the freezer until analysis.

Microbiological Analysis

The microbiological analysis of seaweed samples was performed under sterile conditions. One (1) g of sample was mashed into small pieces using a sterile razor blade and placed in 9 ml diluents. Saline peptone water (0.1% peptone with 0.85% sodium chloride) was used as diluents. The microbiological cultivation was performed by taking 1 ml from the appropriate dilutions (up to eleventh dilution), consisting of 1 g of sample homogenized in 9 ml of saline peptone water, using the spread plate technique in triplicate. From each dilution, an aliquot of 0.1 ml was spread-plated on the marine agar and incubated at room temperature for 2 days (Chellaram et al., 2013). Colonies were counted manually. Colony-forming units (CFU g^{-1}) of culturable heterotrophic marine bacteria were calculated using the formula below (FDA, 2001):

$$N = \frac{\Sigma c}{[(1 \times n_1) + (0.1 \times n_2) + (0.01 \times n_3) \times (d)]} \quad (1)$$

Where, N= number of colonies for ml or g of sample,

Σc=sum of all colonies on all plates counted,

n_1=number of plates in first dilution counted,

n_2=number of plates in second dilution counted,

d=dilution from which the first counts were obtained.

Data Analysis

The data expressed in colony-forming units (CFU g^{-1}) were analyze using one-way analysis of variance using SPSS version 20.

Results and Discussion

Bacterial counts (CFU g^{-1}) from *U. lactuca* associated with farmed seaweeds are shown in Table 1. Bacterial counts from *U. lactuca* associated with farmed seaweed *K. striatus* were found to be higher in colony-forming units (1.14×10^{12} CFU g^{-1}) than that associated with *K. alvarezii* (2.48×10^{10} CFU g^{-1}) and *E. denticulatum* (1.32×10^{11} CFU g^{-1}). However, statistically, there were no significant differences among the samples from three farmed seaweeds.

Table 1. Bacterial counts (colony forming units) from *U. lactuca* associated with farmed seaweeds *Kappaphycus* spp. and *E. denticulatum*

Farm	Bacterial Count (CFU g^{-1})
K. alvarezii	2.48×10^{10}
K. striatus	1.14×10^{12}
E. denticulatum	1.32×10^{11}

The average number of bacterial counts in *U. lactuca* obtained in healthy branches of *Kappaphycus* spp. and *E. denticulatum* ranged from 10^{10} to 10^{12} CFU g^{-1}. Our results were three-fold lower than the previous study of Tahiluddin et al., (2021), where the thalli of nutrient-enriched *K. striatus* had heterotrophic marine bacterial abundance of 10^{16} CFU g^{-1}. The average number of colony-forming units of heterotrophic marine bacteria from *K. alvarezii* in healthy and ice-ice disease thalli were determined as 10^4 to 10^6 CFU g^{-1} (Largo et al., 1995a). Bacterial counts in healthy and ice-ice disease branches of *E. denticulatum* were 10^3 to 10^5 and 10^6 to 10^7, respectively. *U. lactuca* attached from *K. alvarezii* recorded the lowest bacterial counts, and this may be due to the antibacterial properties in *K. alvarezii* (Largo et al., 1995a). *K. striatus* had the highest bacterial counts, perhaps due to the slow water movement (0.06 m s^{-1}) in the farm compared with *K. alvarezii* (0.11 m s^{-1}) and *E. denticulatum* (0.14 m s^{-1}) farms. It is possible that the motile bacteria were able to colonize the *U. lactuca* and attached the farmed seaweeds when the water movement was slow (Largo et al., 1999).

Agar-digesting bacteria are reported to be associated with ice-ice disease in *Kappaphycus* (Largo et al. 1999; Tahiluddin et al., 2021) and in diseased *Gracilariopsis heteroclada* (Martinez & Padilla, 2016). In this study, agar-digesting bacteria were observed in the marine agar and created depression and liquefied after 2-3 days. These were present in both *U. lactuca* associated with *K. alvarezii* and *K. striatus* that could be one of the promoters to the development of ice-ice disease in the farmed seaweeds. Agar-digesting bacteria were not present in *U. lactuca* associated with *E. denticulatum* compared to *Kappaphycus* spp. This is maybe due to the chemical defense mechanism of *E. denticulatum,* which excretes volatile hydrocarbons that serve as a defense mechanism against epiphyte infestation and ice-ice disease (Pang et al. 2015).

Other studies identified these agar-digesting bacteria as the *Vibrio* group isolated from *K. alvarezii* (Largo et al. 1995a) and *Gilvimarinus chinensis* isolated from *K. striatus* (Tahiluddin et al., 2021). During the attack of pathogenic bacteria in farm seaweeds, after 48-72 hours, agar-digesting *Vibrio* species entered the tissue of *K. alvarezii* and *K. striatus* until the medullary seam by pumping the carraginase enzyme causing the thalli to become pale and white, and the soft tissue was easily disintegrated leading to ice-ice disease (Yulianto & Mira, 2009). This may indicate that agar-digesting bacteria from *U. lactuca* may contaminate the farmed *Kappaphycus* spp. that can cause ice-ice disease once they dominate, especially when the farmed *Kappaphycus* spp. are stress due to unfavorable environmental conditions.

Conclusion

The abundance of culturable heterotrophic marine bacteria in *Ulva lactuca* associated with farmed seaweeds *K. alvarezii*, *K. striatus*, and *E. denticulatum* were 2.48×10^{10} CFU g^{-1}, 1.14×10^{12} CFU g^{-1}, and 1.32×10^{11} CFU g^{-1}, respectively. The presence of agar-digesting bacteria in *U. lactuca* from healthy *K. alvarezii* and *K. striatus* farms may be an indicator of pathogenic bacteria that cause ice-ice disease. Therefore, *U. lactuca* may serve as a vector for these potential pathogens to farmed *Kappaphycus* spp. that can contribute to ice-ice disease development but still need to be validated and further studied.

Acknowledgment

The authors are thankful to Rosita T. Jumdain, Rosalinda P. Shariff, and Maria Liza B. Toring-Farquerabao.

Conflict of Interest

The authors declare that they have no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION

https://agriprojournal.com ISSN: 2757-6620

RESEARCH ARTICLE

Alleviation of the Germination Inhibitory Effect of Salt Stress in Pepper (*Capsicum annuum* L.) Seeds by Serotonin

Oguzhan Araz • Ertan Yildirim* • Melek Ekinci

Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Turkey

ARTICLE INFO

Article History:
Received: 24.11.2021
Accepted: 13.12.2021
Available Online: 14.12.2021

Keywords:
Germination
Pepper
Salt
Serotonin
Stress

ABSTRACT

Salinity stress is one of the important factors affecting all growth processes, from seed germination to seedling development, plant growth, yield and quality. In this study, the effects of serotonin treatments on the germination of pepper seeds (*Capsicum annum* L.) under salt stress were investigated. Different doses of salt (0, 75 and 150 mM NaCl) and serotonin (S0:0 μM, S1:5 μM, S2:10 μM, S3:15 μM, S4: 20 μM) were used. The applied seeds were placed between the papers in petri dishes, watered with the prepared salt solutions and left to germinate at 25 °C. In the study, parameters related to germination percentage, germination speed, mean germination time, daily mean germination time, peak value and germination value were investigated. As a result of the research, it was determined that the germination of pepper seeds decreased in parallel with increasing salt concentrations, and this negative effect of salt stress decreased with serotonin applications. Although it changes depending on the serotonin doses, it has been observed that significant effects occur on the measured germination parameters, and the best germination was observed at S1 and S2 doses. It is thought that the application of serotonin will have positive effects on the germination of pepper seeds under salt stress, and these effects may also occur during the plant growth period.

Please cite this paper as follows:

Araz, O, Yildirim, E., & Ekinci, M. (2021). Alleviation of the germination inhibitory effect of salt stress in pepper (*Capsicum annuum* L.) seeds by serotonin. *Journal of Agricultural Production*, 2(2), 48-54. https://doi.org/10.29329/agripro.2021.360.2

Introduction

Plants are exposed to numerous stresses from seed germination to their entire life cycle. Salinity, which is one of these factors, affects all processes including seed germination, root, leaf, fruit and seed formation etc.

Salinity causes various morphological, physiological and biochemical reactions in plants by showing osmotic and ionic effects on plants. High salt concentration causes a decrease in the water potential in the soil and a decrease in the absorption of water by the plant roots. With the osmotic effect of salinity, water deficiency occurs and causes a decrease in water potential and turgor, fading, stomatal closure and decreased cell growth (Parihar et al., 2015; Ibrahimova et al., 2021). The ionic effect of salinity, on the other hand, changes the K^+/Na^+ ratio in plant cells, causing an increase in Na^+ concentrations and disruption of cellular homeostasis (James et al., 2011). With these effects, salinity can cause deterioration of plant metabolism and death of the plant.

Many researchers have done research on the effects of salinity stress on different plants, and it has been tried to determine the tolerance levels of plants to salt stress and ways to increase their tolerance. However, in recent studies, it has been focused on that some substances applied to plants can alleviate the damage caused by salt stress on the plant. The use of exogenous hormones to increase plant stress tolerance is one of these applications.

Serotonin (5-hydroxytryptamine; 5-HT) is an indoleamine (derived from tryptophan) with a strong antioxidant function, and it was first identified as a neurotransmitter signaling molecule in mammals and later determined to be present in all living things. Different amounts of serotonin accumulate in different parts of plants such as roots, stems, leaves, fruits and seeds (Abbasi et al., 2020; Roychoudhury, 2021). Serotonin is

* Corresponding author
E-mail address: ertanyil@atauni.edu.tr

involved in all vital activities in plants, affects plant growth and development, and also responds to biotic and abiotic stress in the plant (Erland et al., 2016; Roychoudhury, 2021). Serotonin, as a plant growth regulator and a stress defense molecule, affects plant morphogenesis, vegetative growth, reproduction, seed germination and ensures survival in abiotic and biotic stress conditions (Erland & Saxena, 2017).

The biosynthesis of serotonin in the plant takes place in the chloroplasts and mitochondria of various tissues, where highly reactive oxygen species (ROS) are produced in the tissue. With the antioxidative effect of serotonin, it detoxifies the ROS in the organelles and ensures better development (Roychoudhury, 2021).

The effects of serotonin in increasing tolerance or reducing the damage level against various abiotic stresses in plants have been determined in some plant species. In the studies, it was determined that the effect of serotonin in plants is important in the case of salt stress in sunflower (Mukherjee et al., 2014) and rapeseed (Liu et al., 2021), cold stress in rapeseed (He et al., 2021), and heat stress in soybean (Kumar et al., 2021). Exogenous application of 200 µmol/L serotonin in *Brassica napus* L. under salt stress alleviated the growth inhibition caused by salinity, increased the fresh and dry weights of roots and shoots, and activated the enzyme system (Liu et al., 2021). It has been determined that salinity prevents oxidative damage in the integrity of chlorophyll and cell membrane in the plant, with the application of serotonin, clearing the reactive oxygen species and osmotic pressure regulation, thus promoting growth (Liu et al., 2021). There are findings that serotonin application could alleviate the effects of salt stress on plants (Mukherjee et al., 2014; Liu et al., 2021). However, it is not known exactly what the effect of serotonin application is on alleviating the damage of salt stress in pepper. For this reason, the effects of serotonin application on the germination of pepper seeds under salt stress were investigated in this study.

Materials and Methods

Pepper (*Capsicum annuum* L. cv. Yalova) seeds were used as plant material in the study. Firstly, the seeds were kept in 3% sodium hypochlorite for 5 minutes for surface disinfection and then washed several times with distilled water. Surface disinfected seeds were kept in solutions of different serotonin (Sigma, CAS: 153-98-0) doses (S0: 0 µM, S1: 5 µM, S2: 10 µM, S3: 15 µM and S4: 20 µM) for 14 hours. Afterwards, the seeds were washed with distilled water and left to dry, and then placed between the paper in the petri dish. The seeds sown in petri dishes were irrigated with 10 ml solutions with different salt levels (I0: 0, I1: 75 and I2: 150 mM NaCl). The sown seeds were left to germinate at 25 °C. The study was performed in 4 replications for each application and 50 seeds in each replication according to ISTA (1996), and germination counts were started on the 7th day and completed on the 14th day. Germination percentage, germination speed, mean germination time, average daily germination, peak value and germination value were calculated by using the formulas given below by counting the germinated seeds every day (Czabator, 1962; Ellis & Roberts, 1981; Gairola et al., 2011).

$$Germination\ percentage\ (\%)(GP) = \left(\frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\right) x\ 100 \quad (1)$$

$$Germination\ speed\ (GS) = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{s3} + \cdots \quad (2)$$

$$Mean\ germination\ time\ (MGT) = \frac{n1xd1 + n2xd2 + n3xd3 + \cdots}{Total\ days} \quad (3)$$

$$Mean\ daily\ germination\ (MDG) = \frac{Total\ number\ of\ germinated\ seeds}{Total\ days} \quad (4)$$

$$Peak\ value\ (PV) = \frac{Highest\ germination}{Number\ of\ days} \quad (5)$$

$$Germination\ value\ (GV) = PVXMDG \quad (6)$$

Where, n: number of germinated seeds, d: days.

After the Arcsin transformation of germination percentages, statistical analysis was performed. Variance analysis was made by SPSS. The average of the obtained data was taken and the comparison was made according to the Duncan Multiple comparison test at a confidence interval of 95.0% (SPSS, 2010).

Results and Discussion

In the study, the effects of serotonin treatments on seed germination properties under salt stress were investigated, and the results are given in Table 1 and Figure 1-5. Significant reductions in germination percentage, speed, daily germination, peak value and germination value have occurred with salt stress, and the mean germination time has increased (Table 1). When the application averages of serotonin were evaluated, the application of serotonin caused an increase in germination characteristics compared to the control without application, and significant increases in germination occurred especially with S1 and S2 applications (Table 1).

Table 1. Mean values of serotonin and salt treatment on parameters of seed germination

		GP	GS	MGT	MDG	PV	GV
Serotonin Mean	S0	83.50 c	2.60 b	13.32 ns	2.89 b	0.79 b	2.50 b
	S1	92.17 a	3.05 a	12.14	3.19 a	1.21 a	3.77 a
	S2	91.33 a	2.99 a	12.37	3.16 a	1.25 a	3.82 a
	S3	88.36 b	2.86 a	12.28	3.15 a	1.03 ab	3.66 a
	S4	88.31 b	2.91 a	12.47	3.12 a	0.86 b	3.53 a
Salt Mean	I0	89.80 A	3.12 A	11.70 B	3.19 A	1.33 A	4.36 A
	I1	90.60 A	3.01 A	12.48 B	3.12 AB	1.12 B	3.97 B
	I2	85.80 B	2.52 A	13.38 A	2.99 B	0.63 C	2.03 C

There is no statistical difference between means shown with the same lowercase and uppercase letters in the same column ($p<0.001$).
S0: 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin, **I0:** 0 mM NaCl, **I0:** 75 mM NaCl, **I0:** 150 mM NaCl

The germination percentage (GP) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on GP in salty conditions. In the highest salt (150 mM) condition, while the GP was 79% in control, it was 91% with S1, 91% with S2, 83.5% with S3 and 84.5% with S4 (Figure 1).

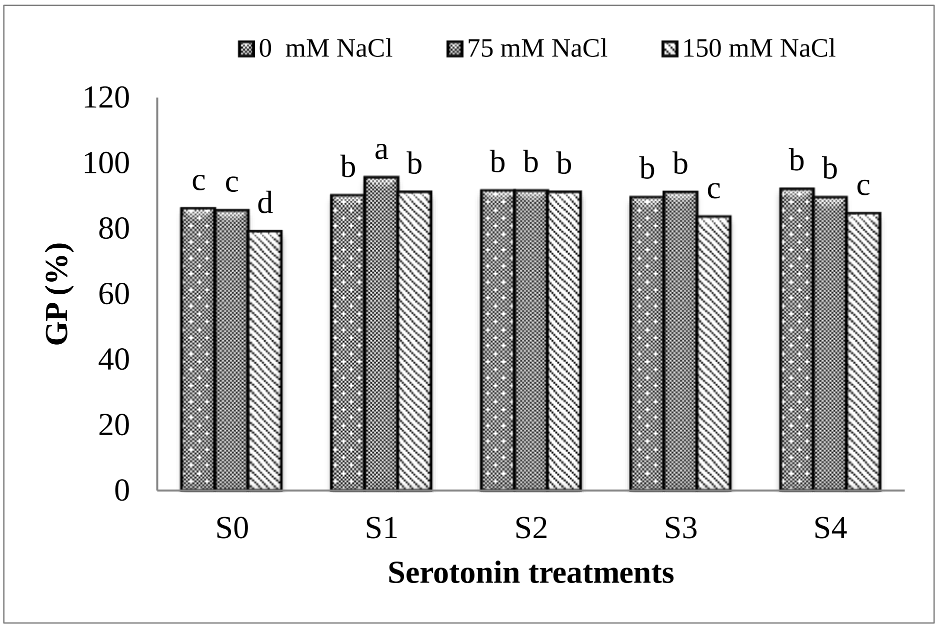

Figure 1. Effects of serotonin and salt treatment on seed germination percentage. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

In the study, it was determined that the germination speed (GS) decreased with salinity, and serotonin applications alleviated this negative effect on GS in salty conditions. While the GS was 2.11 in control, were 2.81, 2.82, 2.41 and 2.45 in the S1, S2, S3 and S4 treatments, respectively under 150 mM condition (Figure 2).

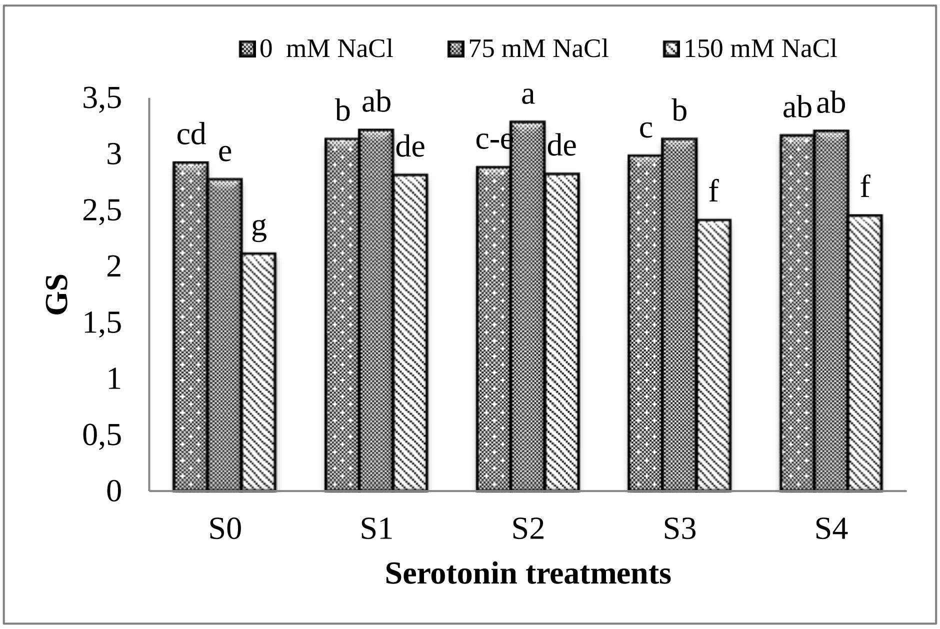

Figure 2. Effects of serotonin and salt treatment on seed germination speed. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

Mean germination time (MGT) increased with salinity, MGT was 11.37 at 0 mM NaCl, 12.08 at 75 mM NaCl and 13.76 at 150 mM NaCl. However, this increase in MGT was lower than the control with serotonin treatments. Especially in S3 application, MGT was lower in 150 mM salt environment compared to other treatments (Figure 3).

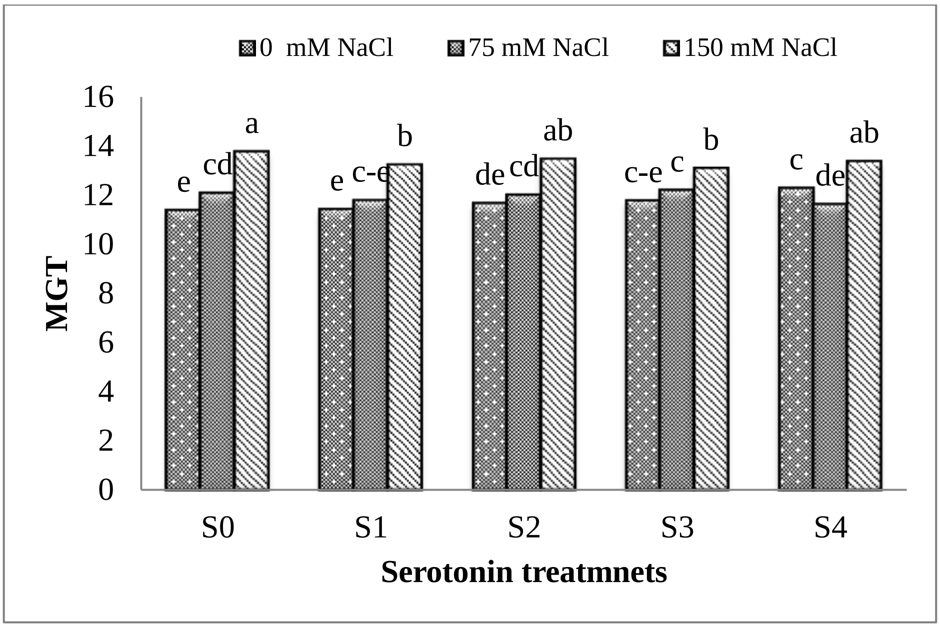

Figure 3. Effects of serotonin and salt treatment on mean germination time. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

In the study, it was determined that mean daily germination (MDG) decreased with salinity, and serotonin applications alleviated this negative effect on MDG in salty conditions. While MDG was 2.74 in control, were 3.07, 3.25, 2.96 and 2.93 in the S1, S2, S3 and S4 treatments, respectively under 150 mM condition (Figure 4).

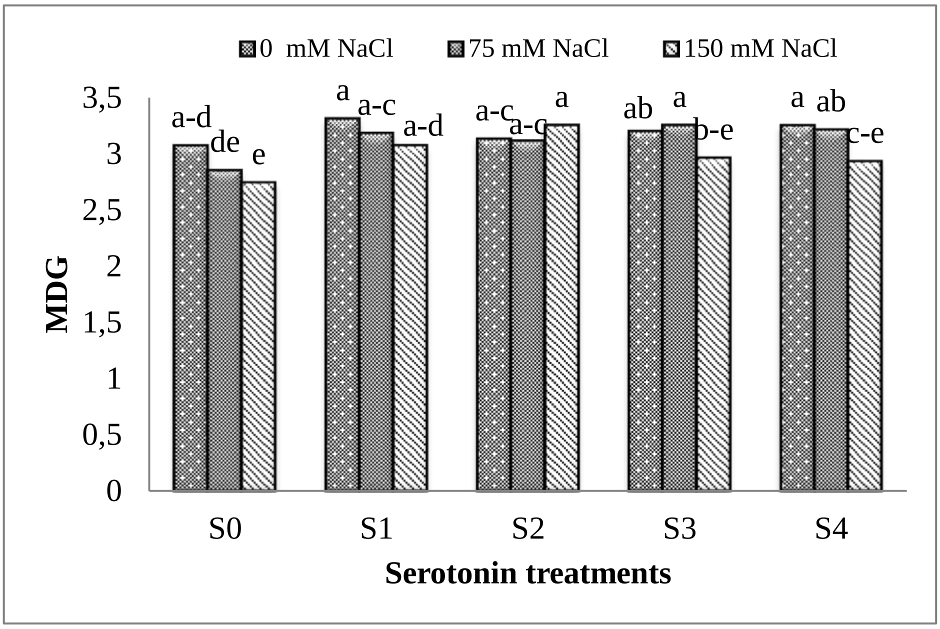

Figure 4. Effects of serotonin and salt treatment on mean daily germination. There is no statistical difference between means shown with the same letters on bars (p<0.001). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

The peak value (PV) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on PV in salty conditions. PV was 1.15 at 0 mM NaCl, 0.95 at 75 mM NaCl and 0.28 at 150 mM NaCl. PV increased with increasing salinity in serotonin treatments, in the highest salt (150 mM) condition, PV was 0.73 with S1, 0.88 with S2, 0.58 with S3 and 0.67 with S4 (Figure 5).

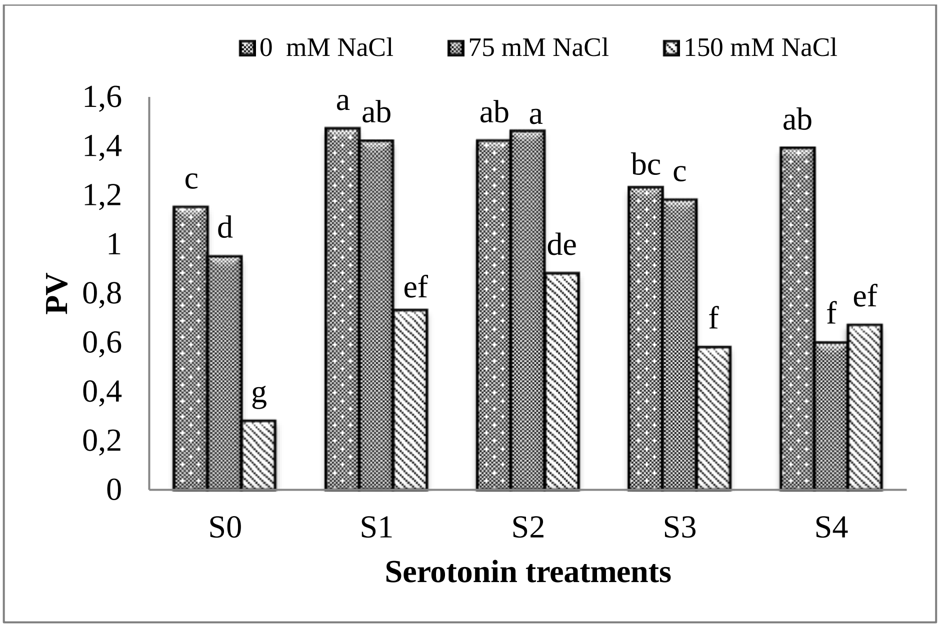

Figure 5. Effects of serotonin and salt treatment on peak value. There is no statistical difference between means shown with the same letters on bars ($p<0.001$). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

Similarly, germination value (GV) of pepper seeds decreased with salinity, and serotonin applications alleviated this negative effect on GV in salty conditions. GV was 3.51 at 0 mM NaCl, 3.12 at 75 mM NaCl and 0.88 at 150 mM NaCl. GV increased with increasing salinity in serotonin treatments, in the highest salt (150 mM NaCl) condition, GV was 2.48 with S1, 2.85 with S2, 1.84 with S3 and 2.12 with S4 (Figure 6).

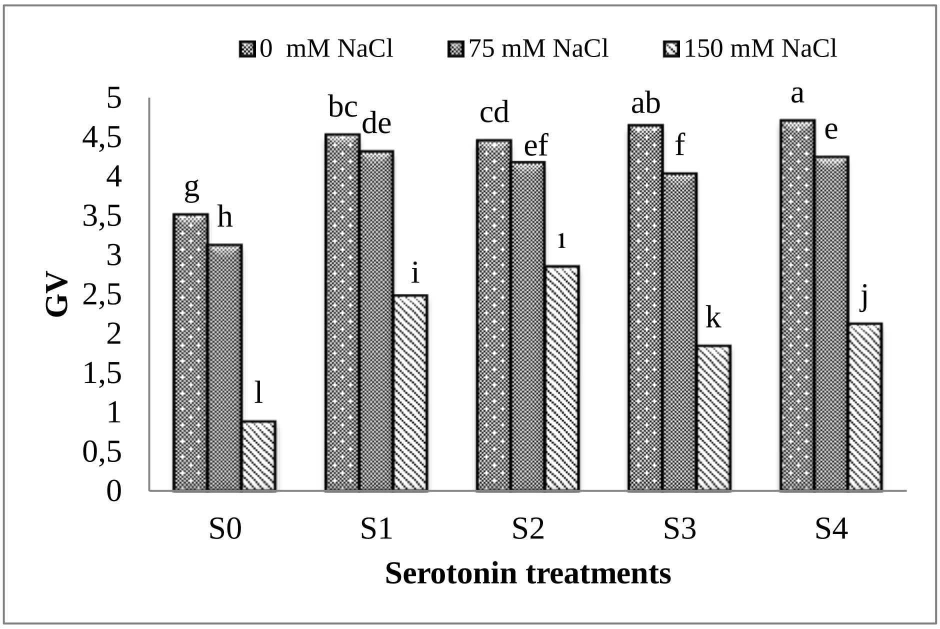

Figure 6. Effects of serotonin and salt treatment on germination value. There is no statistical difference between means shown with the same letters on bars ($p<0.001$). **S0:** 0 µM Serotonin, **S1:** 5 µM Serotonin, **S2:** 10 µM Serotonin, **S3:**15 µM Serotonin, **S4:** 20 µM Serotonin

There was a significant decrease in the germination properties of pepper seeds with salinity. This negative effect of salt stress on germination of pepper seeds was also determined by Yilmaz et al. (2004), Aloui et al. (2014) and Aminifard & Bayat (2020). Salt stress shows its effects on the germination and germination percentage, which is the first and most important stage of plant development, and causes changes in some important processes in plant development such as root dry weight, shoot dry weight, Na^{+}/K^{+} ratio (Parida & Das, 2005). Salinity causes osmotic or ion toxicity and changes in enzyme activities in seed germination stage (Singh et al., 2012). However, it was determined that the negative effect of salt stress on germination was alleviated with the treatment of exogenous serotonin in this study. As a matter of fact, serotonin plays a role as a plant growth regulator at every stage of plant life such as germination, vegetative phase, reproduction and aging, and provides a defense or anti-stress effect with its antioxidative effect (Roychoudhury, 2021). As a plant growth regulator with cytokinin-like activity, serotonin is involved in various growth processes such as root and shoot formation, cell division and differentiation, germination, somatic embryogenesis and senescence (Roychoudhury, 2021). Serotonin can also provide salt stress tolerance by reducing ROS detoxification with the effect of antioxidant enzyme activity in the plant. It has been stated that serotonin provides its effect in reducing salinity in rapeseed seedlings by scavenging ROS, regulating osmotic pressure and promoting growth (Liu et al., 2021). Similarly, serotonin, with its ability to mediate the influx of ions into the chloroplasts, has been suggested to have the capacity to improve survival in response to salinity challenge (Erland & Sexana, 2017).

Conclusion

The effects of serotonin application on the germination of pepper seeds under salt stress were investigated. The germination of pepper seeds decreased in parallel with increasing salt concentrations, and this negative effect of salt stress decreased with serotonin applications. Although it is known that serotonin is involved in various metabolic, physiological and biochemical processes in plants and has an effect against various stresses in plants, its effect on abiotic stresses, especially salt stress, is not well known. In this study, it is concluded that serotonin could alleviate the effects of salinity in pepper seeds. No study has been found on the application of serotonin in salt stress in pepper. Therefore, the data obtained from this study can be a reference for other studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION
https://agriprojournal.com ISSN: 2757-6620

RESEARCH ARTICLE

The Comparison of Structure of Existing Stable with Standards of Stable Requested in IPARD Program in Erzurum Central Districts, Turkey

Erkan Efekan • Bahar Kocaman*

Atatürk University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, Erzurum/Turkey

ARTICLE INFO

Article History:
Received: 06.12.2021
Accepted: 24.12.2021
Available Online: 27.12.2021

Keywords:
EU standards
IPARD
Rural development
Stables

ABSTRACT

Erzurum is one of the provinces in Turkey where the Instrument for Pre-Accession Assistance Rural Development (IPARD) program will be implemented in the first stage. In this study, we determined the status of the existing enterprises in Erzurum and measured their capacity to comply with the IPARD program in relation to the "Investments for Restructuring Agricultural Enterprises and Reaching Community Standards" measure. This was conducted in 33 barns in 11 villages in the central districts of Erzurum Province. The current situation of animal shelters in central districts of Erzurum province was compared with European Union (EU) standards required in animal shelters. In addition, rural development and the basis on which documents give directions in Turkey, IPARD, and IPARD measures related to the subject of study are given general information. It has been concluded that the existing animal shelters in Erzurum are generally far from the EU standards required within the scope of the IPARD program, and it is challenging for existing animal shelters to meet EU standards by modernizing them. Therefore, in Erzurum province, it will be appropriate for the livestock enterprises that want to take advantage of the IPARD program's "Investments for the Restructuring of Agricultural Enterprises and Reaching Community Standards" measure, instead of modernizing their existing shelters, which the Program allows, by building new animal shelters. In addition, the Agriculture and Rural Development Support Institution, which is the implementer of the IPARD program, should inform the enterprises wishing to benefit from the program to build new animal shelters.

Please cite this paper as follows:

Efekan, E., & Kocaman, B. (2021). The comparison of structure of existing stable with standards of stable requested in IPARD program in Erzurum central districts, Turkey. *Journal of Agricultural Production*, 2(2), 55-66. https://doi.org/10.29329/agripro.2021.360.3

Introduction

Farming has an important place and potential in Turkey's agricultural sector. Carrying out animal production activities causes positive effects such as the evaluation of some herbal and by-products, increasing labor productivity and operating profit, reducing the risk factor arising from natural and economic conditions withal it is important in terms of balanced nutrition and public health (Vural & Fidan, 2007).

There are two available ways to increase animal productions. The first is to increase the number of animals. However, this situation will reveal the need for new shelter and area which requires high costs due to the increasing number of animals. The second and recommended way is to ensure that animal shelters have suitable environmental conditions for animals (Kayar, 2011).

Environmental conditions in traditional barns are primitive and unhealthy in terms of animal welfare. Small and medium businesses have handicaps in securing the necessary financing for the modernization of shelter, feeding, parlor equipment, and cooling facilities due to difficulties in obtaining commercial loans (Ekmekyapar, 1991).

Turkish agriculture is faced to a serious competition problem against both EU and world markets, so it has to carry out especially the institutional structuring and programming

* Corresponding author
E-mail address: bkocaman@atauni.edu.tr

that will use in the most effective way the EU resources to improve the rural and agricultural infrastructure and agricultural business structures (Akyüz, 1998). It is a misunderstanding that the IPARD program will be the solution to all the problems of the agricultural sector and rural living areas in Turkey. However, if the needs analysis is done correctly and realistically, the institution that will operate the implementation mechanism is created on a careful and appropriate basis, and the planning is prepared in accordance with the needs with wide participation, this financial support, which can be considered in significant amounts for Turkey's agriculture and rural areas will be used appropriately and effectively (Can, 2007).

IPARD provides support to small and medium-sized enterprises to improve their production techniques and operations in order to ensure their economic efficiency and long-term sustainability, and continuity of their existence. In terms of opening Turkish agriculture to the EU Common Market, it will also help agricultural businesses meet community standards for quality management, hygiene, food safety, animal welfare, environmental protection, and occupational safety (Anonymous, 2008).

The Instrument for Pre-Accession Assistance (IPA) was established by the EU to support Candidate and potential candidate countries within the framework of Council Regulation No 1085/2006. IPA support includes five components. Since Turkey is in the status of candidate country in Annex 1 of the IPA regulation, it can benefit from all components. IPARD (IPA's Rural Development component) supports the preparations for harmonization and policy development for the implementation and management of the EU's Common Agricultural Policy, Rural Development Policy, and related policies. IPARD support within the scope of this study was implemented in 2007-2013. The IPARD program has been designed considering the country's priorities and needs in the pre-accession period in the context of rural development. The program included a seven-year period between 2007-2013 (Anonymous, 2008).

Materials and Methods

Erzurum Province is located between 39.10°- 40.57° north latitudes and 40.15°- 42.35° east longitudes. Erzurum constitutes the western half of the Erzurum-Kars section, which is located in the northeastern part of the Eastern Anatolia Region. The total land size of the province, which is located in the starting area of the Çoruh, Fırat, and Aras basins, is 25,330.9 km^2. Erzurum, which has 966 villages, has a total of 35 municipalities. There are 20 districts in Erzurum, 3 of which are the Central District (Anonymous, 2011).

This research was carried out in 33 barns in 11 villages of 3 Central Districts (Palandoken, Yakutiye, and Aziziye) in Erzurum Province, Turkey. The villages that are the subject of the research and some of their characteristics are shown in Table 1.

Table 1. Some characteristics of the researched villages

Number	District Name	Village Name	Distance to District Center (km)	Number of Households of the Village	The Population of the Village	Altitude	Lowest and Highest Temperatures (°C)	
							Lowest	Highest
1	Aziziye	Pasayurdu	7	59	330	1850	-30	30
2	Aziziye	Gelinkaya	18	110	355	1855	-31	30
3	Aziziye	Kabaktepe	80	33	178	1910	-32	29
4	Aziziye	Sirli	69	28	113	1910	-32	29
5	Yakutiye	Gungormez	35	22	53	2000	-34	30
6	Yakutiye	Akdag	25	50	297	2000	-34	30
7	Yakutiye	Guzelyayla	35	50	168	2000	-34	30
8	Yakutiye	Karagobek	32	50	179	2000	-34	30
9	Yakutiye	Koşk	30	60	345	2000	-34	30
10	Palandoken	Derebogazi	25	200	967	1953	-28	34.1
11	Palandoken	Guzelyurt	27	65	294	1953	-28	34.1

Erzurum province has hot and dry summers and cold and snowy winters. The average number of days with snowfall is 51.8, and the average number of days covered with snow is 112.3. The average annual precipitation of Erzurum Province is 33.96 kg/m^2, and the highest precipitation is in May with an average of 68.1 kg/m^2, and the least precipitation is in August with 17 kg/m^2 (Anonymous, 2012).

The area of Erzurum is 2,533,090 ha. 64% of its total land is meadow and pasture land, and it constitutes 13% of the existing pastures in Turkey. Total agricultural land is 460,252 ha, 305,636 ha of which are suitable for irrigation; however, the irrigated area is only 15,672 ha (Anonymous, 2011).

Animal production, which constitutes 64% of Erzurum's agricultural economy, is one of the main livelihoods. However, since the province's rural areas are not developed enough, the animals have not reached adequate care, feeding, and welfare conditions. The number of the total cattle stock of the province is 538,000 as of 2010. The number of animals in the researched villages is shown in Table 2 in detail (Anonymous, 2011).

Table 2. Animal assets of the research subject villages (Anonymous, 2011)

Number	District Name	Village Name	Cattle Presence									Number of Businesses
			Culture Breed				Hybrid Breed				Native Breed	
			Cow >24 month	Heifer 12-24 month	Calf 6-12 month	Calf 0-6 month	Cow >24 month	Heifer 12-24 month	Calf 6-12 month	Calf 0-6 month	Cow >24 month	
1	Aziziye	Pasayurdu	56	7	5	30	170	65	42	76	59	54
2	Aziziye	Gelinkaya	24	4	-	46	252	113	6	108	134	105
3	Aziziye	Kabaktepe	59	11	-	69	257	5	8	94	137	65
4	Aziziye	Sirli	61	8	-	30	161	64	8	142	189	61
5	Yakutiye	Gungormez	30	30	15	25	289	55	45	30	-	22
6	Yakutiye	Akdag	72	27	30	45	626	160	29	47	95	80
7	Yakutiye	Guzelyayla	72	39	10	111	508	84	117	25	-	47
8	Yakutiye	Karagobek	63	30	8	70	320	95	5	202	52	68
9	Yakutiye	Koşk	90	120	35	80	620	140	35	90	110	86
10	Palandoken	Derebogazi	56	7	5	30	170	65	42	76	59	54
11	Palandoken	Guzelyurt	24	4	-	46	252	113	6	108	134	105

According to the IPARD Program, within the scope of the Investments for Restructuring of Agricultural Enterprises and Reaching Community Standards (Measure Code: 101), the agricultural enterprises that will benefit from this measure must be located in the rural area of the province where the investment will be implemented. For this reason, settlements that were previously in village status but are now in neighborhood status are not included in the scope of the study because they are outside the IPARD program.

In this context, the lists of animal assets of all enterprises in these villages were obtained from the Provincial Directorate of Agriculture through TÜRKVET (Veterinary Information System). Then, the animal assets of the agricultural enterprises in the villages included in the IPARD program in the central districts of Erzurum were examined.

Agricultural Enterprises Producing Milk and Meat are supported separately within the scope of the Investments for Restructuring of Agricultural Enterprises and Reaching Community Standards (Measure Code: 101).

The specific eligibility criteria of these sub-measures were examined. According to this dairy-producing enterprises must have at least 10 and no more than 100 animals, and those producing meat must have at least 30 and no more than 250 animals. For this reason, in order for the enterprises to benefit from the IPARD program, it was selected enterprises which have at least 30 and at most 100 animals in the villages subject to the research.

In this context, a total of 11 villages were determined 3 from Palandöken district (Derebogazi, Guzelyurt), 5 from Yakutiye district (Gungormez, Akdag, Guzelyayla, Karagobek, Koşk) and 4 from Aziziye district (Pasayurdu, Gelinkaya, Kabaktepe, Sirli). Then, 3 barns that could represent each village were selected and a total of 33 barns were studied.

The research was carried out in January, February, and March of 2011. During the period of the research, 33 barns were visited every month. In January, the locations and altitudes of the barns were determined with a hand-held GPS device. In February, the physical properties of the barns were measured with a laser meter. In January, February, and March, temperature, light, and humidity measurements of the barns were made.

Then, the values measured during the study with the EU standard values required by the IPARD program were separately compared, and similar and different points were determined.

Finally, the facilities and problems that will arise in the implementation of the IPARD support program in the region have been determined based on these differences and similarities.

Results and Discussion

It has been observed that closed type and tied-stall systems are applied in all of the barns. The measurements made in the barns were prepared in the form of general tables in order to see the current situation quickly and effectively. The tables prepared for the measured values are given in Tables 3, 4, and 5.

Table 3. The physical properties of the research barns

Number	Barn Dimensions			Feeder Dimensions				Wall Thickness (cm)	Roof Style (cm)	Free stall Dimensions	
	Width (cm)	Length (cm)	Height (cm)	Width (cm)	Depth (cm)	Height from Ground (cm)	Height of Feeder (cm)			Range (cm)	Number
1	625	935	360	32	26	67	41	60 Stone	Wooden Triangle without Slab	103	17
2	600	1140	330	26	26	74	48	25 Briquette		90	18
3	810	2130	335	37	30	70	40	50 Stone	Triangle with Concrete Slab	100	42
4	612	904	350	43	20	54	34	60 Stone	Wooden Triangle without Slab	100	16
5	623	1027	385	34	20	60	40	60 Stone	Wooden Pentagon without Slab	100	20
6	680	709	300	46	23	61	42	50 Stone	Wooden Porch without Slab	100	13
7	1260	2020	372	165	30	50	20	60 Stone	Under the House	125	64
8	530	800	375	32	22	68	48	60 Stone	Wooden Triangle without Slab	100	14
9	615	1280	373	44	31	77	46	60 Stone		105	24
10	774	1355	360	45	28	76	48	60 Stone		95	28
11	844	1865	400	47	34	47	13	25 Briquette		65	30
12	742	1113	345	47	24	80	56	60 Stone		125	18
13	640	1178	309	41	30	69	39	70 Stone		120	18
14	650	1620	370	41	29	70	41	80 Stone		100	30
15	635	2285	404	40	31	55	24	70 Stone	Triangle with Concrete Slab	110	41
16	640	720	348	34	29	79	50	70 Stone	Wooden Triangle without Slab	115	11
17	715	1480	395	37	30	47	17	60 Stone		120	24
18	840	1640	340	40	27	63	36	70 Stone	Triangle with Concrete Slab	110	27
19	600	1500	340	44	32	65	33	50 Stone	Wooden Triangle without Slab	105	35
20	418	645	327	42	28	68	40	60 Stone		120	9
21	720	950	363	44	44	55	11	60 Stone		95	19
22	630	1430	300	33	25	67	42	70 Stone	Triangle with Concrete Slab	82	32
23	940	2260	310	43	32	62	30	60 Stone	Wooden Triangle without Slab	88	50
24	880	1900	300	44	30	59	29	70 Stone	Triangle with Concrete Slab	90	47
25	740	1050	335	46	27	65	38	70 Stone	Wooden Triangle without Slab	105	20
26	785	836	334	43	35	55	20	70 Stone		76	22
27	-	-	-	-	-	-	-	-	-	-	-
28	617	1200	370	57	23	63	40	60 Stone	Wooden Triangle without Slab	100	21
29	790	2620	480	40	30	50	20	30 Briquette		180	25
30	755	1540	410	31	20	51	31	40 Brick		110	26
31	600	1200	400	34	27	69	42	60 Stone		110	21
32	550	1110	350	36	30	68	38	60 Stone		110	16
33	654	1695	400	37	26	64	38	60 Stone		110	29

Table 4. Door, window, chimney dimensions, waterer, and lighting conditions of barns

Number	Door		Window			Flue			Waterer/ Lighting	
	Width (cm)	Height (cm)	Width (cm)	Height (cm)	Number	Width (cm)	Height (cm)	Number	Number	Number
1	100	180	59	97	2	50	40	2	1 F	2 B
2	100	172	68	144	1	-	-	-	1 F	1 B
3	120	190	80	90	7	40	30	3	1 F	2 B
4	120	180	50	50	1	50	50	1	1 F	1 B
5	114	180	70	100	3	50	70	1	-	1 B
6	100	163	100	50	2	-	-	-	-	1 B
7	322	210	110	90	10	25	25	4	33 W	9 B
8	90	185	50	80	1	50	80	3	-	1 B
9	87	175	100	100	1	60	60	1	-	1 B
10	100	200	100	100	4	120	100	1	1 F	1 B
11	130	220	77	190	6	40	40	3	1 F	3 B
12	120	195	100	100	1	-	-	-	1 F	1 B
13	100	180	70	90	3	80	50	1	1 F	2 B
14	120	190	80	100	3	-	-	-	1 F	2 B
15	110	180	130	120	6	30	30	3	1 F	5 B
16	110	170	60	70	1	20	20	1	1 F	1 B
17	120	170	-	-	-	130	70	2	1 F	2 Fl
18	100	190	70	100	2	60	70	1	1 F	2 Fl
19	110	180	100	50	3	100	40	2	1 F	3 B
20	100	170	80	80	1	-	-	-	1 F	1 B
21	100	170	50	120	1	80	100	1	1 F	2 B
22	130	190	80	90	1	-	-	-	1 F	2 B
23	120	250	80	100	4	70	70	1	1 F	2 B
						50	70	2		
24	155	195	80	90	3	50	50	2	1 F	2 B
25	100	180	50	70	2	50	60	1	1 F	1 B
26	100	200	70	40	2	-40	40	1	1 F	1 B
27	-	-	-	-	-	-	-	-	-	-
28	120	190	50	80	2	70	80	1	1 F	1 B
29	170	200	90	170	7	100	170	3	25 W	3 B
30	110	190	100	100	2	50	50	1	1 F	1 B
31	110	180	100	90	2	100	60	1	1 F	2 B
32	100	180	100	50	2	50	50	1	1 F	1 B
33	110	190	90	110	3	100	100	1	1 F	1 B

F: Faucet, W: Waterer, B: Bulb (100W), Fl: Florasan

Table 5. Temperature, light, and humidity measurements inside the barn

No	Date			Indoor humidity %			Outdoor humidity %			Indoor Temperature (°C)			Outdoor Temperature (°C)			Indoor Light (Lux)			Outdoor Temperature (x100 Lux)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	22.01	23.02	25.03	75	78	58	39	52	49	17.9	18.8	16.9	2.3	6	1	187.5	150	250	226	190	250
2	22.01	23.02	25.03	87	90	100	39	50	52	17	20.2	17.7	2	6	1	75	54	68	225	185	260
3	22.01	23:02	25.03	85	85	89	42	50	50	15.7	18	15.2	2.3	5.2	1.5	37	20	23	265	177	230
4	22.01	23.02	25.03	87.5	94	99	39.2	49	50	17.9	17.4	16	2.6	4.7	1	54	10	30	230	120	200
5	22,01	23.02	25.03	78	99	94	37.5	55	49	19.3	21	17.5	2	5	1	25	15	18	225	125	180
6	22.01	23.02	25.03	82	83	82	42	52	51	17.2	17.2	17	1.5	4.8	1.2	19	10	13	160	80	100
7	23.01	24.02	26:03	87.5	97	55	52	58	42	13	16.4	21	0.5	3	6	126	45	138	680	120	750
8	23.01	24.02	26.03	71.5	77.5	58.5	43	50	38	18.1	17	18.5	1	2.8	5.7	5	1	4	650	125	620
9	23.01	24.02	26.03	70.5	84	66	40	53	41	17	18	14	1	3	5.5	55	1	50	670	90	650
10	23.01	24.02	26.03	82.5	96.5	81.5	30	53	42	19.8	19	18.8	1	5	5	105	90	100	700	600	680
11	23.01	24.02	26.03	82.5	95.5	81.5	27	51	42	14.4	16.2	16.5	1.5	3	4.5	230	205	190	710	650	570
12	23.01	24.02	26.03	75.5	89	95	29	47	43	17.6	19.1	16.2	1.5	3.3	5	9	1	5	685	500	580
13	23.01	24.02	26.03	70	98	85	38.5	68	56	17.6	16	17	0	1	2	13	10	15	500	465	750
14	23.01	24.02	26.03	98	95	95	37	65	52	16	18.5	18	-1	1	2	5	7	10	350	450	720
15	23.01	24.02	26.03	72.5	82	89	39	62	50	11.1	14.2	15.7	-1	1	2.5	43	40	51	300	420	700
16	24.01	24.02	26.03	67.5	98	70.5	49	60	56	18.6	15	13	1	1.5	2	7	15	20	325	430	685
17	24.01	24.02	26.03	70.5	83	71.5	45	65	55	11.6	13.3	14	1	1	2	2	1	4	300	420	620
18	24.01	24.02	26.03	72	82	61	48	62	55	12.5	14.5	16	0	1	2	8	12	15	320	450	580
19	24.01	24.02	26.03	75	73	75	46	57	49	18.8	19.2	18.1	1	1	2.5	10	8	15	200	405	690
20	24.01	24.02	26.03	95	72	75	45	58	43	17	18.4	17	1	1	2.5	1	5	8	230	450	700
21	24.01	24.02	26.03	85	85	84	45	51	50	20	20.6	19.3	1	1	3	5	15	25	240	445	745
22	25.01	25.02	27.03	65.5	87	83	48	48	40	16.2	19.1	17.4	1	3	5.5	10	35	50	360	900	1100
23	25.01	25.02	27.03	79.5	84	82.5	48	49	41	12.4	17	16.2	1	3	5.5	15	30	40	350	950	1120
24	25.01	25.02	27.03	78.5	81.5	66.3	49	48	43	15.6	19.5	16.5	1.5	3	6	3	25	35	340	930	1200
25	25.01	25.02	27.03	75.5	87	65	49	47	45	13.6	16.1	17.1	1.5	3.5	5.5	7	17	29	290	920	1100
26	25.01	25.02	27.03	84	92	70	50	49	43	17.7	16.5	20.1	1.5	4	5.5	20	30	45	300	950	1150
27	25.01	25.02	27.03	65.5	--	75	50	--	45	13.6	--	18	2	--	5	11	--	20	240	--	1000
28	25.01	25.02	27.03	74	94	65	50	48	45	17.3	18.2	17.2	2	2	6	2	6	30	100	170	1110
29	25.01	25.02	27.03	97.5	99	83	47	48	47	15.8	19.5	18.5	2	2.5	6.5	30	90	200	110	160	1125
30	25.01	25.02	27.03	76.5	95	68	49	47	46	14.5	18.5	18.1	1.5	2	6.5	1	5	25	90	170	1150
31	25.01	25.02	27.03	68.5	79	52	45	41	42	19.3	18.6	21	0	-1.5	5	0	1	15	10	35	1000
32	25.01	25.02	27.03	67.5	89.5	82	44	44	44	15	16	16.5	0	-1.5	5	0	0	35	8	40	1010
33	25.01	25.02	27.03	85	88	75	46	44	43	16.8	18.5	17.7	-1	-1	4.5	1	6	70	5	50	980

General Condition of the Barns

All but one of the shelters in the cattle farms that are the subject of the research were built as a single storey. Since the settlement in the research area is a collective settlement, the shelters are generally built close to the houses where the households live or adjacent to the dwelling and mostly in the same courtyard. 22% of the barns have ceilings, and the rest are built without ceilings. Roofs were constructed in single, double, and sloping in more than two directions shapes.

Comparison of Barn Features with EU Standards Required in IPARD Program

Flue of natural ventilation

It has been determined that 84% of the barns were less than 0.01 m^2 ventilation shaft opening for a unit m^2 barn floor area, which was not in accordance with EU standards, while 16% of the barns have complied with the EU standards. Furthermore, it has been observed that the natural ventilation chimneys in most of the barns were not opened before the spring months by closing during the winter (Table 6).

Table 6. Comparison of EU standards with barn features

Number	Barn Floor Area m^2	Barn Flue Area m^2	Flue Area per m^2	EU Standards (m^2)
1	58.4	0.4	0.007	
2	68.4	0	0.000	
3	172.5	0.36	0.002	
4	55.3	0.25	0.005	
5	63.9	0.35	0.005	
6	48.2	0	0.000	
7	254.5	0.25	0.001	
8	42.4	1.2	0.028	
9	78.7	0.36	0.005	
10	104.8	1.2	0.011	
11	157.4	0.48	0.003	
12	82.5	0	0.000	
13	75.3	0.4	0.005	
14	105.3	0	0.000	
15	145	0.27	0.002	
16	46	0.04	0.001	
17	105.8	1.82	0.017	**0.01**
18	137.7	0.42	0.003	
19	90	0.8	0.009	
20	26.9	0	0.000	
21	68.4	0.8	0.012	
22	90	0	0.000	
23	212.4	1.19	0.006	
24	167.2	0.5	0.003	
25	77.7	0.3	0.004	
26	65.6	0.16	0.002	
27	-	-	-	
28	74	0.56	0.008	
29	206.9	5.1	0.025	
30	116.2	0.25	0.002	
31	72	0.6	0.008	
32	61	0.25	0.004	
33	110.8	1	0.009	

Window area

It was observed that the windows were positioned on the long side in 31% of the shelters, on the short side in 28%, and on both sides in 25%. In addition, 13% of the shelters have windows located on the roof; however, 3% of shelters do not have windows.

It has been determined that 87.5% of the barns did not meet the EU standards on a window area of 1/20 of the barn floor area for cold regions.Only 12.5% of the barns have complied with the EU standards. In addition, it was observed that there were barns with high light ratio since they have large chimney openings, although the window area was small (Table 7).

Table 7. Comparison of EU standards with barn window areas

Number	Barn Floor Area (m^2)	Barn Window Area (m^2)	Ratio of Floor Area to Window Area	EU Standards (m^2)
1	58.4	1.2	1/49	
2	68.4	2.4	1/29	
3	172.5	5.0	1/35	
4	55.3	0.3	1/184	
5	63.9	2.1	1/30	
6	48.2	1.0	1/48	
7	254.5	13.2	1/19	
8	42.4	0.4	1/106	
9	78.7	1.4	1/56	
10	104.8	4.0	1/26	
11	157.4	8.8	1/18	
12	82.5	1.0	1/83	
13	75.3	1.9	1/40	
14	105.3	2.4	1/44	
15	145	9.4	1/15	
16	46	0.4	1/115	
17	105.8	0.0	0	**1/20**
18	137.7	2.6	1/53	
19	90	1.5	1/60	
20	26.9	0.6	1/45	
21	68.4	0.6	1/114	
22	90	2.0	1/45	
23	212.4	3.4	1/62	
24	167.2	2.3	1/73	
25	77.7	0.7	1/111	
26	65.6	0.6	1/109	
27	-	0.0	0	
28	74	0.8	1/93	
29	206.9	10.7	1/19	
30	116.2	2.0	1/58	
31	72	1.8	1/40	
32	61	1.0	1/61	
33	110.8	3.5	1/32	

Door dimensions

It has been determined that the door sizes of all the researched barns were not in accordance with the standards of the EU.

Barn dimensions

In all the barns, the animals are tied to the feeders with chains from their necks. Free stalls were built in any of the barns, and dividing irons separating the free stalls from each other were not found in the existing free stalls.

When the widths of the free stalls in the barns are examined, it has been determined that 19% of the barns existing free stalls widths were in accordance with the relevant EU standards; however, 81% of the barns were not in accordance with these standards (Table 8).

Table 8. Comparison of EU standards with some dimensions of barn

Number	Animal Compartment (m^3)	EU Standards (m^3)	Free stall Width (cm)	EU Standards (cm)	Barn Height (m)	EU Standards (m)
1	12		103		3.6	
2	13		90		3.3	
3	14		100		3.35	
4	12		100		3.5	
5	12		100		3.85	
6	11		100		3	
7	15		125		3.72	
8	11		100		3.75	
9	12		105		3.73	
10	13		95		3.6	
11	10		65		4	
12	16		125		3.45	
13	13		120		3.09	
14	13		100		3.7	
15	14		110		4.04	
16	15		115		3.48	
17	17	**18 m^3**	120	**120 cm**	3.95	**3m**
18	17		110		3.4	
19	9		105		3.4	
20	10		120		3.27	
21	13		95		3.63	
22	8		82		3	
23	13		88		3.1	
24	11		90		3	
25	13		105		3.35	
26	10		76		3.34	
27	-		-		-	
28	13		100		3.7	
29	40		180		4.8	
30	18		110		4.1	
31	14		110		4	
32	13		110		3.5	
33	15		110		4	

According to EU standards, the minimum animal section in the barns should be 18 m^3. In this study, 6% of the barns have complied with EU standards; however, 94% were below these standards. The heights of the barns have complied with EU standards.

Feeder dimensions

Most of the barns' (62.5%) feeder building materials were used wood, while 37.5% of the barns were concrete. Although it causes many problems in feeding and cleaning processes, 85% of the barns' feeders were built adjacent to the walls (Table 9).

Table 9. Comparison of EU standards with feeder dimensions of barn

Number	Height from Ground (cm)	EU Standards (cm)	Feeder Length (cm)	EU Standards (cm)	Feeder Width (cm)	EU Standards (cm)
1	67		103		32	
2	74		90		26	
3	70		100		37	
4	54		100		43	
5	60		100		34	
6	61		100		46	
7	50		125		165	
8	68		100		32	
9	77		105		44	
10	76		95		45	
11	47		65		47	
12	80		125		47	
13	69		120		41	
14	70		100		41	
15	55		110		40	
16	79		115		34	
17	47	**20-50 cm**	120	**60-120 cm**	37	**55 cm**
18	63		110		40	
19	65		105		44	
20	68		120		42	
21	55		95		44	
22	67		82		33	
23	62		88		43	
24	59		90		44	
25	65		105		46	
26	55		76		43	
27	-		-		-	
28	63		100		57	
29	50		180		40	
30	51		110		31	
31	69		110		34	
32	68		110		36	
33	64		110		37	

The height of the feeder should be between 20-50 cm according to EU standards. It has been found that only 10% of the barns have complied with these standards. The feeders in all barns have complied with the feeder lengths specified in EU standards in terms of feeder length. Only 6% of the barns have the width of the feeder in accordance with the relevant EU standards.

Temperature, light, and humidity values

It has been determined that there was no homogeneous light distribution in large part of the barns that were the subject of the research. Therefore, light measurements were made three times in the barns in January, February, and March. Measurements were made in at least three different parts of the barns, and the average of these measurements was taken. The proportional results obtained from the measurements are shown in Table 10.

Table 10. Comparison of EU standards with measured light values

	January (%)	February (%)	March (%)
Ratio of barn remaining between EU standard range	24	29	45
Ratio of barn remaining under EU standard range	67	65	43
Ratio of barn remaining above EU standard range	9	6	12

It was observed that the light intensity in the barns increased as the summer months approached due to the increase in light intensity.

Measured temperatures inside the barn during the research have been found to be in the range of the highest and lowest critical temperature levels specified in the EU standards in all of the barns.

Humidity measurements in barns varied. In January, it was determined that only 15% of the barns were in the ideal relative humidity range specified in EU standards, while other barns were above the highest value specified in the relevant standards. Furthermore, it was observed that the humidity values measured in February were not in accordance with the relevant EU standards in all of the barns. In March, it was determined that 27% of the barns were in the ideal relative humidity range specified in EU standards.

When the measurements were made, the humidity and temperature values in the external environment were also recorded since it is thought that outside humidity and temperature will affect indoor humidity and temperature.

Conclusion

It is clear that the current situation of animal husbandry in Turkey is far from competing with the EU.

It can be foreseen that there will be a great development in animal husbandry and other branches of agriculture in the country, and its ability to compete will increase by dint of implementation at the desired level in Turkey's IPARD program, which supports the compliance preparations for the implementation and management of the EU's Common Agricultural Policy, Rural Development Policy and related policies, and policy development in this context.

Table 11. Compliance rates of research barns to EU standards

Number	Comparing EU Standards	Compliance Rate of Existing Barns with EU Standards (%)		
1	Natural Ventilation Flue	16		
2	Window Area	12.5		
3	Door Width	0		
4	Door Hight	0		
5	Least Animal Compartment in Barns	6		
6	Barn Hight	100		
7	Free stall Width	19		
8	Feeder Lenth	100		
9	Feeder Width	4		
10	Feeder Hight from Ground	10		
11	Light Intensity Range for Cows	**January**	**February**	**March**
		24	29	45
12	Ideal Humidity Range in Barns	**January**	**February**	**March**
		15	0	27
13	Lowest and highest critical temperature levels in Barns	**January**	**February**	**March**
		100	100	100

As shown in Table 11,almost all structural features of the existing barns need to be changed in order to adapt them to EU standards. It would be wrong to choose modernizing the barns to raise the agricultural structures in this region to EU standards because there is not enough space for the expansion of the barns since the existing barns are built within the settlement. In this case, modernizing these barns will not be cost-effective. On the other hand, If ARDSI directs rebuilding instead of modernizing those willing to invest, it will be effective in cost and time savings.

Conflict of Interest

The authors declare that they have no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION
https://agriprojournal.com ISSN: 2757-6620

RESEARCH ARTICLE

Heavy Metal Levels in the Beyler Dam Lake, Kastamonu (Turkey)

Khalifa Moftah Abdelali*

Higher Institute of Agricultural Techniques, Trhona, Libya

ARTICLE INFO

Article History:
Received: 13.10.2021
Accepted: 27.12.2021
Available Online: 29.12.2021

Keywords:
Heavy metal
Environment
Pollution
Water
Toxicity

ABSTRACT

In this study, some heavy metal concentrations (Cu, Cd, Pb, Cr, Mn and Zn) were seasonally determined in water to evaluate of the quality of the Beyler Dam Lake. The average concentrations of heavy metals analyzed in water samples for cold and hot season were Cu: 11.53, 13.23; Cd: 0.85, 0.97; Pb: 13.77, 16.26; Cr: 2.88, 3.48; Mn: 1.94, 1.71; and Zn: 7.57, 8.20 ppb, respectively. The annual average (AA) concentrations of heavy metals were Cu: 12.38; Cd: 0.91, Pb: 15.01, Cr: 3.18; Mn: 1.82; and Zn: 7.88 ppb. In general, the heavy metal concentrations were found to be higher in hot season than the cold season. It was observed that none of the heavy metals assessed exceeded the limits specified in the Turkish Water Pollution Control Regulation. The Beyler Dam Lake was classified as Class I for all the heavy metals except Pb for which it was classified as Class II. These results indicate that the lake is not polluted by the heavy metals. However, relatively high Pb levels seek further research to identify the source. The lake water can be used for agricultural activities but should continue to be monitored.

Please cite this paper as follows:

Abdelali, K. M. (2021). Heavy metal levels in the Beyler Dam Lake, Kastamonu (Turkey). *Journal of Agricultural Production*, *2*(2), 67-71. https://doi.org/10.29329/agripro.2021.360.4

Introduction

Water is arguably the most important compound on earth. It is an essential resource for all organic life. Lakes and surface water reservoirs are the most important water resources on earth, as they are used for many purposes such as drinking and irrigation and provide ecosystems for aquatic life, especially fish farming, and thus provide a good source of protein. It also has important social and economic benefits as a result of tourism and recreation, and is culturally and aesthetically important to people all over the world (Dirican, 2015; Arain et al., 2008). Studying some physical and chemical parameters and estimating heavy metals are very necessary and important for water quality testing, before using it for any purpose (Elmacı et al., 2010). Water quality analysis is also important for protecting the natural ecosystem (Karim & Panda, 2014; Patil et al., 2012).

The toxicity of heavy metals depends on the type of metal, the amount of material in which it is deposited and the time. The temperature is also directly proportional to the toxicity of some heavy metals (Terzi & Verep, 2012). The water quality of rivers and lakes varies with the change of seasons and the existing aquatic life (Suski et al., 2006). There are basic scientific standards and information on water quality assessment and environmentally relevant toxicants' threshold values to protect the physical and chemical factors that have an impact on the aquatic environment such as temperature, precipitation, pH and heavy metal contaminants. The quality of rivers and lakes changes with seasons and biota (Suski et al., 2006; Lawson, 2011). The presence of minerals in aquatic ecosystems is mainly influenced by direct or indirect human activities. The pH affects the quality of the water body through the concentration of many minerals by changing their availability and toxicity, so the adverse environmental effects of cadmium (Cd), for example, increases at low pH. Temperature and pH are two important factors that control the methylation of elements such as lead (Lawson, 2011).

The selection of the tested parameters depends on the purpose of using that water and the extent to which we need its quality and purity. (Hisseien et al., 2015). Heavy metals, especially those which are toxic even at low concentrations, play a crucial role in the aquatic environment (Elmaci et al., 2007). According to the water quality guidelines directive (2008/105/EC) of the EPC (2008), Cd and Pb are on the list of

* Corresponding author
E-mail address: km_allwaty@yahoo.com

priority, while Cu and Cr on the list of other specific dangerous substances. Therefore, their content should be known for the evaluation of the ecological and overall status of a particular water body. Metals are omnipresent in the aquatic ecosystems in trace amounts and their natural concentration depends on the type of rocks and soil in the respective basin (Evenset et al., 2007). However, various anthropogenic activities could considerably increase their level. It is important to assess the heavy metal levels of a water body to understand whether the water of it can be used for any purpose. Therefore, in the present study, it was aimed to determine the heavy levels in the water of the Beyler Dam Lake.

Materials and Methods

Study Area

Beyler Dam Lake in the district Devrekani of Kastamonu (Turkey) is a dam that was built for irrigation between 1987-1994 on İncesu river. Water samples were taken from four locations determined on the lake as shown in Figure 1.

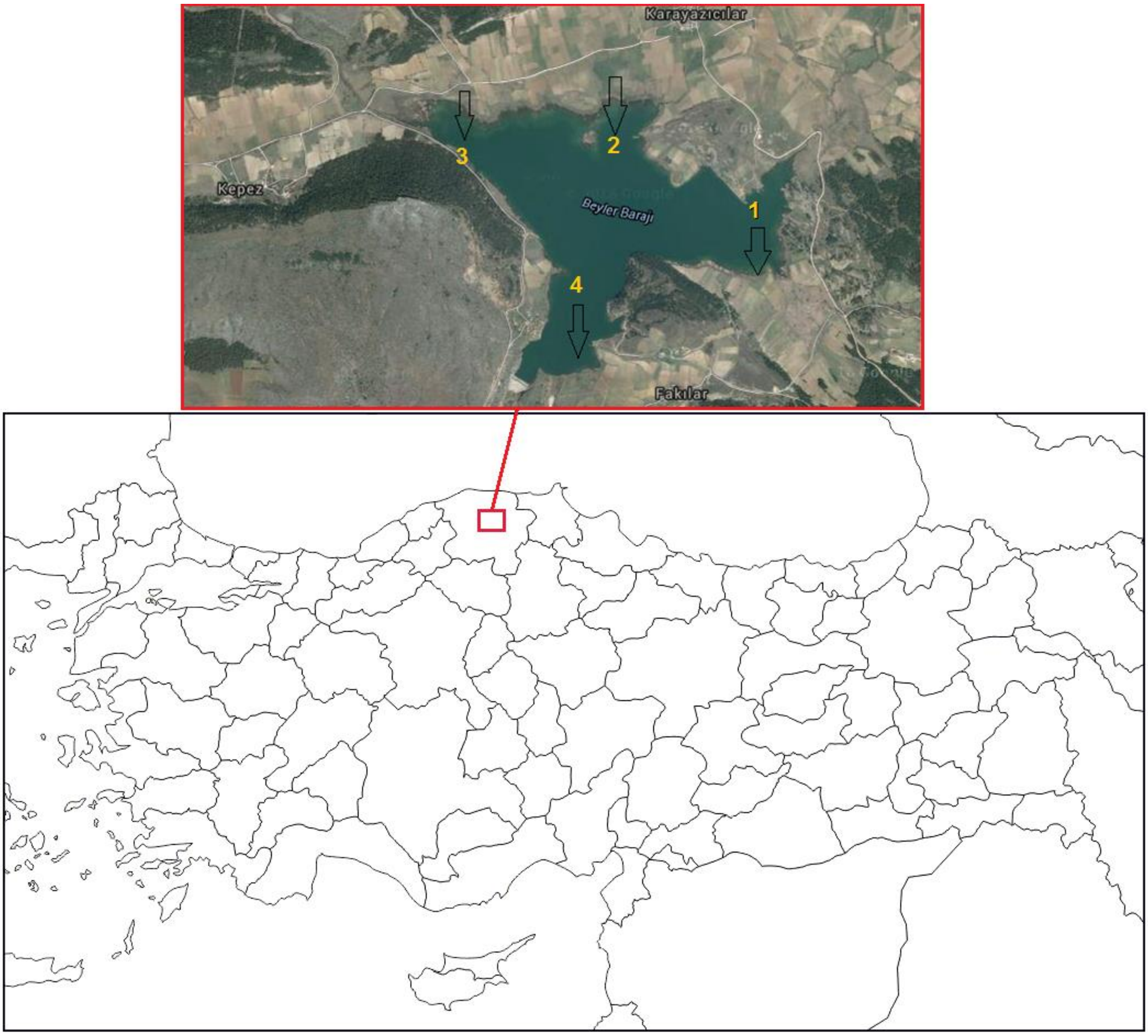

Figure 1. Sampling locations on the Beyler Dam Lake (41°41'06"N, 33°47'48"E).

Sampling and Determination of Heavy Metal Levels

Samplings were carried out in December 2016, January and February 2017 for cold season, and in July, August and September 2017 for hot season. The water samples were taken with sterile glass containers by submerging the enclosed container to 0.1 m below the surface and uncapping under the water. The containers were pre-treated with HNO_3 and rinsed with the ambient water 3 times before taking the samples (Alam et al., 2001; Sönmez et al., 2012). After taking the samples, 1 ml of NHO_3 was immediately added at the collection site to acidify the sample. The samples were transported to the laboratory in maximum two hours and filtered through 0.45 µm membran filters (Sönmez et al., 2013). The heavy metal concentrations (Cu, Cd, Pb, Cr, Mn and Zn) were assessed using ICP/OES (SpectroBlue, Spectro) at the Central Laboratory of Kastamonu University on the sampling day. The mean values for the data obtained were computed using descriptive statistics in SPSS version 25.0 for Windows (IBM).

Results and Discussion

The Lakes are one of the most important freshwater resources and also, they are very important natural areas with many features such as natural beauties, biological diversity, fishing, tourism and recreation. However, technological developments, rapid population growth, industrial and agricultural pollutants are major constraints on the water quality of the lakes. The most common ecological problem is human activity induced pollution worldwide and lakes' pollution caused by neighboring agricultural activities which eventually leads to worsening of water quality and significant reduction in biodiversity (Kristensen & Hansen 1994; Dodson et al., 2000). A large part of surface water resources are the areas where industrial, agricultural and domestic wastes are discharged in developing countries and they are sources used in drinking, irrigation and aquaculture. Therefore, it is important to know the physical, chemical and biological properties of these waters and these should be monitored periodically (Yılmaz Öztürk & Akköz, 2014).

The heavy metal concentrations assessed in the present study are as shown in Table 1.

Table 1. Heavy metals levels in the water of the Beyler Dam Lake (ppb)

		Cu	Cd	Pb	Cr	Mn	Zn
Cold Season	**Mean±SE**	11.53±1.15	0.85±0.13	13.77±1.37	2.88±0.87	1.94±0.48	7.57±1.17
	Min-Max	9.62-13.40	0.65-1.31	13.71-16.06	2.54-3.16	1.84-2.00	4.67-8.62
Hot Season	**Mean±SE**	13.23±1.32	0.97±0.32	16.26±1.62	3.48±0.48	1.71±0.37	8.20±1.21
	Min-Max	11.35-16.01	0.83-2.67	15.64-24.17	3.05-4.00	1.17-1.99	7.72-8.71
AA		12.38	0.91	15.01	3.18	1.82	7.88

Water Pollution Control Regulation (WPCR, 2004)

	Cu	Cd	Pb	Cr	Mn	Zn
Class I	20	3	10	20	100	200
Class II	50	5	20	50	500	500
Class III	200	10	50	200	3000	2000
Class IV	>200	>10	>50	>200	>3000	>2000

AA: Annual average, SE: Standard error.

In the present study, the annual average (AA) value of copper (Cu) was found to be 12.38 ppb. The lake was classified as Class I according to the WPCR (2004). In a similar study, Kayrak and Ozan (2018) found that the average Cu concentration in the Kovada Lake water was 0.54 ppb. On the other hand, Nergiz and Şamat (2019) observed that the Cu concentration in the Hazar Lake water was 8.1 ppb and in the Kara Lake, the Cu was below the detection limit (Keser et al., 2020). These results show that the Cu concentration in the Beyler Dam Lake is higher than the other lakes reported in the literature but still within the permissible limits.

The AA of the cadmium (Cd) in the Beyler Dam Lake was found to be 0.91 ppb. According to the WPCR (2004), the lake was classified as Class I in terms of cadmium. Various levels of Cd have been reported from different lakes. In the Kovada Lake, the average Cd concentration was 0.19 ppb (Kayrak & Ozan, 2018). It was reported to be below the detection limit in the Kara Lake (Keser et al., 2020) and 9.1 ppb in the Hazar Lake (Nergiz & Şamat, 2019).

The AA of the lead (Pb) in the present study was 15.01 ppb. While it was reported to be below the detection limit in the Kovada Lake (Kayrak & Ozan, 2018) and Kara Lake (Keser et al., 2020), Nergiz and Şamat (2019) documented that the average concentration of the Pb was 4.6 ppb in the Hazar Lake. The average Pb value determined in the present study is higher compared to the literature data and the Beyler Dam Lake is classified as Class II in terms of Pb according to the WPCR (2004).

The AA of chromium (Cr), on the other hand, was calculated as 3.18 ppb and the lake was classified as Class I according to the WPCR (2004). Similarly, the average Cr values in the waters of Kovada Lake and Hazar Lake were 0.92 and 7.7 ppb, respectively (Kayrak & Ozan, 2018; Nergiz & Şamat, 2019).

In the present study, the AA of manganese (Mn) was found to be 1.82 ppb. In similar studies, the Mn was reported to be 14.56 ppb in the Kovada Lake (Kayrak & Ozan, 2018) and 3 ppb in the Hazar Lake (Nergiz & Şamat, 2019). It seems that the Mn concentration in the Beyler Dam Lake water is lower than the data presented in the literature and the lake is classified as Class I as per the WPCR (2004).

Lastly, the AA value of the zinc (Zn) was found to be 7.88 ppb. Zn was found to be below detection limit by Keser et al. (2020) in the Kara Lake. In the Hazar Lake, it was reported to be 14.6 ppb (Nergiz & Şamat, 2019) and the average Zn concentration in the Kovada Lake was reported to be 5.18 ppb

(Kayrak & Ozan, 2018). According to the WPCR (2004), the Beyler Dam Lake is also classified as Class I for zinc.

These results show that the Beyler Dam Lake is not contaminated by copper, cadmium, chromium, manganese, or zinc. However, there seems to be a low level of lead contamination in the lake. Lead has no biological role, is toxic even at very low concentrations (Bryan, 1976), and may affect a variety of mechanisms in aquatic organisms such as nervous system, blood parameters, immunity, and enzyme activities (Elbeshti et al., 2018; Lee et al., 2019). Lead may enter the environment through various ways but the main source of the excessive lead concentration is human activities such as smelting, mining, industrial waste, and domestic waste (EPA, 2021). Cosmetics, ammunition, batteries, gasoline, solders, plumbing materials, pipes, ceramics, and paint may contain lead and lead compounds (EPA, 2021). Since there is no industrial activity that may have caused lead pollution near the Beyler Dam Lake, the high concentrations of lead in the water may have been resulted from domestic waste discharged into the lake by the local community.

Furthermore, for all the heavy metals assessed, the variation in the observed results in the stations did not show a significant difference, indicating the spatial and temporal homogeneity in the lake. The results also showed that the average concentration of heavy metals was higher in the hot season than it was in the cold season, except for Mn, which was low in the hot season (1.71 ppb) and high in the cold season (1.94 ppb).

Conclusion

In this study carried out in the Beyler Dam Lake, according to the analysis results of the water samples taken for six months, it was determined that the dam lake was classified as Class I for all heavy metals analyzed (Cu, Cd, Cr, Mn, Zn) except for Pb. The lake was classified as Class II for Pb. These results suggest that the lake is a clear water body that is not contaminated by heavy metals. Therefore, the water is safe for agricultural activities such as aquaculture and irrigation. However, since the Pb is very toxic at low concentrations, the relatively high concentrations of Pb indicate that the source of the Pb in the lake needs identification. Further studies should monitor the water quality of the lake to track the status of the water.

Conflict of Interest

The author declares that he has no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION
https://agriprojournal.com ISSN: 2757-6620

REVIEW ARTICLE

Flavor in a Tea Glass to Present from Past: Safely Organic Production and Health Effects of Tea

Halit Karagöz[1] • Fazilet Parlakova Karagöz[2*] • Erdim Erduran[1] • Ramazan Çakmakçı[3]

[1]Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Turkey
[2]Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Turkey
[3]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crops, Çanakkale/Turkey

ARTICLE INFO

Article History:
Received: 02.12.2021
Accepted: 24.12.2021
Available Online: 29.12.2021

Keywords:
Camellia sinensis (L.) O. Kuntze
Chemical content
Microbial fertilizer
Organic tea

ABSTRACT

Tea (*Camellia sinensis* (L.) O. Kuntze) is the second most-consumed non-alcoholic beverage in the world after water. The health-beneficial properties of tea, known to contain more than 4000 bioactive substances, of which about one-third consist of polyphenols, are increasingly well understood. The medicinal properties of the tea plant have been proven by laboratory and clinical studies to have an anti-cancer effect, benefits for dental health, protect against Alzheimer with anti-paralytic, anti-diabetic, and antiparkinson properties, and its use against skin diseases. However, it is known that the tea plant, which requires plenty of fertilizer, can cause excessive pollution of the groundwater when chemical fertilizers are washed away with precipitation in the areas where it is grown. In order to eliminate this negative situation, studies regarding organic and microbial fertilizers that are more environmentally friendly and do not harm the soil and human health that could be substituted for chemical fertilizers as much as possible or mitigate their use and enable to grow products of adequate amount and quality should be accelerated. The aim of this review is to bring together scientific information about the characteristics and health effects of tea and organic tea cultivation.

Please cite this paper as follows:

Karagöz, H., Parlakova Karagöz, F., Erduran, E., & Çakmakçı, R. (2021). Flavor in a tea glass to present from past: Safely organic production and health effects of tea. *Journal of Agricultural Production*, 2(2), 72-85. https://doi.org/10.29329/agripro.2021.360.5

The Origin and History of Tea

The beverage known as tea, *Camellia sinensis* (L.) O. Kuntze, can be described as the infusion of the leaves of one of the varieties of an evergreen shrub that has been variously processed. The plant is a member of the *Camellia* genus of the Parietales order of the Theaceae family and grows in warm climates and areas rich in precipitation (Namita et al., 2012). Tea is known as the second most consumed beverage in the world among non-alcoholic beverages after water (Singh et al., 2011; Hayat et al., 2015). Many studies have demonstrated that green and black teas have numerous pharmaceutical properties, including antihypertensive (Henry & Stephens-Larson, 1984), antioxidant (Ding et al., 1992; Zhu et al., 1999; Leung et al., 2001), antiarteriolemic (Hertog et al., 1993): anticarcinogenic (Shi et al., 1994; Wang et al., 2009), and hypocholesterolemic (Imai & Nakachi, 1985; Kono et al., 1992; Yang et al., 2001). Tea is a rich source of flavonoids and other polyphenols, like grapes, apples, and cocoa. However, the flavonoid content can be affected by different processing modes. The degree of oxidation affects the polyphenol profile of tea (Zhu et al., 2002). Three specific types of tea, namely green, oolong, and black tea, are obtained from freshly harvested tea leaves depending on the fermentation process. Green tea is dried and heated to avoid enzymatic oxidation. Oolong tea is semi-fermented to allow moderate enzymatic oxidation and then dried. Black tea is enzymatically fully oxidized (Mahmood et al., 2010).

According to botanical classification (Barua, 1965), there are three varieties of tea grown in South-East Asia which are specific to the geographical regions of China, Assam, and Indo-

* Corresponding author
E-mail address: f.parlakova@atauni.edu.tr

China. The first two are believed to be *C. sinensis* L., and the third is considered to be *C. assamica* Masters. The third one, known as the 'Southern' (or Cambod) form, is considered a subspecies of the Assam type. “Chinese species” are characterized as small-leaved cold-resistant shrubs, while “Assam species” are tall trees with large leaves and are less resistant to cold (Sealy, 1958). The "Cambod type" (Kingdom-Ward, 1950; Roberts et al., 1958) is considered to be of medium height between the Chinese and Assam types. In general, all teas are classified under the name *C. sinensis* (L.) O. Kuntze, regardless of taxonomic variation (Sealy, 1937; 1958; Barua, 1965; Visser, 1969).

The homeland of the tea plant is considered to be a fan-shaped region between the hills of Nagaland, Manipur, and Lushai with the Assam-Burmese border in the west, China in the east, and the hills of Burma and Thailand in the south extending into Vietnam. The name tea comes from the Chinese word ‘ça’. Tea is called “cha” by the Japanese, ‘shaye’ by Arabs, while Russians call it ‘chay’. The word was transposed into Turkish from Chinese as ‘çay’, ‘the well-known leaf that is boiled and drunk’ (Alikılıç, 2016). ‘Red Emperor’ Shen Nung, one of the three legendary emperors of Chinese medicine, estimated to have lived about 5000 years ago who holds an important place in Chinese history and mythology claims to have discovered tea for the first time in 2737 BC when some tea leaves accidentally fell into boiling water. The aroma and flavor of the mixture of different colors generated by tea leaves falling into boiling water were appreciated and spread first throughout China and then to the whole world (Üstün & Demirci, 2013).

The first mention of tea in Europe took place in 1559, and 1606 is the year that tea was introduced to Europe. After 1635, the Netherlands and France were known as the pioneers of tea consumption in Europe. The first teapot samples from China reached Europe in the 1650s. The name that brought tea to America was Peter Stuyvesant. The Dutch colonies, which settled in New Amsterdam, now known as New York, were known as the first theics in the history of America. In the 1800s, the tea industry gradually began to emerge in Europe and America. Thomas Lipton's first shop opened in 1871 in Glasgow, England. In 1890, Thomas Lipton bought his first tea plantation in Ceylon. Richard Blechynden, who struggled to sell tea in hot weather in the United States, came up with the idea of offering cold tea. The American concept of Ice Tea was born with this coincidence. Tea bags were discovered in 1908 (Anonymous, 2015).

Tea Production

According to the 2017 FAO statistics, China has the highest tea area and production in the world. China, which has 35.30% of the world’s total tea area, counters 28.84% of the world’s total tea production (Table 1). 78% of the consumed tea is black tea, 20% is green tea, and 2% is oolong tea (Singh et al., 2015). As shown in Table 1, India has ranked second in the country’s ranking with the highest tea area and production in the world. Turkey has a tea production area of 82000 ha and an amount of dry tea production of 234 000 tons.

Table 1. World tea production area and amount of dry tea production (Anonymous, 2019)

Countries	Area (1000 ha)	Production (1000 tons)
China	2224	2473
India	622	1325
Sri Lanka	234	350
Kenya	219	440
Indonesia	114	139
Vietnamese	123	260
Turkey	82	234
Total of Other Countries	2682	3354
Grand total	**6300**	**8575**

The Aroma of Black Tea and the Chemical Composition of Tea Leaves

Although one-third of the tea plant consists of polyphenols, it is known to contain more than 4000 bioactive components (Tariq et al., 2010). Polyphenols, mostly composed of flavonoids, are a large group of plant chemicals containing catechins, and polyphenols have traditionally been considered responsible for the health benefits of tea and especially green tea (Sumpio et al., 2006). Green tea is richer in catechins than black tea. Oolong tea has a mixture of catechins and complex polyphenols. Green tea, black tea, and oolong tea are extremely good sources of vitamin C (Mukhtar & Ahmad, 2000; Wu & Yu, 2006).

Although there is a similarity between the chemical composition of black tea and green tea, chemical changes (fermentation and processing conditions) that occur in the production process lead to some differences between black and green tea and oolong and white tea. The polyphenols of green and black tea are shown in Figure 1.

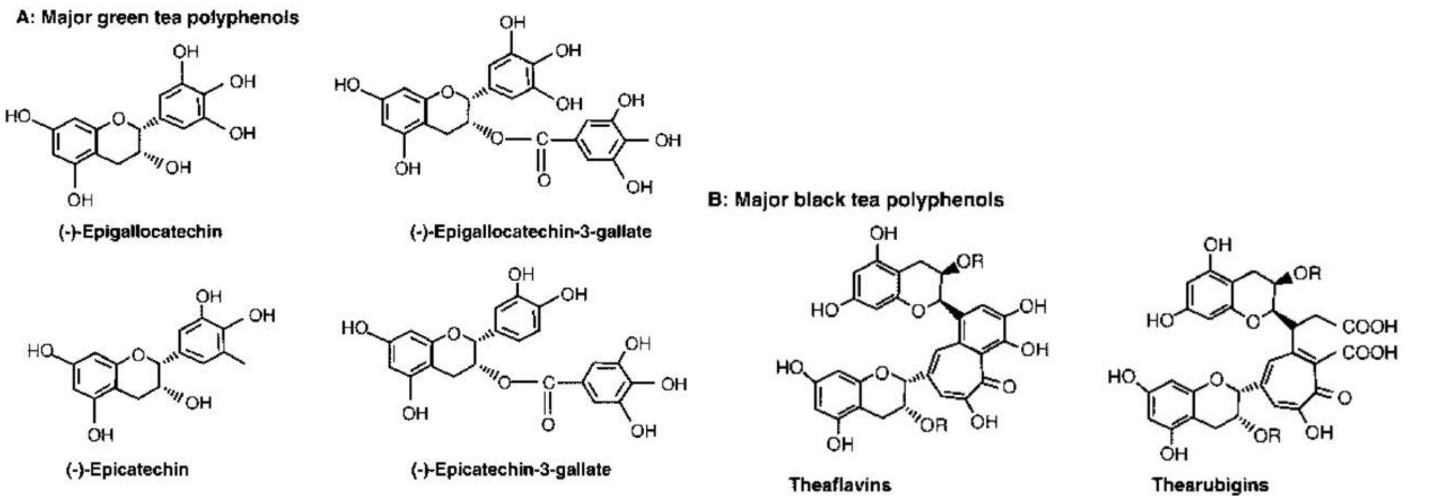

Figure 1. Polyphenols in tea (*Camellia sinensis*). A: The major polyphenol in green tea. B: The major polyphenol in black tea (Anesini et al., 2008).

Carbonyls, which are effective in the aroma of black tea, make up more than 50% of the total aroma and are the compounds with the greatest contribution to sensory properties. Volatile acids make up 10-30% of total black tea volatiles. They are natural or formed in a secondary way (Renner & Melcher, 1978). Some of the phenolic compounds in black tea are formed as natural biosynthesis products. However, some phenolic volatiles, especially phenolic acid derivatives, occur in the drying process (Schreier, 1988). Alcohols are present in small amounts in fresh leaves. Quantitatively significant amounts occur in different processes in black tea production (Hatanaka & Harada, 1972). **Furans** are found in heat-treated foods. The main products of this class of compounds in black tea are furfural, 5-methyl furfural, and furfuryl alcohol (Çalıkoğlu & Bayrak, 2006). There are trace amounts of aromatic and aliphatic hydrocarbons in the tea. Aromatic hydrocarbons are formed by the thermal decomposition of carotenoids (Kawashima & Yamanishi, 1973). The thermal decomposition of amino acids, pyrolysis of amadori compounds, and the reaction of amino acids with carbonyls form pyridine. Pyrazines are components with low sensing thresholds that occur in the heat treatment process of foods (Suyama & Adachi, 1980; Çalıkoğlu & Bayrak, 2006). **Esters** cannot reach the sensing threshold due to low concentration; therefore, they can not directly participate in the aroma (Schreier, 1988). Polyphenols, on the other hand, are considered to be the most biologically active group of tea components with antioxidative, antimutagenic, and anticarcinogenic effects (Yao et al., 2004). Polyphenols and their oxidized derivatives (mainly theaflavins and thearubigins) are important chemical components responsible for the color formation and the aroma of black tea during infusion (Venkatesan & Ganapathy, 2004). The catechins of the flavan-3-ol type constituting 25-30% of the dry weight of tea leaves are the main flavonoid compounds of tea, which are beneficial to human health and have a high antioxidant capacity (Higdon & Frei, 2003). High catechin ratios intensify the tea content, increase the bitter taste and thus affect the sensory quality of the tea. Nutrients have a significant effect on the production of catechins (Chen et al., 2011; Ruan et al., 2013; Li et al., 2016). For example, nitrogen forms (NH_4 or NO_3) affect not only growth but also catechin and flavonoid composition in tea plants (Fallovo et al., 2011; Kovacik & Klejdus, 2014). Furthermore, tea contains other compounds that are beneficial to human health, such as fluoride, caffeine, and essential minerals (Cabrera et al., 2003). Total free amino acid concentrations in tea range from 1-5% and are highly influenced by many factors, including variety, climatic conditions, soil properties, and fertilization. The most common amino acids found in tea are theanine (Thea), glutamine (Gln), glutamic acid (Glu), and arginine (Arg), and theanine accounts for 70% of the total free amino acid in tea (Wang & Ruan, 2009; Ruan et al., 2011). Theanine has many beneficial properties, such as preventing certain cancers and cardiovascular diseases, promoting weight loss, and strengthening the immune system (Vuong et al., 2011). The chemical structure of tea leaves is given in Table 2.

Table 2. Chemical composition of tea leaves (Tosun & Karadeniz, 2005)

Component	Dry matter (%)	Component	Dry matter (%)
Flavanols (Catechins)	17-30	Polyphenolic acid and Deposits	5
Epicatechin (EC)	1-3	Total polyphenols	30-36
Epicatechallate (ECG)	3-6	Caffeine	3-4
Epigallocatechin (EGC)	3-6	Amino acid and protein	15-19
Epigallocatechin gallate (EGCG)	9-13	Simple Carbohydrate	4
Catechin	1-2	Polysaccharides	13
Gallocatechin (GC)	3-4	Ash	5
Flavanols and flavanol glycosides	3-4	Cellulose	7
Lignin	6	Lipid	2-3
Organic Acid	0.5-1.5	Pigment	0.5
Anthocyanins	2-3	-	-

Tea Culture

Tea is a beverage that is consumed almost all over the world. Different consumption patterns have developed in different cultures. Some of these consumption models are as follows. In the USA, tea is consumed as hot and cold in large amounts, especially in southern states; tea is either brewed with cold water or left to cool after brewing with hot water. The British preferred to sweeten their strong tea with milk and sugar, thus reducing the astringency in taste, and the concept of '5 o'clock tea' was conceived by the British. In Europe, where black tea is generally preferred, tea is brewed in a short time, mostly in the form of glass sachets, and herbal, fruit, and aromatic teas are also great favorites in this region. In Turkey, black tea is generally brewed in double-layered kettles (in the samovar fashion): it is strong and consumed mostly with sugar in small, slender waisted glasses, and it is available throughout the day. In North Africa, mostly green tea is preferred; tea prepared with milk and lots of sugar can be served in glass cups and consumed at any time of the day. Green tea is also preferred in Japan; it has an important place in the culture of the country and is served with a ritual from its preparation to drinking and is consumed with a light and soft consistency. Tea is very important in China, where green tea and other regional teas are in high demand. In China, tea is brewed in cups with lids and consumed with these cups. In Tibet, tea is brewed with milk or water for quite a long time and is prepared and drunk after churning in wooden churns with butter. In India, local teas with a strong aroma are prepared and drunk with plenty of sugar, milk, and cinnamon (Anonymous, 2015).

Tea for Health

Anti-Cancer Effect

It has been reported that catechins which are available in abundance in green tea, inhibit tumor cell proliferation and also promote the destruction of leukemia cells (Smith & Dou, 2001). In a study conducted in China, it was found that consumption of green tea was effective in the struggle against gastric cancer, which is the second most common cancer type and chronic gastritis (Setiawan et al., 2001). A study was carried out in Japan with 8552 subjects to investigate whether green tea is an effective anti-carcinogen (Nakachi et al., 2000). According to the results of this study, a relative risk of cancer was reduced for those who consumed more than ten glasses of green tea per day compared to those who consumed less than three glasses. The results of a study conducted on 69 thousand women aged 40-70 showed that consumption of green tea helps protect against colorectal cancer (Chung et al., 2003).

Anti-Alzheimer Effect

Although there is no epidemiological evidence in human studies of the benefit of green tea for Alzheimer's disease, many studies in animal and cell culture models have shown that catechins from green tea can have an impact on the progression of Alzheimer's disease. Catechins provide protection against beta-amyloid-induced neurotoxicity in hippocampal neurons due to their antioxidant properties. Epidemiological studies of the prevalence of Parkinson's disease and the consumption of green tea have shown that it is encountered 5-10% less in Asian populations (Zhang & Roman, 1993; Pan & Le, 2003).

Anti-Stroke Effect

As a result of an 11-year study conducted on 40,530 Japanese people aged 40-79 years without a history of cardiovascular disease, an association was demonstrated between green tea consumption and low stroke risk (Kuriyama et al., 2006). Another study showed that there was an inverse relationship between strokes and the consumption of 5 cups of green tea per day and that those who drink less green tea are at least twice as likely to die from stroke or cerebral hemorrhage (Sato et al., 1989). Another study found that people who consume 3 or more cups of green or black tea are 21% less likely to have a stroke (Arab et al., 2009).

Anti-Parkinson Effect

Some studies based on animal models have shown that catechins in green tea significantly inhibit Parkinson-causing pathologies (Levites et al., 2001). Catechins have been reported to inhibit iron, and alpha-synuclein accumulation in MPTP-treated mice. These effects have been associated with antioxidant activity and the iron-binding properties of catechins (Mandel et al., 2004).

Anti-Diabetic Effect

Green tea has an anti-diabetic effect. In a study carried out with mice, the glucose levels in the bloodstream of diabetic mice were reduced without affecting insulin levels (Tsuneki et al., 2004). When administered to fructose-fed rats, it was determined that green tea extract also prevented the development of insulin resistance, hyperglycemia, and other metabolic defects (Wu et al., 2004).

Tea for Dental Health

Green tea extract is effective in preventing tooth decay. Polyphenols have an antiplaque activity. Sesquiterpene hydrocarbons (delta cadenen and caryophyllene) show a synergistic effect of 128 to 256 times when combined with indole (Duke, 2000); tea leaves have been reported to be rich in fluoride, known to improve dental health and prevent tooth decay (Onisi et al., 1981): and catechins in tea suppress the growth of bacteria that induce 10 types of tooth decay (Sakanaka et al., 1989). In a human study, it was shown that washing the mouth with 0.05 to 0.5% green tea polyphenols for 3 days after meals prevents tooth plaque formation by 30 to 43% (Sakanaka, 1997).

Tea for Skin Care

In laboratory studies using animal experiments, it has been shown that green tea extract taken orally or applied to the skin inhibits the formation of skin tumors caused by chemical carcinogens or ultraviolet radiation. Many cosmetic and pharmaceutical companies support their skincare products with green tea extracts (Katiyar & Elmets, 2001).

Tea Plant Characteristics and Growing Requirements

The tea plant has a strong taproot and sequential hairy roots, which begin to form after the third year. The taproots go very deep, while the hairy roots are located near the surface of the soil. The ramification feature is high, and there are many gemma buds in the trunk and branches. The first shoots are green, starting from the lower parts with the onset of lignification; the annual shoots darken and become brown. The shoots are made of wood buds located in the axil part of the ripe leaves. The shoots in the upper part of the branches are superior to the shoots in the lower part, but after the offshoots in the upper part are harvested, the superiority passes to the developing bud in the lower part (Figure 2). The leaves of a growing tea offshoot are different in shapes and named differently from each other. Growing tea offshoots are named as follows: Bud (floveri-piko), first leaf (oranj - piko), second leaf (piko), third-fourth leaf (sukong), fifth-sixth leaf (kon) (Anonymous, 2013a) (Figure 2 (b)). The flower buds of the tea plant are seen with two or three eyes at the end of the short stem on the leaf axils. The stem of these buds grows, and flamboyant white flowers appear. There are 5 calicles and 5 petals in the tea flower; the flowers are in a whorled array with a combination of male and female organs. After pollination, the petals are shed, and small fruits form at the end of the flower stem. These fruits start to swell in the first days of autumn and then reach their normal size; they are bright green in color, three-eyed and thick-shelled, and contain 3 seeds. When the seeds ripen, the fruit peel opens, and the seeds spontaneously emerge. The seeds contain 20-30% oil, and this oil contains saponin (Anonymous, 2013a).

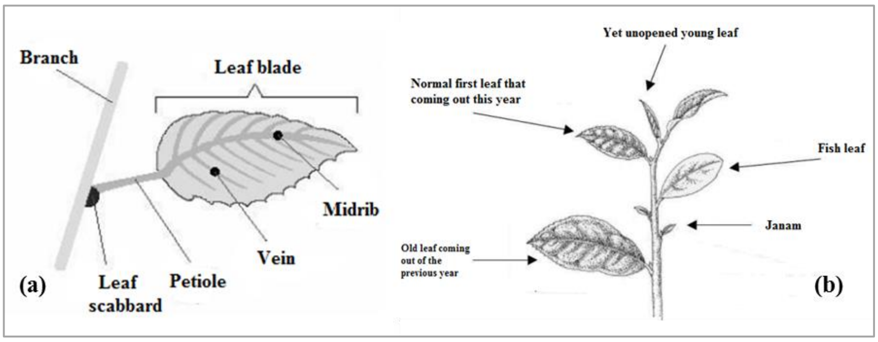

Figure 2. Leaf shape of the tea plant (a); actively growing tea shoot (b).

The tea plant is a perennial plant and can survive up to 100 years. However, after reaching 50, the plant yield decreases gradually. After 50 years, the yield of Assam teas decreases by 1% every year. Chinese tea survives for up to 100 years (Tekeli, 1976).

The most important factors affecting the growth of tea plants are climate and soil properties. The annual average temperature must not fall below 14 ^{0}C, the total annual rainfall must not be less than 2000 mm and its distribution throughout the months must be regular, and the relative humidity must be at least 70% to ensure the necessary climate conditions for the normal development of tea plants. The tea plant, which likes acid-reacting soils in terms of soil properties, shows optimum development in soils with a pH ranging between 4.5-6 and low active lime (Gökhale, 1952; Sharma & Ranhanathan, 1985). While soil pH is adversely affected by acid or alkali changes, when the soil pH falls below 4, the product with the desired yield and quality becomes unavailable (Tekeli, 1962; Tekeli, 1976; Kacar, 1984). The tea plant, which can be grown in soil textures ranging from sand to clayey, likes the soil to be deep and rich in plant nutrients, while plant growth is negatively affected in areas with heavy clay, lime, and a high water table. Therefore, while flat areas where tea is cultivated needs to be rich in organic matter and have high permeability, it is recommended that slopes in inclining areas are not more than 50%, and slope terracing is recommended to reduce the effect of slopes (Özyazıcı et al., 2014).

Shading is an important cultural practice as it is effective in increasing the amount of product in the tea plant as well as the pruning material. In shaded tea plantations, the surface width of the leaves is larger, and their dry weight increases as well. Shading prevents negative changes in soil temperature and causes better root development in tea plants (Kacar, 1984).

Thanks to good nutrition, the roots, branches, and organs of the plant develop and expand better. When fertilization is done in full, and according to the method and maintenance, pruning, leaf collection is done according to the proper methods, seedlings develop quite well, and the plants fill the entire tea plantation with full coverage. Plantations are fertilized at a rate of 70 kg per ac. Fertilizer is not sprinkled on the seedbed but applied to the roots of the tea plant (Haznedar & Sekban, 2012).

In the tea plant, the product is taken from fresh shoots showing periodic development during the whole vegetation on the collection table. The shoot consists of the active peak bud during the growth period and the subsequent fresh 2-3 leaves. Currently, three types of collection systems are in demand in the world of tea cultivation: Janam collection, fish-on-leaf collection, and fish-on-leaf collection leaving one leaf. It is important to note that the harvested leaves must not be coarse. Coarse leaves damage the quality of the product. Tea harvesting is started when the temperature rises above 11˚C in the region to be harvested. The collection period is 7-15 days. The harvested fresh tea products consist of buds, a bud with a leaf, a bud with two leaves, a bud with three leaves, a tender bud, a tea flower, and fresh single leaves (Turna et al., 2008).

Fertilization of Tea Plants and Applications of Organic Fertilizers

The nutrients used by the plant and reduced in the soil by washing must be returned to the soil for the plants to yield high quality and abundant products and healthy growth, and fertilizer application is the second most expensive agricultural input in tea production (Bonheure & Willson, 1992; Zaman et al., 2016). The basic nutritional needs of tea plants are nitrogen, phosphorus, potassium, calcium, and magnesium. Micronutrient needs consist of manganese, boron, copper, zinc, molybdenum, and iron.

It is known that nitrogen, a plant nutrient, plays an important role in improving the vegetative growth of the plant, and its presence in the soil is directly reflected in the yield. Nitrogen is an important element affecting the formation of essential organic compounds such as amino acids, proteins, coenzymes, nucleic acids, ribosomes, chlorophyll, cytochrome, and some vitamins (Marschner, 1995). Phosphorus is the second plant nutrient required by the plant after nitrogen. The availability of phosphorus depends on the mineralization and immobilization processes in which biological processes are effective in the soil. It is fixed even if it is high in the soil or if it is applied in high amounts and regularly, and it turns into a form that is not obtainable as Fe and Al phosphates in acidic soils and Ca phosphates in alkaline soils (Çakmakçı et al., 2010). Potassium is another important nutrient required by the plant (Ranganathan & Natesan, 1985). Potassium increases root growth, stronger stalk formation, resistance to cold and water stress, links directly with improving product quality, reduces crop and disease effects by increasing crop resistance, and is the main nutrient that affects the quality of marketable tea (Venkatesan et al., 2006; Bagyalakshmi et al., 2012).

Tea production is significantly influenced by the N: K ratio because these nutrients play a direct or indirect role in all stages of metabolic processes from absorption to assimilation, which not only increases productivity but also improves the biochemical properties of leaves (Venkatesan et al., 2004). When there is a deficiency of these elements in the tea plant, the leaves weaken, the green leaves become yellowish, leaves fall off, and changes incur in the physical structure of the plant. The better and equal amounts of nutrition the tea plant has, the better the growth and development. Improper fertilization damages product health. There are technical requirements for fertilization in accordance with the requirements.

Tea plants are known to grow in acidic soils; the low pH of these soils, high Al concentration, and small amounts of nutrients indicate strong washing (Singh et al., 2010). Soil acidification threatens the sustainability of the agricultural system. Low soil pH has an adverse effect on the availability of plant nutrients, physiological deterioration in the absorption of calcium, magnesium, and phosphorus, decreased molybdenum availability, and reduced phosphorus availability as a result of reacting with aluminum and iron. Therefore, it is reported that organic fertilization should be carried out in order to maintain sustainability in tea plantations and thus mitigate soil acidification in tea plantations (Li et al. 2016). Plants that are grown in acidic soils often experience severe mineral stress, and such effects include toxic (Al and Mn) deficiency symptoms (Marschner, 1995; Fang et al., 2014). Excess Al is detrimental to root growth and overall growth, especially for plants that grow on acidic soils (Ding & Huang, 1991). Reducing N fertilizer moderately and adding organic matter can be effective in protecting plants from the risk of Al toxicity (Fang et al., 2014).

However, the soil of tea plantations is often strongly acidified due to the high nitrogen fertilizer applied, and the acidity in the soil of tea plantations gradually increases with the age of the plantation (Konishi, 1991; Hayatsu & Kosuge, 1993; Nioh et al., 1993; Tachibana et al., 1995; Shi et al., 1994). Likewise, soil pH may be affected by a variety of anthropogenic factors, including dense plant production, which tends to lower soil pH (Ok et al., 2007).

Globally, anthropogenic N_2O emissions are approximately 10 times higher in tea plantations than forests. N_2O gas has been reported to have ozone-depleting potential similar to that of hydrochlorofluorocarbons and is equal to approximately 300 times the global warming potential of CO_2 (Hall & Matson, 1999; Akiyama et al., 2006; Ravishankara et al., 2009; Li et al., 2013). Nitrogen fertilizer also affects soil nitrification significantly. N_2O flow after winter precipitation was higher than during non-rainfall periods (Zou et al., 2014). Chemical nitrogen fertilizers, controlled-release fertilizers, and organic fertilizers are different from the N-release feature (Fernandez et al., 2007). Compared to chemical fertilizers, organic fertilizers have a slow release of N, thus reducing N_2O gas emissions by preventing high emissions (Ball et al., 2004; Burger et al., 2005; Syväsalo et al., 2006; Petersen et al., 2006; Meijide et al., 2007; Deng et al., 2017). In particular, it has been reported that the nitrate-nitrogen (NO_3, -N) pollution in water sources is caused by the over-fertilization of tea plantations, and the main cause of regional water pollution in tea production areas is tea production (Kemmit et al., 2005) and environmental protection measures should be taken in basins where environmental pollution is observed and that the reduction of excessive N implementation has become mandatory (Morita et al., 2002; Kanazawa et al., 2005; Çakmakçı et al., 2015; Zaman et al., 2016).

It is possible to group fertilizers used in the fertilization of tea plants as chemical, organic, and microbial fertilizers. As a result of studies carried out in Turkey, it has been determined that the most appropriate chemical fertilizer to be used for tea is 25: 5: 10 (N P K) composite fertilizer, and the recommended dosage of this fertilizer is 70 kg per ac per year (Anonymous, 2013b). However, prolonged and excessive use of chemical fertilizers cause deterioration of the herbal properties of tea and a decrease in growth (Müftüoğlu et al., 2010). Research has shown that the high rate of nitrogen fertilizer applied to tea plantations increases economic costs and soil acidity, leads to deterioration, underground and surface water pollution, affects the rate of nitrification, and causes high environmental pollution by causing low nitrogen use efficiency (Ruan et al., 2004; Guo et al., 2010; Bagyalakshmi et al., 2012; Yuan et al., 2013; Fang et al., 2014; Çakmakçı et al., 2016). Furthermore, the intensive use of chemical fertilizers in tea production causes a decrease in soil organic matter content and beneficial soil-borne microorganisms, which affect product yield and soil sustainability (Bagyalakshmi et al., 2012).

The high cost of chemical fertilizers, the deep gap between supply and demand, and the negative impact on the environment have led growers to seek alternative strategies. Organic fertilizers have many agricultural and environmental advantages over chemical fertilizers (Bouldin, 1988). Fertilizers from organic sources play a critical role in the short-term availability and long-term protection of soil organic matter (Pang & Letey, 2000). Soil organic matter, nutrients, biological activity, soil structure, and nutrient content increase are important for productivity (Palm et al., 2001). The addition of organic fertilizers in any way helps to protect the soil organic matter and fertility levels of applied fertilizers and enhances yield (Bokhtiar & Sakurai, 2005).

With the organic management system, it is possible to improve the quality characteristics of tea (epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC), and thus to improve the important catechin concentrations depending on the quality of the tea with accompanying benefits to human health as well as the environment (Han et al., 2018). The significantly higher soil pH, organic carbon (C), and total nitrogen (N) contents in organically managed tea agro-ecosystems also increase the benefits of organic fertilizer (Han et al., 2013; Subramanian et al., 2013). The benefits ensured by controlling insect damage and their effects on biodiversity are also manifested by organically managed tea systems (Saikia et al., 2014).

Tea yields in organically managed agricultural ecosystems are typically 10-20% lower than in traditional agricultural ecosystems (Bisen & Singh, 2012; Han et al., 2013; Das et al., 2016). However, in addition to yield, the quality of tea is equally important in countering consumer preferences (Ahmed et al., 2014). Hallmann et al. (2007) and Kazimierczak et al. (2013), who determined that organic teas had a higher concentration of polyphenols than conventional teas, stated that organic tea contains much higher levels of polyphenolic flavonol and tannin. Roussos (2011), Fernandes et al. (2012), and Baranski et al. (2014) emphasized that organic crops and foods have statistically higher antioxidant concentrations than those grown with conventional systems.

The use of integrated organic and inorganic fertilizers also helps to improve the productivity of various crops and improves soil health. It has been reported that the application of organic fertilizers together with chemical fertilizers increases the absorption of N, P, and K in sugar cane compared to chemical fertilizers alone and leads to higher sugar yield (Bokhtiar & Sakurai, 2005). A study carried out to investigate the effect of using organic (vermicompost, pig litter, and Azolla biomass) and inorganic nitrogen sources (urea) on young tea plants (Clone TV22) determined that the nitrogen supply in the YTD mixture (a mixture of NPK recommended for young tea at the rate of 2: 1: 3) through organic and inorganic and only through organic sources produced comparable results in the initial years as that of conventional treatment. Thereafter (+3 years), the integrated treatments showed better results. Higher number and thickness of pruning sticks, pruning litter weight, canopy spread, plucking point density, and yield were recorded in treatment with vermicompost and urea (T2): followed by pig litter and urea (T4) and *Azolla* biomass with urea (T6). Nitrogen, when supplied through only organic sources, the highest record was in vermicompost (T3), followed by pig litter (T5) and *Azolla* biomass (T7). Nitrogen in YTD mixture as 50% through organic and 50% through urea can be recommended for satisfactory growth, development, and yield of young tea in an integrated nutrient management system (Baruah et al., 2019).

The application of organic fertilizers and bio-inoculants in tea plantations can minimize these problems as they are advantageous over chemical fertilizers in increasing soil fertility. Bio-inoculants are environmentally safer and a cost-effective complement to chemical fertilizers. The literature on the use of organic and biological fertilizers on tea crops is very limited (Karthikeyini, 2002). Therefore, the number of studies examining the effects of single or integrated applications of organic fertilizers and bio-inoculants should be increased, especially for the possible reduction of the amount of inorganic fertilizer used in tea production. The use of organic fertilizers and microbial inoculants or bio-fertilizers is a sustainable alternative to the high input of chemical fertilizers used in conventional production systems (Tanwar et al., 2013).

Bio-fertilizers are apparently eco-friendly and non-bulky, farmer-friendly renewable resources with low-cost organic agricultural input. Although *Rhizobium*, blue-green algae (BGA), and *Azolla* are crop-specific, bio-inoculants such as *Azotobacter, Azospirillum*, phosphorus-soluble bacteria (PSB), vesicular arbuscular micorisers (VAM), arbuscular mycorrhizal fungi (AMF) (Songachan, 2012; Sharma & Kayang, 2017) can be accepted as broad-spectrum bio-fertilizers. Since bio-fertilizers are based on renewable energy sources, they are cost-effective, environmentally friendly, and support chemical fertilizers (Baby et al., 2001). Therefore, fertile organism species must be found, folded, and included in soil. Chemical fertilizers can be significantly reduced in fertilization programs with the application of bio-fertilizers (Gebrewold, 2018).

Bio-agents such as *Trichoderma viride, T. harzianum,* and *Bacillus subtilis* are currently used in many tea plantations. Bio-fertilizer is the main source of N and P inputs in tea. Biological N is an attractive and economic resource to counter the N requirements of a tea plantation. As biotic N fixators, *Azotobacter* and *Azospirillum* are most suitable for growing plants such as tea. However, it is clear that bio-fertilizers alone cannot meet all the nitrogen requirements in tea. It is estimated that 20-50 kg N ha^{-1} can be supplied by N fixers. PSBs such as *Bacillus* and *Pseudomonas* spp. play an important role in increasing P nutrient activity for the total development of tea plants. At the same time, they also increase the solubility/usability of P available in tea soil which is in an insoluble form (Baby et al., 2001). There are reports of the application of bacteria that promote the dissolution and uptake of elements/minerals to increase yield without affecting the quality of black tea (Mandal et al., 2007). Çakmakçı et al. (2017) stated that the use of stable formulations developed with plant-promoting bacteria is promising for sustainable tea cultivation.

Conclusion

Although tea was discovered by chance by one of the three legendary emperors of Chinese medicine, it is a medicinal plant that has been loved by people since its discovery. Laboratory and clinical studies have proven that the plant provides protection against skin diseases as well as cancer, Alzheimer's disease, paralysis, diabetes, and Parkinson's prevention and is beneficial for dental health. In addition, the fact that it is the most consumed beverage after water in the world shows how important this plant is. It is known that the tea plant grows in regions where the rainfall regime is regular, and annual rainfall is more than 2000 mm. Such areas are exposed to soil washing due to the intensity of rainfall. Therefore, in addition to the known hazards of chemical fertilizers for human health, they also lead to excessive groundwater pollution. In recent years, scientists have accelerated their studies on organic and microbial fertilizers that are more environmentally friendly and do not harm human health yet enable equal or more yields than chemical fertilizers in order to eliminate this negative situation.

Such studies are very important so that tea, which is so much in our lives, can be consumed safely without posing a threat to human health. In terms of health, transforming a product that has all the important features mentioned above into a product that is harmful to human and environmental health instead of an organically healthy and environmentally friendly product is an important issue for everyone whether they are interested parties or not. Once man loses his health to have more product and more gains; unfortunately, those gains will not restore his lost health.

Conflict of Interest

The authors declare that they have no conflict of interest.

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